Interaction of HLA-DRB1*1501 and TNF-Alpha in a Population-based Case-control Study of Multiple Sclerosis

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Abstract  This study was conducted to determine whether single nucleotide polymorphisms (SNPs) in nine genes (human leukocyte antigen (HLA), T cell receptor beta (TCR), tumor necrosis factor alpha (TNFα), tumor necrosis factor beta (TNFβ), interleukin 7 receptor alpha chain (IL7RA), interleukin 2 receptor alpha chain (IL2RA), myelin basic protein (MBP), vitamin D receptor (VDR)) associated with multiple sclerosis (MS) could be replicated in a population-based sample, and to determine if these associations are modified by presence of HLA DRB1*1501. DNA was available from 722 individuals (223 with MS and 499 controls) who participated in a population-based case-control study. Cases and controls were matched on ancestry, age, gender and geographic area. HLA DRB1*1501 risk allele (T) was confirmed in this population using a genotypic test, controlling for multiple comparisons. Examining the effect of each SNP in the presence or absence of the HLA DRB1*1501 risk allele identified significant associations with TNFα-1031 (rs1799964) among those without the HLA risk allele. No additional interactions were significant in a cases-only analysis. Our results indicate that an interaction between SNPs in TNFα and HLA DRB1*1501 may influence the risk of developing MS.

Keywords  Case-Control Study, Genetic Susceptibility, Multiple Sclerosis, Tumor Necrosis Factor-Alpha, A-DRB1*1501, Gene–Gene Interaction

1. Introduction

Family studies have demonstrated that genetic and non-genetic factors are involved in the etiology of multiple sclerosis (MS) [1-2]. Heritability estimates of up to 76% from twin studies provide the most convincing evidence for a genetic component [3]. Studies of multiplex families, half-siblings and adoptees also provide evidence for a genetic contribution to disease susceptibility [1]. The sole consistent genetic association identified with MS is the human leukocyte antigen (HLA) [4]. Other immune-mediated genes such as immunoglobulin heavy chain (IgH), T-cell receptor (TCR), tumor necrosis factor (TNFα) and myelin basic protein (MBP) have also been associated with MS [5-6]. Classic linkage and affected sib-pair linkage studies have been conducted examining these genes as potential risk factors but the results have been conflicting [1,3].

Genome-wide association studies have also been conducted to localize genomic regions that may harbor novel MS susceptibility genes in unrelated individuals [7-10] and parent-affected child trios [11]. These studies used large sets of polymorphic markers that spanned the genome and identified additional risk loci, supporting the hypothesis that MS is polygenic and genetically heterogeneous. Overall, HLA DRB1*1501, interleukin 2 receptor alpha chain (IL2RA) and interleukin-7 receptor alpha chain (IL7RA) genes have been the most consistently replicated and have shown the strongest associations [6,7].

The aims of this study were to determine whether single nucleotide polymorphisms (SNPs) in nine genes previously reported to be associated with MS could be replicated in a population based sample and to determine if these associations are modified by having the HLA DRB1*1501 risk allele.

2. Materials and Methods

2.1. Ethics Statement

Written informed consent was obtained from all participants. This study was approved by the Institutional Review Boards of the Centers for Disease Control and Prevention, Duke University Medical Center, Michigan Public Health Institute, the Texas Department of State Health Services, and the Cleveland Clinic.

2.2. Study Population
Study participants were individuals with MS and controls who participated in a population-based case-control study conducted from November 2004 to September 2009 [12]. Cases were identified from a prevalence study conducted in four geographic areas ( metro Atlanta, Georgia; Lorain County, Ohio; the cities of Independence and Sugar Creek, Missouri; or the 19-county area surrounding Lubbock, Texas) and were classified as having definite MS according to both the Poser [13] and McDonald [14] criteria by a study neurologist [12]. Controls were recruited by random digit dialing and are matched to cases on sex, age, race and geographic area.

