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ORIGINAL ARTICLE

# Replication study of GWAS risk loci in Greek multiple sclerosis patients

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## Abstract

**Objectives** To validate in an ethnically homogeneous Greek multiple sclerosis (MS) cohort, genetic risk factors for the disease, identified through a number of previous multi-ethnic genome-wide association studies (GWAS).

**Methods** A total of 1228 MS cases and 1014 controls were recruited in the study, from 3 MS centers in Greece. We genotyped 35 susceptibility SNPs that emerged from previous GWAS or meta-analyses of GWAS. Allele and genotype single locus regression analysis, adjusted for gender and site, was performed. Permutation testing was applied to all analyses.

**Results** Six polymorphisms reached statistical significance (permutation $p$ value $< 0.05$). In particular, rs2760524 of LOC105371664, near RGS1 (permutation $p$ value 0.001), rs3129889 of HLA-DRA, near HLA-DRB1 (permutation $p$ value $<$ 1.00e-04), rs1738074 of TAGAP (permutation $p$ value 0.007), rs703842 of METTL1/CYP27B1 (permutation $p$ value 0.008), rs9596270 of DLEU1 (permutation $p$ value $<$ 1.00e-04), and rs17445836 of LincRNA, near IRF8 (permutation $p$ value 0.001) were identified as susceptibility risk factors in our group.

**Conclusion** The current study replicated a number of GWAS susceptibility SNPs, which implies that some similarities between the examined Greek population and the MS genetic architecture of the GWAS populations do exist.

**Keywords** Multiple sclerosis · SNPs · Susceptibility · Risk factors · Genetic architecture · Genetic variants

## Introduction

Multiple sclerosis (MS) is a an immune-mediated disease of the central nervous system (CNS) characterized by focal or diffuse inflammation, demyelination, axonal loss, and neurodegeneration [1].MS susceptibility is considered to stem from an interplay between exposure to environmental factors, epigenetic alternations, and an individual's genetic background [2–5]. Each genetic factor is expected to only have a modest contribution to disease risk, either independently or by interacting with other polymorphic sites or environmental factors [6, 7].

On-going research around MS reveals new subtypes, classifications, and manifestations of the disease [8, 9]. Apart from the few therapeutic agents already in use for MS management, new potential targets for MS therapy can be further identified through recent studies [10]. Moreover, the genetic architecture of adverse drug reactions from MS therapy has already been reported [11]. Consequently, taking into consideration the complexity of making a therapeutic decision, the qualitative and quantitative characterization of the genetic architecture of

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MS may prove to be of great use for clinicians in the immediate future.

The genetic architecture of MS has been investigated with candidate gene approaches, linkage and genome-wide linkage analyses, genome-wide association studies (GWAS), meta-analyses of GWAS, and hypothesis-driven studies with extensive genome coverage [10, 12]. These efforts have yielded, till now, more than 110 genetic risk factors of MS [13], and it is generally expected that the genetic loci will soon be over 400 [14]. However, these variants, along with HLA loci, can only account for about 27% of the apparent MS heritability [15].

Genetic studies including GWAS have yielded inconsistent findings, with limited replication of genetic variants in other studies or GWAS. Genetic heterogeneity among different ethnic groups may be one reason for the low reproducibility of MS loci in different studies [10]. It has been shown that homogeneous ethnic populations may harbor disease-specific pathogenic variations in different rates compared to other populations [16] and thus, MS genetic architecture may be different among various ethnic groups. It is, therefore, important for genetic polymorphisms identified in GWAS or meta-analyses of GWAS to be validated in different ethnic groups. In fact, none of the previous 14 MS GWASs has included Greek MS patients [10].

In the present national study, we genotyped 35 single nucleotide polymorphisms (SNPs) in a relatively large number of ethnically homogeneous Greek MS patients. SNPs were selected from a previous meta-analysis of 7 GWAS, which highlighted SNPs with either genome-wide significance or suggestive evidence of association with MS [17]. Validation of these susceptibility SNPs in a Greek population may imply that similarities with the MS genetic architecture and the rest of the GWAS populations exist.

