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HLA-DR and -DQ Associations with Multiple Sclerosis in Turkey

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ABSTRACT: The DRB, DQA, and DQB subregions of the major histocompatibility complex (MHC) were investigated by polymerase chain reaction and sequence-specific oligonucleotide probe hybridization (PCR/SSO) in 103 multiple sclerosis (MS) patients and 101 healthy controls from Turkey. Significant differences were detected between MS and control populations in the frequencies of DRB1*1501 [29 vs. 14, $p = 0.02$, odds ratio (OR) = 2.4], DRB1*04 (35 vs. 18, $p = 0.01$, OR = 2.3), DQB1*0302 (30 vs. 15, $p = 0.02$, OR = 2.3), DQB1*0602 (27 vs. 10, $p = 0.005$, OR = 3.2), DQB1*0501 (10 vs. 24, $p = 0.01$, OR = 0.3), DQA1*0101 (16 vs. 31, $p = 0.02$, OR = 0.4), and DQA1*0103 (7 vs. 19, $p = 0.02$, OR = 0.3). These results confirm the proposed positive association of the Dw2 (DRB1*1501 DQA1*0102 DQB1*0602) haplotype with MS in Caucasians in our Turkish population (25 vs. 8, $p = 0.003$, OR = 3.7). Furthermore, the "putative" haplotype supposed to be more frequent in the MS population of Mediterranean countries, namely DRB1*04 DQA1*03 DQB1*0302, is also associated with MS in Turkey (29 vs. 12, $p = 0.006$, OR = 2.9). The presence of two different haplotypic associations in MS emphasizes the complexity of the genetic susceptibility to MS in different populations. *Human Immunology 55, 59–65 (1997).* © American Society for Histocompatibility and Immunogenetics, 1997. Published by Elsevier Science Inc.

ABBREVIATIONS

CI	confidence interval
MS	multiple sclerosis
MHC	major histocompatibility complex
OR	odds ratio
PCR	polymerase chain reaction
PP	primary progressive MS
RR	relapsing/remitting MS
SP	secondary progressive MS
SSO	sequence-specific oligonucleotide

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system widely believed to be a multifactorial disease of autoimmune nature. It is well established that genetic determinants are implicated in predisposition to MS [1, 2]. The extensive polymorphism and the functional relevance of major histocompatibility complex (MHC) genes to an immune response made them the focus of many disease-association studies [3]. If MS is considered as a T cell mediated autoimmune process [4–6], MHC gene products may be involved in presenting self-antigens such as myelin to T lymphocytes. In the experimental autoimmune encephalomyelitis animal model of MS, the relevance of T lymphocytes and MHC genes as susceptibility elements has been convincingly demonstrated [7].

Attempts to identify the susceptibility markers for MS in humans have resulted in many population-based studies. Various human leukocyte antigen (HLA) class II associations are shown in different ethnic groups, including predominantly DR2 in northern European and American Caucasians [8, 9], DR4 in Arabs [10] and Sardinians [11], and DR6 in Japanese [12] and Mexicans [13]. By using DNA-based typing techniques, associations of MS have also been described with different DQ

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alleles [14], combinations of DQ alleles [15], or groups of alleles based on their sequence [16]. However, because of the strong linkage disequilibrium between class II loci, the susceptibility is most easily defined with the "putative" haplotypes including the associated alleles. The most frequently reported association of MS is with the HLA class II haplotype DR15 (Dw2) DQ6 (DRB1*1501 DQA1*0102 DQB1*0602) [17]. The haplotype DR4 DQ8 is also found significantly associated with MS in a Sardinian population [18] and in some clinical subgroups of MS [19]. This association, however, could not be verified in other populations.

In this study, MS patients and healthy controls from the population of Turkey are evaluated in terms of HLA DRB, DQA, and DQB alleles by genotyping and comparisons of the distribution of the alleles and the "putative" haplotypes are made.