The case-control study enrolled 276 cases and 590 matched controls (n=866) [12]. Because allele frequencies and disease risk differ across racial and ethnic groups [15] we restricted our analysis to Whites to ensure a homogeneous population. Data from 60 non-White individuals were excluded from the analysis as were data from 84 white participants who did not provide a blood sample (24 cases and 60 controls). This resulted in a total of 722 individuals (223 cases and 499 controls) in 212 strata for the analyses presented here. The majority of the strata were either 1:1 or 1:2 matched (62.3%).

2.3. Specimen Collection and DNA Extraction

Each participant was asked to provide a blood sample (3 EDTA Starstedt tubes of whole blood) for genetic analysis. All samples were labeled by a unique identifier so technicians were blinded to case/control status. The Center for Human Genetics at Duke University Medical Center conducted the genotyping. DNA was extracted using the PUREGENE system (QIAGEN, Germantown, MD).

2.4. Candidate Gene and SNP Selection

Seven candidate genes were selected for confirmatory analysis based on published evidence that they play a role in the development of MS: human leukocyte antigen (HLA), T cell receptor beta (TCA receptor β), tumor necrosis factor α (TNF α), tumor necrosis factor β (TNF β), apolipoprotein E (APOE), interleukin 7 receptor alpha chain (IL7RA) and interleukin 2 receptor alpha chain (IL2RA). We selected 13 SNPs (HLA, n=1; TCA receptor β, n=2; TNF α/β, n=6; APOE, n=2; IL7RA, n=1; and IL2RA, n=1) that had been previously reported as associated with MS [4-7, 16].

Two additional candidate genes were selected for exploratory analysis based on biological plausibility: myelin basic protein (MBP) and vitamin D receptor (VDR) [17-20]. For VDR and MBP genes, haplotype tagging SNPs were identified using LDSelect v1.0 (30) based on data from the CEU population in the HapMap project (www.hapmap.org). To minimize redundancy among SNPs in high linkage disequilibrium, single SNPs were selected to represent each haplotype block, as defined by r² > 0.64. SNPs were prioritized based on the potential for biological effect (coding SNPs, 5’/3’ untranslated and regulatory regions), physical position, and allele frequency. Forty-five exploratory SNPs were selected in the VDR and MBP genes (n=22 and n=23 respectively).

2.5. Genotyping

Genotyping was conducted using the Applied Biosystems Taqman platform at the Center for Human Genetics, Duke University Medical Center. Quality control measures applied for all genotyping assays included the genotyping of a series of blinded duplicate samples and Centre d’Etude du Polymorphism Humain (CEPH) controls. To pass quality control, all duplicate samples in an assay had to match 100%.

2.6. Statistical Analysis

Differences in demographic characteristics between cases and controls were analyzed using a chi-square test. Tests for deviations from Hardy-Weinberg equilibrium (HWE) were conducted in controls using PLINK [21]. SNPs that failed HWE (p ≤ 0.005) were excluded from further analyses. We controlled for population stratification using questionnaire data regarding ancestry information. Participants were asked to list up to three countries where their maternal ancestors came from and up to three countries where their paternal ancestors came from. Responses were grouped into geographic areas (Western Europe, Eastern Europe, Mediterranean, Scandinavia, US/Canada/Don’t know). Each participant’s proportion of ancestry from each geographic region was entered as a covariate in the analysis. Education was dichotomized as high school education or less versus post high school education.

