Published in final edited form as:

Hum Immunol. 2009 November ; 70(11): 910–914. doi:10.1016/j.humimm.2009.08.005.

ASSOCIATION OF HUMAN LEUKOCYTE ANTIGENS (HLA) WITH NASOPHARYNGEAL CARCINOMA (NPC) IN HIGH-RISK MULTIPLEX FAMILIES IN TAIWAN

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Abstract

Association between specific human leukocyte antigens (HLA) alleles and NPC have been reported for sporadic NPC but studies of familial NPC are lacking. We evaluated this association with familial NPC in a study of 301 NPC cases and 1010 family and community controls from Taiwan. Class I HLA alleles were characterized using a sequence-based typing protocol. Allele frequencies between case and control groups were compared by chi-square or exact tests. For alleles associated with NPC, odds ratios (OR) and 95% confidence intervals (CI) were calculated. Similar allelic frequency distribution and HLA associations were found as those previously reported for sporadic NPC: protective effect for HLA-A*1101 and increased risk for HLA-A*0207, HLA-A*3303, HLA-B*3802, and HLA-B*5801. Overall, the magnitude of observed associations was weakest when cases were compared to sibling controls and strongest when compared to unrelated community controls. Evaluating the joint effect of HLA-A*0207 and HLA-B*4601, individuals who were carriers of HLA-A*0207 with or without the presence of HLA-B*4601 had a 1.9-fold (95% CI = 1.0-3.4) and 2.1-fold (95% CI = 0.83-5.3) risk of NPC, respectively. Conversely, carriers of HLA-B*4601 in the absence of HLA-A*0207 had a 50% reduction in NPC risk (95% CI = 0.27-0.93). Comparable

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findings from our family study and those from previous sporadic studies were found with the notable exception of a lack of positive association between HLA-B*4601 and familial NPC in the absence of HLA-A*0207. This finding requires replication in larger studies.

Keywords

Human leukocyte antigens; nasopharyngeal carcinoma; epidemiology; genetics

INTRODUCTION

Epstein-Barr virus (EBV) infection, an infection that is nearly ubiquitous worldwide and establishes lifelong infection, is known to be closely linked to nasopharyngeal carcinoma (NPC) development [1,2]. Since most EBV-infected individuals do not develop NPC, it is widely accepted that additional factors (both exogenous and host susceptibility factors) play a role in NPC development.

Among host susceptibility factors, arguably the most extensively studied in the context of NPC risk are the major histocompatibility complex (MHC) genes located on chromosome 6. These genes encode for the very polymorphic human leukocyte antigens (HLA) as well as other genes. Because nearly all NPC tumors are EBV positive, it is postulated that individuals who inherit HLA alleles with a decreased ability to present EBV antigens to the immune system might be at an increased risk for developing NPC and vice versa. In fact, studies have confirmed the association between HLA and NPC [3-7]. Several HLA alleles and haplotypes have been convincingly and consistently shown to be associated with altered risk of NPC, including HLA-A*0207, HLA-A*1101, HLA-B*4601, HLA-B*5801, and haplotypes containing these alleles [3-7]. While the associations reported might be attributable to linkage disequilibrium between specific HLA alleles or haplotypes and other NPC susceptibility genes on chromosome 6, a direct involvement of HLA in NPC development is also plausible given the important role that HLA plays in the presentation of foreign antigens (including viral antigens) to the host immune system [7-10].

Most studies conducted to date to evaluate the association between HLA and NPC have been association studies of largely sporadic NPC but studies of familial NPC is lacking [3-7]. The link between HLA and NPC has not been extensively investigated among high-risk individuals such as those with a family history of NPC. One small study that did directly evaluate HLA and NPC in the context of affected siblings found evidence for linkage on chromosome 6 (i.e., within families affected siblings shared HLA alleles more often than would be expected by chance), but there was no apparent association between specific class I HLA alleles and NPC across families [11]. Subsequent family-based linkage studies [12-14], however, failed to confirm evidence for linkage in the MHC region of chromosome 6. One important limitation of the study by Lu and colleagues was the inability to test for class I HLA using methods that permit evaluation at the individual genotype level.

