

HLA Markers and Clinical Characteristics in Caucasians with Primary Sjögren's Syndrome

ANNE ISINE BOLSTAD, RALF WASSMUTH, HANS-JACOB HAGA, and ROLAND JONSSON

ABSTRACT. Objective. To explore the association between HLA genotypes and clinical and immunological characteristics in Caucasians with primary Sjögren's syndrome (pSS).

Methods. HLA genotyping for DRB1, DQA1 and DQB1 was carried out in 62 single case patients with pSS and 64 healthy controls. The specific amino acid residues at DQA1 position 34 (DQ α -34Q) and DQB1 position 26 (DQB-26L) in addition to the DQ-DI (AA59-AA69) motif were determined. Subsequently, the relative contribution of individual HLA markers to clinical and immunologic characteristics of pSS was assessed by group comparisons.

Results. No significant associations were seen between HLA markers and histopathological or clinical features of pSS. Significant positive associations with HLA Class II markers were restricted to the formation of different autoantibodies. Formation of an anti-Ro/SSA and anti-La/SSB autoantibody response was positively associated with DRB1*03, DQB1*02 and DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygosity. Patients positive for anti-La/SSB also showed a strong positive anti-La/SSB association with DQA1*0501. Considering the contribution of individual DQA1 and DQB1 amino acids and sequence motifs to the formation of anti-Ro/SSA and anti-La/SSB autoantibodies, a dose dependent positive influence was detected for DQ α -34Q and DQB-26L. For DQB-DI, the largest difference between patients and controls was seen for the presence of a single copy of this motif after selecting patients with either anti-Ro/SSA or anti-La/SSB autoantibodies.

Conclusion. The association of HLA Class II markers with pSS may concern the anti-Ro/La response rather than the disease itself. The strongest contributors to the formation of an anti-Ro/La response included components of the DRB1*03-DQB1*02-DQA1*0501 haplotype also encompassing the transethnic-associated DQB-DI motif. In addition, the dose dependent contribution of DQ α -34Q and DQB-26L argue for a recessive contribution of HLA-DQ to the formation of an anti-Ro/La response. Given the prominent associations with DRB1*03 and the complex dose dependent interactions at HLA-DQ, a joint contribution of HLA-DR and DQ is likely to be relevant for the formation of anti-Ro/La autoantibodies in patients with pSS. (J Rheumatol 2001;28:1554-62)

Key Indexing Terms:

SJÖGREN'S SYNDROME HLA GENOTYPING ANTI-RO/SSA ANTI-LA/SSB

Sjögren's syndrome (SS) is a chronic autoimmune rheumatic disease mainly characterized by xerostomia and

From the Broegelmann Research Laboratory, University of Bergen; the Center for Medical Genetics and Molecular Medicine, and the Section of Rheumatology, Institute of Medicine, Haukeland University Hospital, Bergen, Norway; the Institute for Clinical Immunology, Department of Medicine III, University of Erlangen-Nuremberg, Erlangen, Germany.

Supported in part by grants from the EU - Biomed II BMH4 CT96-0595, EU - Biomed II BMH4 CT98-3489, The Research Council of Norway; Project 115563/320, Aslaug Andersens fond for revmatologisk forskning i Bergen, L. Meltzers høyskolefond and Norske Kvinners Sanitetsforening.

A.I. Bolstad, DDS, PhD, Broegelmann Research Laboratory, University of Bergen and Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital; R. Wassmuth, MD, PhD, Institute for Clinical Immunology, Department of Medicine III, University of Erlangen-Nuremberg; H.-J. Haga, MD, PhD, Section of Rheumatology, Institute of Medicine; R. Jonsson, DMD, PhD, Broegelmann Research Laboratory.

Dr. Bolstad and Dr. Wassmuth contributed equally to this article.

Address reprint requests to Dr. A.I. Bolstad, Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, N-5021 Bergen, Norway.

Submitted August 28, 2000 revision accepted November 10, 2000.

keratoconjunctivitis sicca with lymphocyte infiltration in the salivary and lacrimal glands¹. The spectrum of the disease extends from an organ-specific disorder affecting exocrine glands to a systemic disorder with pulmonary, musculoskeletal, hematological, gastroenterological, dermatological, renal and neurological involvement. SS can occur alone (primary SS, pSS) or in combination with a systemic rheumatic disease (secondary SS, sSS) like rheumatoid arthritis or systemic lupus erythematosus (SLE). An important diagnostic criterion for pSS is the detection of autoantibodies. Anti-Ro/SSA is found in 50 to 80% of the patients and anti-La/SSB in 30 to 60%². Antinuclear antibodies (ANA) and rheumatoid factors (RF) are also commonly present. About 5% of pSS patients develop lymphoid malignancy, most commonly non-Hodgkin's lymphoma³. Primary SS is a common rheumatic disease found worldwide with an estimated prevalence of about 0.2-0.6% depending on the criteria used for diagnosis⁴. Ninety percent of the patients are women.

The polymorphic major histocompatibility complex

(MHC) genes are the best documented genetic risk factors for the development of autoimmune diseases^{5,6}, and the most relevant MHC genes involved in susceptibility to pSS are the Class II genes, most specifically HLA-DR and DQ alleles⁷. A primary association between pSS and HLA-DR3, which is in linkage disequilibrium with HLA-B8, is well recognized for Caucasians⁸⁻¹². In addition, an association with HLA-DR2 has been reported in Scandinavians¹³, DR5 in Greeks¹⁴, and DRw53 in Japanese¹⁵. Recently, the HLA-DRB1*0301-*1501 heterozygous genotype has been suggested to play a role in susceptibility to pSS¹⁶. Increased frequency of the haplotype HLA-DRB1*0301-DRB3*0101-DQA1*0501-DQB1*0201 was found in Caucasian patients with pSS, DRB1*0405-DRB4*0101-DQA1*0301-DQB1*0401 in Japanese patients, and DRB1*0803-DQA1*0103-DQB1*0601 in Chinese patients¹⁷.