Matched strata contained between 1-4 cases and 1 – 12 controls. Genotype was coded as a quantitative variable that counts the number of minor alleles for each individual. HLA DRB1*1501 risk was defined as having at least one copy of the risk allele (T). Because cases and controls were matched, data were analyzed using conditional logistic regression. For genetic main effects, we fit models that included SNP genotype while adjusting for education and ancestry. For analyses of interaction, we fit models that included SNP genotype, HLA DRB1*1501 risk, and a multiplicative SNP x HLA DRB1*1501 risk interaction term, while again adjusting for education and ancestry. An unmatched cases-only analysis was conducted using unconditional logistic regression with HLA DRB1*1501 risk as the outcome and each SNP genotype as a predictor; also included in each model were education and ancestry. Assuming independence (i.e., no linkage disequilibrium) between a SNP and HLA DRB1*1501, an association found among cases would be evidence for gene x gene interaction. SNPs in TNF α and TNF β were not included in this analysis because they are in linkage disequilibrium (LD) with HLA DRB1*1501. Since this analysis makes an extra assumption about linkage disequilibrium, it may be more powerful than the matched analysis [22].

Analyses involving the 13 SNPs previously found
associated with MS were considered as confirmatory, and were not adjusted for multiple comparisons. Analyses involving the 22 VDR and 23 MBP SNPs are considered exploratory; for these SNPs, adjustments for multiple comparisons were made using the Bonferroni correction (by adjusting the p value cutoff to correspond to a size of 0.05/45). SAS, version 9.2 (SAS Institute, Cary, NC, USA) was used for data management and statistical analysis.

### Table 1. Characteristics of the study population

| Characteristic          | Cases (n=223) | Controls (n=499) | χ² (p value) |
|-------------------------|--------------|-----------------|-------------|
| State of Residence      |              |                 |             |
| Georgia                 | 72 (32.3)    | 151 (30.2)      | 1.13 (0.77) |
| Missouri                | 18 (8.1)     | 51 (10.2)       |             |
| Ohio                    | 75 (33.6)    | 161 (32.3)      |             |
| Texas                   | 58 (26.0)    | 136 (27.3)      |             |
| Sex (n, %)              |              |                 |             |
| Female                  | 179 (80.3)   | 393 (78.8)      | 0.21 (0.64) |
| Male                    | 44 (19.7)    | 106 (21.2)      |             |
| Age (years) (n, %)      |              |                 |             |
| < 39                    | 2 (0.9)      | 34 (6.8)        |             |
| 40 – 49                 | 57 (25.6)    | 106 (21.2)      | 12.96 (0.01) |
| 50 – 59                 | 99 (44.4)    | 196 (38.9)      |             |
| 60 – 69                 | 34 (15.2)    | 127 (25.5)      |             |
| 70+                     | 11 (4.9)     | 38 (7.6)        |             |
| Educational Status      |              |                 |             |
| Attained (n, %)         |              |                 |             |
| < High School           |             |                 |             |
| High School graduate    | 8 (3.6)      | 19 (3.8)        | 2.6 (0.47)  |
| Some                    | 48 (21.5)    | 84 (16.8)       |             |
| College/Technical School| 69 (30.9)    | 154 (30.9)      |             |
| College Graduate        | 98 (44.0)    | 242 (48.5)      |             |
| Number of missing genotypes |         |                 |             |
| 0                       | 168 (75.3)   | 409 (82.0)      | 9.14 (0.10) |
| 1                       | 38 (17.0)    | 65 (13.0)       |             |
| 2                       | 4 (1.8)      | 13 (2.6)        |             |
| 3                       | 3 (1.4)      | 4 (0.8)         |             |
| 4                       | 4 (1.8)      | 2 (0.4)         |             |
| >5                      | 6 (2.7)      | 6 (1.2)         |             |

*a* At time of interview

### Table 2. Characteristics of the individuals with MS

| Type of MS              | Cases (n=223) | Controls (n=499) | Mean | Standard deviation |
|-------------------------|--------------|-----------------|------|--------------------|
| Relapsing remitting     | 160 (71.8)   | 21 (9.4)        |      |                    |
| Primary progressive     | 21 (9.4)     |                 |      |                    |
| Secondary progressive   | 5 (2.2)      |                 |      |                    |
| Don’t know              | 37 (16.6)    |                 |      |                    |
| Age at onset of symptoms|              |                 | 32.7 | 9.4                |
| Duration of MS since symptom onset*| | | | |
| < 5 years               | 8 (3.6)      |                 |      |                    |
| 5-10 years              | 38 (17.1)    |                 |      |                    |
| > 10 years              | 176 (79.3)   |                 |      |                    |
| Age at diagnosis        |              |                 | 37.2 | 8.94               |
| Duration of MS since diagnosis*| | | | |
| < 5 years               | 15 (6.8)     |                 |      |                    |
| 5-10 years              | 69 (31.1)    |                 |      |                    |
| > 10 years              | 138 (62.1)   |                 |      |                    |

*a* One individual did not know their date of symptom onset and was excluded.

