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HLA antigens in Behçet's disease: a reappraisal by a comparative study of Turkish and British patients

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SUMMARY We have compared the distribution of histocompatibility antigens of 22 Turkish and 14 British patients with Behçet's disease with those of their respective controls. There was a strong association of B5, specifically its Bw51 split, with the disease. A modest increase in the incidence of HLA B27 was noted in British patients, but numbers were too small for analysis. The incidence of DRw2 and DRw4 antigens among Turkish and British patients did not differ from that of their respective controls.

Regional differences have been reported for the associations between the HLA antigens and Behçet's disease. Ohno *et al.*[1] from Japan initially reported a strong association with HLA B5. Confirmatory reports appeared from Turkey,[2 3] Israel,[4] southern France,[5] Japan,[6] and Northern Africa.[7] Two reports from Britain[8 9] and 1 from the United States[10] failed to find an association with any of the alleles tested. Another paper from Britain[11] reported an association with HLA B5 in male patients only and a weak association in the entire group with HLA B27. None of those studies contained data on the DR locus antigens.

The purpose of the studies in this report was to re-examine the frequencies of the HLA antigens in patients with Behçet's disease and their controls in 2 Caucasoid populations, especially with regard to the newly defined splits of HLA B5[12] and the HLA DR locus antigens which have not been studied previously.

Materials and methods

The probands came from Istanbul, Turkey, and from Leeds, England. Most of them had been tissue typed previously in the local laboratories. They were called back for retyping on the basis of availability irrespective of the results of their previous typings.

The diagnostic criteria of Mason and Barnes[13] had originally been used for the patients in Leeds and those of O'Duffy[14] for those in Istanbul. For uniformity the entire group was re-evaluated according to O'Duffy's criteria. Four patients from the British group were excluded. There were 22 Turkish (19 males, 3 females) and 14 British (6 males, 8 females) patients that fulfilled the criteria.

Unrelated healthy laboratory technicians, medical students, house staff, and faculty members were used as controls. There were 52 controls from Istanbul and 34 from Leeds.

All HLA typings were performed in the Department of Immunohaematology of the University Hospital, Leiden, Holland. The typings were performed centrally in order to obviate any factors related to different antisera and methodology as causes for the differences in the previously mentioned studies.

Typing for the HLA A, B, and C specificities was performed with the standard NIH microcytotoxicity technique[15] utilising a set of antisera for 13 A locus, 28 B locus, and 6 C locus antigens. DR (D related) typing was performed with the 2-colour fluorescence test[16] using antisera for 10 DR specificities. Heparinised blood samples in siliconised bottles were

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transported by air from Istanbul and Leeds on the day of collection.

Differences in the antigen frequencies between patients and controls in the 2 populations were examined by means of the Woolf-Haldane tests.[17 18]

Results

The clinical findings for the patient groups are summarised in Tables 1, 2a, and 2b. There were no major differences in the 2 patient populations except for a male predominance among the Turkish patients (19/22) as compared to the English patients (6/14). A breakdown of the age and sex information is set out in Table 3.

Table 4 lists the distribution of HLA A, B, C, and DR specificities in the patient and control groups of both populations. The results of the Woolf-Haldane analysis of B5 and its splits (Bw51 and Bw52) are set out in Table 5.

Although the distributions of the antigens B5, Bw51, and Bw52 in the Leeds population were not significantly different in the patient and control groups, they were not significantly heterogeneous when compared to the Istanbul population (all heterogeneity chi-squares less than 3·84). Consequently, the significance levels of Y (relative risk) for B5 and Bw51 may be interpreted as reflecting true differences in the frequencies of those antigens in the patient and control groups of both populations.

Table 1 *Incidence of various features in patients with Behçet's disease*

	Istanbul		*Leeds*	
Aphthous stomatitis	22	(100%)	14	(100%)
Genital ulceration	18	(82%)	14	(100%)
Eye (anterior and posterior segments)	13	(59%)	4	(28%)*
Skin (erythema nodosum, pyoderma, acne)	21	(95%)	10	(71%)
Arthritis	12	(54%)	8	(57%)

*Including conjunctivitis in 1 British patient.

