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ORIGINAL COMMUNICATION

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Multiple sclerosis and autoimmune diseases

Epidemiology and HLA-DR association in North-east Italy

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Abstract An autoimmune background is thought to characterize the families of multiple sclerosis (MS) patients, but disease patterns and HLA-DR association seem to vary considerably among different ethnic groups. We investigated the prevalence of autoimmune diseases in 245 MS patients and 245 age- and sex-matched normal controls (NC), originating from and living in North-east Italy, and their first degree relatives, using a case-control method. Further, HLA-DRB1 expression was analysed in MS and NC. The following significant findings were observed: 1) a significant excess of autoimmunity in first-degree relatives of MS patients ($p = 0.000$), 2) an association of MS with Type 1 diabetes mellitus (T1DM) ($p = 0.02$), 3) an increase in DR4 expression (namely DRB1*0401) in MS patients from families with multiple autoimmune pathology compared with reference MS patients ($p = 0.02$) and NC ($p = 0.01$). We conclude that the risk of autoimmune disease is higher in first-degree relatives of MS patients and that disease association and HLA-DR expression in North-east Italy differs from other geographic regions of Europe.

Key words multiple sclerosis · autoimmune disease · epidemiology · HLA-DR

Introduction

A generalized autoimmune background seems to characterize the families of multiple sclerosis (MS) patients. An excess of autoimmunity, expecially autoimmune thyroid disorders, in first-degree relatives of MS patients was observed in an English cohort [1], a significant increase in prevalence of autoimmune diseases in MS families compared to controls was noticed in Australia [2] and France [3], and the risk of having type I diabetes mellitus (T1DM) was found to be significantly higher in Sardinian MS patients and their first degree relatives [4]. Nevertheless, data in the literature are contradictory [5–8]. Autoimmunity patterns and HLA genotype association seem to vary considerably among different ethnic groups, and the role played by HLA antigens in autoimmune susceptibility has not yet been clarified [9–11]. For instance, in Sardinia MS is associated with the expression of HLA-DR3/HLA-DR4 [12] that predispose individuals to T1DM, while in Northern Europe MS is associated with HLA-DR2, which is known to protect against T1DM [13]. These findings, however, do not necessarily apply to people of other geographic regions of Europe, such as North-east Italy, where migratory fluxes have been more pronounced compared to Sardinia and Scandinavia. On the basis of an epidemiological study recently carried out in the Province of Padova [14] we have conducted a case-control study aimed at defining the prevalence of autoimmune diseases in MS patients and their first-degree relatives. HLA-DRB1 phenotypic frequencies in

MS patients with or without an autoimmune background was also investigated.

Material and methods

245 MS patients and 245 age- and sex-matched normal controls (NC) were included in the study (Table 1). All cases (167 F, 78M, F/M = 2.08; mean age = 39 ± 11; age range = 16–69) and controls (unrelated to MS cases; 150 F, 95 M, F/M = 1.58 [MS vs NC: $\chi^2 = 2.287$, p = 0.13]; mean age = 37 ± 12 [MS vs NC: p = 0.06]; age range = 15–74) were natives of and living in North-east Italy, where MS shows a prevalence of 81.5/100,000 and an incidence of 4.2/100,000 [14]. Ethical approval was obtained from the University Hospital of Padova Ethic Committee and informed consent was obtained from all the subjects (patients and controls). All MS patients included in this study are regularly followed by neurologists of the Multiple Sclerosis Centre of Veneto Region, and meet the Poser criteria for a diagnosis of clinically definite MS [15]. Information on the occurrence of both autoantibodies-associated and putative T-cell mediated autoimmune diseases were obtained from almost all (> 95 %) the first degree relatives of both groups by a direct interview. Selected common non-autoimmune diseases (i.e., hypertension, hearth attack, headache) were considered in order to control for reporting bias. When an autoimmune disease was recorded, all relevant clinical and paraclinical informations about the disease were requested, and, when possible, the affected individual was directly examined. Therefore, only documented diseases were accepted.

On the basis of the most recent Italian and European epidemiological estimates for the most important autoimmune diseases [16–19], the overall population prevalence of autoimmune diseases in our geographic region was estimated to range from 5 % to 6 %.

Genomic DNA was extracted from peripheral blood using the Qiamp DNA mini kit (Qiagen). Cases and controls were typed for HLA-DRB1 by means of a commercially available Kit (Genovision) consisting in a PCR-SSP (PCR-sequence specific primers) method. All subjects gave informed consent. HLA-DR patterns of MS patients from multiple autoimmune families were compared with those from MS patients with no autoimmune background and from NC and the Veneto (the Region of North-east Italy where the city of Padova is located) registry of bone marrow donors (BMD).