HLA-DR2 and DR3 have frequently been reported to be associated with autoantibody production to Ro/SSA and La/SSB autoantigens¹⁸⁻²⁰, and stronger associations have been found with HLA-DR2 and HLA-DR3 and the Ro/La antibody responses than with the disease itself¹⁸. A role of HLA-DQ locus (particularly DQ1-DQ2 heterozygotes) in anti-Ro/SSA and anti-La/SSB production has been demonstrated²¹⁻²³. Fei *et al*²⁴ reported a correlation of anti-Ro/SSA and anti-La/SSB with DR3, DQA4 and DQA4/DQA1 heterozygotes in Caucasians. Heterozygotes for HLA-DQw1/DQw2 were found to have the highest level of anti-Ro/SSA and anti-La/SSB in both pSS and SLE²⁰⁻²². Specific amino acid residues in the second hypervariable region of HLA-DQA1 and DQB1, glutamine (Gln, Q) in position 34 of DQA1 and leucine (Leu, L) in position 26 of DQB1 may promote the anti-Ro/SSA and anti-La/SSB responses^{22,23}. A shared amino acid motif in the DQB1 first domain, aspartic acid as amino acid 66 (Asp, D) and isoleucine as amino acid 67 (Ile, I), has been demonstrated in a disease-associated haplotype in different ethnic groups (Caucasian, Chinese and Japanese patients with pSS)¹⁷. There is also an increasing interest in the role of HLA-markers for the severity of autoimmune disease^{12,25}. The DR3-DQ2 haplotype has been indicated as a possible marker for a more active immune response in Finnish patients with pSS²⁶, and distinct HLA haplotypes have been associated with different degrees of autoantibody diversification in pSS²⁷.

Given the broad spectrum of clinical manifestations of pSS and the complexity of the HLA markers incriminated in pSS, we assessed the influence of HLA markers on the clinical and immunological manifestations of the disease.

MATERIALS AND METHODS

Patients and controls. Our study included 62 patients with pSS (59 females and 3 males) followed at the Department of Rheumatology at Haukeland University Hospital, and 66 healthy blood donors (35 females and 31 males) at the same hospital (Table 1). All were Norwegian Caucasians except for one Russian Caucasian and one patient from Pakistan. They all

met the classification criteria for pSS proposed by the European study group for SS²⁸. The patients were seen for the first time during the years 1992-7, and have subsequently been followed once a year since 1996. The mean followup period was 49.1 months. The mean age was 59.3 years (median 61.0 years). The Schirmer-I test was used (≤ 5 mm wetting of the paper strips in 5 min) to test for ocular signs. Salivary gland biopsies were taken from the lower lip of 55 of 62 patients (88.7%) and histological evaluation of focus score was performed²⁹. Unstimulated salivary flow should be ≤ 1.5 ml in 15 min to achieve the diagnosis of salivary gland involvement. The study was approved by the Committee of Ethics at the University of Bergen.

DNA extraction. DNA was extracted from peripheral venous blood either by QIAamp[®] blood kit (Qiagen, Germany) or by Genepure[™] 341 nucleic acid purification system (Applied Biosystems, CA, USA). DNA from each patient was quantified in a spectrophotometer and run on a 1% agarose gel to check the quality.

HLA genotyping. HLA genotyping was performed by oligonucleotide hybridization of enzymatically amplified DNA. Low resolution HLA-DRB1 typing comprising the DRB1*01 to DRB1*17 specificities was done by sequence-specific hybridization of a panel of oligonucleotide probes to PCR products as published³⁰. Similarly, DQB1 and DQA1 alleles were determined following the XI.IHWC protocol³¹. Using this approach, most of the DQA1 (DQA1*0101/04, *0102, *0103, *0201, *03, *0401, *0501-*0503, *0601) and DQB1 (DQB1*0501-*0504, *0601-*0604, *0605/06, *02, *0301-*0304) alleles can be differentiated. To assess the importance of individual amino acid positions, DQA1 position 34 (DQ α -34Q [DQA1*0102, *0103, *0401, *0501-*0503, *0601] vs DQ-34E [DQA1*0101, *0104, *0201, *0301, *0302]), DQB1 position 26 (DQB-26L [DQB1*0602-*0606, *0606, *02, *0302, *0303] vs DQB-26G/26Y [DQB1*05, *0601, *0301, *0304]), and the DQB motif AA59-69 (EYWN-SQKDI~~L~~E), termed DQB-DI, seen in DQB1*0601, *02, *04, were determined based on the sequence information and the genotyping results.

Biometric analyses. Odds ratios (OR) were calculated according to Woolf's method as cross-product ratios of a 2 \times 2 contingency table [OR = (a \times d) / (c \times b)]^{32,33}. Haldane's correction for the OR was used when either all patients were positive or all controls were negative for a particular specificity or allele³⁴. The level of significance was assessed by Yates-corrected chi-square analysis, or Fisher's exact test where appropriate. Stratification analysis (Mantel-Haenszel test) was used to detect relative influences of individual HLA markers³⁵. For the comparison of different groups the Wilcoxon rank sum test or the two-sample t test was used where appropriate³⁶. Statistical significance was assessed using the chi-square heterogeneity test, or the Fisher's exact test where appropriate. McNemars' test was used to test the null hypothesis of an equal probability of a positive result under the adjacent pair of tests in the different stratified groups of patients as described³⁷.