Table 2a *Features of patients with Behçet's disease in Istanbul*

Patient no.	*Age*	*Sex*	*Aphthous stomatitis*	*Eye lesions*	*Genital lesions*	*Skin lesions*	*Arthritis*	*Special features*	*HLA-typing*		
									ABC	*DR*	
1	30	M	+	+	+	+	+		A2, A11, Bw51 B 15B, w4, w6, Cw3	w1	LB47
2	23	M	+	+	+	+	+		A2, A9, Bw51, B40, w4, w6, Cw3	w6	LB47
3	21	M	+	+	+	+	+	Left ventricular aneurysm	A2, Aw24, Bw51, B27, w4, Cw2	w6	w7
4	36	M	+	+	+	+	−	Thrombophlebitis	A3, A11, Bw51, w4, Cw2, Cw4	LB47	LB58
5	37	M	+	+	+	+	−		A2, A3, Bw51, B18, w4, w6	w2	w5
6	22	M	+	+	−	+	−		A2, A3, Bw51, B14, w4, w6, Cw6	w5	w6
7	27	M	+	+	+	+	−	Superior vena caval syndrome	A1, Aw24, Bw51, Bw35, w4, w6, Cw1	w2	w5
8	47	F	+	+	+	+	−		A1. A11, Bw51, Bw35, w4, w6, Cw4	nd	
9	45	M	+	−	−	+	+		A26, Aw33, B18, B17, w4, w6, Cw3	w3	w5
10	36	M	+	−	+	+	−	Thrombophlebitis	A2, Bw51, B17, W4	w4	w8
11	29	M	+	+	+	+	+		Aw24, Bw5·1, Bw35 w4, w6, Cw4	w5	
12	33	F	+	+	−	−	+		Aw24, B5, Bw35, w4, w6, Cw4	nd	
13	32	M	+	−	+	+	+		A1, Aw24, Bw51, B8, w4, w6	w3	w7
14	41	M	+	−	+	+	−		A3, A29, Bw51, w4, Cw6	w4	w5
15	37	M	+	+	+	+	+		Aw30, Aw32, Bw52, B7, w4, w6	w4	w6
16	47	F	+	−	+	+	+		A29, Aw30, B14, w6	w1	
17	30	M	+	+	+	+	+	Haemoptysis	Aw24, Aw32, Bw51 Bw44, w4	w5	LB58
18	37	M	+	−	+	+	−		A1, A2, B17 w4, Cw6	w5	w7
19	27	M	+	+	+	+	−		A3, Aw24, Bw51, Bw42, w4, w6	w2	w5
20	37	M	+	−	+	+	+	Sinus bradycardia	A2, Bw5·1, Bw53, w4, Cw4	w6	
21	23	M	+	−	+	+	−		A28, Aw32, Bw51. B18, w4, w6	w5	w6
22	68	M	+	−	−	+	+		A1, A3, Bw51, Bw44, w4, Cw5	nd	

Table 2b *Features of patients with Behçet's disease in Leeds*

Patient no.	*Age*	*Sex*	*Aphthous stomatitis*	*Eye lesions*	*Genital lesions*	*Skin lesions*	*Arthritis*	*Special features*	*HLA typing*	
									ABC	*DR*
1	33	M	+	+	+	+	+	Thrombophlebitis Macular haemorrhage	A1, A11, Bw52, B7, w4, w6	w4
2	29	M	+	+	+	+	+		A1, A26, B8, B27, w4, w6, Cw1	w1
3	41	F	+	+	+	+	+	Arytenoid ulcer	A2, Bw44, B15B, 16 w4, w6, Cw3, Cw5	w5 w7
4	45	M	+	−	+	+	−	Thrombophlebitis	A2, Aw32, Bw51, B7, w4, w6, Cw2	w4
5	46	M	+	−	+	+	+	Thrombophlebitis Tracheoesophageal fistula	A2, Bw51, 17, W4, w6, Cw1, Cw5	w6
6	26	F	+	−	+	+	−		A2, A11, Bw44, B15B, 2 w4, w6, Cw1, Cw5	w4
7	38	F	+	−	+	+	−	Nonspecific caecal ulceration	A1, B7, B17, w4, w6, Cw6	w4
8	43	F	+	−	+	+	−		Aw24, A28, Bw44, w4, Cw5	nd
9	14	M	+	−	+	+	+		A2, A11, Bw22, B40, w6, Cw3	w2
10	51	F	+	+ (c)	+	−	−		A2, A11, Bw35, B27, w4, w6, Cw2, Cw4	w4 w6
11	35	F	+	−	+	−	+		A1, Aw24, B8, B27, w4, w6, Cw1	w1 w4
12	66	F	+	−	+	−	+		A2, Aw32, B18, B40 w6, Cw3	nd
13	38	F	+	−	+	−	+	Thrombophlebitis	A2, B27, B40 w4, w6, Cw3	w4
14	60	M	+	+	+	+	−	Paget's disease of skull also	A1, 2, Bw51 37, w4, w4, Cw6	w3 w4

(c)=Conjunctivitis only.