MATERIALS AND METHODS

Patients and Controls

A total of 103 unrelated patients with clinically definite MS diagnosed according to Poser criteria [20] were investigated. In this group, 64 of the patients had relapsing/remitting (RR), 8 had primary progressive (PP), and 31 had secondary progressive (SP) MS. All patients and the controls were of Turkish descent with mixed ethnic origin, selected randomly. The control group consisted of 101 unrelated healthy individuals, including blood donors of the blood bank and the hospital staff.

DNA Typing of DRB, DQA, and DQB

DNA was prepared from anticoagulated venous blood by using a salting-out method [21]. The second exons of MHC DRB, DQA1, and DQB1 loci were amplified by polymerase chain reaction (PCR) using the primer pairs of the XI International Histocompatibility Workshop (XI.IHWS) [22]. Specifically, the following primer pairs were used: for the DRB region, 2DRBAMP-A and 2DRBAMP-B; for DR4 subtyping, 2DRBAMP-4 and 2DRAMP-B; for DR2 subtyping, 2DRBAMP-2 and 2DRAMP-B; for the DQA1 region, 2DQAAMP-A and 2DQAAMP-B; and for the DQB1 region, 2DQBAMP-A and 2DQBAMP-B. For PCR, 100–300 ng of genomic DNA, 25 pmol of each primer, and 1.25 units of *Taq* polymerase (Promega, USA) were used in a reaction volume of 50 μl with 200 μM of each dNTP and 1.5–2.5 μM $MgCl_2$ (DRB 2–2.5 μM, DQB 2 μM, DQA 1.5 μM). The reaction program consisted of 30 cycles of 95°C, 55°C (DQA1, DQB1) or 58°C (DRB), and 72°C for 1 min each, after an initial denaturation step of 4 min at 95°C. A final extension step of 7 min at 72°C was added. Successful PCR amplification was confirmed by agarose electrophoresis.

For hybridization experiments, 2 μl of each PCR product was dotted onto positively charged nylon membranes (Amersham International, Amersham Place, Little Chalfont, Bucks, UK) and immobilized by UV irradiation. The respective membranes were hybridized with 29 sequence-specific oligonucleotide (SSO) probes for DRB, with 10 SSO for DQA1, and with 17 SSO for DQB1, allowing a relatively high resolution of the known alleles [22]. The 5′-biotinylated, 18-mer SSO probes were based on those of the XI.IHWS and were produced by the British Society for Histocompatibility and Immunogenetics. All membranes for DRB, DQA, and DQB had appropriate control DNA samples (from XI.IHWS cell lines). Hybridizations were performed after Kimura and Sasazuki [22] with some modifications according to the manufacturer's instructions for a nonradioactive detection system (Enhanced Chemoluminescence, Amersham International). Briefly, the membranes were prehybridized at 43°C for 30 min in ECL hybridization buffer [0.5% blocking agent (Amersham International), 0.1% *N*-lauroyl sarcosine, 0.02% sodium dodecyl sulfate (SDS) in 5× standard saline citrate (SSC)]. Biotinylated probes were added at 40 ng/ml of hybridization buffer, and incubation was continued for an additional 75 min at 43°C. After two washes in 5× SSC, 0.1% SDS at room temperature (RT) and one stringently in TMAC solution [3 M tetramethylammoniumchloride (TMAC), 50 mM Tris, pH 8.0, 2 mM NaEDTA, 0.1% SDS] at temperatures between 50 and 66°C depending on the Tm of the SSOs, the membranes were blocked for nonspecific binding (0.5% blocking agent in 0.15 M NaCl, 0.1 M Tris, pH 7.5) at RT for 30 min. The conjugate of streptavidin–horseradish peroxidase (Amersham International) was added at 1:7500. After 30 min at RT, membranes were washed four times and developed with a chemiluminescent detection system (ECL; Amersham International).

Removal of the probes for rehybridization with the same membranes was achieved by washing the membranes in 5× SSC for 5 min at RT, then in 0.1× SSC, 0.1% SDS for 60 min at 65°C, and repeating the first wash. With the set of SSO probes, DQA1*0301/0302 cannot be distinguished because they have identical sequences in the amplified region and the DQA1*0102 reaction pattern is overlapped in the presence of DQA1*0103. The haplotype assignments were made according to reported associations of these loci [22, 23].