## Patients and methods

### Study population

Greek MS patients and healthy controls were recruited from three MS centers in Greece: the University Hospital of Larissa in Central Greece, the Eginition Hospital of the University of Athens Medical School in Athens, and the AHEPA Hospital of Aristotle University in Thessaloniki in North Greece. In total, 1228 MS cases and 1014 controls were recruited for the study. Patients were included in the study if they had clinically definite MS according to the 2005 revised McDonald criteria. Cases with primary progressive MS were excluded, so that our cohort will be phenotypically homogenous. The control group consisted of healthy volunteers without any neurological disease from the same regions, and was matched with cases for age and gender. The study protocol was approved by the ethics committees of University Hospital of Larissa, of the Eginition Hospital of the University of Athens Medical School in Athens, and of the AHEPA Hospital of Aristotle University in Thessaloniki in North Greece. Written informed consent was obtained from all participants included in the study.

### Isolation of DNA, SNP selection, and genotyping

Genomic DNA was extracted from peripheral blood using the standard methods as previously described [18].

Thirty-five SNPs that reached a genome-wide significance or had suggestive evidence of association with MS were selected from a previous meta-analysis of 7 GWAS [17]. These SNPs, along with the details about their respective gene, chromosome position, and minor allele frequency in Hapmap TSI population (Toscans in Italy), which is considered genetically closer to the Greek population [19], are presented in Table 1.

TaqMan allelic discrimination assays were used to genotype SNPs, by obtaining specific assays for each SNP and then performing allelic discrimination assays on the ABI 7900HT Real-Time System (Applied Biosystems, Foster City, CA, USA). Genotype call rates were above 95%. However, three SNPs (rs170934, rs4680534, and rs1250542) failed to be sufficiently genotyped and were consequently not included in the analysis. As a quality control, a random 15% of samples were re-genotyped and no inconsistencies were observed.

### Statistical analysis

Hardy–Weinberg equilibrium (HWE) was evaluated with the Pearson's chi square test. Power calculation was performed by means of the CaTS software (http://www.sph.umich.edu/csg/abecasis/cats/). Allele and genotype frequencies between MS patients and controls were calculated using the Fisher's chi square test. Allele single locus regression analysis (adjusted by gender and site) was performed using the SHEsis Plus software (http://shesisplus.bio-x.cn/SHEsis.html) [20]. A total of 10,000 permutations were carried out in order to correct the $p$ value, and values smaller than 0.05 were considered as statistically significant.

## Results

The clinical characteristics of MS and control groups are presented in Table 2. Females represented 63% of our MS patients with a female: male ratio of 1.7:1. The mean age of the MS cohort was 40 years.

The distribution of SNPs' genotypes in MS patients and controls and the respective HWE among controls are presented in Supplementary Table 1. Rs9260489 and rs12722489 presented a deviation from the HWE ($p < 0.001$) and were therefore excluded from further analysis. According to power

