The Association Between HLA DR, DQ Antigens, and Vulval Lichen Sclerosus in the UK: HLA DRB1*12 and its Associated DRB1*12/DQB1*0301/04/09/010 Haplotype Confers Susceptibility to Vulval Lichen Sclerosus, and HLA DRB1*0301/04 and its Associated DRB1*0301/04/DQB1*0201/02/03 Haplotype Protects from Vulval Lichen Sclerosus

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Lichen sclerosus (LS) is considered to have an immunogenetic background. Several small studies, using serological typing, have reported that HLA-DR11, DR12, and DQ7 were increased in LS, with DR17 less frequent. This study aimed to validate and detect new HLA-DR and DQ associations with LS in females and its characteristic clinical parameters. The cases, 187 female LS patients, and 354 healthy controls were all UK North Europeans. PCR-sequence specific primers method was applied to genotype the HLA-DR, DQ polymorphisms that correspond to 17 serologically defined DR and seven DQ antigens. Statistical analysis was performed with two-tailed Fisher's exact test with Bonferroni adjustment (p value after Bonferrroni adjustment, Pc). We found increased frequency of DRB1*12 (DR12) (11.2% *vs* 2.5%, pc < 0.01) and the haplotype DRB1*12/DQB1*0301/04/09/010 (11.2% *vs* 2.5%, p < 0.001, pc < 0.05), and a lower frequency of DRB1*0301/04 (DR17) (11.8% *vs* 25.8%, pc < 0.01) and the haplotype DRB1*03/DQB1*02DRB1*0301/DQB1*0201/02/03 (11.2% *vs* 24.6%, pc < 0.0001) in patients compared with controls. HLA DR and DQ antigens were not associated with time of onset of disease, site of involvement, structural changes of genitals, and response to treatment with potent topical steroids. In conclusion, HLA-DR and DQ antigens or their haplotypes appear to be involved in both susceptibility to and protection from LS.

Key words: genotyping/HLA-DQ/HLA-DR/lichen sclerosus/susceptibility
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Lichen sclerosus (LS) is a chronic inflammatory disease that has a predilection for the anogenital area. It is characterized clinically by plaques of ivory white atrophic or thickened skin. In the majority of patients, there is progressive disease with genital structural changes or scarring (Ridley, 1988). Although it is common in postmenopausal women, there are increasing numbers of reports of girls with LS (Powell and Wojnarowska, 2001). The current management of LS is topical potent steroids that relieve the symptoms and sometimes reverse the clinical signs (Dalziel *et al*, 1991; Sinha *et al*, 1999; Cooper *et al*, 2004).

There has been accumulating evidence that immunological reactions are involved in the onset and pathogenesis of LS (Farrell *et al*, 1999; Carlson *et al*, 2000; Lukowsky *et al*, 2000). The association of autoimmune diseases with LS has been well documented in clinical studies (Meyrick Thomas *et al*, 1988; Marren *et al*, 1995; Lewis, 1998; Powell *et al*, 2000). The recent demonstration that skin autoantibodies, targeting extracellular matrix protein 1 and basement membrane zone antigens, are present in LS which further supports an immunological basis for LS (Oyama *et al*, 2003; Howard *et al*, 2004). A genetic basis for disease susceptibility is suggested by case reports of familial aggregation of LS and other autoimmune disease, which has been reported in up to 17% of female children with LS (Sahn *et al*, 1994; Powell and Wojnarowska, 2001). Two studies showed that HLA DQ3 and specifically DQ7 were more frequent in females with LS than controls (Marren *et al*, 1995; Powell *et al*, 2000), especially in girls with LS (Powell *et al*, 2000), suggesting a role in susceptibility to LS. DR 17 was less frequent in girls with LS than the controls (Powell *et al*, 2000). Further analysis suggested that the DR4/DQ8 haplotype was less frequent in vulval LS with structural changes than those without, suggesting a role in disease progression (Marren *et al*, 1995). Another study of LS in adult men showed increased frequencies of DR11, DR12, and DQ7 (Azurdia *et al*, 1999).

Abbreviations: LS, lichen sclerosus; Pc, p value after Bonferrroni adjustment

In this study, we have collected and genotyped the largest cohort of ethnically homogeneous North European female LS patients with the aim of replicating the associations between polymorphic HLA DR, DQ antigens, and LS. Possible novel associations between DR, DQ polymorphisms, and clinical parameters of LS were also investigated.

Results

Clinical data Clinical information was available from 187 LS patients, but was incomplete in a minority. The results are summarized in Table I.

HLA-DR and HLA-DQ frequencies in LS and controls

Single loci The allele frequencies of the 187 female LS and 354 controls are shown in Table II. There were more patients than controls with DRB1*12 genotypes (11.2% *vs* 2.5%, $pc < 0.005$). There were fewer patients than controls with DRB1*0301/04 (11.8% *vs* 25.8%, $pc < 0.005$). These data suggest that the former might be a susceptibility gene, and the latter a protective gene for LS. There were no other significant differences in frequency of alleles between the patients and controls.

