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ORIGINAL COMMUNICATION

Interaction of loci within the HLA region influences multiple sclerosis course in the Sardinian population

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Abstract We examined the influence of alleles at the HLA loci, previously found to be associated with multiple sclerosis (MS) in Sardinia, on the clinical course of the disease in 835 relapsing (R) and 100 primary progressive (PP) patients. Multivariate analysis was carried out on predisposing 0301 or non-associated DPB1 alleles, susceptible or non-associated DRB1-DQB1 haplotypes, both predisposing and non-predisposing, and negatively and non-negatively associated D6S1683 alleles, taking interaction between them into account. Intra-patient analysis showed that the presence of the susceptible or protective D6S1683 allele interacting with predisposing DP 0301 modulated risk of PP disease. These findings suggest that a locus telomeric to HLA class I exerts an effect on alleles at the DPB1 locus in modulating disease course.

Key words multiple sclerosis · genetics · MHC · HLA · course

Introduction

Recurrence risk for multiple sclerosis (MS), higher in relatives of MS patients and dropping quickly from 1st-degree relatives to succeeding degrees of parental relationship, and the evidence that risk declines in adopted as opposed to natural offspring, demonstrate that familial aggregation of the disease is genetic in nature [6]. Despite this evidence, the involvement of several chromosomal regions in disease susceptibility appears, at present, only modest [4, 7, 11, 14, 15, 31], and the relative contribution of genes contained in these loci largely conjectural. Whether genes not only influence susceptibility but also disease course, thus contributing to MS outcome, is at present controversial. In experimental autoimmune encephalomyelitis induced by immunization with myelin oligodendrocyte glycoprotein (MOG), an animal model of MS, clinical course and central nervous system pathology are driven by the major histocompatibility complex (MHC) haplotypes in a hierarchical and allele-specific manner [37]. While in humans the involvement of human leukocyte antigen (HLA) class II alleles in MS predisposition has been clearly established, the contribution of these molecules to disease course and severity has not yet been definitively accepted [1, 5, 13, 22, 36].

We have recently reported that several HLA loci, namely the DPB1, DRB1, DQB1 loci and a locus defined by the D6S1683 microsatellite, telomeric to the classical class I region, were associated with MS in Sardinian patients [21]. At the DR-DQ locus, five DRB1-DQB1 haplotypes, including DRB1*0405-DQB1*0301 and DRB1*0301-DQB1*0201, previously found in Sardinian MS [20] and DRB1*1303-DQB1*0301 DRB1*1501-DQB1*0602 and DRB1*0405-DQB1*0302, were positively associated with the disease [21], demonstrating that predisposition to MS carried by HLA is locus- but not allele-specific. In that report, the independence of the associated alleles at the DPB1 and DRB1-DQB1 loci and at the D6S1683 marker was underlined, but neither interaction between loci nor differences in association among clinically-different MS courses was examined.

The present study was undertaken to examine the contribution of HLA MS-associated loci to clinical phenotypes of MS, defined as relapsing (R) and primary progressive (PP) disease.

All affected subjects came from Sardinia, a Mediterranean island which, with about 140 MS cases per 100,000 inhabitants [10], has one of the highest incidences of MS in Europe. Hence it was possible to collect clinical and genetic data from a large cohort of patients, all observed for more than 20 years by the same team of neurologists at a single neurological clinic. Because the Sardinian population is unmixed and is characterised by a highly-homogeneous genetic structure [16], it is an ideal population for studying the modifying influence of genes while avoiding confounding factors due to genetic admixture [17].

Methods

Subjects

Each patient included in the study had to be born and living in Sardinia and of Sardinian origin for at least three generations. Informed consent was obtained from all patients,as well as the approval of the local Ethics Committee.

The study comprised 935 MS patients (302 men and 633 women), all frequenting the MS Clinic at the University of Cagliari (Italy) from 1 January 1976 to 31 December 2002. Three hundred and seventy-nine patients had previously been reported [21], while 556 constituted a new cohort. Mean present age of patients was 40.6 years (range 12–79), while mean age at onset was 28.3 (range 8–71). All patients and affected relatives included in the study met MS criteria [23].

Disease duration, considering onset as its starting point, ranged from 1 to 51 years (mean 12.4 years, 95% CI 11.8–13). Disease course was classified as relapsing-remitting (RR) and secondary progressive (SP), according to Lublin and Reingold's criteria [18]. Patients having RR and SP course were combined in the R group (n = 835). PP patients (n = 100) were defined according to the criteria proposed by Thompson et al. [33].