*b* Four individuals did not know their date of diagnosis and were excluded.

The chromosomal location, gene name, SNP reference sequence (rs) number, minor allele and minor allele frequencies for each SNP included in this analysis are shown in Table 3. The minor allele frequencies in this population varied dramatically across SNPs and ranged from 0.02 to 0.49. None of the SNPs selected failed HWE. Two confirmatory SNPs were significantly associated with MS at the 0.05 level: VDR β (rs909253) and HLA DRB1*1501 (rs3135388). Five exploratory SNPs in the MBP gene (rs9676113, rs595997, rs2974260, rs12967023, and rs8096433) were significant at the 0.05 level, but did not remain significant after correction for multiple comparisons.

We also examined the effect of each SNP in the presence or absence of the HLA DRB1*1501 risk allele (T) (Table 4). One of the confirmatory SNPs was associated with MS among participants with no HLA DRB1*1501 risk allele (TNFα rs1799964, odds ratio per copy of the minor allele (OR) 1.77, 95% confidence interval (CI) 1.17, 2.69). Two exploratory SNPs were associated with MS among participants with no HLA DRB1*1501 risk allele: VDR rs2189480 (OR 1.54, 95% CI 1.07, 2.23) and MBP rs9676113 (OR 0.48, 95% CI 0.30, 0.76). No SNPs were associated with MS among participants with the HLA DRB1*1501 risk allele. In the cases-only analysis for interaction between HLA DRB1*1501 risk and the other loci (Table 4), two SNPs were significant at p≤ 0.05 level (VDR rs2189480 and MBP rs3794848). However, none of the exploratory interactions were statistically significant after adjustment for multiple comparisons. Finally, when we repeated our analyses without adjusting for ancestry, we found similar p-values and effect estimates (data not shown).

### 3. Results

Most study participants were female, ≥50 years old and had attended or graduated from college (Table 1). The female-to-male ratio was 4.09:1. The majority (80%) of study participants had complete genotype information, while approximately 6% were missing genotype information for two or more SNPs. Nearly three-quarters of the cases reported having relapsing remitting MS (Table 2). Most reported the onset of symptoms in their early thirties and were diagnosed in their late thirties, and had had MS for over 10 years.
Table 3. Genotyping information including gene and SNP identification, minor allele, minor allele frequencies (MAF), testing for Hardy-Weinberg equilibrium (HWE) and genotypic trend test results