To address this limitation and to further evaluate the association between HLA and NPC among individuals in families at high risk of this disease, we conducted a study of 1311 individuals (301 NPC cases from multiplex families; 693 unaffected family members; 317 unrelated community controls) in Taiwan. Direct sequencing methods were used to test for class I HLA at the individual genotype level, and both linkage and association based analyses were performed to assess the role of HLA in NPC within our high-risk population.

MATERIALS AND METHODS

Details of the NPC Cancer Genetics (CAGEN) Multiplex Family Study have been described elsewhere [15-16]. Briefly, NPC cases diagnosed between 1980 and 2003 were identified through the National Cancer Registry, ten tertiary care hospitals, and selected outpatient NPC treatment clinics and queried for a family history of NPC. Of the 20,450 NPC cases identified, 10,178 (49.8%) were successfully screened. Through this screening, 802 NPC cases with a family history of ≥ 2 NPC were identified and further screened for eligibility, assessed by the number of NPC cases that were alive and available for sampling and the relationship between the affected individuals. Presence of two or more NPC cases was confirmed and family members recruited for a total of 358 NPC multiplex families from which 3216 individuals were recruited, including 404 live NPC cases and 2557 unaffected family members. Informed consent was obtained from participating subjects. Institutional Review Boards at both the National Institutes of Health and National Taiwan University approved the study protocol and informed consent.

For the present study, all cases with consented unaffected relatives (i.e., siblings and/or spouses) whereby cases and relatives all have DNA sample available were selected for testing ($n=301$; 71.0% male; mean age = 52.3 years). For each case, up to two siblings without NPC were also selected ($n=498$). When selecting sibling controls for testing, those of the same gender and closest in age to the NPC case but older were given priority. When matching on both gender and age was not possible, matching on gender was prioritized over matching on age given the 2.5:1 male: female ratio of NPC. To this group of 498 sibling controls (49.6% male; mean age = 49.9, SD=11.9), we added a set of 139 siblings from these same families who had previously been HLA tested in the same laboratory using the same testing methods in an initial pilot effort within our family study. Inclusion of this second group of siblings was justifiable to increase study power since they were largely comparable to the first sibling set with respect to gender (matched: 48.7% male versus unmatched: 52.3% male, p -value=0.47) and age (matched: mean 49.7 years, SD=12.0 versus unmatched: mean age=50.4 years, SD=11.6, p -value=0.57). As a separate unrelated family member control group, we selected for testing all spouses of NPC cases for whom DNA was available for testing ($n=212$).

As a final, unrelated control group we included in our study a set of 317 community controls previously recruited and HLA typed as part of a case-control study of NPC conducted in Taiwan by our group [7]. Details of the criteria for selection of these community controls have been described elsewhere [17-18]. Briefly, these controls represent individuals identified through the National Household Registration System who were individually matched to NPC cases from the case-control study at a 1:1 ratio on age (within 5 years), sex, and district/township of residence. Eligibility requirements for controls included residency in Taipei city or county for at least 6 months and no prior history of NPC.

DNA Extraction and HLA Genotyping

HLA class I typing for HLA-A and HLA-B was performed as previously described [19]. Class I HLA alleles were characterized using a sequence-based typing protocol. HLA genes were selectively amplified with locus-specific PCR primers for HLA-A, B and C separately. The PCR product contains exon 2, intron 2 and exon 3 and was used as the template for the sequencing analysis. For each HLA gene two sequencing reactions (one for exon 2 and one for exon 3) were applied with exon-specific sequencing primers. The sequencing analysis was performed using the ABI Big Dye Terminator Cycle Sequencing Kit and ABI3730xl DNA analyzer (Applied Biosystems, Foster City, CA). HLA alleles were assigned on the basis of the sequence database of known alleles with the help of the ASSIGN software developed by Conexio Genomics (Conexio Genomic, Western Australia). Ambiguous heterozygous genotypes were resolved by extra PCR and sequencing procedures using allele-specific PCR