The control population consisted of 471 ethnically-matched individuals (315 women and 156 men, mean age 34.7, range 18–62) and 121 pseudo-controls consisting of affected family-based control (AFBAC [34]) genotype frequencies from singleton MS families from the island itself.

AFBAC frequencies are based on chromosomes never transmitted from parents to affected children and therefore provide a source of unequivocally-established haplotypes based on family data [20]. The AFBAC cohort has previously been described [21], while the control population of ethnically-matched individuals was a de novo typed cohort.

Genotyping

The chromosomal location and intra-marker distance of DPB1, DRB1, DQB1 and D6S1683 are reported in Fig. 1. Linkage-disequilibrium patterns between DPB1, DRB1, DQB1 and D6S1683 have been calculated as previously reported [21], and ranged from 0 to 1, with 0 reflecting perfect independence between alleles at the two loci we compared, and 1 reflecting complete linkage disequilibrium. The DPB1-DQB1 intermarker D' value was 0.29, DPB1-DRB1 D' value was 0.37, DPB1-D6S1683 D' was 0.16, DQB1-D6S1683 D' was 0.27 and DRB1-D6S1683 was 0.33.

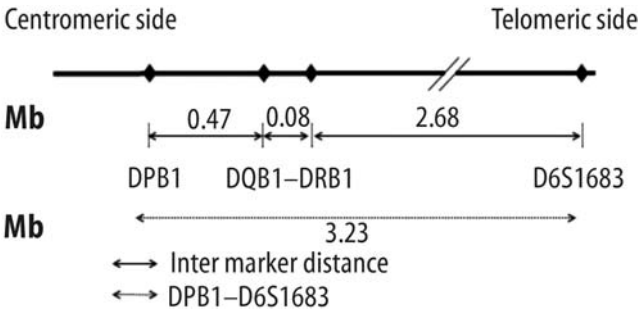

Fig. 1 Schematic representation of chromosomal location of DPB1, DQB1-DRB1 loci and the D6S1683 microsatellite and intramarker distances

Genotyping of HLA-DRB1, -DQB1, DPB1 loci and of the D6S1683 microsatellite was performed as previously described [21].

The attribution of haplotypes in MS patients was established following the co-segregation of alleles in both parents of patients and using computer program TDTPHASE by F. Dudbridge (see http://www-gene.cimr.cam.uk/tdt/). Only some haplotypes from parental genotype data were considered in the analysis.

Genotype frequencies in AFBAC were derived from the Hardy-Weinberg equilibrium using non-transmitted allele frequency, as described by Thomson [34].

In all, 935 patients and 592 controls were fully-typed at all loci.

Statistical analysis

Using one logistic model comparing R and PP patients, the aim was to detect possible genetic differences between the two clinical phenotypes.

Analysis was performed comparing R and PP by means of one multivariate logistic regression, in order to highlight differences between the two clinical phenotypes (intra-case analysis).

Secondarily, in order to highlight the impact of these differences on disease risk, the two clinical phenotypes were individually compared with controls, again using multivariate logistic regression. Obviously, the relationship between the two separate case-control studies arises directly from intra-case analysis.

Alleles at the DRB1-DQB1, DPB1*0301 and D6S1683 loci on MS were considered as independent variables, and the individual's genotype carrying at least one copy of the predisposing/protective allele was evaluated.

At DRB1-DQB1 locus, the five predisposing haplotypes in Sardinian patients, DRB1*1303-DQB1*0301 (DR13), DRB1*0405-DQB1*0301 (DR4-DQ 3.1), DRB1*0301-DQB1*0201 (DR3), DRB1*1501-DQB1*0602 (DR2) and DRB1*0405-DQB1*0302 (DR4-DQ 3.2), were considered as a whole as associated (DR+) vs other non-predisposing (DR–) haplotypes. Similarly, we considered the DPB1*0301 (DP+) positively associated allele vs all other non-associated alleles (DP–).

Alleles at the D6S1683 microsatellite were considered as positively-associated (allele 4, 1683+) vs other non-positively associated alleles (1683–), and as negatively-associated (allele 3, 1683p) vs other non-negatively associated alleles (1683p–).

According to Selvin [28], it is reasonable to delete the interaction term from the model only when its influence cannot be clearly differentiated from chance variation (i. e., p-value > 0.20). Statistical analysis was performed using SPSS software.

Results

Genotype frequencies of DP+, DP–, DR+, DR–, 1683+, 1683– and 1683p in R, PP, control and AFBAC are reported in Table 1. Because the two sets of controls and AFBAC showed similar frequencies (Table 1), they were combined in order to simplify subsequent analyses.