| Chr | Gene          | SNP                  | Minor Allele | MAF   | HWE p value  | p value     |
|-----|---------------|----------------------|--------------|-------|--------------|-------------|
| 5   | IL7RA         | rs6897932            | A            | 0.25  | 0.2826       | 0.8375      |
|     |               | rs1799964            | C            | 0.21  | 0.1802       | 0.7952      |
|     |               | rs1800629            | A            | 0.15  | 1            | 0.2243      |
| 6   | TNF α         | rs3093671            | A            | 0.02  | 0.2154       | 0.3277      |
|     | TNF β         | rs769178             | T            | 0.08  | 0.4088       | 0.2501      |
|     | HLA DRB1*1501 | rs769177             | T            | 0.02  | 1            | 0.2170      |
|     |               | rs909253             | A            | 0.50  | 1            | 0.0176      |
|     |               | rs3135388            | T            | 0.21  | 0.3126       | < 0.0001    |
| 7   | TCA receptor β| rs17133575           | G            | 0.16  | 0.4811       | 0.4994      |
|     |               | rs17243              | G            | 0.43  | 0.6453       | 0.7979      |
| 10  | IL2RA         | rs2104286            | C            | 0.25  | 0.2873       | 0.5194      |
|     |               | rs5795128            | A            | 0.43  | 0.4637       | 0.8362      |
|     |               | rs2249098            | A            | 0.47  | 1            | 0.3754      |
|     |               | rs2239182            | T            | 0.49  | 0.7872       | 0.2035      |
|     |               | rs2107301            | A            | 0.26  | 0.0677       | 0.2594      |
|     |               | rs1540339            | T            | 0.36  | 0.9205       | 0.2017      |
|     |               | rs2239179            | C            | 0.44  | 0.855        | 0.8074      |
|     |               | rs2189480            | T            | 0.38  | 1            | 0.1975      |
|     |               | rs3819545            | G            | 0.38  | 0.4992       | 0.6966      |
| 12  | VDR           | rs3782905            | G            | 0.34  | 0.618        | 0.3914      |
|     |               | rs2239186            | G            | 0.20  | 0.7777       | 0.6424      |
|     |               | rs2228570            | T            | 0.39  | 0.5128       | 0.7020      |
|     |               | rs2254210            | A            | 0.38  | 0.5668       | 0.7964      |
|     |               | rs2238136            | T            | 0.25  | 0.8098       | 0.9835      |
|     |               | rs2853564            | C            | 0.40  | 0.4481       | 0.2479      |
|     |               | rs760648             | T            | 0.42  | 0.9266       | 0.4309      |
|     |               | rs11168287           | G            | 0.49  | 0.6517       | 0.6395      |
| 18  | MBP           | rs4328262            | G            | 0.41  | 0.78         | 0.7742      |
|     |               | rs4237855            | G            | 0.36  | 0.6267       | 0.2850      |
|     |               | rs7136534            | T            | 0.25  | 0.1503       | 0.5520      |
|     |               | rs7299460            | A            | 0.30  | 0.0645       | 0.8243      |
|     |               | rs4760658            | G            | 0.35  | 1            | 0.9057      |
|     |               | rs4516035            | C            | 0.44  | 0.5222       | 0.5843      |
|     |               | rs17026              | C            | 0.29  | 0.1238       | 0.3912      |
|     |               | rs470724             | T            | 0.31  | 0.2421       | 0.2491      |
|     |               | rs470350             | T            | 0.50  | 0.1769       | 0.6393      |
|     |               | rs6767112            | G            | 0.26  | 1            | 0.0073      |
|     |               | rs1661054            | A            | 0.46  | 0.2771       | 0.9498      |
|     |               | rs4890785            | T            | 0.22  | 1            | 0.1244      |
|     |               | rs1661755            | A            | 0.40  | 0.3487       | 0.5430      |
|     |               | rs9675994            | T            | 0.23  | 0.7057       | 0.7465      |
|     |               | rs8090438            | T            | 0.43  | 0.8546       | 0.8376      |
|     |               | rs8094402            | G            | 0.34  | 0.1291       | 0.4380      |
|     |               | rs12456341           | G            | 0.38  | 0.5654       | 0.7153      |
|     |               | rs17576751           | T            | 0.39  | 1            | 0.9220      |
|     |               | rs3794848            | A            | 0.30  | 0.6664       | 0.1911      |
|     |               | rs4890875            | G            | 0.33  | 0.222        | 0.9386      |
|     |               | rs595997             | G            | 0.22  | 0.8008       | 0.0433      |
|     |               | rs2974260            | T            | 0.43  | 0.1131       | 0.0375      |
|     |               | rs2051344            | A            | 0.31  | 0.0477       | 0.9369      |
|     |               | rs470681             | C            | 0.26  | 0.8202       | 0.1010      |
|     |               | rs12967023           | A            | 0.30  | 0.2205       | 0.0040      |
|     |               | rs4890788            | A            | 0.36  | 0.6252       | 0.7949      |
|     |               | rs7232502            | A            | 0.39  | 0.0128       | 0.1068      |
|     |               | rs8096433            | A            | 0.44  | 0.8536       | 0.0239      |
|     |               | rs17660901           | G            | 0.34  | 0.6911       | 0.3750      |
|     |               | rs429358             | C            | 0.14  | 1            | 0.8762      |
| 19  | APOE          | rs7412               | T            | 0.08  | 0.7709       | 0.8794      |