primers to selectively isolate one of the two alleles. HLA-A typing was successful for 301 NPC cases and 693 unaffected family members. HLA-B typing was successful for 295 NPC cases and 681 unaffected family members. For community controls from our previously conducted case-control study, HLA Class I typing for HLA-A and HLA-B was performed by PCR-based co-amplification of the polymorphic exon 2 and exon 3 of each locus followed by detection using a reverse line-blot typing system, as previously described [7].

EBV Testing

Serum obtained from study participants was tested for antibodies against several EBV antigens known to be associated with NPC: anti-VCA IgA, anti-DNase antibodies, and EBNA-1 IgA. Anti-VCA IgA was tested by the indirect immunofluorescence assay [20]. VCA results were positive when anti-VCA IgA antibody was detected in a 1:10 or greater dilution of serum. Anti-DNase antibody levels were evaluated via an enzyme neutralization assay [21]. One unit DNase activity was defined as the amount of enzyme required to convert 1 microgram of dsDNA into acid-soluble material in 10 minutes at 37⁰ C. A positive result for anti-DNase antibody was considered when 2 units or more of DNase activity were neutralized by 1 ml serum. Anti-EBNA-1 IgA antibodies were detected by ELISA [22]. A positive result for anti-EBNA-1 IgA was defined as OD₄₀₅ >0.20. The positivity cutoffs described above for the various assays were chosen based on data from previous studies that have demonstrated their sensitivity and specificity in distinguishing NPC cases from unaffected individuals [22-24].

Statistical Analysis

Allele frequencies were computed and compared between case and control groups with Pearson's chi-square test or Fisher's exact test (when the number of subjects in a cell is <5) [25]. For alleles associated with NPC and those consistently reported in previous literature, odds ratios (ORs) were computed and 95% confidence intervals (CIs) were calculated to determine the statistical significance of the findings between cases who are carriers for the HLA of interest and controls, unless where specified. To account for lack of independence among individuals from the same family, logistic regression that accounted for family as a repeated measure was used when NPC cases were compared against sibling or spouse controls [26]. A second regression method that conditions on family was also employed and yielded comparable results (data not shown). Unconditional logistic regression methods were used when NPC cases were compared against unrelated, community controls [27].

We also performed analysis stratified on EBV seropositivity. For this analysis all NPC cases were considered EBV seropositive since it is well established that altered EBV seroprofile is evident in >90% of NPC cases [1-2]. Controls were considered seropositive if they tested positive for one or more of the three anti-EBV assays described above. To ensure that findings were not due to confounding by ethnicity (population stratification), analyses were also performed restricted to individuals of Fukienese descent; results were largely comparable to those observed overall (data not shown). For linkage analysis, we used SIBPAL from S.A.G.E. 4.2 to model the sib-pair covariance of the trait as a function of marker allele identity-by-descent (IBD) sharing. Our analyses used estimated IBD information from the GENIBD procedure to perform single-point linkage analysis in which the weighted combination of squared trait difference and squared mean-corrected trait sum was regressed onto the IBD information [28]. All statistical tests were 2-sided. Analyses were performed using SAS release 9.0 (SAS Institute, Cary, NC) except where specified otherwise.

RESULTS

Both studies were comprised primarily of Fukienese Chinese (69.8% of cases, 73.5% of family controls, and 72.8% of community controls).

