HLA and nasopharyngeal carcinoma in Malays

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Summary HLA associations were observed in unrelated Malay patients with nasopharyngeal carcinoma (NPC). HLA-B18 was observed in 18/45 (40%) Malay NPC patients compared to 22/167 (13%) Malay normals ($P=0.0001$; $P_c=0.0027$, $RR=4.4$). The frequency of HLA-B17, one of the antigens associated with Chinese NPC, was also increased among Malay NPC (13/45 29%) compared to controls (18/167 11%; $P=0.003$, $P_c=0.07$ $RR=3.4$). Similar to the findings among Chinese NPC, the frequency of B17 was higher in early onset (≤ 30 years) Malay NPC resulting in a higher relative risk ($RR=5.0$).

Nasopharyngeal carcinoma (NPC) is a geographically restricted tumour that has attracted considerable attention recently because of the unique opportunity to study the genetic and environmental factors in the pathogenesis of this tumour. Implicated environmental factors include the Epstein Barr virus, nitrosamine and plant extracts (Prasad *et al.*, 1983). The data of different incidences among different populations, incidence among migrants and family clustering suggested the involvement of genetic factors in addition to environmental factors.

HLA associations have been reported in NPC. With the highest incidence populations, similar HLA associations have been reported among the Chinese from Singapore (Simons *et al.*, 1976; Chan *et al.*, 1983) Malaysia, Hong Kong (Simons *et al.*, 1977), China (Cui & Lin, 1982) and California (Jing *et al.*, 1977). In Singapore, the associations among Chinese NPC were with A2 BW46 among older onset NPC and AW19 B17 among total NPC but this association was especially strong among younger onset NPC (Chan *et al.*, 1983). Among the mid-incidence populations, reports of HLA associations have been inconsistent. HLA-A29 was associated with NPC among East African Blacks ($RR=19.5$; Hall *et al.*, 1982). Among North African NPC, the frequencies of A3, B5 and B15 were increased and AW33, B14 and DR4 decreased (Herait *et al.*, 1983) and yet in another North African population, the Tunisians, no HLA association was found (Betuel *et al.*, 1975). Among low incidence populations, reports of HLA associations in NPC were equally mixed. An

increase in the frequencies of A3 and B5 was observed among German NPC (Kruger *et al.*, 1981), A3 among NPC from Australia (Simons & Shanmugaratnam, 1982) while no associations was reported in other Caucasian populations (Jing *et al.*, 1977; Moore *et al.*, 1983; Beigel *et al.*, 1983). In South East Asia, besides the high NPC incidence Chinese population, there are ethnic groups with mid range incidence of NPC and here we report that results of HLA typing in NPC from one such ethnic group, the Malays.

Materials and methods

Subjects

A total of 45 unrelated Malay NPC patients and 167 unrelated Malay normal controls was studied. The patients attended the Ear, Nose and Throat Departments of the Singapore General Hospital, Singapore and the University Hospital, Kuala Lumpur. Most of the patients were bled within 1.5 years after diagnosis but some had survived for varying periods (1.5 to 8 years) at the time of bleeding. The patients could be divided into early (≤ 30 years of age) onset or late (> 30 years) onset groups at the time of diagnosis. There were 8 early and 37 late onset patients. The controls were normal Malays and consisted of 106 Malays reported previously (Chan *et al.*, 1978) and an additional 61 who were the normal spouses of Malay renal patients (live transplantation program) from Singapore and Malaysia.

HLA typing

Lymphocyte separation and HLA typing were performed from freshly drawn venous blood as

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discussed previously (Simons *et al.*, 1976). The panel of antisera used to define the 26 locus A and B antigens consisted of over 200 sera of Malay, Chinese, Japanese, Filipino and Caucasian in origin. Antigens of the C and DR locus were not typed for. The significance of the difference in antigen frequencies between patients and controls was calculated by the Fisher's exact *P*-value and this value was multiplied by the number of antigens typed for (26) to give the corrected *P*(Pc) value. The relative risk (RR) was calculated by the odds ratio in the classical 2 × 2 table (Breslow & Day, 1980). If a, b, c, d represented patients with marker, patients without marker, controls with marker and controls without marker respectively, the relative risk was calculated as a × d divided by b × c.

Results

The HLA locus A and B antigen frequencies of the total NPC patients, patient subgroups and normal controls are shown in Table I. Compared to controls, total Malay NPC patients had a higher frequency of B17 and B18 and a lower frequency of

A11, B15 and B40. There was no increase in the frequency of BW46, one of the antigens found to be associated with Chinese NPC. All the B17 in this study was of the split BW58. Similarly all B17 in the Chinese is also BW58. However, for ease of comparison between this study and previously published Chinese data where only B17 was defined we have retained the definition of B17.

HLA-B18 was observed in 18/45 (40%; Table II) NPC patients compared to 22/167 (13%) controls ($\chi^2=16.7$; $P=0.0001$, $P_c=0.0027$; relative

Table II HLA antigen frequencies of Malay NPC patients and controls

Antigen	NPC <i>n</i> =45	Control <i>n</i> =167	<i>P</i>	<i>P_c</i>	RR
B18	18 ^a (40%)	22 (13%)	0.0001	0.0027	4.4
B17	13 (29%)	18 (11%)	0.003	0.07	3.4
B40	3 (7%)	37 (22%)	0.009	NS	0.25
B15	12 (27%)	74 (44%)	0.014	NS	0.46

^aNumber (%)

P – by Fisher's exact test

P_c – corrected *P* value (*P* × 26)