Table 3 *Distributions of age and sex in the Istanbul and Leeds Behçet patient populations*

		No.	*Age*		
			Range	*Mean*	*Median*
Istanbul	Males	19	21–68	33·6	30
	Females	3	33–47	42·3	47
Leeds	Males	6	14–60	36·2	33
	Females	8	26–66	42·3	41

Table 4 *Distribution of HLA A, B, C, and DR specificities in patient and control groups*

HLA	*Istanbul*		*Leeds*	
	Patients N=22	*Controls N=52*	*Patients N=14*	*Controls N=34*
A1	5*	10	5	13
A2	8	27	9	19
A3	5	13	0	9
A9	7	16	2	6
A10	1	7	1	1
A11	3	5	3	6
A28	1	5	1	1
A29	2	0	0	3
Aw30	2	3	0	1
Aw31	0	1	0	4
Aw32	3	4	2	0
Aw33	2	4	0	0
Aw34	0	0	0	2

*Number of positive individuals.

Table 4b

HLA	*Istanbul*		*Leeds*	
	Patients N=22	*Controls N=52*	*Patients N=14*	*Controls N=34*
B5	19	22	4	6
Bw51	18	12	3	4
Bw52	1	9	1	2
B7	1	4	3	12
B8	1	5	2	9
B13	0	3	0	2
B14	2	1	0	0
B15 A	0	1	0	1
B15 B	1	0	2	1
B17	3	6	2	1
B18	3	7	1	1
Bw22	0	2	1	5
B27	1	3	4	1
Bw35	4	11	1	5
B37	0	1	1	1
Bw38	0	6	0	0
Bw39	0	2	0	1
B40	1	4	3	4
Bw41	0	2	0	1
Bw42	1	0	0	0
Bw44	2	6	3	9
Bw45	0	0	0	0
Bw47	0	0	0	0
Bw48	0	1	0	0
Bw49	0	5	0	1
Bw50	0	4	0	1
Bw53	0	2	0	0

Table 4c

HLA	*Istanbul*		*Leeds*	
	Patients N=22	*Controls N=52*	*Patients N=14*	*Controls N=34*
Cw1	1	2	3	1
Cw2	2	4	2	1
Cw3	3	6	4	9
Cw4	5	14	1	3
Cw5	1	2	3	1
Cw6	3	9	2	5
	N=19	*N=49*	*N=12*	*N=28*
DRw1	2	11	2	6
DRw2	3	13	1	8
DRw3	2	10	1	5
DRw4	3	11	8	12
DRw5	10	18	1	2
DRw6	6	12	2	10
DRw7	3	11	1	5
LB8	1	1	0	0
LB47	3	6	0	0
LB58	2	3	0	0

It is interesting to note that all of the English Behçet's patients with B5 are male.

The number of observations for the DR antigens were small. They have been analysed and listed in Table 5 because of the reported linkage disequilibrium between B5 and DRw2 and DRw4 in North American caucasoids.[19] There is no difference in the incidence of DRw2 and DRw4 in Turkish and British patients when compared with their controls.

Since the numbers of observations were rather small for the remaining HLA and A, B, C, and DR specificities, the frequency differences were not analysed, in order to avoid the effect of sampling errors.

The data in this study were insufficient to confirm our previously reported finding of a weak association between B27 and English patients with Behçet's disease.

Discussion

The results of this study demonstrate significant conformity in the different incidence of HLA B5, specifically its Bw51 split, in patients with Behçet's disease in 2 Caucasoid populations. The modest increase in the frequency of HLA B27 in British patients is suggested by the data but was not analysed because of the small sample sizes.

The striking increase in the frequency of HLA B5 among the Turkish patients is confined to Bw51, the split of B5 found commonly among Caucasians.[12] It is interesting that the relative risk statistic for B5 in Caucasians was reported as 7·43 by the HLA and Disease Registry in 1977.[20] The value is 7·55 for the same antigen in the Istanbul population. However, it is almost twice as large for Bw51 in the same population (see Table 4). These observations suggest that other investigators should type their Behçet patients for the B5 splits. This would be especially important for the Japanese patients, since Bw51 and Bw52 seem to be common alleles in the Japanese population.[12]

An inspection of the report of the HLA and Disease Registry reveals very few disease associations with antigenic splits. In view of the findings in this report it is perhaps pertinent to suggest that a reappraisal of HLA and disease associations with special attention to antigenic splits may reveal new associations or strengthen presently recognised associations.