We first compared allele frequencies for HLA-A and HLA-B alleles among NPC cases and unaffected family members from our family study. Results are summarized in Table 1. When NPC cases were compared to all family controls combined, significantly higher allelic frequencies were observed among cases for HLA-B*6701. Significantly lower allelic frequencies were observed among cases for HLA-A*1101, HLA-B*2704, and HLA-B*5502. The magnitude of the observed differences was larger when NPC cases were compared to spouse controls. Additional significant differences in allelic frequencies were observed when NPC cases were compared to spouse controls for HLA-A*0101, HLA-A*0207, HLA-B*3802, HLAB*5701, and HLA-B*5801. Two rare alleles (HLA-B*5201 and HLA-B*5504) were only found to differ in frequency when NPC cases were compared to sibling controls.

Based on results from Table 1, the following HLA alleles (whose distribution differed significantly between NPC cases and family controls, and whose allelic frequencies were >5% in either cases or controls) were selected for further evaluation: HLA-A*0207, HLA-A*1101, HLA-B*3802, and HLA-B*5801. Based on the published literature, we also selected HLA-A*3303 and HLA-B*4601 for further evaluation. For these analyses, NPC cases from our family study were compared to family controls and to community controls. Results are summarized in Table 2. Overall, the magnitude of observed associations tended to be higher when NPC cases were compared to spouse (non-blood relatives) than sibling (blood relatives) controls, and were strongest when NPC cases from our families were compared to our non-familial community controls. The most consistent finding observed was a protective effect for HLA-A*1101, an effect that was evident when NPC cases were compared to sibling controls (adj. OR = 0.7, 95% CI: 0.52-0.92), spouse controls (adj. OR = 0.3, 95% CI: 0.22-0.53), or community controls (adj. OR = 0.4; 95% CI = 0.27-0.60). This protective effect was strongest for NPC cases from the family study compared to community controls with homozygous HLA-A*1101 allele (adj. OR = 0.1, 95% CI: 0.048-0.41). Additional significant associations observed included a 2.4-fold elevation in risk of NPC for HLA-B*3802 (95% CI: 1.1-5.2) when cases were compared to spouse controls. Also, elevations in risk was observed for HLA-A*0207 (adj. OR = 2.6; 95% CI = 1.6-4.2), HLA-A*3303 (adj. OR = 1.5; 95% CI = 1.0-2.1), HLA-B*3802 (adj. OR = 2.7; 95% CI = 1.1-7.0), and HLA-B*5801 (adj. OR = 3.1; 95% CI = 1.7-5.4) when cases were compared to community controls. These findings were not meaningfully altered in analyses that restricted by anti-EBV antibody status as described in the Methods (data not shown; supplemental table provided: Table 4).

HLA-A*0207 and HLA-B*4601 are known to be in linkage disequilibrium in Chinese populations, and various studies have reported an association between one or both of these alleles and NPC [7]. We therefore evaluated the joint effect of HLA-A*0207 and HLA-B*4601 in our study population (Table 3). Results are suggestive of a stronger effect for HLA-A*0207 than HLA-B*4601 when cases were compared to either spouse or community controls. The most striking effects were observed when cases were compared to community controls: individuals who were carriers of HLA-A*0207 with or without the presence of HLA-B*4601 were found to have a 1.9-fold (95% CI = 1.0-3.4) and 2.1-fold (95% CI = 0.83-5.3) risk of NPC, respectively. Interestingly, carriers of HLA-B*4601 in the absence of HLA-A*0207 were found to be at a significantly reduced risk of NPC (adj. OR = 0.5; 95% CI = 0.27-0.93).

Previous linkage studies of NPC and HLA region have generated mixed results. To examine whether our high-risk NPC families are linked to the HLA region on chromosome 6, we performed non-parametric linkage analyses using HLA loci as polymorphic markers. Results from SIBPAL analysis showed no evidence for linkage between NPC and either HLA-A ($p = 0.30$) or HLA-B ($p = 0.19$). This lack of linkage persisted regardless of the number of affected siblings in the sib-pair analysis.