The DR2 subtyping was performed for the DR2 positive samples by group-specific oligotyping with the primer pair indicated above. The PCR products (261 bp) of this group were hybridized with a set of 17 SSO probes of XII.IHWS [24] that distinguish DRB1*1501 to 1505 and *1601 to 1605.

The DR4 subtyping was performed for the DR4 positive samples by group-specific oligotyping with the

TABLE 1 Distribution of DRB1 alleles in MS patients and healthy controls

	Controls (n = 101)		Multiple sclerosis (n = 103)		PP (n = 8)	SP (n = 31)	RR (n = 64)
DRB1	n	%	n	%	n	n	n
*0101	13	12.9	9	8.7	1	5	3
*0102	5	4.8	0	0	0	0	0
*1501	14	13.9	29	28.2[†]	3	9	17
*1502	7	6.9	3	2.9	0	1	2
*1601	8	7.9	7	6.8	1	0	6
*0301	16	15.8	23	22.3	1	7	15
*04	18	17.8	35	34.0[††]	3	9	23[†††]
*0401	4	3.9	1	1.0			
*0402	5	4.8	10	9.7			
*0403	4	3.9	12	11.6			
*0404	1	1.0	7	6.8			
*0405	4	3.9	4	3.9			
*0407	2	2.0	1	1.0			
*0408	0	0	1	1.0			
*11	36	35.6	36	34.9	2	14	20
*12	2	2.0	3	2.9	0	1	2
*13	26	25.7	19	18.4	1	4	14
*14	12	11.9	9	8.7	1	2	6
*07	19	18.8	11	10.7	0	4	7
*08	10	9.9	9	9.4	0	3	6
*0901	2	2.0	0	0.9	0	0	0
*1001	5	4.8	1	2.4	0	0	1

Clinical subgroups of MS are shown: PP, primary progressive; SP, secondary progressive; RR, relapsing/remitting. Sample sizes are indicated in parentheses; numbers of individuals displaying specific alleles are shown.

[†]p = 0.02, OR = 2.4; [††]p = 0.01, OR = 2.3; [†††]p = 0.01, OR = 2.6; OR, odds ratio.

primer pair indicated above. The PCR products (263 bp) of this group were hybridized with a set of 10 SSO probes of the XII.IHWS [24] that distinguish DRB1*0401 to 0419.

Statistical Analysis

The results were compared with the chi-square test and with Fisher's exact test when appropriate. Relative risks were calculated as odds ratios (OR) according to Woolf's formula [25]. Individual associations were compared following Svejgaard et al. [26]. A p value of <0.05 was accepted significant. Confidence intervals (CI) are given at 95%.

RESULTS

DRB1

The frequencies of HLA-DRB1 alleles in the groups of MS patients and the healthy controls are shown in Table 1. DRB1*1501 (29 vs. 14, p = 0.02, OR = 2.4, CI 1.2–5.0) and the grup of DRB1*04 alleles (35 vs. 18, p = 0.01, OR = 2.3, CI 1.2–4.6) were found to be positively assocated with MS.

When the clinical forms of MS were compared with the control group, the positive association with DRB1*04 was confirmed in the RR MS group (23/64 vs. 18/101, p = 0.01, OR = 2.6, CI 1.3–5.3). DRB1*1501 was also increased in this group, but not to the point of statistical significance (17/64 vs. 14/101, p = 0.06, OR = 2.2, CI 1.0–4.9). The frequency of the DRB1*0301 allele was higher in the patients group and in the RR subgroup, with no statistical significance (Table 1). To determine whether a particular allele of DRB1*04 groups was increased in MS, DR4 subtyping was undertaken. As shown in Table 1, the DRB1*04 alleles were fairly evenly distributed in both patients and controls. Although DRB1*0402 (10 vs. 5), *0403 (12 vs. 4), and *0404 (7 vs. 1) alleles were increased in MS patients, no DRB1*04 allele was significantly associated with the disease. No significant association was shown with stratification of the data by clinical subgroups of the disease (data not shown).