When comparing R and PP patients (Table 2), the DR+ variable was eliminated from the model because it had no influence, either as an independent variable or as a confounder.

The final model included two significant interactions: DP+/1683p (OR = 0.08, 95% CI 0.01–0.73, p = 0.025) and DP+/1683+ (OR = 2.55, 95% CI 1.01–6.39, p = 0.047).

According to the model, no variation in risk was observed in DP+ carriers in the absence of 1683+ and 1683, and in 1683+ or 1683p carriers in the absence of DP+.

As a consequence of interactions, the presence or absence of 1683+ or 1683p modified the effect of DP+ on MS course.

As a result of the positive interaction, subjects carrying 1683+ and DP+ showed increased risk of PP course, while patients with the 1683p and DP+ alleles showed decreased risk. Data are reported in Table 2.

Comparing risk in R patients vs total controls (Table 3), the greatest effect was found at the DRB1-DQB1 locus. Subjects with a DP+ genotype had slightly increased risk in respect to those having a DP– genotype. Increased risk was observed in subjects having a 1683+ genotype, while risk decreased in those with a 1683p genotype. We found no evidence of interaction between the loci examined, suggesting that each predisposing/protective allele has an independent effect on disease risk.

Comparing PP patients vs total controls (Table 4), increased risk was observed in subjects having a DR+ genotype. Two interactions are included in the model: between DP+ and 1683+ (value = 2.10, P = 0.12) and between DP+ and 1683p (value = 0.12, P = 0.06). No variation in risk was observed in DP+ carriers in the absence of 1683+ and 1683p, and in 1683+ or 1683 p in the absence of DP+.

As a consequence of interactions, the presence or ab-

Table 1 Genotype frequencies in controls, AFBAC, R, and PP

HLA genotype	Controls N (%)	AFBAC N (%)	Total controls N (%)	R N (%)	PP N (%)
DR–	117 (0.47)	175 (0.51)	292 (49)	227 (27)	27 (27)
DR+	132 (0.53)	168 (0.49)	300 (51)	608 (73)	73 (73)
DP–	143 (0.57)	204 (0.59)	347 (58)	373 (45)	38 (38)
DP+	106 (0.43)	139 (0.41)	245 (42)	462 (55)	62 (62)
1683p–	192 (0.77)	263 (0.77)	455 (77)	727 (87)	92 (92)
1683p	57 (0.23)	80 (0.23)	137 (23)	108 (13)	8 (8)
1683–	134 (0.54)	187 (0.55)	321 (54)	321 (38)	37 (37)
1683+	115 (0.46)	156 (0.45)	271 (46)	514 (62)	63 (63)

DR+ indicates any of the following predisposing haplotypes: DRB1*1303-DQB1*0301 (DR13), DRB1*0405-DQB1*0301 (DR4-DQ3.1), DRB1*0301-DQB1*0201 (DR3), DRB1*1501-DQB1*0602 (DR2) and DRB1*0405-DQB1*0302 (DR4-DQ3.2). DR– indicates all DR-DQ haplotypes other than those previously listed
DP+ indicates the DPB1*0301 allele; DP– indicates absence of the DPB1*0301 allele
1683+ and 1683– respectively indicate the presence or absence of allele 4 at the D6S1683 marker
1683p and 1683p– respectively indicate the presence or absence of allele 3 at the D6S1683 marker

Table 2 Multivariate analysis in 100 primary progressive vs 835 relapsing multiple sclerosis patients calculated according to carriage of the HLA genotype at the DPB1, DRB1-DQB1 and D6S1683 loci

HLA genotype	Odds Ratio	95% CI	p	PP N (%)	R N (%)
Interaction DP+ and 1683+	2.55	1.01–6.35	0.047	–	–
Interaction DP+ and 1683p	0.08	0.01–0.73	0.025	–	–
DP+ in the absence of 1683+ and 1683p	0.91	0.44–1.88	0.79	14 (14)	127 (15)
1683+ in the absence of DP+	0.58	0.29–1.14	0.11	16 (16)	211 (25)
1683p in the absence of DP+	1.42	0.59–3.44	0.44	7 (7)	47 (5.6)
DP+ and 1683+	2.31	1.26–4.24	0.007	47 (47)	303 (36)
DP+ and 1683p	0.08	0.01–0.86	0.03	1 (1)	61 (7)