**Table 1** Characteristics of the studied SNPs

| | SNP | Gene | Chr | Chr location | Chr position | SNP function | Risk allele | MAF (TSI/CEU) | MAF in our control group |
|---|---|---|---|---|---|---|---|---|---|
| 1 | rs2300747 | CD58 | 1 | 1p13.1 | 116,561,593 | Intron variant | G | 0.131 (TSI) | 0.092 |
| 2 | rs2760524 | LOC105371664, near RGS1 | 1 | 1q31.2 | 192,561,418 | Intron variant | A | 0.170 (TSI) | 0.210 |
| 3 | rs12122721 | KIF21B | 1 | 1q32.1 | 201,015,352 | Intron variant | A | 0.278 (TSI) | 0.217 |
| 4 | rs6718520 | LOC100506047 | 2 | 2p21 | 43,098,432 | Intron variant | A | 0.403 (TSI) | 0.449 |
| 5 | rs7592330 | PLEK/FBXO48 | 2 | 2p13.3 | 68,419,651 | Regulatory region variant | G | 0.435 (CEU) | 0.353 |
| 6 | rs170934 | near EOMES | 3 | 3p24.1 | 28,037,594 | Intergenic variant | C | 0.435 (TSI) | – |
| 7 | rs1132200 | TMEM39A | 3 | 3q13.33 | 119,431,989 | Missense variant (Ala487Thr) | T | 0.222 (TSI) | 0.117 |
| 8 | rs4680534 | IL12A-AS1 | 3 | 3q25.33 | 159,981,157 | Intron variant | C | 0.258 (CEU) | – |
| 9 | rs2681424 | ILDR1 | 3 | 3q13.33 | 122,050,675 | Upstream gene variant | T | 0.296 (CEU) | 0.488 |
| 10 | rs6897932 | IL7RA | 5 | 5p13.2 | 35,874,473 | Missense variant (Thr244Ile) | T | 0.222 (TSI) | 0.203 |
| 11 | rs2546890 | LOC285626, near IL12B | 5 | 5q33.3 | 159,332,892 | Non coding transcript exon variant (ncRNA) | A | 0.415 (TSI) | 0.420 |
| 12 | rs10866713 | near IL12B | 5 | 5q33.3 | 159,491,886 | Intergenic variant | A | 0.244 (TSI) | 0.206 |
| 13 | rs4613763 | near PTGER4 | 5 | 5p13.1 | 40,392,626 | Regulatory region variant | C | 0.102 (TSI) | 0.064 |
| 14 | rs9260489 | HLA-W, near HLA-A | 6 | 6p22.1 | 29,952,555 | Upstream gene variant | T | 0.384 (CEU) | 0.53 |
| 15 | rs3129889 | HLA-DRA, near HLA-DRB1 | 6 | 6p21.32 | 32,445,768 | Downstream gene variant | G | 0.080 (TSI) | 0.03 |
| 16 | rs9277535 | HLA-DPB1 | 6 | 6p21.32 | 33,087,084 | 3 prime UTR variant | G | 0.295 (TSI) | 0.217 |
| 17 | rs1738074 | TAGAP | 6 | 6q25.3 | 159,044,945 | 5 prime UTR variant | T | 0.409 (TSI) | 0.374 |
| 18 | rs2150702 | MLANA/KIAA2026 | 9 | 9p24.1 | 5,893,861 | Intron variant | A | 0.471 (TSI) | 0.491 |
| 19 | rs12722489 | IL2RA | 10 | 10p15.1 | 6,060,049 | Intron variant | T | 0.142 (TSI) | 0.094 |
| 20 | rs7089861 | near IL2RA | 10 | 10p15.1 | 6,068,363 | Downstream gene variant | G | 0.367 (CEU) | 0.286 |
| 21 | rs1250542 | ZMIZ1 | 10 | 10q22.3 | 79,274,913 | Intron variant | A | 0.340 (CEU) | – |
| 22 | rs17824933 | CD6 | 11 | 10q12.2 | 60,993,140 | Intron variant | G | 0.195 (CEU) | 0.274 |
| 23 | rs1800693 | TNFRSF1A | 12 | 12p13.31 | 6,330,843 | Non coding transcript exon variant | C | 0.392 (TSI) | 0.447 |
| 24 | rs703842 | METTL1/ CYP27B1 | 12 | 12q14.1 | 57,768,956 | Missense variant (Ile11Thr) for CYP27B1 gene, 3 prime UTR variant for METTL1 gene | G | 0.259 (TSI) | 0.267 |
| 25 | rs1790100 | MPHOSPH9 | 12 | 12q24.31 | 123,172,178 | Intron variant | G | 0.200 (CEU) | 0.282 |
| 26 | rs9596270 | DLEU1 | 13 | 13q14.2 | 50,268,304 | Intron variant | C | 0.036 (CEU) | 0.129 |
| 27 | rs2119704 | lincRNA, near GALC | 14 | 14q31.3 | 88,021,345 | Intron variant | A | 0.099 (TSI) | 0.066 |
| 28 | rs17445836 | lincRNA, near IRF8 | 16 | 16q24.1 | 85,984,057 | Intron variant | A | 0.267 (CEU) | 0.214 |
| 29 | rs12708716 | CLEC16A | 16 | 16p13.13 | 11,086,016 | Intron variant | G | 0.438 (TSI) | 0.410 |
| 30 | rs7191700 | RMI2 | 16 | 16p13.13 | 11,312,946 | Intron variant | T | 0.335 (TSI) | 0.302 |
| 31 | rs744166 | STAT3 | 17 | 17q21.2 | 42,362,183 | Intron variant | G | 0.415 (TSI) | 0.383 |
| 32 | rs2293152 | STAT3 | 17 | 17q21.2 | 42,329,511 | Intron variant | G | 0.388 (CEU) | 0.588 |
| 33 | rs8070463 | TBKBP1 | 17 | 17q21.32 | 47,691,470 | Upstream gene variant | C | 0.494 (TSI) | 0.466 |
| 34 | rs10411936 | EPS15L1 | 19 | 19p13.11 | 16,437,564 | Intron variant | A | 0.222 (TSI) | 0.197 |
| 35 | rs6074022 | CD40 | 20 | 20q13.12 | 46,111,557 | Regulatory region variant | C | 0.318 (TSI) | 0.314 |

