Human leukocyte class I antigen alleles A2 and A11 are not associated with nasopharyngeal carcinoma in West Malaysia

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ABSTRACT

Introduction: Nasopharyngeal carcinoma (NPC) is the second most common cancer among Malaysian Chinese males. We determined the frequencies of 17 human leukocyte antigens (HLA), HLA-A and HLA-B, alleles in 88 Malaysian Chinese with NPC.

Methods: Using polymerase chain reaction sequence-specific primers, the frequencies of 17 HLA-A and HLA-B alleles were analysed. They were A1, A2, A11, A31, A32, A33, B8, B13, B27, B38, B39, B44, B46, B55, B58, B61 and B71.

Results: Three of the 17 alleles were detected in NPC patients. They were A1 (0.6 percent), A2 (56.3 percent) and A11 (43.2 percent). Three of the 17 alleles were detected in age- and sex-matched healthy individuals. They were A2 (50.0 percent), A11 (50.0 percent) and B27 (4.7 percent). The A2 and A11 alleles were evenly distributed in both groups, while A1 was only found in one NPC patient and B27 exclusively in healthy individuals.

Conclusion: We conclude that A1 is very rare, and A2, A11, A31, A32, A33, B8, B13, B38, B39, B44, B46, B55, B58, B61 and B71 alleles have no associations with the occurrence of NPC in Malaysia, while allele B27 is negatively associated.

Keywords: human leukocyte antigen, nasopharyngeal carcinoma, polymerase chain reaction sequence-specific primers

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) is one of the major cancers in Malaysia. According to the 2003 report of the National Cancer Registry, NPC ranked second in terms of cancer prevalence among Malaysian Chinese males.[1] NPC is more common among people originating from the Southeastern provinces of China;[2] Malaysian Chinese are largely southern Chinese.[3] High incidence of NPC has also been reported among the natives of Sarawak, East Malaysia.[4] Multiple factors have been reported to be associated with this disease, including Epstein-Barr virus (EBV),[5] environmental factors,[6] food carcinogens,[7] and host genetic factors.[8]

Since the early 1970s, numerous studies on the association of the human leukocyte antigens (HLA) with NPC were undertaken mainly to identify and establish possible cancer markers. However, in many of such studies, disparities between ethnic groups in addition to geographical locations were evident,[9,10] while the statistical significance of the associations between NPC and HLA types remained doubtful. In a study, HLA-B13 was thought to display protective effects among the southern Chinese.[11] However, this finding did not corroborate with results from another study from Morocco, where there was a significantly higher manifestation of the allele in young NPC patients.[12] Alleles that have been positively correlated to NPC are A1, A2, A3, A10, A19, A28, A33, B5, B8, B13, B14, B17, B18, B38, B46, B51 and B58.[9-19] Alleles that are known to confer protective effects against NPC are A9, A11, A23, A31, B13, B22, B27, B39, B44 and B55.[9-13,18,20] Two-loci analyses have shown that individuals with A2(+)B17(+),[15] A2(+)B38(+), A2(+)B46(+),[18] and A19(+)B13(+)[11] were at greater risk of succumbing to NPC.

METHODS

In this study, we investigated the association of NPC with HLA-A and HLA-B alleles in Malaysia. Alleles A1, A2, A11, A31, A32, A33, B8, B13, B27, B38, B39, B44, B46, B55, B58, B61 and B71, which were commonly associated with Chinese NPC patients, were selected for HLA typing. These 17 alleles were selected based on published reports indicating either a positive or negative association with NPC. The study cohort included 60 Chinese male NPC patients from the

University of Malaya Medical Centre, Kuala Lumpur, and 28 Chinese male NPC patients from the Nilai Cancer Institute, Negeri Sembilan. All of the 88 NPC patients enrolled in this study were seropositive for EBV and had elevated IgA titres for viral capsid antigen. Blood from 86 healthy Chinese males were used as age- and sex-matched, non-NPC controls. All patients and individuals were typed for the selected HLA-A and HLA-B alleles by polymerase chain reaction sequence-specific primers (PCR-SSP) as described by Bunce et al.[21] The chi-square (χ^2) test using a standard 2 × 2 contingency table was used to measure the difference between the NPC patients and healthy individuals, and the Fisher's exact test was applied in cases where the number of subjects in a group was less than five.