DR+ indicates any of the following predisposing haplotypes: DRB1*1303-DQB1*0301 (DR13), DRB1*0405-DQB1*0301 (DR4-DQ3.1), DRB1*0301-DQB1*0201 (DR3), DRB1*1501-DQB1*0602 (DR2) and DRB1*0405-DQB1*0302 (DR4-DQ3.2). DR– indicates all DR-DQ haplotypes other than those previously listed
DP+ indicates the DPB1*0301 allele
1683+ indicates the presence of allele 4 at the D6S1683 marker
1683p indicates the presence of allele 3 at the D6S1683 marker

Table 3 Multivariate analysis in 835 relapsing multiple sclerosis patients vs 592 total controls calculated according to carriage of the HLA genotype at the DPB1, DRB1-DQB1 and D6S1683 loci

HLA genotype	Odds Ratio	95% CI	p	R N (%)	Controls N (%)
DR+	2.08	1.63–2.66	< 0.0001	608 (73)	300 (51)
DP+	1.30	1.03–1.64	0.03	462 (55)	254 (43)
1683+	1.45	1.15–1.82	0.001	514 (62)	271 (46)
1683p	0.58	0.44–0.78	0.0003	108 (13)	137 (23)

DR+ indicates any of the following predisposing haplotypes: DRB1*1303-DQB1*0301 (DR13), DRB1*0405-DQB1*0301 (DR4-DQ3.1), DRB1*0301-DQB1*0201 (DR3), DRB1*1501-DQB1*0602 (DR2) and DRB1*0405-DQB1*0302 (DR4-DQ3.2). DR– indicates all DR-DQ haplotypes other than those previously listed
DP+ indicates the DPB1*0301 allele
1683+ indicates the presence of allele 4 at the D6S1683 marker
1683p indicates the presence of allele 3 at the D6S1683 marker

Table 4 Multivariate analysis in 100 primary progressive multiple sclerosis patients vs 592 total controls calculated according to carriage of the HLA genotype at the DPB1, DRB1-DQB1 and D6S1683 loci

HLA genotype	Odds Ratio	95% CI	p	PP N (%)	Controls (%)
DR+	1.88	1.11–3.19	0.02	73 (73)	300 (51)
Interaction DP+ and 1683+	2.10	0.82–5.41	0.12	–	–
Interaction DP+ and 1683p	0.12	0.01–1.10	0.06	–	–
DP+ in the absence of 1683+ and 1683p	1.25	0.59–2.66	0.56	14 (14)	81 (14)
1683+ in the absence of DP+	0.93	0.46–1.86	0.84	16 (16)	139 (23)
1683p in the absence of DP+	0.68	0.29–1.62	0.38	7 (7)	89 (15)
DP+ and 1683+	2.63	1.37–5.04	0.004	47 (47)	132 (22)
DP+ and 1683p	0.15	0.02	0.09	1 (1)	48 (8)

DR+ indicates any of the following predisposing haplotypes: DRB1*1303-DQB1*0301 (DR13), DRB1*0405-DQB1*0301 (DR4-DQ3.1), DRB1*0301-DQB1*0201 (DR3), DRB1*1501-DQB1*0602 (DR2) and DRB1*0405-DQB1*0302 (DR4-DQ3.2). DR– indicates all DR-DQ haplotypes other than those previously listed
DP+ indicates the DPB1*0301 allele
1683+ indicates the presence of allele 4 at the D6S1683 marker
1683p indicates the presence of allele 3 at the D6S1683 marker

sence of 1683+ or 1683p modified the effect of DP+ on PP risk, and the presence or absence of DP+ modified the effect of 1683p and 1683+. DP+ individuals carrying 1683+ showed greater risk of PP course, while this was not observed in individuals with both DP+ and 1683p alleles.

Discussion

The course of MS is heterogeneous and basically divided into two main groups which, in the absence of biological markers, are defined on the basis of clinical characteristics. While the majority of patients (defined in the present study as R) experience an onset bout, followed by total or partial remission in the initial phase of the disease and then increasing disability, about 10–15% of patients (here designated PP) have a progressive from onset course (reviewed in [24]). From the clinical point of view, PP MS is characterised by poor response to treatment, a more severe outcome and faster disability from onset than in R patients (reviewed in [24]). Several lines of evidence suggest that PP MS represents one of the entities of the MS spectrum rather than a separate disease. Recent studies using magnetic resonance imaging have demonstrated that the major differences between PP and R MS lie in less or no inflammation [29, 32], greater tissue destruction of normal-appearing white matter [9], higher levels of creatine, a gliosis marker, in lesions, and normal-appearing white matter [30] in PP patients. The less intense inflammation in PP MS has been confirmed by pathological findings, which also showed marked oligodendrocyte depletion in plaque and periplaque white matter [3, 19, 26]. Understanding the possible genetic mechanisms influencing the various disease courses might have implications for future treatment of these patients.