DQA1 and DQB1

The frequencies of the alleles at the DQA1 and DQB1 loci are shown in Tables 2 and 3. The DQA1*03 group,

TABLE 2 Distribution of DQA1 alleles in the MS patient and control groups

	Multiple sclerosis (n = 103)		Controls (n = 101)			
DQA1	n	%	n	%	p	OR
*0101	16	15.5	31	30.7	0.02	0.4
*0102	40	38.8	26	25.7		
*0103	7	6.8	19	18.8	0.02	0.3
*0201	11	10.6	19	18.8		
*0301/2	35	33.9	21	20.8	0.05	1.9
*0401	4	3.8	5	4.9		
*0501	67	65.0	52	51.4		
*0601	5	4.8	4	3.9		

Sample sizes are indicated in parentheses; numbers of individuals displaying specific alleles are shown. OR, odds ratio.

consisting of *0301 and *0302, was increased in the MS group (35 vs. 21, $p = 0.05$, OR = 1.9, CI 1.0–3.6). Although found with higher frequency (40/103 vs. 26/101), DQA1*0102 of the Dw2 (DRB1*1501 DQA1*0102 DQB1*0602) haplotype alone was found not significantly associated with MS. DQA1*0101 and *0103 were decreased in the MS group (16 vs. 31, $p = 0.02$, OR = 0.4 and 7 vs. 19, $p = 0.02$, OR = 0.3). Moreover, when all DQA1 alleles with glutamine at amino acid (aa) position 34 were investigated (DQA1*0102, 0103, 0401, 0501, and 0601) [15], no significant increase was observed in the MS group (92 vs. 83).

TABLE 3 Distribution of DQB1 alleles in MS patients and controls

	Multiple sclerosis (n = 103)		Controls (n = 101)			
DQB1	n	%	n	%	p	OR
*0501	10	9.7	24	23.7	0.01	0.3
*0502	9	8.7	11	10.9		
*0503	7	6.7	8	7.9		
*0504	0	0	1	0.9		
*0601	4	3.9	10	9.9		
*0602	27	26.2	10	9.9	0.005	3.2
*0603	4	3.9	10	9.9		
*0604	3	2.9	10	9.9		
*0605	2	1.9	1	0.9		
*02	31	30.0	33	32.6		
*0301	59	57.3	47	46.5		
*0302	30	29.1	15	14.8	0.02	2.3
*0303	2	1.9	4	3.9		
*0401	1	0.9	0	0		
*0402	5	4.8	4	3.9		

Sample sizes are indicated in parentheses; numbers of individuals displaying specific alleles are shown. OR, odds ratio.

TABLE 4 Frequencies of some "putative" DRB1-DQA1-DQB1 haplotypes significantly or not significantly associated with both groups

DRB1 DQA1 DQB1	Multiple sclerosis (n = 103) n	Controls (n = 101) n	p	OR
*1501*0102*0602	25	8	0.003	3.7
*1502*0103*0601	2	6		
*04*0301/2*0302	29	12	0.006	2.9
*0401*03*0301	0	2		
*0401*03*0302	1	2		
*0402*03*0301	1	0		
*0402*03*0302	9	4		
*0403*03*0301	1	0		
*0403*03*0302	11	4		
*0404*03*0302	6	1		
*0404*03*0401	1	0		
*0405*03*0301	2	2		
*0405*03*0302	2	2		
*0407*03*0301	1	1		
*0407*03*0302	0	1		
*0408*03*0302	1	0		
*040X*03*0302	1	0		
*0101*0101*0501	7	12		
*0301*0501*02	22	15		
*07*0201*02	8	16		
*1101*0501*0301	23	15		
*1301*0103*0603	1	5		
*1302*0102*0604	1	9		

Sample sizes are indicated in parentheses; numbers of individuals displaying specific haplotypes are shown. OR, odds ratio.

The distribution of the DQB1 alleles indicated two positive associations with MS at this locus (Table 3): DQB1*0602 (27 vs. 10, $p = 0.005$, OR = 3.2, CI 1.4–7.1) and DQB1*0302 (30 vs. 15, $p = 0.02$, OR = 2.3, CI 1.2–4.7). DQB1*0501 (10 vs. 24, $p = 0.01$, OR = 0.3, CI 0.2–0.8) was negatively associated with MS.