Information regarding location of SNPs in genes and chromosome positions according to GRCh38.p2 (Annotation Release:107). SNP function according to Ensembl (GRCh38.p5). Minor allele frequencies (MAF) in TSI population (when available; otherwise in CEU as indicated) according to Hapmap (Data Rel 27 phaseII + III, Feb09, on NCBI assembly, dbSNP b126)

**Table 2** Demographic and clinical characteristics of study participants

| | Total patients | Controls |
|---|---|---|
| *n* | 1228 | 1014 |
| Female, *n* (%) | 771 (62.8) | 668 (65.9) |
| Male, *n* (%) | 457 (37.2) | 346 (34.1) |
| Female:male ratio | 1.69:1 | 1.93:1 |
| Age at time of analysis, mean (range) | 39.9 (19–76) | 37.6 (20–73) |

analysis, our sample size had more than 80% power to detect an association of a SNP with a relative risk of 1.35, under the assumption of a multiplicative model, minor allele frequency (MAF) of 6% (the lowest MAF in controls for the rs2119704), and a type I error level of 0.05.

Results from multiple regression analysis, either for alleles or for genotypes, under the recessive model, with the respective adjusted *p* values and the permutation *p* values are presented in Table 3. Significant associations were found for six polymorphisms. In particular, in allelic comparisons, statistically significant MS susceptibility risk factors in our group were the rs2760524 of LOC105371664, near RGS1 (permutation *p* value 0.001), the rs3129889 of HLA-DRA, near HLA-DRB1 (permutation *p* value < 1.00e-04), the rs1738074 of TAGAP (permutation *p* value 0.007), the rs703842of METTL1/ CYP27B1 (permutation *p* value 0.008), the rs9596270 of DLEU1 (permutation *p* value < 1.00e-04), and the rs17445836 of LincRNA, near IRF8 (permutation *p* value 0.001). When genotypes between cases and controls were compared under the recessive model, significant associations were found for the rs2760524 of LOC105371664, near RGS1 (permutation *p* value 0.006) and the rs703842 of METTL1/ CYP27B1 (permutation *p* value 0.013).

## Discussion

The genetic architecture of Greek MS patients may share either many, or few similarities with the known genetic MS risk factors identified in a number of multi-ethnic GWAS [13]. In the present study, we have performed a replication study of 35 GWAS-emergent susceptibility SNPs in an ethnically distinct group of Greek MS patients, and we found significant associations for six of them, namely rs2760524, rs17445836, rs703842, rs1738074, rs9596270, and rs3129889.

The 35 SNPs analyzed in our study were selected from a previous meta-analysis of 7 GWAS in a total of 5545 cases and 12,153 controls [17]. These numbers of participants, according to simulations [21], were sufficiently powered to identify common susceptibility variants of modest effect. In addition, the authors, in order to combine the data from the different genotyping platforms of the 7 GWAS, implemented genotype imputation based on shared haplotype linkage disequilibrium; consequently, the tested loci substantially extended form approximately 750,000 to 2.5 million SNPs. This study highlighted SNPs with either genome-wide significance or suggestive evidence of association with MS [17].

Rs2760524, which reached the statistical significance threshold in allelic and genotypic comparisons in our group, is an intronic variant of LOC105371664, near the regulator of the G protein-signaling receptor 1 (RGS1), which regulates several signaling cascades [22]. Altered RGS1 expression affects B-cells' migration and their recruitment into the CNS [23]. It is possible that functional variants of the RGS1 gene may influence B-cells' phenotype and consequently MS risk. In addition, treatment strategies may affect the RGS1 expression. Treatment with IFN-β leads to RGS1 induction in peripheral blood mononuclear cells (PBMCs), monocytes, T-cells and B-cells, so regulation of G protein, apart from MS susceptibility, may also be involved in MS activity response [24].

The intronic rs17445836 of LincRNA, near IRF8, in chromosome 6, conferred susceptibility to MS in our group. IRF8 is an interferon-response gene, as its function was affected by IFN-β or glatiramer acetate treatment [25]. It was also described that the G allele of rs17445836 influences IRF8 gene expression in PBMC of MS patients [25].