Statistical analysis was carried out using the proportion of families in whom one or more relatives had the specified condition. Two-tailed (TT) Fisher's test and χ^2 were applied for comparison between two groups. The odds ratio was used to calculate the risk.

Results

The prevalence of MS patients suffering from other autoimmune diseases (23/245, 9.4 %) was not significantly increased compared with that observed for NC (15/245, 6 %) (MS vs NC: χ^2, p = 0.24) (Table 2). Four families with multiple autoimmune pathology (autoimmune families, AF) were identified in the NC group (2 %) and 42 in the MS group (17 %) (χ^2, p = 0.000). Including the MS multiplex families (n = 6), the significance did not change (χ^2, p = 0.000). In MS patients and their first-degree relatives, a total number of 73/984 (8.7 %) subjects suffering from autoimmune disorders were identified, while only 36/1002 (≅ 3.6 %) were scored in the NC group and their first-degree relatives (χ^2, p = 0.000). The occurrence of autoimmune thyroid diseases (Hashimoto thyroiditis and Graves' disease) in MS patients (9 cases = 3.6 %; increased anti-thyroid antibody levels during interferon beta therapy were not taken into consideration) was similar to that of NC (7 cases = 2.8 %; prevalence in North-east Italy = 3–5 %), while T1DM was much more frequent in MS patients (9 cases) than in NC (only 1 case) and the difference was significant (MS vs NC: χ^2:5.002, p = 0.02, OR = 9.3) (Table 2). In first-degree relatives of MS patients, clinically manifest thyroid autoimmunity and T1DM were also more frequent (13 and 8 cases, respectively) than in first-degree relatives of NC (10 and 3 cases, respectively), but differences were not significant. Taken all together, these findings clearly show a significant clustering of autoimmune diseases in families of MS patients and a significant association of MS with T1DM in North-east Italy.

DR2 (DR15) expression was higher in MS patients suffering from, or having first degree relatives affected by autoimmune diseases (34 %) than in MS patients with no other autoimmune diseases in their family (32 %) or in NC (25 %). These differences, however, were not significant (two-tailed Fisher, p = 0.32 for both comparisons) (Fig. 1).

Table 1 Age and sex distribution of the MS patients and NC subject included in the study

	MS	NC	
N°	245	245	
F/M (ratio)	167/78 (2.08)	150/95 (1.58)	$\chi^2 = 2.287$, p = 0.13
Mean age (years)	39 ± 11	37 ± 12	p = 0.06
Age range (years)	16–69	15–74	n.s.

Table 2 Twenty-three MS patients had 25 autoimmune diseases (AD). * and ° = two MS patients with T1DM and autoimmune thyroid pathology. No case of mixed connective tissue inflammation, SLE, Coeliac disease, Sjøgren syndrome, Horton's arteritis, anemia perniciosa, myasthenia gravis, and ankylosing spondilitis were found in these MS and NC series

	MS with AD	Controls with AD	χ^2
Total number	23/245 (9.4 %)	15 (6 %)	ns
Females/Males	16/7 (ratio 2.3)	11/4 (ratio 2.7)	ns
Type 1 DM	9*°	1	p = 0.02
Graves' Disease	5*	3	ns
Hashimoto's Disease	4°	4	ns
RA	2	2	ns
Alopecia	1	0	ns
Psoriasis	1	2	ns
Vitiligo	1	3	ns
Uveitis	1	0	ns
Autoimmune Glomerulonephritis	1	0	ns