A significant difference was detected for the presence of the DQB1 alleles with shared sequencs (DQB1*0602, *0603, *0604, *0302, and *0303) [16], suggesting a positive association with the disease state (60 vs. 42, $p = 0.02$, OR = 1.9). However, the reported associations of MS with DQB1 alleles with leucine at aa position 26 (DQB1*0602, *0603, *0604, *0605, *0302, *0303, and *0201) [27] was not confirmed in our results (79 vs. 68). The presence of any "susceptible" DQ (*cis* or *trans*) heterodimer [15] was also not associated with MS significantly (68 vs. 56, OR = 1.6).

Haplotype and Genotype Associations

When the combinations of HLA class II alleles were considered as "putative" haplotypes and compared, significant differences were demonstrated between the MS and control groups (Table 4). The MS-associated Dw2

(DRB1*1501 DQA1*0102 DQB1*0602) haplotype was significantly increased in the MS group (25 vs. 8, $p = 0.003$, OR = 3.7, CI 1.6–8.7). The haplotypes with DRB1*04 were also significantly more frequent in MS patients than in controls (29 vs. 12, $p = 0.006$, OR = 2.9, CI 1.4–6.1), but the distribution of the haplotypes with different DRB1*04 alleles did not show any significant difference between the two groups because of small numbers in each group (Table 4). The haplotype DRB1*0404 DQA1*03 DQB1*0302 was found positively associated with clinical subgroup RR MS, with a relative risk of 10.7 (6 vs. 1, $p = 0.01$, CI 1.3–91.3). When the haplotypes were compared as DQA and DQB combinations only, the DQA1*03 DQB1*0302 haplotype was also significantly increased in the MS group (31 vs. 14, $p = 0.008$, OR = 2.7, CI 1.3–5.4).

If DRB1 genotypes of groups were compared, as has been done recently by others [26], the increase of the DRB1*1501/04 genotype was not significant in the MS group (5 vs. 2). In the MS group we found four individuals "homozygous" in DRB1*04 and three in DRB1*1501, whereas among the controls only two individuals were "homozygous" for DRB1*04 and there were no DRB1*1501 homozygotes.

DISCUSSION

In this study, we examined an MS population from Turkey that has not been typed for HLA genes before. We found two distinct haplotypic positive associations with MS in our population. Among DRB1 alleles, DRB1*1501 and DRB1*04 have shown the highest risk for developing MS (OR = 2.4 and 2.3, respectively). The comparisons of DQA1 and DQB1 alleles also demonstrated associations of DQA1*0301/2 and DQB1*0602 and *0302 with MS, suggesting a role of combined presence, because these alleles are in linkage disequilibrium with the associated DRB1 alleles. Consistent with this approach, two "putative" haplotypes, namely DRB1*1501 DQA1*0102 DQB1*0602 (25 vs. 8, $p = 0.003$, OR = 3.7) and DRB1*04 DQA1*03 DQB1*0302 (29 vs. 12, $p = 0.006$, OR = 2.9), were significantly increased in MS patients compared with controls.

Numerous studies had previously demonstrated a strong association of Dw2 and DR2 with MS in Caucasian patients from Europe and North America [8]; the association was later confirmed in other ethnic groups such as Chinese [29], Japanese [30], and Asian Indians and Afro-Caribbeans [31]. This association is defined more precisely as DR2 haplotype DRB1*1501 DQA1*0102 DQB1*0602. Our results extend this haplotypic disease association to the Turkish population. However, as in most other studies, the primary locus on the associated haplotype responsible for the susceptibility could not be delineated. Confirming combined presence as the major susceptibility marker, in the absence of DRB1*1501 carrying individuals, DQB1*0602 hardly reaches statistical significance ($p = 0.05$) [26], although the single presence of DQB1*0602 confers a higher risk of 3.2.

