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HLA alleles in British Caucasians with mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is an autoimmune mucosal scarring disease having severe ocular morbidity [1]. Disease susceptibility is associated with increased frequencies of human leukocyte antigen (HLA)-DQB1*03:01, HLA-DRB1*11, and HLA-DRB1*04 and decreased frequencies of DQB1*02 [2]. To explore correlations between clinical involvement and HLA-class-II alleles, we prospectively phenotyped a cohort of 55 British MMP patients, and 41 age/sex-matched controls (ethics approval reference 09/H0721/54).

Inclusion criteria have been described [3] and were pre-agreed clinical criteria with or without a positive direct immunofluorescence (DIF) study. HLA-typing used allele-specific sequencing protocols (Protrans S3 HLA-DRB1* and HLA-DQB1* Cyclerstrips, Protrans, Hockenheim, Germany). Statistical analysis used Fisher's exact test (p), with Benjamini–Hochberg correction (p_c) defined as significant when $p_c < 0.05$.

The study dataset, with clinical and laboratory results, is provided in Table and Legend S1. Fifteen patients, 14 of them with ocular involvement, were DIF-negative of whom seven had antibodies to basement membrane zone epitopes. MMP-affected sites varied: eight were ocular only, ten oral only, 15 oral and ocular only and 22 multisite. Exons 2 and 3 in HLA-DQB1 were fully analysed in 54/55 patients and 39/41 controls (Table 1a). The frequency of HLA-DQB1*03:01 was increased ($p_c = < 0.01$) in 36/54 (67%) of MMP patients (13 homozygous and 23 heterozygous) compared to 13/39 (33%) controls (two homozygous and 11 heterozygous, Table 1a). Exon 2 of HLA-DRB1 was also fully analysed in 54/55 patients and 40/41 controls; HLA-DRB1*03:01 was decreased ($p = < 0.01$, $p_c = 0.045$, Table 1b). Additionally, we compared the frequency of HLA-DQB1*0301 with controls for different sites of

Table 1a Distribution of HLA-DQB1 among patients and controls

	Patients		Controls		p-value	p_c
	($n = 54$)	in %	(n = 39)	in %		
HLA-DQB1						
HLA-DQB1*02	9	16.6	18	46.2	**<0.01**	**<0.01**
HLA-DQB1*03	48	88.9	20	51.3	**<0.01**	**<0.01**
HLA-DQB1*04	4	7.4	6	15.4	0.31	0.39
HLA-DQB1*05	13	24	11	28.2	0.81	0.81
HLA-DQB1*06	13	24	18	46.2	**0.04**	0.07
*HLA-DQB1*02*						
HLA-DQB1*02:01	6	11.1	9	23.1	0.15	ND
HLA-DQB1*02:02	3	5.5	7	17.9	0.09	ND
*HLA-DQB1*03*						
HLA-DQB1*03:01	36	66.6	13	32.1	**<0.01**	**0.01**
HLA-DQB1*03:02	7	13	2	5.1	0.29	0.63
HLA-DQB1*03:03	4	7.4	2	5.1	1	1
HLA-DQB1*03:05	0	0	1	2.6	0.42	0.63
HLA-DQB1*03:19	1	1.8	0	0	1	1
HLA-DQB1*03:22	0	0	1	1.3	0.42	0.63

The submission complies with the tenets of the Declaration of Helsinki and patients have provided appropriate ethical approval for publication.

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Table 1b Distribution of HLA-DRB1 among patients and controls