* IL7R= Interleukin-7 receptor; TNFα = Tumor necrosis factor alpha; TNFβ = Tumor necrosis factor beta; IL2RA= Interleukin-2 receptor alpha; TCA-β = T cell antigen receptor beta; VDR= Vitamin D receptor; MBP= myelin basic protein; APOE = Apolipoprotein E;  
* in controls  * adjusted for level of education (≤HS graduate vs. post-HS education) and ancestry
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Table 4. *P*-values from tests of association between individual SNPs and HLA-DRB1*1501 risk allele among the entire study population and cases-only

| Chromosome | Gene                | SNP                | Entire Study Population | Case Only |
|------------|---------------------|--------------------|-------------------------|-----------|
|            |                     |                    | HLA-                  | HLA+      |           |
| 5          | IL7RA               | rs6897932          | 0.4836                 | 0.2314    | 0.9272    |
|            |                     | rs1799964          | 0.0066                 | 0.2114    | na        |
|            |                     | rs1800629          | 0.6871                 | 0.9739    | na        |
|            |                     | rs3093671          | 0.8454                 | 0.2170    | na        |
|            |                     | rs769178           | 0.1747                 | 0.7644    | na        |
|            |                     | rs769177           | 0.1642                 | 0.7722    | na        |
|            |                     | rs909253           | 0.0888                 | 0.8057    | na        |
| 6          | TNF α               | rs17133575         | 0.2956                 | 0.6961    | 0.0886    |
|            |                     | rs17243            | 0.8861                 | 0.6881    | 0.3510    |
|            | TNF β               | rs2104286          | 0.4797                 | 0.3509    | 0.2122    |
|            |                     | rs7975128          | 0.9328                 | 0.7525    | 0.2110    |
|            |                     | rs2248098          | 0.1923                 | 0.9697    | 0.8575    |
|            |                     | rs2239182          | 0.2709                 | 0.6560    | 0.9620    |
|            |                     | rs2107301          | 0.1360                 | 0.6497    | 0.5112    |
|            |                     | rs1540339          | 0.0884                 | 0.7865    | 0.1406    |
|            |                     | rs2239179          | 0.9898                 | 0.7155    | 0.5786    |
|            |                     | rs2189480          | 0.0197                 | 0.9669    | 0.0229    |
|            |                     | rs3819545          | 0.4513                 | 0.9154    | 0.4739    |
|            |                     | rs3782905          | 0.3676                 | 0.9466    | 0.6754    |
|            |                     | rs2239186          | 0.2952                 | 0.9836    | 0.3504    |
|            | TCA receptor β      | rs2228570          | 0.8150                 | 0.7191    | 0.7562    |
|            |                     | rs2254210          | 0.6710                 | 0.6681    | 0.4874    |
|            |                     | rs2238136          | 0.6522                 | 0.8489    | 0.7503    |
|            |                     | rs2833564          | 0.6881                 | 0.3770    | 0.9648    |
|            |                     | rs4760648          | 0.7002                 | 0.5703    | 0.3755    |