Haplotypes Among 57 estimated haplotypes, only haplotypes DRB1*12/DQB1*0301/04/09/010 (11.2% *vs* 2.5%, $pc < 0.05$, $RR = 4.84$) and DRB1*0301/04/DQB1*0201/02/03 (11.2% *vs* 24.6%, $pc < 0.0001$, $RR = 0.39$) reached statistical differences between patients and controls after Bonferroni correction. Haplotype DRB1*15/DQB1*06 was less frequent in LS than controls (17.1% *vs* 28.6%, $p < 0.01$, $RR = 0.52$), and DRB1*13/DQB1*06 was more frequent in LS than controls (24.6% *vs* 16.4%, $p < 0.05$, $RR = 1.66$); their statistical differences were lost after Bonferroni correction. Other 53 haplotype frequencies were similar between patients and controls ($p > 0.05$).

Table I. Clinical data on 187 female patients with lichen sclerosus

Clinical parameter		Number (%)	Number of patients data available on
Age of onset	<0.5–86 y (mean value 44.6 y)		187
	Pre-menarche	35 (21)	168
	Reproductive years	41 (24)	
	Postmenopausal	92 (55)	
Sites	Anogenital	187 (100)	187
	Extragenital	20 (11)	
Structural Change	Severe	52 (31)	166
	Moderate	66 (40)	
	None	48 (29)	
Treatment	Resolution	25 (16)	152
Response	Partial	109 (72)	
Signs	Poor	18 (12)	
Autoimmune disease		60 (34)	179

DR and DQ polymorphism in association with clinical parameters Different DR, DQ phenotypes or haplotypes might influence clinical parameters in females with LS. We therefore analyzed whether there were any associations between DR, DQ phenotypes and haplotypes, and certain clinical parameters using Fisher's exact test.

Age and timing of onset The patients were grouped into onset in childhood (pre-menarche) and post-menarche. As shown in Table I, data on the age and timing of onset in relation to menarche were obtained from 168 patients. There were significantly increased frequencies of DRB1*12 as compared with the controls in both LS girls with onset pre-menarche (17.1% *vs* 2.5%, $p < 0.001$, $pc < 0.05$, $RR = 7.91$) and women with LS onset post-menarche (9.8% *vs* 2.5%, $p < 0.01$, $pc < 0.05$, $RR = 4.141$). There was a significantly decreased frequency of DRB1*0301/04 in LS of post-menarche onset, as compared with the controls (9.0% *vs* 25.8%, $p < 0.0001$, $pc < 0.001$, $RR = 0.286$). But there were no significant differences of DR or DQ allele frequencies between patients with onset in childhood or later in life.

Site of involvement The patients were grouped into those with anogenital involvement alone and those with anogenital involvement and extragenital involvement. There were no DR, DQ phenotype associations with site of involvement.

Structural changes or scarring The patients were grouped into those patients without structural changes and those with structural changes. The DRB1*04/DRB1*08 haplotype was present in 34 (26.3%) patients with structural changes, and seven (14.5%) patients without structural changes, ($p > 0.05$). We were not able to confirm our finding in a previous smaller study that this haplotype was less common in LS patients with structural changes compared with those without (Marren *et al*, 1995).

Steroid treatment response As the clinical signs objectively reflect the treatment response by steroid, we analyzed the influence of DR, DQ polymorphisms on those patients with good improvement, partial improvement, and poor response to topical steroid. Different DR, DQ phenotypes had no association with improvement of clinical signs by topical steroids.

Patients with other autoimmune disorders The patients were grouped into those with and without systemic or organ-specific autoimmune disease. There were fewer DRB1*13 positive LS patients (10 of 60, 16.7%) with other autoimmune diseases than those without other autoimmune diseases (36 of 119, 30.3%), ($p < 0.05$) indicating DR13 might have a protective role against autoimmunity in female LS patients.