DISCUSSION

Our results suggest that the associations between HLA and NPC in high-risk families in Taiwan do not differ appreciatively from those previously noted from sporadic NPC studies. We observed a consistent protective effect of HLA-A*1101 on risk of NPC. This effect was strongest in the comparison between cases from the NPC Family study and community controls who were homozygous for the allele. Previously identified HLA risk alleles were also found to confer increased risk in our study population, e.g., HLA-A*3303 as well as HLA-B*3802 and 5801.

Associations observed in our study were consistently strongest in analysis that compared cases to unrelated community controls. As expected, associations tended to be intermediate when cases were compared to spouse controls, who are not genetically related to cases but are likely to be more similar to cases with respect to genetic background than community controls. The weakest associations were observed when cases were compared to genetically related siblings who by definition share 50% of their genes. This observation is not surprising given that individuals from the same family share genes and environmental factors that may play a role in NPC risk, especially given that our families are high-risk by definition.

Previous studies have consistently observed association between HLA-A*0207, HLA-B*4601 and NPC. HLA-A*0207 and HLA-B*4601 are known to be in strong linkage disequilibrium. This has increased the difficulty in determining whether only one of these two alleles is important for NPC development, whether both are associated with risk, or whether haplotypes containing these two alleles are markers of susceptibility alleles on the MHC region of chromosome 6. To the extent that it has been possible to evaluate the separate effects of these two alleles, both appear to play a role, with individuals who carry both having the strongest risk of NPC [7]. In our study of high-risk NPC families, we did not observe the same effect. When comparing cases and community controls, we observed an elevated risk of NPC for HLA-A*0207, when alone or in combination with HLA-B*4601. On the other hand, a protective effect was observed in those with HLA-B*4601 alone. This suggests that the risk associated with the joint effect of HLA-A*0207 and HLA-B*4601 may be due to the effects of HLA-A*0207 and not HLA-B*4601. Given the strong linkage disequilibrium between HLA-A*0207 and HLA-B*4601, larger studies will be required to carefully evaluate the individual and joint effects of these two alleles on NPC risk.

Unlike association studies of largely sporadic NPC, linkage studies of HLA and NPC conducted within high-risk, multiplex families have yielded conflicting results. An initial evaluation by Lu *et al.* saw strong evidence for linkage to chromosome 6 in a small study of 36 total sibling pairs and trios in Chinese populations from Singapore, Hong Kong, and southern provinces of China [11]. In contrast, others have failed to show the increase in relative risk between human major-histocompatibility-complex (MHC) and NPC [12-13]. In addition, Feng *et al.* [14] failed to find linkage to chromosome 6 after performing a genome-wide scan for linkage to NPC in 20 Cantonese-speaking families from Guangdong Province, China. The study included 65 affected individuals and 54 were genotyped for the analysis. In our study, no evidence for significant linkage was observed, despite the significant evidence for association observed where specific HLA alleles were evaluated and compared to community controls. There may be multiple possibilities to explain the discrepant results from linkage and association analyses. It is well known that linkage analysis is powerful in detecting rare and high risk alleles but has limited power in identifying common genetic variants with low-penetrance [29-30]. In addition, multiple alleles of HLA genes appear to be associated with NPC risk, which makes it more challenging to detect linkage.

A strength of our analysis is that it is the largest family NPC study to date, although sometimes limited by small number when individual alleles or combination of alleles were evaluated. We were able to maximize study power by using different family control groups (i.e., sibling and spouse controls) and by utilizing unrelated community controls in our analysis. Also, by comparing our cases to community controls from the same population, we were able to derive a more generalizable estimate of risk than would have been possible with the use of family controls alone. While some of our results might reflect false positive finding as a result of multiple comparisons, the fact that our findings are consistent with the large body of literature on the association between HLA and sporadic NPC is reassuring. Finally, our study was strengthened by the use of PCR-based high-resolution genotyping that allowed for the evaluation of individual HLA alleles. A limitation of our study is the issue of multiple comparisons to evaluate the polymorphic HLA genes and its association with NPC. Nonetheless, our findings are consistent with previous findings and therefore, the concern with chance findings is less worrisome.