|            |                     | rs11168287         | 0.3992                 | 0.9917    | 0.9166    |
|            |                     | rs4322862          | 0.8502                 | 0.6609    | 0.3199    |
|            |                     | rs4227855          | 0.6404                 | 0.1878    | 0.4151    |
|            |                     | rs7136534          | 0.5978                 | 0.7833    | 0.4731    |
|            |                     | rs7299460          | 0.8531                 | 0.9014    | 0.1911    |
|            |                     | rs4760658          | 0.5264                 | 0.5559    | 0.5192    |
|            |                     | rs4516035          | 0.3622                 | 0.9808    | 0.6188    |
|            | VDR                 | rs17026            | 0.5056                 | 0.3909    | 0.6979    |
|            |                     | rs470724           | 0.3036                 | 0.1167    | 0.4938    |
|            |                     | rs470550           | 0.6057                 | 0.5359    | 0.9642    |
|            |                     | rs9676113          | 0.0022                 | 0.2961    | 0.0652    |
|            |                     | rs11661054         | 0.7590                 | 0.8274    | 0.9543    |
|            |                     | rs4890785          | 0.3192                 | 0.3699    | 0.6510    |
|            |                     | rs11661755         | 0.7217                 | 0.9191    | 0.7300    |
|            |                     | rs9675994          | 0.1899                 | 0.3787    | 0.1337    |
|            |                     | rs8090438          | 0.7118                 | 0.8427    | 0.6176    |
|            |                     | rs8094402          | 0.6018                 | 0.9749    | 0.6815    |
|            |                     | rs12456341         | 0.7136                 | 0.4851    | 0.8624    |
|            |                     | rs17576751         | 0.9898                 | 0.5746    | 0.6133    |
|            |                     | rs3794848          | 0.0538                 | 0.9609    | 0.0384    |
|            |                     | rs4890875          | 0.1850                 | 0.0906    | 0.0798    |
|            |                     | rs595997           | 0.0919                 | 0.4793    | 0.7481    |
|            |                     | rs2974260          | 0.5131                 | 0.0524    | 0.1300    |
|            |                     | rs2051344          | 0.3225                 | 0.3575    | 0.3576    |
|            |                     | rs470681           | 0.1942                 | 0.5718    | 0.5790    |
|            |                     | rs12967023         | 0.1094                 | 0.0514    | 0.3978    |
|            |                     | rs4890788          | 0.3221                 | 0.4180    | 0.4621    |
|            |                     | rs7232502          | 0.4015                 | 0.4850    | 0.5410    |
|            |                     | rs8096433          | 0.1877                 | 0.1294    | 0.3215    |
|            |                     | rs17660901         | 0.7039                 | 0.3997    | 0.6958    |
|            | MBP                 | rs429358           | 0.6850                 | 0.6695    | 0.4511    |
|            |                     | rs7412             | 0.4733                 | 0.7039    | 0.4345    |
| 19         | APOE                | rs17473            | 0.5131                 | 0.0524    | 0.1300    |