RESULTS

Study population. Clinical data from the study population are summarized in Table 1. All 62 patients (100%) had oral symptoms, 56% had lower labial salivary gland focus score > 1 , 37% had a history of salivary gland enlargement, and salivary gland involvement with unstimulated salivary flow ≤ 1.5 ml/15 min was found in 47% patients. While 95% had ocular symptoms, objective ocular signs were found in 65%. Autoantibodies were a frequent finding (87%) with antinuclear antibodies being the most common (87%); 45% had rheumatoid factors, 34% were anti-Ro/SSA positive, and 24% were anti-La/SSB positive. Dry skin was reported by 44% of the patients, skin manifestations by 45%, dry nose by 61%, and dry cough was common (74%). One major

Table 1. Characteristics of the study population with pSS.

Variable	All Patients (n = 62) n (%)	Anti-Ro+ Patients (n = 21) n (%)
Male/female patients	3/59	1/20
Age at first symptoms, yrs, mean/median	43/51	44/48
Age at diagnosis, yrs, mean/median	56/59	54/40
Duration of disease, yrs, mean/median ^a	6/19	7/19
Followup, mo, mean/median	36/56	68/51
Ocular symptoms	59 (95)	19 (91)
Oral symptoms	62 (100)	21 (100)
Ocular signs	40 (65)	14 (67)
Focus score > 1 ^b	31 of 55 (56)	17 of 17 (100) ^d
Salivary gland involvement ^c	29 (47)	11 (52)
Salivary gland enlargement	23 (37)	8 (38)
Autoantibodies	54 (87)	21 (100)
Anti-Ro/SSA antibodies	21 (34)	21 (100)
Anti-La/SSB antibodies	15 (24)	15 (100)
Rheumatoid factor (pos. ≥ 1:32)	28 (45)	19 (91) ^e
ANA (pos. ≥ 1:32)	54 (87)	21 (100) ^f
Dry cough	46 (74)	17 (81)
Dry nose	38 (61)	14 (67)
Dyspareunia	34 (55)	11 (52)
Extraordinary fatigue	51 (82)	17 (81)
Low grade fever	8 (13)	2 (10)
Arthralgias	46 (74)	11 (52)
Arthritis	22 (35)	6 (29)
Raynaud's phenomenon	34 (55)	13 (62)
Lung involvement	10 (16)	3 (14)
Kidney involvement	8 (13)	6 (29) ^g
Liver involvement	5 (8)	0 (0)
Thyroid involvement	9 (15)	3 (14)
Vasculitis	2 (3)	2 (10) ^h
Peripheral nerve involvement	5 (8)	1 (5)
Dry skin	27 (44)	8 (38)
Skin manifestations	28 (45)	9 (43)
Myositis	0 (0)	0 (0)
CNS involvement	0 (0)	0 (0)
Leukopenia	3 (5)	1 (5)

Data are median, mean, or number (%). ^aTime from diagnosis until closing the study. ^bThe preliminary criteria for classification of Sjögren's syndrome was followed²⁸ except that only focus scores > 1 were accepted as positive. Percentage with focus score is calculated from total number of biopsies in the respective group (55 and 17 respectively). ^cPresent if unstimulated salivary flow ≤ 1.5 ml/15 min. ^dChi-square = 19.05, df 1, p = 0.00001, CI = 0.24–0.56; ^eChi-square = 26.33, df 1, p = 0.00000, CI = 6.59–173.08; ^fChi-square = 4.70, df 1, p < 0.05, CI = 0.69–0.94; ^gChi-square = 6.94, df 1, p < 0.01, CI = 1.41–43.01; ^hChi-square = 4.03, df 1, p < 0.05, CI = 0.96–1.27. CI = confidence interval. p values are given only when statistically significant. The statistical data are derived from comparisons of frequencies of anti-Ro/SSA positive vs anti-Ro/SSA negative patients with pSS.

feature of pSS is the extraordinary fatigue (82%). Other common characteristics were dyspareunia (55%), arthralgias (74%) and Raynaud's phenomenon (55%). Some had arthritis (35%), while thyroid (15%), lung (16%), kidney (13%), liver (8%), and peripheral nerve (3%) involvement was more seldom seen. When patients were divided over the presence of anti-Ro/SSA autoantibodies (anti-Ro/SSA positive vs anti-Ro/SSA negative) and compared for the presence of disease characteristics, significant differences were seen for the focus score exceeding 1 (100 vs 56%; p = 0.00001), RF (91 vs 45%; p = 0.00000), ANA (100 vs 54%,

p < 0.05), kidney involvement (29 vs 13%; p < 0.01) and vasculitis (10 vs 3%; p < 0.05).

HLA associations. HLA DRB, DQA1 and DQB1 genotypes were determined successfully in all 62 patients and 64 healthy controls. In addition to the comparison of all patients (SS) with the controls, subsets of patients identified based on the presence of autoantibodies (ANA, and/or anti-Ro/SSA or anti-La/SSB, RF) or combinations of individual autoantibody specificities were also selected for comparison. Also, the comparison included the amino acid variability at position 34 of the DQα chain (DQα-34Q vs

DQ α -34E), at position 26 of the DQB β chain (DQB β -26L vs DQB β -26G/26Y) and included a sequence motif at position

59 through 69 (EYWNSQKDILE) of the Dq β chain comprising DQB1*0504, *0601, *02, and *04 (DQB-DI).

Table 2. HLA associations with pSS for HLA markers that show at least one significant association for any of the comparisons made.

