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Gene variants of adhesion molecules predispose to MS: A case-control study

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Abstract

Objective

To examine the effect of variants in genes encoding molecules that are implicated in leukocyte trafficking into the CNS on the development of MS.

Methods

A total of 389 Greek MS cases and 336 controls were recruited by 3 MS centers in Cyprus and Greece. In total, 147 tagging single nucleotide polymorphisms across 9 genes encoding for P-selectin (*SELP*), integrins (*ITGA4*, *ITGB1*, and *ITGB7*), adhesion molecules (*ICAM1*, *VCAM1*, and *MADCAM1*), fibronectin 1 (*FN1*), and osteopontin (*SPP1*) were genotyped. The clinical end point of the study was diagnosis of MS according to the 2005 revised McDonald criteria. Permutation analysis was used for adjusting for multiple comparisons.

Results

Overall, 21 variants across *SELP*, *ITGA4*, *ITGB1*, *ICAM1*, *VCAM1*, *MADCAM1*, *FN1*, and *SSP1* genes were each associated with MS ($p_{perm} < 0.05$). The most significant were rs3917779 and rs2076074 (*SELP*), rs6721763 (*ITGA4*), and rs1250258 (*FN1*), all with a permutation p value of less than 1e-004.

Conclusions

The current study provides preliminary evidence that variants across genes encoding adhesion molecules, responsible for lymphocyte adhesion and trafficking within the CNS, are implicated in the risk of developing MS.

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Glossary

BBB = blood-brain barrier; **CGAS** = candidate gene association study; **DMT** = disease-modifying therapy; **EDSS** = Kurtzke Expanded Disability Status Scale; **GWAS** = genome-wide association study; **LD** = linkage disequilibrium; **MAF** = minor allele frequency; **MSSS** = Multiple Sclerosis Severity Score; **OR** = odds ratio; **SELP** = P-selectin; **SNP** = single nucleotide polymorphism.

MS is a common complex immune-mediated disease of the CNS.[1] Inflammation, demyelination, axonal loss, and neurodegeneration appear during the course of MS.[2] Although the proximal pathogenic mechanisms that lead to MS development still remain largely unclear, there is evidence that hints toward an interplay between genetic background, environment factors, and epigenetic modifications conferring susceptibility to MS.[3]

A few genetic variants that confer susceptibility to MS have been identified through candidate gene association studies (CGASs), linkage and genome-wide linkage analyses, and genome-wide association studies (GWASs).[4] Over 110 genetic risk factors of MS have been identified.[5] However, most studies have yielded inconsistent results, with limited replication of the reported associations.[6]

Several molecules have been implicated in the adhesion and diapedesis of leukocytes via the blood-brain barrier (BBB), as well as their migration from the extracellular matrix to the target tissue.[7] The protective effect of an antibody against α4 integrin (natalizumab) in MS supports the hypothesis that the ingress of leukocytes into the brain parenchyma is a crucial step.[8] We have previously reported that variants of genes coding for proteins implicated in leukocyte trafficking across the BBB influence the severity of MS (namely rs6721763 [*ITGA4*] and rs6532040 [*SPP1*]) and have an effect on disease onset (rs1250249 [*FN1*]).[9] Therefore, we also hypothesize that variants of these molecules may also predispose to MS development.

The present study examines tagging single nucleotide polymorphisms (SNPs) in *P-selectin* (*SELP*), integrins α4, β1, and β7, adhesion molecules *ICAM1*, *VCAM1*, and *MADCAM1*, fibronectin 1, and osteopontin genes for possible association with MS risk.

Methods

Study population

Our cohort consisted of Greek patients with MS and controls from 3 MS centers: the Cyprus Institute of Neurology and Genetics in Cyprus, the University Hospital of Larissa, Greece, and the AHEPA Hospital of Aristotle University in Thessaloniki, Greece, that have been previously described in detail.[9] In short, a total of 389 MS cases and 336 controls have participated in the study. The diagnosis of MS was assessed according to the 2005 revised McDonald criteria.[10] All participants were aged ≥18 years and had a disease duration of ≥5 years. We did not include primary progressive MS cases.