In the present study, we attempted to determine the effect of alleles at loci within the HLA region on disease course. An effect of these alleles on MS predisposition and protection had previously been found in a population of Sardinian patients [21], without considering disease type. Multivariate analysis of R patients vs controls confirms previously-reported findings [21], showing that the relative risk carried by genes in the HLA region is multilocus and multiallelic and that each predisposing/protective allele at the loci examined exerts an independent effect on disease risk. As previously found [21],

the main effect on risk was exerted by the DRB1-DQB1 locus, with other minor effects at the DPB1 and D6S1683 loci. By contrast, analysis of PP patients vs controls showed that only DR+ independently affected risk, to an extent similar to that found in the R group, but neither alleles at the D6S1683 locus nor those at DPB1 had an effect on PP course risk, independent of other alleles. However, in a DP+ background, the presence of predisposing/protective 1683 alleles increased or decreased the risk of PP course as an effect of interaction between alleles at those loci. Intra-patient analysis confirmed such an effect, supporting the hypothesis that loci in the HLA region not only confer risk in cases of MS predisposition but also contribute to modulating its course. This hypothesis is supported by the joint effect of the DP+ and 1683+ alleles, which increase risk of PP disease more than twofold, while individuals carrying the DP+/1683p genotype seem more prone to R than to PP course. However, we cannot exclude the possibility that, rather than the D6S1683 microsatellite itself, another gene in linkage-disequilibrium with D6S1683 influences the effect of alleles at the DPB1 locus. Obvious candidates are genes within the HLA class I region, in linkage-disequilibrium (D' < 0.4 [21]) with D6S1683. The HLA class I region has been reported to contain certain alleles modifying the action of the HLA-DR2 MS-associated allele in humans [9, 12] and exercising a protective effect in EAE induced in rats [25]. Another possible gene involved in disease modulation is the MOG gene, located 0.47 Mb from D6S1683 [21], which codes for a candidate autoantigen of MS [35]. A marker of the MOG gene, the MOG51 microsatellite has been found in linkage disequilibrium (D' 0.29) with D6S1683 in the Sardinian population [21].

There is some evidence of an effect of DPB1 alleles in modulating the clinical course of MS. The DPB1*0301 allele (called DP+ in the present report) was found to preferentially restrict T-cell responses to an epitope within myelin proteolipid protein, one of the candidate autoantigens of MS, and to contribute to epitope spreading and the clinical progression of MS [39]. Another DPB1 allele, namely DPB1*0501, has been associated with the opticospinal form of MS, a clearly-distinct clinical variant of the disease [38]. However, in neither report have the effects of DPB1 alleles with other loci been examined.

Because multivariate analysis of PP vs R patients showed that the DR+ genotype did not differ in the two patient populations, the DRB1-DQB1 locus does not appear to exert an effect on the clinical course of the disease, thus supporting the idea that the relative risk conferred by predisposing DRB1-DQB1 alleles is equally distributed in the two forms of the disease. This finding is in line with other reports in populations ethnically different from Sardinians, showing no difference in R and PP MS risk due to DRB1-DQB1 alleles. Aside from negative results in small association studies (reviewed in [24]), a recent extensive analysis of Northern European patients showed no differences in carriage of the DRB1*1501-DQB1*0602 or DR2 MS-associated molecule between PP and R individuals [22]. Although in a small group of patients "severe" MS has been associated with carriage of two copies of the DR2 allele and "mild" disease with only one copy, no DR2-dependent differences on MS course were detected [2].

Interaction between the DPB1 allele and other predisposing/protective alleles at a still-unidentified locus telomeric to the HLA class I region might support an epistatic mechanism between them and deserves further study to clarify the biological significance of this finding. Precise dissection of the telomeric HLA region to establish the exact locus/gene involved might be useful in identifying the genetic mechanisms underlying the various MS courses.

As a whole, the present data may support the idea that genes involved in MS within the HLA region are locus and allelic heterogeneous in promoting either susceptibility or clinical phenotypes of the disease. However, given the small number of PP patients, this study should be considered an exploratory one, and the suggested modulatory role of DPB1 and D6S1683 alleles should be confirmed in a larger dataset of patients.

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