HLA Marker	Patient Group	n (%)	Controls n (%)	OR	Chi-square	p	CI
	All	n = 62					
DRB1*03		29 (46.8)	19 (29.7)	2.08	3.21	0.07	1.00–4.33
DQA1*0501		34 (54.8)	28 (43.8)	1.56	1.14	0.29	0.77–3.15
DQ α -34Q		52 (83.9)	54 (84.4)	0.96	< 0.001	1	0.37–2.5
DQB1*02		32 (51.6)	26 (40.6)	1.56	1.12	0.29	0.77–3.16
DQB β -26L		57 (89.1)	58 (93.5)	1.78	0.33	0.56	0.49–6.42
DQB-DI		36 (58.1)	26 (40.6)	2.02	3.17	0.08	0.99–4.11
	FS > 1	n = 31					
DRB1*03		16 (51.6)	19 (29.7)	2.53	3.42	0.06	1.04–6.12
DQA1*0501		17 (54.8)	28 (43.8)	1.56	0.63	0.43	0.66–3.70
DQ α -34Q		23 (74.2)	54 (84.4)	0.53	0.83	0.36	0.19–1.52
DQB1*02		18 (58.1)	26 (40.6)	2.02	1.9	0.17	0.85–4.83
DQB β -26L		28 (90.3)	57 (89.1)	1.15	0.03	1	0.28–4.77
DQB-DI		19 (61.3)	26 (40.6)	2.31	2.38	0.09	0.96–5.57
	AAB+	n = 54					
DRB1*03		27 (50)	19 (29.7)	2.37	4.26	0.039*	1.1–5.05
DQA1*0501		32 (59.3)	28 (43.8)	1.87	2.23	0.135	0.90–3.90
DQ α -34Q		46 (85.2)	54 (84.4)	1.07	< 0.001	1	0.39–2.92
DQB1*02		28 (51.9)	26 (40.6)	1.57	1.07	0.301	0.76–3.27
DQB β -26L		50 (92.6)	57 (89.1)	1.54	0.16	0.73	0.42–5.55
DQB-DI		32 (59.3)	26 (40.6)	2.13	3.36	0.07	1.02–4.44
	ANA+	n = 54					
DRB1*03		27 (50.0)	19 (29.7)	2.37	4.26	0.04*	1.11–5.05
DQA1*0501		32 (59.3)	28 (43.8)	1.87	2.23	0.14	0.90–3.9
DQ α -34Q		45 (85.2)	54 (84.4)	1.07	< 0.001	1	0.39–2.92
DQB1*02		28 (51.9)	26 (40.6)	1.57	1.07	0.30	0.76–3.27
DQB β -26L		50 (92.6)	57 (89.1)	1.54	0.11	0.73	0.42–5.55
DQB-DI		32 (59.3)	26 (40.6)	2.13	3.36	0.07	1.02–4.44
	Ro/SSA+	n = 21					
DRB1*03		15 (71.4)	19 (29.7)	5.92	9.81	0.002*	2.00–17.58
DQA1*0501		15 (71.4)	28 (43.8)	3.21	3.8	0.051	1.11–9.35
DQ α -34Q		18 (85.7)	54 (84.4)	1.11	< 0.001	1	0.26–4.49
DQB1*02		16 (76.2)	26 (40.6)	4.68	6.64	0.01*	1.52–14.35
DQB β -26L		19 (90.5)	57 (89.1)	1.17	< 0.001	1	0.22–6.11
DQB-DI		17 (81)	26 (40.6)	6.21	8.74	0.003*	1.88–20.58
	La/SSB+	n = 15					
DRB1*03		13 (86.7)	19 (29.7)	15.4	15.35	< 0.001*	3.43–80.14
DQA1*0501		13 (86.7)	28 (43.8)	8.36	8.15	0.004*	1.89–42.90
DQ α -34Q		13 (86.7)	54 (84.4)	1.32	< 0.001	1	0.24–6.17
DQB1*02		13 (86.7)	26 (40.6)	9.50	10.31	0.001*	1.98–45.67
DQB β -26L		13 (86.7)	57 (89.1)	0.80	< 0.001	1	0.15–4.30
DQB-DI		13 (86.7)	26 (40.6)	9.50	10.31	0.002*	1.98–45.67
	RF+	n = 28					
DRB1*03		19 (67.9)	19 (29.7)	5.00	10.18	0.001*	1.92–13.02
DQA1*0501		19 (67.9)	28 (43.8)	2.70	3.62	0.06	1.07–6.91
DQ α -34Q		23 (82.1)	54 (84.4)	0.85	< 0.001	1	0.16–2.77
DQB1*02		20 (71.4)	26 (40.6)	3.65	6.21	0.013*	1.40–9.54
DQB β -26L		26 (92.9)	57 (89.1)	1.60	0.03	0.86	0.31–8.22
DQB-DI		21 (75.0)	26 (40.6)	4.39	7.81	0.005*	1.65–11.8

FS > 1 - Focus score greater than 1; AAB+ - autoantibody-positive patients, including ANA, Ro/SSA+, La/SSB+; Ro/SSA+ - Ro/SSA-positive patients; La/SSB+ - La/SSB-positive patients; RF+ - rheumatoid factors-positive patients. Odds ratio (OR); Chi-square; p- value (p), and the confidence interval (CI) for the odds ratios are given. *Statistically significant.

Table 2 summarizes HLA associations with pSS for those HLA markers that showed at least one significant association for any of the following comparisons made.