* IL7R= Interleukin-7 receptor; TNFα=Tumor necrosis factor alpha; TNFβ=Tumor necrosis factor beta; IL2RA=Interleukin-2 receptor alpha; TCA-β=T cell antigen receptor beta; VDR= Vitamin D receptor; MBP=myelin basic protein; APOE=Apolipoprotein E;

1 adjusted for level of education (≤HS graduate vs. post-HS education) and ancestry na: not calculated because SNPs are in LD with HLA DRB1*1501
4. Discussion

In this population based case-control study, we examined the association of 58 SNPs in nine previously reported candidate genes and having MS. We confirmed an association with two SNPs previously identified in the literature (TNF β and HLA DRB1*1501) and found an association with a TNF α SNP that was limited to individuals who lack the HLA DRB1*1501 risk allele. Because we only examined statistical interaction, it is impossible to determine if this is a true biological interaction, or simply that the smaller TNF α effect is easier to detect among persons without the HLA DRB1*1501 risk allele. If this is the case, it may be reasonable to accept the nominal p-value for this “interaction” without adjustment for multiple comparisons.

Further, because there is linkage disequilibrium between TNF α and the HLA region, we cannot rule out the possibility that the TNF α effect we see is actually the result of different risk allele in the HLA region.

Associations between polymorphisms in the TNF-α -1031 T/C (rs1799964) polymorphism and immune-mediated diseases such as Crohn’s disease, rheumatoid arthritis and Graves’ disease have been reported [23-25]. Studies examining the association between TNF α and MS have been conflicting, and have usually focused on the TNF α -308 polymorphism (rs1800629) [26-34]. A study in men of Russian and Tatar ethnic origin found that susceptibility to MS was influenced by the HLA DRB1*1501 SNP rs909253 and TNF α SNP rs1800629 [35]. Another case-control study conducted with an Iranian population reported an interaction between HLA-DRB1*1501 and TNF α SNP rs1800629, with a 7-fold increased odds of MS among individuals with at least one copy of the risk allele [26]. We also found an interaction between HLA DRB1*1501 and TNF-α but with at a different locus (rs1799964). However, rs1799964 is in strong LD with rs1800629 in our study population (D’ = 1), and the haplotype having the minor allele at both loci is completely absent in our study population. Strong LD in the region suggests that different SNPs in TNF α may be associated with MS in different populations. Taken together, these studies strongly suggest a causal SNP resides somewhere in this region.

Tumor necrosis factor (TNF) is an important pro-inflammatory cytokine which plays a role in the regulation of cell differentiation, proliferation and death, as well as in inflammation, innate and adaptive immune responses [23]. The exact role of the TNF superfamily is unclear although they are the principal mediators in immune defense, inflammation and the development and maintenance of the immune system [36]. TNF-α is a pleiotropic cytokine with wide-ranging actions on the immune system and is an important mediator in immune-mediated inflammatory disease states, including multiple sclerosis [36]. The presence of DNA sequence variations in the regulatory region might interfere with transcription of TNF gene, influencing the circulating level of TNF and consequently susceptibility to autoimmune and other neurodegenerative diseases [23]. In addition, clinical observations have implicated dysregulation of the TNF-α pathway in susceptibility to MS. In a clinical trial, patients who received leneroxcept, a p55 tumor necrosis factor receptor fusion protein, had increased relapse activity compared to patients who received a placebo [37]. A clinical trial of an anti-TNF antibody was associated with increased disease activity, and demyelinating disease has emerged in persons treated with monoclonal antibodies to TNF-α for indications such as inflammatory bowel disease and rheumatoid arthritis [38-39]. It is unclear whether the interaction between HLA and TNF α is biological or merely that the weaker effect of TNF α is more easily detected when the HLA risk allele is absent. However, HLA haplotypes are known to affect the synthesis of TNF-α, with some haplotypes increasing TNF α synthesis and others decreasing TNF α synthesis [40-41].

We found five SNPs in the myelin basic protein gene that were associated with MS. One of these (rs9676113) had a p-value of 0.0073 when testing its main effect and a p-value of 0.0022 among persons with no HLA risk. While these results were not significant after adjusting for multiple comparisons, the small p-values we observed suggest that association between SNPs in MBP (especially rs9676113) and MS warrants further research.

Since MS predominantly affects individuals who are white and female, most research conducted in this field has focused specifically on this population. A limitation of our study was that we identified very few individuals who were non-white and thus could not evaluate whether the identified association was also present across populations. It is unknown if the genetic contributions to this disease identified in this study will be confirmed in other racial/ethnic groups. Another limitation in this study was that we looked at common variations in the selected candidate genes. Our results do not rule out the possibility that rare variants may also contribute in these and other genes.

5. Conclusions

Research has shown that a single locus model for MS is unlikely. Our work adds to the emerging literature regarding gene-gene interactions, particularly those related to immune regulation. Future efforts should focus on evaluating this finding in both population-based and family studies, and on evaluating this finding in non-White populations. Consistent replication of these results in different populations would support causality. Evaluation of the potential effects of an interaction between TNF-α and HLA, and the possible role of MBP, may offer insights into the mechanisms of disease as well.

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Disclaimer

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