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References

[1] Ohno S, Aoki K, Sugiura S, Nakayama E, Itakura K, Aizawa M. HL-A5 and Behçet's disease. *Lancet* 1973; **2**: 1383-4.

Table 5 *Woolf-Haldane analysis of HLA B5 and its splits [Bw51 and Bw52] in 2 patient populations and their controls*

	Istanbul					*Leeds*							
	Patients		*Controls*			*Patients*		*Controls*			*Chi squares*		
	Pos.	*Neg.*	*Pos.*	*Neg.*	*Y*[1]	*Pos.*	*Neg.*	*Pos.*	*Neg.*	*Y*	*Y*	*Het.*[2]	*Total*
B5	19	3	22	30	7·552	4	10	6	28	1·879	9·437[3]	2·293	11·730
Bw51	18	4	12	40	13·320	3	11	4	30	2·063	16·085[4]	3·783	19·869
Bw52	1	21	9	43	0·319	1	13	2	32	1·444	0·673	1·420	2·093
DRw2	3	16	13	36	0·574	1	11	8	20	0·315	2·248	0·314	2·561
DRw4	3	16	11	38	0·710	8	4	12	16	2·493	0·324	1·824	2·148

[1]Y=the relative risk statistic. [2]Het.=Heterogeneity. [3]P=0·009. [4]P=0·0003.

[2] Ersoy F, Berkel A I, Firat T, Kazokoglu H. HLA antigen associated with Behçet's disease. *HLA and Disease.* Paris: Editions INSERM, 1976.

[3] Yazici H, Akokan G, Yalcin B, Muftuoglu A. The high prevalence of HLA-B5 in Behçet's disease. *Clin Exp Immunol* 1977; **30:** 259–61.

[4] Chajek T, Brautbar C, Cohen T, Lamm L U. A study of genetic factors in patients with Behçet's disease in Israel. *Aba* No. 907, 1977.

[5] Godeau P, Terre D, Campinchi R, *et al.* HLA-B5 and Behçet's disease. *HLA and Disease,* 101. Paris: Editions INSERM, 1976.

[6] Takano M, Miyajima Y, Kiuchi M. *et al.* Behçet's Disease and the HLA system. *Tissue Antigens* 1976; **8:** 95–9.

[7] Hamza M. *International Symposium on Behçet's Disease.* Istanbul, 1977.

[8] Chamberlain M A. (1975). Behçet's disease. *Ann Rheum Dis* 1975; **34:** Suppl 1, 53–4.

[9] Jung R T, Chalmers T M, Joysey V C. HLA in Behçet's disease. *Lancet* 1976; **1:** 694.

[10] O'Duffy J D, Taswell H F, Elvebach L R. HLA antigens in Behçet's disease. *J Rheumatol* 1976; **3:** 1–8.

[11] Chamberlain M A. Behçet's syndrome in 32 patients in Yorkshire. *Ann Rheum Dis* 1977; **36:** 491–9.

[12] Dick H. HLA-A, B, and C serology and antigen report. In *Histocompatability Testing 1977.* Joint Report, 157. Copenhagen: Munksgaard, 1978.

[13] Mason R M, Barnes C G. Behçet's syndrome with arthritis. *Ann Rheum Dis* 1969; **28:** 95–103.

[14] O'Duffy J D. Suggested criteria for diagnosis of Behçet's disease. *Book of Abstracts,* Toronto, Canada: VI Pan American Congress of Rheumatic Disease, 18, 1974.

[15] *Manual of Tissue Typing Techniques* (1976–77). Department of Health, Education and Welfare. Publications No. (NIH) 77–545.

[16] Rood J J van, Loouwen A van, Ploem J S. Simultaneous detection of two cell populations by two-colour fluorescence and application to the recognition of B-cell determinants. *Nature* 1976; **262:** 795–7.

[17] Woolf B. (1955). On estimating the relation between blood groups and disease. *Ann Hum Genet* **19,** 251–253.

[18] Haldane J B S. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1955; **20:** 309–11.

[19] Pickbourne P, Piazza A, Bodmer F. Population Analysis. In *Histocompatibility Testing 1977.* Joint Report, 259 Copenhagen: Munksgaard, 1978.

[20] Svejgaard A, Ryder L P. HLA and disease, In: Ferrara G B, ed. *HLA system–new aspects.* Amsterdam: North Holland, 1977; 143–51.