Table II. Frequencies of individual or grouped alleles in LS and controls

Alleles	Phenotypes	Number of subjects with allele frequencies (%)		p-value	pc-value	RR
		LS (187) number (%)	Controls (354) number (%)			
DRB1*0101/02/04–07	DR1	26 (13.9)	67 (19.0)	0.151	NS	0.689
DRB1*0103	DR103	2 (1.1)	13 (3.7)	0.10	NS	0.283
DRB1*04	DR4	80 (42.8)	131 (37.1)	0.228	NS	1.267
DRB1*0701/03/04	DR7	71 (38.0)	91 (25.8)	0.004	NS	1.762
DRB1*08	DR8	9 (4.8)	13 (3.7)	0.503	NS	1.322
DRB1*0901	DR9	5 (2.7)	10 (2.8)	1	NS	0.942
DRB1*1001	DR10	0	0		NS	
DRB1*11	DR11	25 (13.4)	59 (16.7)	0.322	NS	0.769
DRB1*12	DR12	21 (11.2)	9 (2.5)	0.00009	0.002	4.835
DRB1*13	DR13	48 (25.7)	70 (19.8)	0.191	NS	1.396
DRB1*14	DR14	9 (4.8)	21 (5.9)	0.691	NS	0.799
DRB1*15	DR15	32 (17.1)	101 (28.6)	0.00324	NS	0.515
DRB1*16	DR16	4 (2.1)	3 (0.8)	0.242	NS	2.550
DRB1*0301/04	DR17	22 (11.8)	91 (25.8)	0.000096	0.002	0.384
DRB5*01/*02	DRw51	36 (19.3)	103 (29.2)	0.013	NS	0.579
DRB3*01/*02/*03	DRw52	107 (57.2)	214 (60.6)	0.462	NS	0.869
DRB4*01	DRw53	126 (67.4)	222 (62.9)	0.345	NS	1.219
DQB1*02 01/02/03	DQ2	72 (38.5)	144 (40.8)	0.645	NS	0.909
DQB1*0401/02	DQ4	7 (3.7)	13 (3.7)	1	NS	1.017
DQB1*0501/02–4	DQ5	43 (23.0)	96 (27.2)	1	NS	0.799
DQB1*06	DQ6	74 (39.6)	148 (42.0)	0.646	NS	0.907
DQB1*0301/04/09/010	DQ7	80 (42.8)	147 (41.6)	0.855	NS	1.048
DQB1*0302/07/08	DQ8	43 (23.0)	59 (16.7)	0.083	NS	1.488
DQB1*0303/06	DQ9	21 (11.2)	33 (9.3)	0.547	NS	1.227

pc-value, after Bonferroni adjustment (factor = 24); NS, not significant; LS, lichen sclerosus.

Discussion

We report positive associations in vulval LS with DRB1*12 (11.2% *vs* 2.5%, $pc < 0.005$) and the haplotype DRB1*12/DQB1*0301/04/09/010 (11.2% *vs* 2.5%, $pc < 0.05$). In addition, negative associations are reported with DRB1*0301/04 (11.8% *vs* 25.8%, $p < 0.05$) and the haplotype DRB1*0301/04/DQB1*0201/02/03 (11.2% *vs* 24.6%, $pc < 0.0001$).

There are several possible risks and errors in carrying out and interpreting an association study, for example, small sample size, multiple testing, poorly matched control group, failure to detect linkage disequilibrium with adjacent loci (Cardon and Bell, 2000; Gambaro *et al*, 2000). Although we collected the largest ever female LS cohort for HLA class II association study, more samples are needed to ensure an analysis with sufficient power. We ensured that patients and controls were exclusively North European white Caucasians to ensure a homogenous population.

In this study, we found that DQB1*301/04/09/010 was carried by 42.8% of the whole LS cohort and by 50.0% of patients with disease of pre-menarche onset. Previous studies showed that DQ7 (DQB1*301/04/09/010) was present in 50% of female LS and 66% of girls with LS (Marren *et al*, 1995; Powell *et al*, 2000). There were more DQB1*301/04/09/10 positive subjects in our control cohort (41.6%) than the controls of previous study, which were renal transplant donors and not ethnically matched (25%) (Marren *et al*, 1995; Powell *et al*, 2000). This explains why we could not demonstrate an increased prevalence of DQB1*30104/09/010 in our vulval LS patients.

Interestingly, we found that the DRB1*12/DQB1*03 haplotype was more prevalent in vulval LS patients than in controls ($pc < 0.05$). These data suggest that there may be several susceptibility genes for LS. In contrast, the lowered frequency of the DRB1*0301/04/DQB1*0201/02/03 ($pc < 0.0001$) haplotype may indicate a protective role in the pathogenesis of LS. A further study with high-resolution genotyping is required to pinpoint the exact allelic haplotype of these haplotypes.

Elderly women have LS more often than younger women, and there are increasing numbers of young females and pre-

menarche girls affected by LS, when levels of sex hormones are low (Ridley, 1988; Powell and Wojnarowska, 1999, 2001; Powell *et al*, 2000). in this cohort 75.9% had disease onset either pre-menarche or postmenopausal. Polymorphisms of HLA may influence the level of sex hormones (Ollier *et al*, 1989; Larsen *et al*, 2000). We could not find any DR, DQ haplotypes that influenced the stage of reproductive life at which onset of LS occurred. LS patients with different ages of onset might have exposure to different antigenic stimuli, such as virus, bacteria, or even self-antigen peptides. These antigenic factors might require different polymorphisms of HLA class II molecules to elicit or block an immune response (Phelps *et al*, 1996; Burg *et al*, 2001).