For the comparison of all patients with the controls, no significant associations were seen for individual HLA-DRB1 specificities as well as DQA1 and DQB1 alleles. Also, there was no difference between patients and controls for the presence of at least one of the HLA-DRB1 types DRB1*02, DRB1*03 or DRB1*05 (64.5 vs 65.2%). Similarly, there was no significant heterogeneity detected for the distribution of DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygotes (14.5 vs 4.5%; OR = 3.57; chi-square = 2.66; p = 0.103). Also, the variability at individual DQA1 or DQB1 amino acid positions was not different in distribution between patients and controls. When patients (n = 54) with autoantibodies (ANA, anti-Ro/SSA, anti-La/SSB) were selected for comparison, a positive association was seen only for DRB1*03 (OR = 2.37; chi-square = 4.26; p = 0.04). Restricting the comparison to patients who are ANA-positive (n = 28), a positive association was seen for DRB1*03 only (OR = 2.37; chi-square = 4.26; p = 0.04). Considering patients with anti-Ro/SSA (n = 21), positive associations were seen for DRB1*03 (OR = 5.92; chi-square = 9.81; p = 0.002), DQB1*02 (OR = 4.68; chi-square = 6.64; p = 0.01), and the predisposing effect of DQB-DI motif (OR = 6.21; chi-square = 8.74; p = 0.003) as well as DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygosity (33.3 vs 4.5%; OR = 10.5; chi-square = 10.3; p = 0.001). Similar patterns were observed for patients with an anti-La/SSB response (n = 15): DRB1*03 (OR = 15.4; chi-square = 16.37; p < 0.001), DQB1*02 (OR = 9.5; chi-square = 10.31; p = 0.001), the DQB-DI motif (OR = 9.5; chi-square = 10.31; p = 0.002) and in addition DQA1*0501 (OR = 8.36; chi-square = 8.97; p = 0.004). Significant heterogeneity was detected for the distribution of DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygotes (40.0 vs 4.5%; OR = 14.0; chi-square = 12.17; p = 0.001). As all patients showing an anti-La/SSB response carried autoantibodies against Ro/SSA, the association patterns for the group of patients being positive for either antibody were identical to the situation seen for the anti-Ro/SSA response. Similarly, the patterns for the presence of both autoantibodies were identical to the situation for the anti-La/SSB response. A positive association was also seen for DRB1*03 (OR = 5.00; chi-square = 10.18; p = 0.001), DQB1*02 (OR = 3.65; chi-square = 6.21; p = 0.013), DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygosity (25.0 vs 4.5%; OR = 7.0; chi-square = 6.63; p = 0.001), and the DQB-DI motif (OR = 4.39; chi-square = 7.89; p = 0.005) when patients (n = 28) who were RF positive were considered. However, it has to be noted that 19 out of these 28 patients carried anti-Ro/SSA or anti-La/SSB autoantibodies.

Next, patients and controls were compared for the total number of copies (range 0-4) of DQ α -34Q, DQ β -26L and

Table 3. Gene dosage of DQ α -34Q and DQ β -26L in patients with pSS and controls. The distribution of the number of alleles with glutamine DQA1 position 34 (DQ α -34Q) and leucine in DQB1 position 26 (DQ β -26L) in patients and controls is shown. For the patients, the distinction was made between all patients (A), autoantibody-positive patients (B), anti-Ro/SSA-positive patients (C) and anti-La/SSB-positive patients (D) when compared with the controls. For each group comparison, the chi-square, degrees of freedom (df), and the p value (p) are given.

A					
All	Allele no.	pSS		Controls	
		n	%	n	%
	0	3	4.8	5	7.8
	1	2	3.2	4	6.3
	2	18	29.0	23	35.9
	3	17	27.4	22	34.4
	4	22	35.5	10	15.6
Total		62	100	64	100

Chi-square = 6.88, df 4, p = 0.142.

B					
AAB+	Allele no.	pSS		Controls	
		n	%	n	%
	0	3	5.6	5	7.8
	1	1	1.9	4	6.3
	2	15	27.8	23	35.9
	3	16	29.6	22	34.4
	4	19	35.2	10	15.6
Total		54	100	64	100

Chi-square = 6.93, df 4, p = 0.140.

C					
Ro/SSA+	Allele no.	pSS		Controls	
		n	%	n	%
	0	2	9.5	5	7.8
	1	0	0	4	6.3
	2	4	19.0	23	35.9
	3	2	9.5	22	34.4
	4	13	61.9	10	15.6
Total		21	100	64	100

Chi-square = 18.76, df 4, p = 0.001.

D					
La/SSB+	Allele no.	pSS		Controls	
		n	%	n	%
	0	2	13.3	5	7.8
	1	0	0	4	6.3
	2	1	6.7	23	35.9
	3	2	13.3	22	34.4
	4	10	66.7	10	15.6
Total		15	100	64	100

Chi-square = 19.06, df 4, p = 0.001.

DQB-DI (range 0-2). No significant heterogeneity could be observed for the comparisons of all patients and autoantibody-positive patients with the controls (Table 3). However, in comparisons made after selecting patients with either anti-Ro/SSA or anti-La/SSB autoantibodies, significant heterogeneity was seen (chi-square = 18.76, 4 degrees of freedom (df), $p = 0.001$ and chi-square = 19.06, 4 df, $p = 0.001$, respectively). The largest contribution to the heterogeneity came from the overrepresentation of patients carrying in total four copies of DQA-34Q and DQB-26L. Similarly, heterogeneity for the presence of the different copy numbers of the DQB-DI motif was seen after selecting patients with either anti-Ro/SSA or anti-La/SSB autoantibodies (chi-square = 10.83, 2 df, $p = 0.04$ and chi-square = 11.59, 2 df, $p = 0.03$, respectively) (Table 4). For DQB-DI, the largest difference between patients and controls was seen for the presence of a single copy of this motif.

As for ANA and RF, titer information was available, mean ranks were compared for patients carrying the respective antibody entities after splitting the antibody-positive patients according to the presence or absence of DRB1*03, DQA1*0501, DQA-34Q, DQB1*02, DQB-26L, DQB-DI. While no significant differences were seen for RF, patients who were ANA positive had significantly higher ANA titers when positive for DRB1*03, DQB1*02, and DQB-DI (p values: $p < 0.001$, $p = 0.001$, and $p = 0.001$, respectively).

Considering patients with histologic evidence (focus score > 1) ($n = 31$), no significant association with individual HLA markers was observed. Nevertheless, the frequency of DRB1*03 was higher in the patient group compared to the controls (51.6 vs 29.7%; OR = 2.53; chi-square = 3.42; $p = 0.06$). In contrast, significant heterogeneity was detected for the distribution of DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygotes (22.6 vs 4.5%; OR = 6.13; chi-square = 5.6; $p < 0.018$).

In comparisons using the presence versus absence of arthritis, hepatitis, glomerulonephritis/renal tubular acidosis, pulmonary disease, (poly-) neuritis, and leukopenia ($< 4.000/\mu\text{l}$) as stratifying variables, no significant associations were seen in comparison to the healthy control group.