RR – relative risk

Table I HLA antigen frequencies in Malay NPC patients and controls

	Early onset <i>n</i> =8		Late onset <i>n</i> =37		Total NPC <i>n</i> =45		Normals <i>n</i> =167	
	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
A1	0	—	3	0.081	3	0.067	11	0.066
A2	4	0.500	9	0.243	13	0.289	56	0.335
A3	0	—	1	0.027	1	0.022	4	0.024
A9	7	0.875	22	0.595	29	0.644	106	0.635
A10	0	—	2	0.054	2	0.044	23	0.138
A11	0	—	10	0.270	10	0.222	55	0.329
A28	0	—	0	—	0	—	1	0.006
A29	0	—	0	—	0	—	0	—
AW19	1	0.125	11	0.297	12	0.267	34	0.204
B5	2	0.250	4	0.108	6	0.133	25	0.150
B7	0	—	1	0.027	1	0.022	12	0.072
B8	0	—	2	0.054	2	0.044	0	—
B12	0	—	4	0.108	4	0.089	20	0.120
B13	0	—	3	0.081	3	0.067	14	0.084
B14	0	—	0	—	0	—	0	—
B15	2	0.250	10	0.270	12	0.267	74	0.443
B17	3	0.375	10	0.270	13	0.289	18	0.108
B18	3	0.375	15	0.405	18	0.400	22	0.132
B27	0	—	4	0.108	4	0.089	18	0.108
B37	0	—	0	—	0	—	2	0.012
B40	1	0.125	2	0.054	3	0.067	37	0.222
BW16	0	—	8	0.216	8	0.178	19	0.114
BW21	0	—	0	—	0	—	5	0.030
BW22	0	—	1	0.027	1	0.022	5	0.030
BW35	3	0.375	7	0.189	10	0.222	41	0.246
BW46	0	—	1	0.027	1	0.022	4	0.024

risk=4.4). This difference was still significant after correction for the number of antigens (26) typed for. The frequency of B17 was significantly higher in NPC patients (13/45, 29%) than in controls (18/167, 11%; $P=0.003$, $P_c=0.07$; $RR=3.4$). However the corrected P value just failed to reach significance. The frequencies of B40 and B15 were significantly lower among NPC patients compared to controls but both these differences were not significant when corrected.

Old vs young onset NPC

In Chinese NPC, there was differences in the HLA associations in early and late onset patients. BW46 was associated only with late onset (>30 years) patients whereas B17 was associated in total patients but particularly in early onset (≤ 30 years) patients (Chan *et al.*, 1983). In this study, early onset (≤ 30 years) Malay patients had higher frequencies of A2, A9 and B17 and lower frequencies of A11 compared to late onset patients or to normals (Table I). It is interesting to note that the finding of a higher frequency of B17 among early onset patients observed among the Chinese was also present in the Malays. The frequency of B17 was higher in early onset patients and this reflected in the higher relative risk (5.0; Table III) compared to total patients ($RR=3.4$). HLA-A11 was not detected among early onset patients and the decreased frequency of B40 seen in total patients was even more marked among the late onset patients ($P=0.009$; $RR=0.2$; Table III).

Discussion

This study indicates HLA associations in Malay NPC. The associations were with B18 and B17. The association with B18 was significant even after correction for the number of antigens typed for but with B17 the corrected P value just failed to be significant. B17 is also one of the antigens associated with Chinese NPC. Among Chinese NPC the frequency of B17 was lower in long term survivors compared to newly diagnosed patients (Chan *et al.*, 1981). Unfortunately in this study we did not have sufficient patients to make this comparison and the frequency of B17 could be even higher if only newly diagnosed patients were studied. Among the Chinese, the frequency of B17 was higher in early onset patients and this study showed a similar relationship in Malay NPC.

The frequency of B40 and B15 were lower among the patients compared to controls and the frequency of B40 was even slightly lower in late onset patients. However it could not be determined whether there was a specific decrease in these antigen frequencies or whether the decrease was a compensation for the increases in the frequencies of B18 and B17.

The present findings supported the hypothesis that genes are important in the pathogenesis of NPC and some of these genes are associated with the major histocompatibility complex. There are certain similar findings between Malay and Chinese NPC – the association with B17, the high frequency of B17 among early onset compared to late onset

Table III HLA antigen frequencies in NPC patient subgroups and controls

		<i>Nasopharyngeal carcinoma</i>			
<i>Antigen</i>		<i>Early onset n=8</i>	<i>Late onset n=37</i>	<i>Total n=45</i>	<i>Controls n=167</i>
B18	Freq.	37.5%	40.5%	40.4%	13.2%
	P^a	0.06	0.0002	0.0001	—
	P_c^a	NS	0.0055	0.0027	—
	RR^a	4.0	4.5	4.4	—
B17	Freq.	37.5%	27.0%	28.9%	10.8%
	P	0.048	0.008	0.003	—
	P_c	NS	NS	0.07	—
	RR	5.0	3.1	3.4	—
A11	Freq.	0	27.0%	22.2%	32.9%
	P	0.03	NS	NS	—
	P_c	NS	NS	NS	—
	RR	0	0.7	0.6	—
B40	Freq.	12.5%	5.4%	6.7%	22.2%
	P	NS	0.009	0.009	—
	P_c	NS	NS	NS	—
	RR	0.5	0.2	0.3	—

^aComparison of total NPC patients or patient subgroups with normal controls.

patients and the decrease frequency of A11. This closeness may be due in part to a strong belief that the Malays originated from the Yunan Province in China between 2500–1500 BC as evidenced by their

quadrangular adze culture and unglazed cord-marked pottery which have been traced from China southwards (Omar, 1983). However these findings will have to be confirmed by a larger study.

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