	Patients		Controls		*p*-value	p_c
	(*n* = 54)	in %	(n = 39)	in %		
HLA-DRB1						
HLA-DRB1*01	10	18.5	12	30	0.23	0.48
HLA-DRB1*0301[a]	7	13	15	37.5	**<0.01**	**0.046**
HLA-DRB1*04	20	37	7	17.5	**0.04**	0.15
HLA-DRB1*05	0	0	1	2.5	0.43	0.59
HLA-DRB1*07	5	9.3	10	25	0.05	0.14
HLA-DRB1*08	3	5.5	4	10	0.45	0.59
HLA-DRB1*09	3	5.5	0	0	0.27	0.48
HLA-DRB1*11	25	40.7	7	17.5	**<0.01**	**0.046**
HLA-DRB1*12	3	5.5	1	2.5	0.63	0.75
HLA-DRB1*13	13	24	6	17.5	0.31	0.5
HLA-DRB1*14	1	1.8	0	0	1	1
HLA-DRB1*15	9	16.6	14	35	0.05	0.14
HLA-DRB1*16	1	1.8	0	0	1	1
*HLA-DRB1*11*						
HLA- DRB1*11:01/11:97	12	22.2	5	12.5	0.29	ND
HLA-DRB1* 11:01/11:97 or 11:04	2	3.7	0	0	0.5	ND
HLA-DRB1*11:02 or 11:36 or 11:48	1	1.85	0	0	1	ND
HLA-DRB1*11:03	2	3.7	0	0	0.5	ND
HLA-DRB1*11:03 or 11:11	2	3.7	0	0	0.5	ND
HLA-DRB1*11:04	6	11.1	2	5	0.46	ND

In the first step of the analysis the gene frequencies for the allel groups of HLA-DQB1 (2–6) and HLA-DRB1 (1, 3–5, 7–9, and 11–16) were compared and in the second step, only significant alleles were further analysed regarding specific HLA-protein (e.g., HLA-DRB1*11) and compared. The *p*-value is given for comparisons of HLA gene frequencies, between cases and controls, using Fisher's exact test for each allele. The p_c value is the probability value after using the Benjamini–Hochberg correction for multiple testing.

ND not done

[a] All patients with HLA-DRB1*03 expressed the specific protein HLA-DRB1*0301.

involvement showing that HLA-DQB1*0301 was increased in all subgroups except for ocular only MMP. Compared to controls, DIF-positive MMP had significantly increased HLA-DQB1*0301 ($p = 0.00009$) but this difference was not significant for either DIF-negative MMP ($p = 0.113$) or for DIF-positive ocular only MMP.

Some studies have described a correlation of HLA-DQB1*03:01 with ocular and oral MMP or in ocular only MMP, whereas others have shown HLA-DQB1*03:01 to be associated with multisite MMP [2]. In this study, the association of HLA-DQB1*0301 with MMP was lower than in the largest reported study [2] ($p_c < 0.00000028$), possibly due to our inclusion of eight patients without detectable tissue-bound or serum antibodies, who were excluded from the latter study [2]. In ocular only MMP 50% are DIF-negative but have a phenotype that both progresses and responds to therapy in the same way as DIF-positive cases [3, 4]. DIF-negative ocular MMP may result from inadequate test sensitivity, because of the small volumes of tissue involved, to dominance of an autoreactive T-cell mediated over a autoantibody-mediated disease [4] or because this is a different disease subset. We included our DIF-negative cases because to leave these out of this analysis disregards a group of cases which do not fit criteria for any other disease.

In our prospectively characterized cohort the association with HLA-DQB1*0301, HLA-DQB1*02, and HLA-DRB1*11 was corroborated, whereas HLA-DRB1*0301 was identified as a potentially protective allele, which requires confirmation in a larger study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Dart J. Cicatricial pemphigoid and dry eye. Semin Ophthalmol. 2005;20:95–100.
2. Setterfield J, Theron J, Vaughan RW, et al. Mucous membrane pemphigoid: HLA-DQB1*0301 is associated with all clinical sites of involvement and may be linked to antibasement membrane IgG production. Br J Dermatol. 2001;145:406–14.
3. Ong HS, Setterfield JF, Minassian DC, Dart JK. The MMP Study Group 2009-14 Mucous membrane pemphigoid with ocular involvement: the clinical phenotype and its relationship to direct immunofluorescence findings. Ophthalmology. 2018;125:496–504.
4. Dart JKG. The 2016 Bowman Lecture. Conjunctival curses: scarring conjunctivitis 30 years on. Eye (Lond). 2017;31:301–32. https://doi.org/10.1038/eye.2016.284