The same criteria for collection of clinical and demographic data were applied at each center. Participants' sex, current age, age at disease onset (defined as age of the first neurologic symptom suggestive of MS), use of disease-modifying therapies (DMTs), and duration of treatment were recorded. Using the Kurtzke Expanded Disability Status Scale (EDSS),[11] the disability status was assessed in the absence of relapse for at least 6 months. The time from the first symptom suggestive of MS to the last EDSS assessment was considered as the disease duration (in years) for each patient. The progression index was applied to express the rate of disease progression. By means of the Multiple Sclerosis Severity Score (MSSS), disease severity was estimated.[12]

The unrelated healthy control group was matched for age and sex and consisted of healthy volunteers from the same ethnic regions.

Standard protocol approvals, registrations, and patient consents

The study received approval from the respective institutional ethical standards committee on human experimentation for any experiments using human patients. Written informed consent was obtained from all patients (or guardians of patients) participating in the study (consent for research).

DNA isolation, selection of tagging SNPs, and genotyping procedure

With the puregene DNA purification kit (Qiagen, Valencia, CA), we isolated DNA from peripheral blood samples. Variants across the following genes were genotyped: (1) *SELP*, (2) *ITGA4*, (3) *ITGB1*, (4) *ITGB*, (5) *ICAM1*, (6) *VCAM1*, (7) *MADCAM1*, (8) *FN1*, and (9) *SPP1*.

Tagging SNPs were identified on the basis of linkage disequilibrium (LD) blocks according to the HapMap project. More precisely, the selection was based on the CEU (Utah residents with Northern and Western European ancestry from the CEPH [Centre d'Etude du Polymorphisme Humain collection]) population at the HapMap Release 27 database (Phase II + III, Feb09, on National Center for Biotechnology Information B36 assembly, dbSNP b126). Pairwise r^2 values ≥0.8 and minor allele frequency (MAF) >0.05 were the applied criteria. Thirty-two tagging SNPs in the *SELP* gene, 36 in the *ITGA4* gene, 16 in *ITGB1*, 3 in *ITGB7*, 5 in *ICAM1*, 15 in *VCAM1*, 3 in *MADCAM1*, 38 in *FN1*, and 8 in *SPP1*, which add up to 156 SNPs, were retrieved. Studied tagging SNPs' characteristics and information regarding their genes are available in table e-1 (links.lww.com/NXG/A137).

TaqMan 5′-nuclease assays were used to genotype SNPs by acquiring specific assays for each SNP and then performing allelic discrimination assays on the ABI 7900HT Real-Time System (Applied Biosystems, Foster City, CA). Nine SNPs failed to be genotyped, whereas the genotypic rates were above 96%.

Statistical analysis

The exact test, with a p value of ≤0.05, was considered as indicative for deviation from the Hardy-Weinberg equilibrium in the control group for each SNP.[13] Haploview software version 4.2 (broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) was applied in each gene in order for pairwise D′ and r^2 between variants and LD blocks to be calculated.[14,15] The power of the sample was calculated using CaTS software (csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html).[16] Quality control with a threshold lower than 0.05% was performed by calculating MAFs. Allelic association regression analysis was performed using PLINK 1.07 software (zzz.bwh.harvard.edu/plink/).[17] p values were corrected for multiple testing by means of permutation analysis. A total of 10,000 permutations were performed to correct the p value. Value less than 0.05 was set as the statistically significant threshold.

Data availability statement

Anonymized data will be shared by request from any qualified investigator.