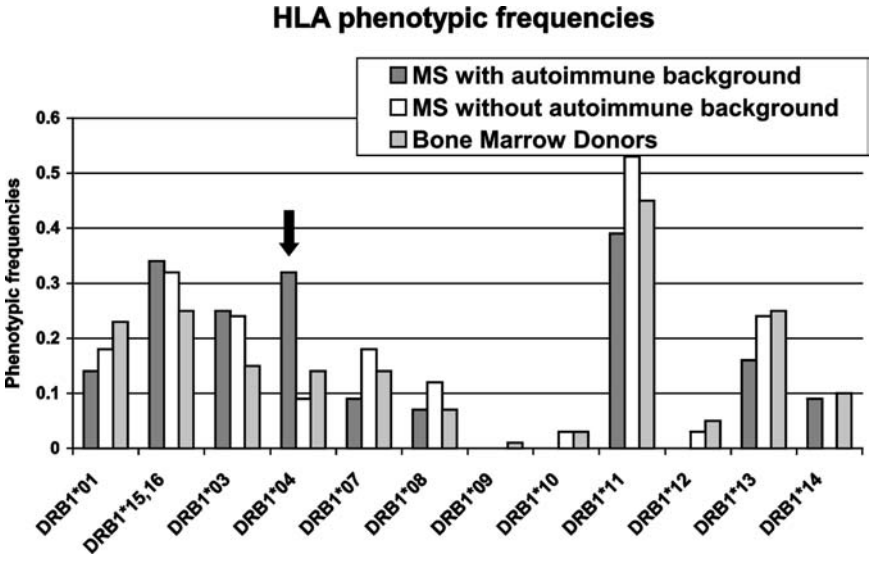

Fig. 1 HLA-DRB1 phenotypic frequencies in MS patients with or without autoimmune background and in bone marrow donors of the Veneto Region of Italy. MS patients suffering from other autoimmune diseases had a significantly higher expression of DRB1*04 (arrow)

DR3 was also more expressed in MS patients from AF (25%) than in MS (24%) and NC (15%), but did not reach significance (χ^2, $p = 0.1$; TT Fisher, $p = 0.1$).

Interestingly, DR4 expression in MS patients from AF was significantly higher (14/42, 33%) than in reference MS patients (9%; χ^2: $p < 0.001$; TT Fisher: $p = 0.02$; OR: 4.7) and NC (14%) (χ^2: $p = 0.004$; TT Fisher: $p < 0.01$; OR 2.82). These findings were only partially explained with the unexpected high number of MS patients suffering from T1DM. Finally, we observed that DR4 and DR2 were mutually exclusive, since only 0.8% of our MS patients expressed the DR2/DR4 phenotype (10.8% was expected).

DR13 was, by contrast, less expressed in MS patients from multiplex autoimmune families (16%) than in reference MS patients (24%) and in healthy controls (25%), but differences were not significant (TT-Fisher, $p = 0.56$ and $p = 0.3$, respectively).

Discussion

Our data confirm previously reported findings of a significant excess of autoimmune pathology in first-degree relatives of MS patients [1–3]. In our control subjects, well matched with the MS population, the prevalence of autoimmune diseases (6%) was in line with figures rising from Italian and European epidemiological studies.

In MS AF, autoimmune thyroid diseases did not contribute particularly to the excess risk of autoimmunity as described elsewhere [1, 3]. However, an underestimation of the real prevalence of thyroid autoimmunity in our study is possible since both case and control populations had a low mean age, and the immunological screening for thyroid antibodies was routinely performed only in MS patients. Since MS patients treated with immunomodulatory drugs (e.g., interferon beta and Campath-1H) [20] may develop autoimmune thyroiditis, the relationship between MS and autoimmune thyroid diseases (especially Graves' disease) need to be further investigated.

We would like to stress two findings of our study that are particularly interesting: 1) the association of T1DM with MS (9 cases, 3.6%; a 9-fold increase in prevalence compared with the NC, but 15–20 times more than in the normal Italian population, i.e., 0.15–0.3%) that was invariably associated with DR4 expression; 2) the high prevalence of DR4 expression in MS patients belonging to AF (33%) compared with the reference MS population (9%). While these data point to a role for DR4 in autoimmune predisposition, they seem to indicate that DR expression in Caucasian MS patients of North-east Italy shows an intermediate pattern compared with Nordic MS (mainly associated with DR2, which actually protects against T1DM) [14–21] and Sardinian MS (mainly DR4-associated) [22, 23], probably reflecting differences in genetic isolation/migratory fluxes of the ethnic groups studied.

Taken all together, our data deserve to be considered because firstly, they reinforce previously reported findings of an excess of autoimmune pathology in first-degree relatives of MS patients, and further suggest the hypothesis that a common genetic susceptibility for autoimmunity co-exists with additional disease-specific factors (non-MHC self-antigen? environmental factors?), which determine clinical phenotype in the individual. Secondly, they point to a role for DR4 in autoimmune predisposition, and support the hypothesis of a preferential association of MS with T1DM, at least in Southern Europe. Thirdly, they show that MS is not necessarily associated with a particular DR phenotype. Indeed, DR expression in Caucasian MS patients of North-

east Italy shows an intermediate pattern compared with Nordic MS and Sardinian MS. Fourthly, they further stress the high heterogeneity of MS, and strongly suggest the opportunity for a more detailed stratification of MS patients not only for genetic studies, but also for their enrolment in clinical trails with immunomodulatory agents/antigens.

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