RESULTS

Four alleles (A1, A2, A11 and B27) were detected, with the A2 and A11 alleles being almost evenly distributed in both groups (Table I). The A2 allele frequency was higher in the NPC group (56.3%) compared to the healthy group (50.0%) (combined odds ratio [OR], 1.29; 95% confidence interval [CI], 0.84–1.96). On the other hand, the A11 allele frequency was lower in the NPC group (43.2%) compared to the healthy group (50.0%) (combined OR, 0.76; 95% CI, 0.50–1.16). However, there is no significant difference in alleles A2 (p = 0.243) and A11 (p = 0.202) among NPC and healthy individuals (Table II). Only p-values less than 0.05 from the χ^2 test were considered significant. Interestingly, although the A1 allele was found exclusively in NPC patients, it was not a statistically significant factor (0.6%). Allele B27 (4.7%) was exclusively found in the healthy individuals and is negatively correlated with the presence of NPC (p = 0.003) (Table II).

Table I. The distribution of HLA-A and HLA-B alleles frequencies.

HLA	NPC (2n* = 176)		Healthy (2n* = 172)	
	count	%	count	%
HLA-A				
A1	1	0.6	0	0
A2	99	56.3	86	50.0
A11	76	43.2	86	50.0
A31	0	0	0	0
A32	0	0	0	0
A33	0	0	0	0
HLA-B				
B8	0	0	0	0
B13	0	0	0	0
B27	0	0	8	4.7
B38	0	0	0	0
B39	0	0	0	0
B44	0	0	0	0
B46	0	0	0	0
B55	0	0	0	0
B58	0	0	0	0
B61	0	0	0	0
B71	0	0	0	0

* the total number of individuals studied in the patient or control group. The effective sample size was 2n because each individual inherited two separate alleles from their parents.

Table II. The HLA-A and HLA-B alleles level of significant associations with NPC.

HLA	OR	95% CI	χ^2 test	p-value#
HLA-A				
A1*				0.506
A2	1.29	0.84–1.96	1.37	0.243
A11	0.76	0.50–1.16	1.63	0.202
HLA-B				
B27*				0.003

* Data were analysed using Fisher's exact test.

\# Only p-value < 0.05 from chi-square (χ^2) or Fisher's exact tests were considered statistically significant.

DISCUSSION

The HLA alleles, A2 and B46, had been reported to be associated with increased risk of NPC among the Chinese in Asia,[10,11,19] but were found to confer protective effects among Caucasians.[9] A subsequent study revealed that the presence of both A2 and B46 confer two-fold increased risks for NPC among the Chinese.[19] Nonetheless, the protective effect of the A11 allele was consistently reported across all races.[9-11] The A2 and A11 allele frequencies in Malaysian NPC patients in the present study were almost similar to those of healthy individuals. However, our results are in agreement with the findings in Moroccan NPC patients.[12] We conclude that A2 and A11 alleles, in contrast to several other studies,[8-11] are not correlated with NPC in Malaysia.

Alteration of HLA class I molecule and the members of the antigen processing machinery are frequent events in many cancers. This is especially an important consideration in NPC where EBV is an aetiological agent. Recently, the prevalence of the HLA-A2 restricted 'epitope-loss variant' of EBV latent membrane protein (LMP-1) was demonstrated in cases of NPC in southern China and Taiwan.[22,23] In addition, a variety of sequence changes in the EBV nuclear antigen

(EBNA-3B), which encodes two immunodominant HLA-A11 epitopes, were also reported.[24] Both scenarios demonstrate the ability of EBV in resisting immune recognition that may nullify the role of a supposedly protective HLA allele.

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