DISCUSSION

We examined the potential influence of different HLA markers on the expression of clinical and serological disease manifestations in a group of Norwegian patients with pSS. Our aim was twofold. In addition to individual HLA DRB1 specificities and DQA1/DQB1 alleles, particular attention was given to combinations, haplotypes and individual amino acids and sequence motifs previously implicated to play a role in susceptibility to pSS. Secondly, clinical and laboratory variables were included to explore the relevance of HLA markers for the clinical presentation and course of the disease.

When clinical and serological features were examined,

Table 4. Gene dosage of DQB-DI in patients with pSS and controls. Distribution of the number of alleles with the DQB motif AA59-69 (EYWNSQKDILE), termed DQB-DI, in patients and controls. For the patients, the distinction was made between all patients (A), autoantibody-positive patients (B), anti-Ro/SSA-positive patients (C) and anti-La/SSB-positive patients (D) when compared with the controls. For each group comparison, the chi-square, degrees of freedom (df), and the p value (p) are given.

A					
All	Allele no.	pSS n	%	Controls n	%
	0	26	41.9	38	59.4
	1	31	50.0	21	32.8
	2	5	8.1	5	7.8
Total		62	100	64	100

Chi-square = 4.14, df 2, $p = 0.126$.

B					
AAB+	Allele no.	pSS n	%	Controls n	%
	0	22	40.7	38	59.4
	1	27	50.5	21	32.8
	2	5	9.3	5	7.8
Total		54	100	64	100

Chi-square = 4.2, df 2, $p = 0.122$.

C					
Ro/SSA+	Allele no.	pSS n	%	Controls n	%
	0	4	19	38	59.4
	1	15	71.4	21	32.8
	2	2	9.5	5	7.8
Total		21	100	64	100

Chi-square = 10.83, df 2, $p = 0.04$

D					
La/SSB+	Allele no.	pSS n	%	Controls n	%
	0	2	19	38	59.4
	1	12	71.4	21	32.8
	2	1	9.5	5	7.8
Total		15	100	64	100

Chi-square = 11.59, df 2, $p = 0.03$.

the presence of anti-Ro/SSA antibodies was found to be associated with focus score > 1 , RF, ANA, kidney involvement, and vasculitis, which is in line with previous studies^{38,39}.

Our key results indicate that overall no significant asso-

ciations of HLA-DQ and DRB1 markers with the disease existed. This lack of significant influence of susceptibility to pSS also extended to the presence of either DRB1*02, *03 or *05 as well as DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygosity. Although the latter heterozygous haplotype combination was increased among patients compared to controls, this heterogeneity was not as prominent as reported^{16,40}.

Anti-Ro and anti-La autoantibody formation is under genetic control by HLA Class II markers^{21,41-43}. Our data support this notion as significant positive associations were found for DRB1*03 and presence of ANA, anti-Ro/SSA or anti-La/SSB, for DQB1*02 and anti-Ro/SSA or anti-La/SSB, for DQA1*0501 and anti-La/SSB, and for DRB1*03/DRB1*15-DQB1*02/DQB1*0602 heterozygosity and anti-Ro/SSA or anti-La/SSB. These associations indicate a prominent role for HLA Class II alleles/specificities which are components of the DRB1*03-DQB1*02-DQA1*0501 haplotype known to play a central role in different autoimmune diseases. The restriction of the association to the anti-Ro/anti-La autoantibody formation is in agreement with most studies^{16,18,44,45}.

The observation that different HLA Class II loci and specificities/alleles may contribute to disease susceptibility and autoantibody formation in pSS as well as the notion that ethnic differences in HLA association patterns exist has prompted the search for a common denominator. As a result, specific amino acid residues in the second hypervariable region of HLA-DQA1 and DQB1, glutamine (Gln) in position 34 of DQA1 and leucine (Leu) in position 26 of DQB1 have been proposed to promote the anti-Ro/SSA and anti-La/SSB responses²². Moreover, gene dosage effects for the number of alleles carrying these amino acids in promoting Ro/SSA and/or La/SSB autoantibody response have been described by some²² but have not been observed by others^{17,23,46}. In our cohort, no significant associations were found between any of these amino acids and pSS, or the presence of either anti-Ro/SSA or anti-La/SSB (Table 2). Furthermore, no heterogeneity for the distribution of different copy numbers of alleles carrying DQ α -34Q or DQ β -26L between controls and both all patients and AAB positive patients was observed. Interestingly, however, a gene dosage effect, indicative of a recessive contribution, was seen for these DQA1 and DQB1 amino acids when patients with either anti-Ro/SSA or anti-La/SSB autoantibodies were compared to the controls, consistent with previous observations^{22,23}. These observations have several implications. Firstly, the gradient in association strength between anti-Ro/SSA or anti-La/SSB positive patients and the whole group of patients suggests a primary role for anti-Ro/SSA and anti-La/SSB autoantibody formation over influencing disease susceptibility. In consequence, disease associations with these amino acids, preferably in high copy number, may be observed in some but not all studies of

Caucasian patients depending on the frequency of anti-Ro/SSA or anti-La/SSB positive patients in the study cohort. Secondly, the increase of DQ1/DQ2, specifically DQB1*02/DQB1*0602, and DRB1*03/DRB1*15 heterozygosity is compatible with a recessive and complementing influence of DQ α -34Q and DQ β -26L, as these combinations of HLA markers can be subsumed under DQ α -34Q-DQ β -26L homozygosity.

