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

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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 (DQα-34Q) and DQB1 position 26 (DQß-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 DQα-34Q and DQß-26L. For DQß-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 transethnically-associated DQß-DI motif. In addition, the dose dependent contribution of DQα-34Q and DQß-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 keratoconjunctivitis sicca with lymphocyte infiltration in the salivary and lacrimal glands[1]. 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 lymphoma[3]. 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 diagnosis[4]. Ninety percent of the patients are women.

The polymorphic major histocompatibility complex

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(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 alleles[7]. A primary association between pSS and HLA-DR3, which is in linkage disequilibrium with HLA-B8, is well recognized for Caucasians[8-12]. In addition, an association with HLA-DR2 has been reported in Scandinavians[13], DR5 in Greeks[14], and DRw53 in Japanese[15]. Recently, the HLA-DRB1*0301-*1501 heterozygous genotype has been suggested to play a role in susceptibility to pSS[16]. 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 patients[17].

HLA-DR2 and DR3 have frequently been reported to be associated with autoantibody production to Ro/SSA and La/SSB autoantigens[18-20], and stronger associations have been found with HLA-DR2 and HLA-DR3 and the Ro/La antibody responses than with the disease itself[18]. A role of HLA-DQ locus (particularly DQ1-DQ2 heterozygotes) in anti-Ro/SSA and anti-La/SSB production has been demonstrated[21-23]. 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 SLE[20-22]. 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 pSS[26], and distinct HLA haplotypes have been associated with different degrees of autoantibody diversification in pSS[27].

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 SS[28]. 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 (≤ 5mm 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 performed[29]. 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 published[30]. Similarly, DQB1 and DQA1 alleles were determined following the XI.IHWC protocol[31]. 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 (DQα-34Q [DQA1*0102, *0103, *0401, *0501-*0503, *0601] vs DQ-34E [DQA1*0101, *0104, *0201, *0301, *0302]), DQB1 position 26 (DQß-26L [DQB1*0602-*0606, *0606, *02, *0302, *0303] vs DQß-26G/26Y [DQB1*05, *0601, *0301, *0304]), and the DQß motif AA59-69 (EYWN-SQKDILE), termed DQß-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 × 2 contingency table [OR = (a × d) / (c × 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 allele[34]. 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 markers[35]. For the comparison of different groups the Wilcoxon rank sum test or the two-sample t test was used where appropriate[36]. 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 described[37].

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 (%). [a]Time from diagnosis until closing the study. [b]The preliminary criteria for classification of Sjögren's syndrome was followed[28] 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). [c]Present if unstimulated salivary flow ≤ 1.5 ml/15 min. [d]Chi-square = 19.05, df 1, p = 0.00001, CI = 0.24–0.56; [e]Chi-square = 26.33, df 1, p = 0.00000, CI = 6.59–173.08; [f]Chi-square = 4.70, df 1, p < 0.05, CI = 0.69–0.94; [g]Chi-square = 6.94, df 1, p < 0.01, CI = 1.41–43.01; [h]Chi-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

DQα-34E), at position 26 of the DQß chain (DQß-26L vs DQß-26G/26Y) and included a sequence motif at position 59 through 69 (EYWNSQKDILE) of the Dqß chain comprising DQB1*0504, *0601, *02, and *04 (DQß-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
DQα-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
DQß-26L		57 (89.1)	58 (93.5)	1.78	0.33	0.56	0.49–6.42
DQß-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
DQα-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
DQß-26L		28 (90.3)	57 (89.1)	1.15	0.03	1	0.28–4.77
DQß-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
DQα-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
DQß-26L		50 (92.6)	57 (89.1)	1.54	0.16	0.73	0.42–5.55
DQß-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
DQα-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
DQß-26L		50 (92.6)	57 (89.1)	1.54	0.11	0.73	0.42–5.55
DQß-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
DQα-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
DQß-26L		19 (90.5)	57 (89.1)	1.17	< 0.001	1	0.22–6.11
DQß-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
DQα-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
DQß-26L		13 (86.7)	57 (89.1)	0.80	< 0.001	1	0.15–4.30
DQß-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
DQα-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
DQß-26L		26 (92.9)	57 (89.1)	1.60	0.03	0.86	0.31–8.22
DQß-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 DQß-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 DQß-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 DQß-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 DQα-34Q, DQß-26L and

Table 3. Gene dosage of DQα-34Q and DQß-26L in patients with pSS and controls. The distribution of the number of alleles with glutamine DQA1 position 34 (DQα-34Q) and leucine in DQB1 position 26 (DQß-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.

DQß-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 DQα-34Q and DQß-26L. Similarly, heterogeneity for the presence of the different copy numbers of the DQß-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 DQß-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, DQα-34Q, DQB1*02, DQß-26L, DQß-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 DQß-D1 (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/μl) as stratifying variables, no significant associations were seen in comparison to the healthy control group.

Table 4. Gene dosage of DQß-D1 in patients with pSS and controls. Distribution of the number of alleles with the DQß motif AA59-69 (EYWNSQKDIILE), termed DQß-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		Controls	
		n	%	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		Controls	
		n	%	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		Controls	
		n	%	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		Controls	
		n	%	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.

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, 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 responses[22]. 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 some[22] 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 DQα-34Q or DQß-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 DQα-34Q and DQß-26L, as these combinations of HLA markers can be subsumed under DQα-34Q-DQß-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 DQß chain (amino acid positions 58-69) to be transethnically associated with pSS[17]. This motif, termed DQß-DI here, overlaps with DQß-26L in this Caucasian cohort only with the DRB1*0301-DQB1*0201-DQA1*0501 haplotype. It is not surprising, therefore, that this DQß-D1 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.

DQα-34Q-DQß-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 severity[26] and enhanced autoantibody production[21], particularly the presence of precipitating anti-Ro/SSA and anti-La/SSB antibodies[18,20,24].

In addition to DQα-34Q, DQß-26L and DQß-DI, a study of Israeli Jewish and Greek non-Jewish patients had implicated a DQß chain sequence motif encompassing amino acids 84-90 (QLELRTT) as part of the DRB1*1101/04-DQB1*0301 haplotype, common to DQ*02, *03 and *04 alleles, in disease susceptibility to pSS[46]. This motif is partly shared by DQß-26L (DQB1*02, *0302, *0303) and DQß-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 DQß-DI motif. In addition, the dose dependent contributions of DQα-34Q and DQß-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.

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