The second group associated with MS, DRB1*04, currently with 19 subtypes [32], is one of the most polymorphic subgroups of DRB1. Our data extend the reported DR4 association with MS in a Sardinian population [11]. Although the alleles *0402, *0403, and *0404 seemed to be more frequent in the MS group, the difference was insignificant, possibly because of small sample sizes. Expansion of the MS group to include more DR4 positive individuals may reveal a significant association with DRB*04 subtypes; this work is under way.

The association of MS with DR4 in the Sardinian population also implied a haplotypic association bearing DRB1*04 with DQA1*0301 and DQB*0302 [18]. Our data also support the positive associations with the DQA1*0301/2 (35 vs. 21, OR = 1.9) and DQB1*0302 (30 vs. 15, OR = 2.3) reported for the Sardinian population. However, because of strong linkage disequilibrium of the DQA, DQB, and DRB loci, we are unable to demonstrate whether the increase of the DQ alleles is related to an increase of DRB1*04.

The relative low frequencies of the DQA*0101 and *0103 alleles in our study can be interpreted either as protective markers for the disease or secondary to the positively associated alleles in the patient group.

To test a previously reported hypothesis with our results, we compared the presence of "shared DQB1 sequences" (DQB1*0602, *0603, *0604, *0302, and *0303) [16] in both groups. A significant difference was detected for the presence of the DQB1 alleles with shared sequences (DQB1*0602, 0603, 0604, 0302, and 0303), suggesting a positive association with the disease state (60 vs. 42, $p = 0.02$, OR = 1.9). If the DRB1*1501 positive individuals are excluded, however, this increase seems to be related to the presence of DRB1*1501. The reported associations of MS with DQA1 alleles with glutamine at aa position 34 (DQA1*0102, *0103, *0401, *0501, and *0601) [15], with DQB1 alleles with leucine at aa position 26 (DQB1*0602, *0603, *0604, *0605, *0302, *0303 and *0201) [27], and DQ $\alpha\beta$ heterodimer associations [15] were also not confirmed in this study.

The hypothesis based on sequence analysis of MS-associated DRB1 alleles claiming the importance of the dimorphic position 86 of the DRβ chain was also tested with our data [33]. In the MS group, 84% of the patients have one or two DRB1 alleles with valine at aa position 86, compared with 70% of the control group ($p = 0.02$, OR = 2.3). After excluding the DRB1*1501 or

DRB1*04 positive individuals, the presence of valine at aa position 86 did not differ significantly in the groups (58 vs. 58 and 59 vs. 62), implying an association secondary to the presence of the susceptible haplotypes with DRB1*1501 or DRB1*04.

Two distinct disease-associated haplotypes may be evaluated in two ways. A "genetic" approach may propose that another HLA or non-HLA gene closely linked to the susceptibility markers causes the disease [34]. This possibility is compatible with our findings, because a common gene carried on both associated haplotypes cannot be ruled out. Furthermore, more than one locus in the MHC could influence susceptibility or individual class II alleles could influence susceptibility in a hierarchical way [2]. An "immunologic" approach searching for a structural basis for the relevant antigen presentation of these gene products is also compatible with our results. In autoimmune response, autoantigenic peptides can be presented by different components of the haplotypes. As shown for DRB1*1501, the candidate autoantigenic peptide of myelin basic protein (MBP) can be presented by the product of this allele [35]. However, T cell responses to different peptide epitopes of MBP are demonstrated in MS [36], and other epitopes can be preferentially presented by the components of the DR4 haplotype. T cell responses of DR4-carrying MS patients need to be evaluated in a similar way.

In conclusion, this study confirms the associations of MS with HLA in a population from Turkey by emphasizing the presence of two different haplotypes. The MS-associated haplotype HLA Dw2 of the Northern European and North American Caucasians was significantly increased in the Turkish MS patient population compared with a control group of the same origin. An overrepresentation of HLA DR4 and related haplotypes was also found in this group of patients. Because the major susceptibility locus has yet to be defined, and alleles of DQA1 and DQB1 loci commonly borne on the same haplotypes are also associated with MS, probably related to linkage disequilibrium, comparisons of the patient group with controls should be evaluated primarily with haplotypes rather than individual alleles [37].

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