The variant rs703842 was associated with MS risk in both allelic and genotypic comparisons in our group. Our results regarding the rs703842 are consistent with those from the recent meta-analysis of Jiang et al. [26]. More precisely, they reported that rs703842 is associated with MS risk in subgroup analysis, including only Caucasian populations (OR = 0.85, 95% CI 0.80–0.90; $p < 0.0001$) [26]. Rs703842 is an upstream variant of the CYP27B1 gene, also neighboring with the methyltransferase-like protein 1 (METTL1) gene. CYP27B1 appears to have a pivotal role in vitamin D metabolism. It converts vitamin D to 1,25-dihydroxyvitamin D3, which is the active form of vitamin D [27]. Vitamin D deficiency is a well-established risk factor of MS [28]. A number of variants across CYP27B1 have been associated with MS [3, 29], and it has been suggested that genotype variants, such as the rs703842, combined with vitamin D blood levels, may be applied as biomarkers of MS risk [26, 30].

The polymorphism rs1738074, located in the 5' UTR region of the TAGAP gene, was also identified in our study as a susceptibility variant. The TAGAP gene encodes the T cell activation Rho-GTPase-activating protein (TAGAP) [31]. TAGAP, therefore, influences T cell activation, and it is suggested to be implicated in a number of autoimmune diseases, such as MS, diabetes mellitus type 1, celiac disease, rheumatoid arthritis, and Crohn's disease [31, 32]. In vitro studies showed that TAGAP gene expression and protein levels in CD4+ T cells are regulated by vitamin D, as the TAGAP gene contains vitamin D response elements (VDREs) [31].

**Table 3** Allelic and genotypic multiple regression analysis

| | | Allelic comparisons | | | Genotypic comparisons (recessive model) | | |
|---|---|---|---|---|---|---|---|
| | SNP | OR [95% CI] | Fisher's *p* value | Perm *p* value | OR [95% CI] | Fisher's *p* value | Perm *p* value |
| 1. | rs2300747 | 1.271 [0.923–1.751] | 0.142 | 0.992 | 2.382 [0.674–8.424] | 0.178 | 0.895 |
| 2. | *rs2760524* | *0.532 [0.410–0.692]* | *3.0e-06* | *0.001* | *0.521 [0.387–0.712]* | *1.46e-06* | *0.006* |
| 3. | rs12122721 | 1.037 [0.85–1.264] | 0.723 | 1 | 0754 [0.378–1.503] | 0.422 | 1 |
| 4. | rs6718520 | 0.883 [0.726–1.073] | 0.210 | 1 | 0.815 [0.959–1.359] | 0.57 | 1 |
| 5. | rs7592330 | 1.133 [0.927–1.384] | 0.223 | 1 | 0.877 [0.570–1.351] | 0.553 | 1 |
| 6. | rs1132200 | 0.619 [0.466–0.822] | 0.002 | 0.61 | 1.142 [0.209–6.251] | 0.878 | 1 |
| 7. | rs2681424 | 1.017 [0.863–1.204] | 0.848 | 1 | 0.965 [0.700–1.330] | 0.828 | 1 |
| 8. | rs6897932 | 0.781 [0.609–1.003] | 0.053 | 0.838 | 0.741 [0.370–1.482] | 0.396 | 1 |
| 9 | rs2546890 | 1.231 [1.012–1.498] | 0.038 | 0.701 | 1.779 [1.259–2.515] | 0.001 | 0.067 |
| 10. | rs10866713 | 0.752 [0.585–0.967] | 0.02 | 0.552 | 1.000 [0.449–2.226] | 1.000 | 1 |
| 11. | rs4613763 | 1.031 [0.702–1.515] | 0.877 | 1 | 1.414 [0.162–12.276] | 0.754 | 1 |
| 12. | *rs3129889* | *4.491 [2.896–6.964]* | *1.96e-11* | *< 1.00e-04* | 4.216 [0.488–36.431] | 0.191 | 0.995 |
| 13. | rs9277535 | 1.003 [0.795–1.267] | 0.978 | 1 | 1.173 [0.642–2.144] | 0.604 | 1 |
| 14. | *rs1738074* | *0.669 [0.544–0.822]* | *1.29e-04* | *0.007* | 0.551 [0.362–0.838] | 0.005 | 0.123 |
| 15. | rs2150702 | 0.886 [0.752–1.044] | 0.154 | 0.993 | 0.725 [0.525–1.000] | 0.25 | 0.437 |
| 16. | rs7089861 | 0.942 [0.767–1.132] | 0.478 | 1 | 1.138 [0.708–1.827] | 0.594 | 1 |
| 17. | rs17824933 | 1.147 [0.957–1.373] | 0.141 | 1 | 1.430 [0.892–2.292] | 0.137 | 0.802 |
| 18. | rs1800693 | 1.089 [0.923–1.284] | 0.313 | 0.964 | 1.394 [0.986–1.971] | 0.06 | 0.787 |
| 19. | *rs703842* | *0.621 [0.493–0.782]* | *4.9e-05* | *0.008* | *0.257 [0.124–0.532]* | *2.53e-04* | *0.013* |
| 20. | rs1790100 | 1.223 [0.961–1.466] | 0.101 | 1 | 1.265 [0.784–2.041] | 0.335 | 1 |
| 21. | *rs9596270* | *0.382 [0.267–0.546]* | *1.21e-7* | *< 1.00e-04* | 0.159 [0.018–1.374] | 0.095 | 0.810 |
| 22. | rs2119704 | 0.787 [0.563–1.155] | 0.275 | 0.984 | 0.388 [0.028–5.429] | 0.482 | 1 |
| 23. | *rs17445836* | *0.523 [0.402–0.680]* | *1.00e-06* | *0.001* | 0.765 [0.356–1.642] | 0.491 | 1 |
| 24. | rs12708716 | 0.839 [0.708–1.135] | 0.121 | 1 | 0.965 [0.654–1.423] | 0.857 | 1 |
| 25. | rs7191700 | 1.112 [0.931–1.328] | 0.257 | 1 | 4.497 [0.598–1.529] | 0.852 | 1 |
| 26. | rs744166 | 1.241 [1.051–1.465] | 0.011 | 0.508 | 1.480 [1.025–2.138] | 0.036 | 0.599 |
| 27. | rs2293152 | 0.883 [0.747–1.042] | 0.15 | 1 | 0.891 [0.590–1347] | 0.585 | 1 |
| 28. | rs8070463 | 1.301 [1.106–1.543] | 0.001 | 0.457 | 1.445 [1.056–1.978] | 0.021 | 0.162 |
| 29. | rs10411936 | 1.279 [1.012–1.617] | 0.039 | 0.987 | 2.254 [1.169–4.343] | 0.015 | 0.507 |
| 30. | rs6074022 | 1.046 [0.871–1.246] | 0.622 | 1 | 0.992 [0.640–1.538] | 0.971 | 0.449 |