Table 1 Demographic and clinical characteristics of study participants

	Total patients	Controls	Cyprus patients	Cyprus controls	Larissa patients	Larissa controls	Thessaloniki patients
n	389	336	104	127	206	209	79
Female, n (%)	251 (64.5)	234 (69.6)	74 (71.2)	88 (69.3)	126 (61.2)	146 (69.9)	51 (64.6)
Male, n (%)	138 (35.5)	102 (30.4)	30 (28.8)	39 (30.7)	80 (38.8)	63 (30.1)	28 (35.4)
Female:male ratio	1.8:1	2.3:1	2.5:1	2.3:1	1.6:1	2.3:1	1.8:1
Age at disease onset, mean (range)	30.4 (18–63)		35.3 (18–58)		29.0 (18–63)		27.9 (18–50)
Age at the time of analysis, mean (range)	43.3 (23–74)	36.3 (23–78)	43.8 (25–66)	47.4 (20–78)	43.0 (23–74)	29.6 (23–58)	41.9 (23–63)
Disease duration (y), mean	12.9		12.44		12.77		13.90
Median (range)	11 (5–52)		11 (5–35)		10 (5–52)		12 (5–38)
Time from 1st to 2nd relapse (mo), mean (SD)	40.1 (52.3)		44.8 (54.1)		36.1 (51.8)		44.3 (50.6)
Monosymptomatic onset, n (%)	268 (74.9)		94 (90.4)		132 (69.8)		42 (64.6)
DMT, n (%)	271 (69.7)		71 (68.3)		131 (63.6)		69 (87.3)
DMT time (mo) (SD)	72.1 (44.2)		73.6 (49.6)		70.4 (43.3)		73.9 (40.2)
EDSS, n (%)							
<3	188 (47.8)		69 (66.3)		84 (40.8)		33 (41.8)
3 to <6	114 (29.3)		27 (26.0)		54 (26.2)		33 (41.8)
6–6.5	40 (10.3)		6 (5.8)		25 (21.1)		9 (11.4)
≥7	35 (9.0)		2 (1.9)		31 (15.0)		2 (2.5)
Mean (SD)	3.40 (2.16)		2.44 (1.54)		3.83 (2.46)		3.61 (1.59)
Progression index, mean (SD)	0.31 (0.24)		0.22 (0.15)		0.34 (0.24)		0.35 (0.31)
MSSS							
Median	4.00		3.00		5.00		5.00
Mean (SD)	4.39 (2.61)		3.18 (1.98)		4.92 (2.89)		4.71 (2.00)
Benign MS (MSSS = [1–2]), n (%)	109 (28.0)		49 (47.1)		51 (24.8)		9 (11.4)
Severe MS (MSSS = [7–10]), n (%)	86 (22.1)		9 (8.7)		61 (29.6)		16 (20.3)

Abbreviations: DMT = disease-modifying treatment; EDSS = Kurtzke Expanded Disability Status Scale; MSSS = Multiple Sclerosis Severity Score.

Results

Detailed characteristics of the recruited cohort have been previously extensively described.[9] In brief, 251 patients (64.5%) were women. Two hundred seventy-one patients (69.7%) were under DMT. Two hundred thirty-four healthy controls (69.6%) were women. The mean age of the control group was 36.3 years. Detailed clinical and demographic data of controls and patients are presented in table 1.

According to the power analysis, our study had a power of 80.0% to detect an association of a variant with a genetic relative risk of 1.729, assuming the multiplicative model, MAF equal to 5% (the lowest in MS cases for the rs3917714), type I error level of 0.05, in a sample of 336 controls and 389 MS cases.

The distribution of the variants that were analyzed in our cohort, in distinct LD blocks across genes, and the pairwise D′ and r^2 values between them are demonstrated in figure e-1 (links.lww.com/NXG/A136).