The comparison of associated HLA Class II haplotypes in Caucasian (DRB1*0301, DQB1*0201, DQA1*0501), Japanese (DRB1*0405, DQB1*0401, DQA1*0301), and Chinese (DRB1*0803, DQB1*0601, DQA1*0103) patients with pSS had indicated a segment of the DQ β chain (amino acid positions 58-69) to be transethnically associated with pSS¹⁷. This motif, termed DQ β -DI here, overlaps with DQ β -26L in this Caucasian cohort only with the DRB1*0301-DQB1*0201-DQA1*0501 haplotype. It is not surprising, therefore, that this DQ β -DI motif is significantly increased among anti-Ro/SSA or anti-La/SSB positive patients and that a single copy of this motif is sufficient to confer susceptibility to autoantibody formation.

DQ α -34Q-DQ β -26L homozygous combinations involving the DR3-DQ2 (DRB1*0301-DQB1*0201-DQA1*0501) haplotype represent an overlap situation of these structural susceptibility requirements in Caucasian patients with pSS. Possibly, synergism plays a role for clinical observations of increased disease severity²⁶ and enhanced autoantibody production²¹, particularly the presence of precipitating anti-Ro/SSA and anti-La/SSB antibodies^{18,20,24}.

In addition to DQ α -34Q, DQ β -26L and DQ β -DI, a study of Israeli Jewish and Greek non-Jewish patients had implicated a DQ β chain sequence motif encompassing amino acids 84-90 (QLELRIT) as part of the DRB1*1101/04-DQB1*0301 haplotype, common to DQ*02, *03 and *04 alleles, in disease susceptibility to pSS⁴⁶. This motif is partly shared by DQ β -26L (DQB1*02, *0302, *0303) and DQ β -DI (*02, *04). However, as DQB1*0301 has not been observed at high frequencies in other populations with pSS including our patient population, and since this motif does not account for DQB1*0601 seen among Chinese patients with pSS, the role of this pSS susceptibility element remains to be determined.

Our study indicates a primary role for HLA Class II markers for anti-Ro/anti-La responses over an involvement in disease susceptibility to pSS. The strongest contributors to the formation of an anti-Ro/La response included components of the DRB1*03-DQB1*02-DQA1*0501 haplotype also encompassing the transethnically-associated DQ β -DI motif. In addition, the dose dependent contributions of DQ α -34Q and DQ β -26L argue for a recessive contribution of HLA-DQ to the formation of an anti-Ro/La response. Given the prominent associations with DRB1*03 and the complex dose dependent interactions at HLA-DQ, a joint

contribution of HLA-DR and -DQ is likely to be relevant for the formation of anti-Ro/La autoantibodies.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Marlis Arnold and Isabel Breunig.