Italic values indicate statistical significance

Therefore, vitamin D seems to be a recurrent element in the assessment of MS predisposition, as two out of the six SNPs that reached the significance threshold in our study, are variously involved with this vitamin.

Another SNP that was also associated with MS in our study is rs9596270, located in the DLEU1 (deleted in lymphocytic leukemia 1) gene. DLEU1 regulates the expression of nuclear factor κB (NF-κB), a transcription factor implicated in the pathophysiology of MS [33, 34]. Moreover, the NF-κB pathway is the target of current immunomodulatory agents. Terfilunomide was found to block the NF-κB transcription factor [35] and dimethyl fumarate inhibits dendritic cell maturation via NF-κB [36].

Finally, the downstream gene variant rs3129889 of HLA-DRA, near the HLA-DRB1 gene, was the most significantly associated with increased risk of MS in our study, revealing the significance of MCH genes in MS susceptibility. HLA-DRB1*1501-bearing haplotypes of the major histocompatibility complex (MHC) class II region are considered to be the major genetic risk factors for MS [37]. A previous study in Greece has also revealed the significance of MCH genes in MS susceptibility [38].

One of the strengths of our study is the clinically well-characterized, phenotypically and ethnically homogenous group. Moreover, we accumulated data from a large number of participants, leading to a well-powered GWAS replication study, sufficient to identify genetic susceptibility of modest effect. Additionally, correction for multiple testing was performed with permutation analysis, a powerful and unbiased method.

Certain limitations of the present report need to be acknowledged, however. Our study carries all the inherent limitations of a retrospective analysis of prospectively collected data. In addition, given the complexity of MS, adjustment for additional cofounders in the regression models [39, 40] might have increased the possibility of disclosure of the net effects of the polymorphisms. Finally, our results would have been more robust if they were accompanied by supportive functional analyses.

In brief, our study replicated a number susceptibility SNPs that emerged from previous GWAS, which insinuates that some similarities between the examined Greek population and the MS genetic architecture of the GWAS populations are present. Results from this GWAS replication study are of significant importance in the effort of validating GWAS genetic associations and gaining a deeper knowledge of MS susceptibility and of potential genetic homogeneity between different ethnicities.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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