According to allelic association regression analysis, significant associations (with $p_{perm} < 0.05$) were found for 21 SNPs in total. Namely, 8 *SELP* polymorphisms (rs3917779, rs3917768, rs2142760, rs3917744, rs2076074, rs3917740, rs3917727, and rs3917709), 7 *ITGA4* polymorphisms (rs12988934, rs11694175, rs17224277, rs155103, rs17225354, rs11689738, and rs6721763), 1 *ITGB1* polymorphism (rs10763902), 1 *ICAM1* polymorphism (rs281437), 1 *VCAM1* polymorphism (rs3176878), 1 *MADCAM1* polymorphism (rs12982646), 1 *FN1* polymorphism (rs1250258), and 1 *SSP1* polymorphism (rs6532040) were found to reach a statistical significant level. The most statistically significant p_{perm} values were reached from rs3917779 and rs2076074 (*SELP*), rs6721763 (*ITGA4*), and rs1250258 (*FN1*), with a permutation *p* value equal to 9.999e-005. The odds ratios [ORs], 95 CIs, and the permutation *p* values after correction for multiple testing for statistically significant variants (stressed according to the permutation *p* value) are presented in table 2. The ORs, 95 CIs, *p* values, and the permutation *p* values after correction for multiple testing for each variant are presented in table e-2 (links.lww.com/NXG/A137).

Table 2 Rs number, gene, OR, 95% CI, and permutation *p* value for the statistical significant variants resulted from allelic association analysis association with MS risk (MS cases vs healthy controls)

rs number	Gene	OR (95% CI)	Permutation *p* value
rs3917779	*SELP*	3.08 (1.943–4.883)	9.999e-005
rs2076074	*SELP*	2.07 (1.594–2.689)	9.999e-005
rs6721763	*ITGA4*	0.5542 (0.448–0.6855)	9.999e-005
rs1250258	*FN1*	0.6427 (0.5155–0.8013)	9.999e-005
rs12982646	*MADCAM1*	1.661 (1.248–2.212)	0.0007999
rs6532040	*SPP1*	1.551 (1.253–1.92)	0.0003
rs17224277	*ITGA4*	0.5479 (0.3718–0.8074)	0.0009999
rs3176878	*VCAM1*	1.667 (1.263–2.2)	0.0009999
rs3917709	*SELP*	1.831 (1.255–2.673)	0.0018
rs17225354	*ITGA4*	0.7332 (0.5918–0.9084)	0.0036
rs10763902	*ITGB1*	1.418 (1.119–1.796)	0.0048
rs11689738	*ITGA4*	1.363 (1.097–1.694)	0.005199
rs11694175	*ITGA4*	1.33 (1.074–1.646)	0.005299
rs3917768	*SELP*	1.357 (1.092–1.685)	0.005399
rs155103	*ITGA4*	0.7048 (0.5454–0.9108)	0.007199
rs3917740	*SELP*	0.6807 (0.5127–0.9038)	0.008099
rs2142760	*SELP*	1.32 (1.059–1.647)	0.0148
rs12988934	*ITGA4*	1.74 (1.11–2.728)	0.016
rs3917727	*SELP*	0.7883 (0.6376–0.9745)	0.0201
rs281437	*ICAM1*	0.7691 (0.6234–0.9488)	0.0405
rs3917744	*SELP*	0.8048 (0.6478–0.9999)	0.0485

Abbreviations: CI = cluster interval; OR = odds ratio.

Discussion

The present research aimed at investigating a possible association between predisposition to MS and variants of genes that are implicated in leukocyte trafficking into the CNS. The 147 tagging SNPs across 9 genes were selected with the LD block method and further analyzed. Our analysis revealed associations between 21 tagging SNPs across *SELP*, *ITGA4*, *ITGB1*, *ICAM1*, *VCAM1*, *MADCAM1*, *FN1*, and *SSP1* and MS. This result needs further replication in ethnically different and even larger groups.

Recently, a renewed effort to identify genetic variants conferring susceptibility to MS has been made. A few variants have emerged through CGASs and GWASs.[5,6,18–20] However, CGASs and GWASs have yielded inconsistent findings, with limited replication of genetic variants.[6] Previous CGASs have focused on pathways related to autoimmunity, immune function, myelin structure, and the human leukocyte antigen, which also yielded inconsistent results.[21,22] It is possible that the complexity of MS pathophysiology,[5] the interplay between environmental and genetic risk factors, epistasis between loci, and the lack of systematic exploration of rare variations in MS susceptibility may be responsible, to some degree, for the low reproducibility of MS loci in different studies.[6,18]