REFERENCES

1. Jonsson R, Haga H-J, Gordon T. Sjögren's syndrome. In: Koopman WJ, editor. *Arthritis and allied conditions — a textbook of rheumatology*. Baltimore: Lippincott, Williams & Wilkins; 2001:1736-59.
2. Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* 1989;44:93-151.
3. Voulgarelis M, Dafni UG, Isenberg DA, Moutsopoulos HM. Malignant lymphoma in primary Sjögren's syndrome: a multicenter, retrospective, clinical study by the European Concerted Action on Sjögren's Syndrome. *Arthritis Rheum* 1999;42:1765-72.
4. Silman AJ, Rooney BK. Epidemiology of Sjögren's syndrome. In: Eriksson E, Jonsson R, editors. *The 100-year anniversary of Henrik Sjögren*. Jönköping: Hygiea; 1999;108:53-7.
5. Merriman TR, Todd JA. Genetics of autoimmune disease. *Curr Opin Immunol* 1995;7:786-92.
6. Campbell RD, Milner CM. MHC genes in autoimmunity. *Curr Opin Immunol* 1993;5:887-93.
7. Reveille JD. The molecular genetics of systemic lupus erythematosus and Sjögren's syndrome. *Curr Opin Rheumatol* 1992;4:644-56.
8. Hinzova E, Ivanyi D, Sula K, Horejs J, Dostal C, Drizhal I. HLA-Dw3 in Sjögren's syndrome. *Tissue Antigens* 1977;9:8-10.
9. Reveille JD, Arnett FC. The immunogenetics of Sjögren's syndrome. *Rheum Dis Clin North Am* 1992;18:539-50.
10. Fye KH, Terasaki PI, Michalski JP, Daniels TE, Opelz G, Talal N. Relationship of HLA-Dw3 and HLA-B8 to Sjögren's syndrome. *Arthritis Rheum* 1978;21:337-42.
11. Chused TM, Kassan SS, Opelz G, Moutsopoulos HM, Terasaki PI. Sjögren's syndrome association with HLA-Dw3. *N Engl J Med* 1977;296:895-7.
12. Foster H, Walker D, Charles P, Kelly C, Cavanagh G, Griffiths I. Association of DR3 with susceptibility to and severity of primary Sjögren's syndrome in a family study. *Br J Rheumatol* 1992;31:309-14.
13. Manthorpe R, Morling N, Platz P, Ryder LP, Svejgaard A, Thomsen M. HLA-D antigen frequencies in Sjögren's syndrome. Differences between the primary and secondary form. *Scand J Rheumatol* 1981;10:124-8.
14. Papasteriades CA, Skopouli FN, Drosos AA, Andonopoulos AP, Moutsopoulos HM. HLA-alloantigen associations in Greek patients with Sjögren's syndrome. *J Autoimmun* 1988;1:85-90.
15. Moriuchi J, Ichikawa Y, Takaya M, et al. Association between HLA and Sjögren's syndrome in Japanese patients. *Arthritis Rheum* 1986;29:1518-21.
16. Guggenbuhl P, Jean S, Jégo P, et al. Primary Sjögren's syndrome: role of the HLA-DRB1*0301-*1501 heterozygotes. *J Rheumatol* 1998;25:900-5.
17. Kang HI, Fei HM, Saito I, et al. Comparison of HLA class II genes in Caucasian, Chinese, and Japanese patients with primary Sjögren's syndrome. *J Immunol* 1993;150:3615-23.
18. Wilson RW, Provost TT, Bias WB, et al. Sjögren's syndrome. Influence of multiple HLA-D region alloantigens on clinical and serologic expression. *Arthritis Rheum* 1984;27:1245-53.
19. Harley JB, Alexander EL, Bias WB, et al. Anti-Ro (SS-A) and anti-La (SS-B) in patients with Sjögren's syndrome. *Arthritis Rheum* 1986;29:196-206.
20. Hamilton RG, Harley JB, Bias WB, et al. Two Ro (SS-A) autoantibody responses in systemic lupus erythematosus. Correlation of HLA-DR/DQ specificities with quantitative expression of Ro (SS-A) autoantibody. *Arthritis Rheum* 1988;31:496-505.
21. Harley JB, Reichlin M, Arnett FC, Alexander EL, Bias WB, Provost TT. Gene interaction at HLA-DQ enhances autoantibody production in primary Sjögren's syndrome. *Science* 1986;232:1145-7.
22. Reveille JD, Macleod MJ, Whittington K, Arnett FC. Specific amino acid residues in the second hypervariable region of HLA-DQA1 and DQB1 chain genes promote the Ro (SS-A)/La (SS-B) autoantibody responses. *J Immunol* 1991;146:3871-6.
23. Scofield RH, Harley JB. Association of anti-Ro/SS-A autoantibodies with glutamine in position 34 of DQA1 and leucine in position 26 of DQB1. *Arthritis Rheum* 1994;37:961-2.
24. Fei HM, Kang H, Scharf S, Erlich H, Peebles C, Fox R. Specific HLA-DQA and HLA-DRB1 alleles confer susceptibility to Sjögren's syndrome and autoantibody production. *J Clin Lab Anal* 1991;5:382-91.
25. Nepom GT. Class II antigens and disease susceptibility. *Ann Rev Med* 1995;46:17-25.
26. Kerttula TO, Collin P, Polvi A, Korpela M, Partanen J, Maki M. Distinct immunologic features of Finnish Sjögren's syndrome patients with HLA alleles DRB1*0301, DQA1*0501, and DQB1*0201. Alterations in circulating T cell receptor gamma/delta subsets. *Arthritis Rheum* 1996;39:1733-9.
27. Rischmueller M, Lester S, Chen Z, et al. HLA class II phenotype controls diversification of the autoantibody response in primary Sjögren's syndrome (pSS). *Clin Exp Immunol* 1998;111:365-71.
28. Vitali C, Bombardieri S, Moutsopoulos HM, et al. Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum* 1993;36:340-7.
29. Greenspan JS, Daniels TE, Talal N, Sylvester RA. The histopathology of Sjögren's syndrome in labial salivary gland biopsies. *Oral Surg Oral Med Oral Pathol* 1974;37:217-29.
30. Wagner U, Kaltenhauser S, Sauer H, et al. HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. *Arthritis Rheum* 1997;40:341-51.
31. Bignon JD, Fernandez-Vina MA, Cheneau ML, et al. HLA DNA class II typing by PCR-SSOP: 12th International Histocompatibility Workshop experience. In: Charron D, editor. *HLA: Genetic diversity of HLA, functional and medical implications*. Paris: EDK, 1997.
32. Miettinen O. Estimability and estimation in case-referent studies. *Am J Epidemiol* 1976;103:226-35.
33. Woolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1955;19:251-3.
34. Haldane S. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956;20:309-11.
35. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719-48.
36. Rosner BA. *Fundamentals of Biostatistics*. Boston: Duxbury Press; 1982.
37. Michelsen B, Wassmuth R, Ludvigsson J, Lernmark A, Nepom GT, Fisher L. HLA heterozygosity in insulin-dependent diabetes is most frequent at the DQ locus. *Scand J Immunol* 1990;31:405-13.
38. Shah F, Rapini RP, Arnett FC, Warner NB, Smith CA. Association of labial salivary gland histopathology with clinical and serologic features of connective tissue diseases. *Arthritis Rheum* 1990;33:1682-7.
39. Alexander EL, Arnett FC, Provost TT, Stevens MB. Sjögren's syndrome: association of anti-Ro(SS-A) antibodies with vasculitis,

- hematologic abnormalities, and serologic hyperreactivity. *Ann Intern Med* 1983;98:155-9.
40. Jean S, Quelvennec E, Alizadeh M, et al. DRB1*15 and DRB1*03 extended haplotype interaction in primary Sjögren's syndrome genetic susceptibility. *Clin Exp Rheumatol* 1998;16:725-8.
41. Buyon JP, Slade SG, Reveille JD, Hamel JC, Chan EK. Autoantibody responses to the "native" 52-kDa SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus, and Sjögren's syndrome. *J Immunol* 1994;152:3675-84.
42. Bell DA, Maddison PJ. Serologic subsets in systemic lupus erythematosus: an examination of autoantibodies in relationship to clinical features of disease and HLA antigens. *Arthritis Rheum* 1980;23:1268-73.
43. Miyagawa S, Shinohara K, Nakajima M, et al. Polymorphisms of HLA class II genes and autoimmune responses to Ro/SS-A- La/SS-B among Japanese subjects. *Arthritis Rheum* 1998;41:927-34.
44. Arnett FC, Hamilton RG, Reveille JD, Bias WB, Harley JB, Reichlin M. Genetic studies of Ro (SS-A) and La (SS-B) autoantibodies in families with systemic lupus erythematosus and primary Sjögren's syndrome. *Arthritis Rheum* 1989;32:413-9.
45. Arnett FC, Bias WB, Reveille JD. Genetic studies in Sjögren's syndrome and systemic lupus erythematosus. *J Autoimmun* 1989;2:403-13.
46. Roitberg-Tambur A, Friedmann A, Safirman C, et al. Molecular analysis of HLA class II genes in primary Sjögren's syndrome. A study of Israeli Jewish and Greek non-Jewish patients. *Hum Immunol* 1993;36:235-42.