The extent of lesional involvement and structural changes are two important parameters with which to assess severity of LS. A previous study reported that DR4/DQ8 was less common in patients with structural changes (Marren *et al*, 1995). We could not confirm this association in this study, because of the small numbers with this haplotype, nor could we find any other DR, DQ polymorphism in associations with these two parameters. It seems that severity of LS is not related to HLA DR and DQ antigens.

Topical steroids are the most widely used option for the management of LS, although the response rate varies (Dalziel *et al*, 1991; Sinha *et al*, 1999; Cooper *et al*, 2004). Different DR or DQ antigens may influence steroids sensitivity in several conditions (Gaag *et al*, 1990; Bakr and El-Chenawy, 1998). We could not find any association of DR or DQ with LS with regard to response of clinical signs to treatment.

A proportion of LS patients had one or more other autoimmune diseases. We found that DRB1*13 was more prevalent in those LS patient without autoimmune diseases than those with autoimmune diseases. Thus DRB1*13 may have a protective role against development of other autoimmune disease in association with LS.

In summary, we found increased frequencies of DRB1*12 and the haplotype DRB1*12/DQB1*0301/04/09/010, in vulval LS patients compared with controls, and a decreased frequency of DRB1*0301/04 and the haplotype DRB1*0301/04/DQB1*0201/02/03. The results suggested that the former have a role in susceptibility to, whereas the latter in protection from the onset of LS. HLA DR and DQ antigens do not seem to influence the age of onset, disease severity, or treatment response of LS.

Materials and Methods

Patients and samples One hundred and eighty-seven female LS patients attending the vulvar clinics of Dermatology Department, Oxford were enrolled in this study. They were all North European (country of origin of four grandparents) white UK Caucasians from Southern England (Oxford region). The criteria for the diagnosis were: (1) characteristic clinical appearance, (2) histological confirmation for adult patients. Patients gave 5–10 mL peripheral blood for DNA extraction, with informed consent (OxREC 97.149). Control blood for DNA was taken from 354 UK North European individuals. The control group was randomly selected from over 10,000 anonymized blood samples collected from nearby health screening clinics (OXCHECK) between 1987 and 1990. All controls were from a similar ethnic (white North European) background. One hundred and eighty of 354 (50.8%) of control individuals were female, average age was 48.6 y (range 35.1–65.0 y).

Relevant clinical parameters were obtained from LS patients (Table I), either by direct questionnaire and clinical examination or by reviewing clinical notes. The recorded parameters include age of onset, time of onset in relation to age of menarche and menopause, site of lesions, signs and their response to treatment with topical steroids, vulvar structural changes, or scarring. The sites of LS involvement were classified as anogenital and extragenital. Clinical signs were mainly pallor, atrophy, hyperkeratosis, and fissuring or ulceration of the anogenital skin. Severe vulvar structural changes or scarring, manifest as labial adhesion, resorption, burying of the clitoris, and narrowing of the introitus, was recorded. Routine management was application of very potent (or potent) topical steroid twice a day for up to 12 wk, then when necessary. Treatment response with regard to objective physical signs was graded as good (complete resolution of signs except scarring or structural changes), partial (partial and/or complete resolution of some signs), minor or poor (minor resolution of signs), no change, or worse. A personal history of systemic and organ-specific autoimmune diseases, including autoimmune thyroid disease, pernicious anaemia, insulin-dependent diabetes mellitus, billiary cirrhosis, rheumatoid arthritis, morphea, lichen planus, alopecia, and vitiligo, was also recorded. Central Oxford Research Ethics Committee approval was given for the study. The study was carried out according to Declaration of Helsinki Principles.

Genotyping A well-established polymerase chain reaction sequence-specific primers method (PCR-SSP) was applied for genotyping of LS patients (Bunce *et al*, 1995). Forty-eight pairs of primers were designed to amplify DRB1, DRB3, DRB4, DRB5, and DQB1 loci that correspond to 17 DR and seven DQ phenotypes (Table II). Genotyping for the control cohort was performed using the same technique, with a difference in primers to allow higher resolution typing, however, the results of patients and controls were made comparable by leveling the resolutions of both groups.

Statistics This was performed using SPSS for Windows (A Pearson Education Company, Massachusetts). Student's *t* test and two-tailed Fisher's exact test was applied. Bonferonni adjustment was also applied where necessary, and the result shown as Pcorrected (Pc). The correction factor for analysis of frequencies of individual or grouped alleles in LS and controls was 24, and that for analysis of haplotype frequencies was 57, cutting-off those large numbers of rare haplotypes with similar small frequencies. The haplotypes were constructed using the OxDelta programme. The oxdelta program uses the standard formulae for calculating haplotype frequency, taking into account linkage disequilibrium (delta).

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