The SELP protein is stored in the alpha-granules of platelets and Weibel-Palade bodies of endothelial cells.[23] During platelet activation and degranulation, SELP is redistributed to the plasma membrane and mediates the interaction between leukocytes and activated endothelial cells or platelets.[23] Eight *SELP* polymorphisms (rs3917779, rs3917768, rs2142760, rs3917744, rs2076074, rs3917740, rs3917727, and rs3917709) have reached the statistically significant threshold in our study. The rs3917779, rs3917768, and rs2142760 are in block 2 according to LD values. This region of the SELP gene, that spans approximately 7.8 kb between the 2 extremes (rs3917786 and rs2142760, with a value of D′ equal to 0.986), appears to represent an important part across the SELP gene and may affect the expression of the gene. Every single SNP within this region may confer an additional risk to the development of MS.

The *ITGA4* gene encodes the α4 subunit of the α4β1 integrin. This integrin is involved in the adhesion and migration procedures of leukocytes through the BBB.[7] ITGA4 is already an approved treatment target in clinical practice in relapsing-remitting MS, with the monoclonal antibody natalizumab.[24] A few variants (rs1143676, rs1449263, and rs6721763) in *ITGA4* have been linked to MS risk.[9,25–27] Of interest, the intronic rs6721763 (intron 17–18), which has been found to be associated with MS risk, has also been associated with disease severity, as evaluated with the MSSS.[9]

Osteopontin consists a secreted matrix protein, which is involved in processes regarding activation, proliferation, and migration of T cells.[28] The intronic rs6532040 has been found to confer susceptibility to MS, based on our results. The rs6532040 of *SPP1* has been previously reported to be a genetic modifier of MS course.[9]

Fibronectin 1 is an extracellular matrix glycoprotein. Its main function includes cytoskeleton organization, cell adhesion, and migration.[29] Previously, the *FN1* rs1250249 has been found to exert a dose-dependent effect on MS onset.[9] However, this polymorphism does not seem to be associated with the risk of developing MS in our study. In contrast, the *FN1* rs1250258 (p_{perm} = 9.999e-005) is strongly associated with MS risk in our cohort. The rs1250258 is 12.9 kb away from the rs1250249, and in low LD (D′ = 0.048), suggesting that additional loci across *FN1* may consist a risk factor for MS.

One polymorphism across each of the *ITGB1*, *ICAM1*, *VCAM1*, and *MADCAM1* genes has been associated with MS in our study. Namely, the polymorphisms rs10763902, rs281437, rs3176878, and rs12982646, respectively, have reached p_{perm} values of <0.05. ITGB1, ICAM1, VCAM1, and MADCAM1 are adhesion molecules expressed in the CNS extracellular space.[7,9,30] They are mainly implicated in the regulation of the reactivation and migration of lymphocytes to myelinated axons.[7,9,30]

The clinically well-characterized MS group is one of the strengths of our study. Moreover, according to the number of participants, our sample was sufficiently powered to reveal common susceptibility variants of modest effect. In addition, the correction for multiple testing was assessed by means of permutation analysis, an unbiased method.

However, our study has certain limitations. First, it carries all the inherent limitations of studies with a case-control design. Moreover, our concluding remarks would have been more robust if our analysis was accompanied by supportive functional analyses, a validation in an independent sample or an in silico analysis.

In brief, our study implicates variants of genes encoding adhesion molecules, which are mainly involved in lymphocyte ingress and trafficking in the CNS, in the risk of developing MS. The investigation of additional genetic factors that predispose to MS may provide physicians with personalized tools for diagnosis or treatment, particularly in patients with clinically or radiologically isolated syndromes.[31]

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Disclosure

Efthimios Dardiotis, Elena Panayiotou, Vasileios Siokas, and Athina-Maria Aloizou report no disclosures. Kyproula Christodoulou has received foundation/society research support from the Research Promotion Foundation (Cyprus), Telethon, the

Appendix 1. Author contributions

Name	Location	Role	Contribution
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