In summary, we found associations between specific HLA markers and NPC in high-risk families that are generally consistent with those reported from previous studies of sporadic NPC. In particular, we observed strong protective effect of HLA-A*1101 on NPC risk. An association was also observed between HLA-A*0207 and NPC, an effect that was most evident when comparing cases to unrelated community controls. In contrast to previous reports, we did not observe positive association between HLA-B*4601 and NPC in the absence of HLA-A*0207, a finding that requires replication in larger studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Brenda Sun, Beth Mittl, and Jeanne Rosenthal, (Westat, Inc., Rockville, MD) for managing the result database and Joseph Danny Carreon (NCI/DCEG/HREB) for assistance with data.

Grant support: This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This Research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

References

1. Cohen JL. Epstein-Barr virus infection. *N Engl J Med* 2000;343:481–492. [PubMed: 10944566]
2. McDermott AL, Dutt SN, Watkinson JC. The aetiology of nasopharyngeal carcinoma. *Clin Otolaryngol* 2001;26:82–92. [PubMed: 11309046]
3. de Campos-Lima PO, Gavioli R, Zhang QJ, Wallace LE, Dolcetti R, Rowe M, et al. HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11+ population. *Science* 1993;260:98–100. [PubMed: 7682013]
4. Gavioli R, Kurilla MG, de Campos-Lima PO, Wallace LE, Dolcetti R, Murray RJ, et al. Multiple HLA A11-restricted cytotoxic T-lymphocyte epitopes of different immunogenicities in the Epstein-Barr virus-encoded nuclear antigen 4. *J Virol* 1993;67:1572–8. [PubMed: 7679748]
5. Burrows JM, Burrows SR, Poulsen LM, Sculley TB, Moss DJ, Khanna R. Unusually high frequency of Epstein-Barr virus genetic variants in Papua New Guinea that can escape cytotoxic T-cell recognition: implications for virus evolution. *J Virol* 1996;70:2490–6. [PubMed: 8642677]
6. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. *Annu Rev Immunol* 1997;15:405–31. [PubMed: 9143694]

7. Hildesheim A, Apple RJ, Chen CJ, Wang SS, Cheng YJ, Klitz W, et al. Association of HLA class I and II alleles and extended haplotypes with nasopharyngeal carcinoma in Taiwan. *J Natl Cancer Inst* 2002;94:1780–9. [PubMed: 12464650]
8. Hildesheim A, Levine PH. Etiology of nasopharyngeal carcinoma: a review. *Epidemiol Rev* 1993;15:466–85. [PubMed: 8174667]
9. Sell, S.; Berkower, I.; Max, EE. Immunology, immunopathology and immunity. Vol. 5th ed.. Appleton & Lange; Stamford (CT): 1996.
10. Yu, MC.; Henderson, BE. Nasopharyngeal cancer. In: Schottenfeld, D.; Fraumeni, JF., editors. *Cancer Epidemiology and Prevention*. Oxford University Press; New York (NY): 1996. p. 603–18.
11. Lu SJ, Day NE, Degos L, Lepage V, Wang PC, Chan SH, et al. Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region. *Nature* 1990;346:470–1. [PubMed: 2377207]
12. Chan SH. Aetiology of nasopharyngeal carcinoma. *Ann Acad Med Singapore* 1990;19:201–7. [PubMed: 2189340]
13. Ooi EE, Ren EC, Chan SH. Association between microsatellites within the human MHC and nasopharyngeal carcinoma. *Int J Cancer* 1997;74:229–32. [PubMed: 9133461]
14. Feng BJ, Huang W, Shugart YY, Lee MK, Zhang F, Xia JC, et al. Genome-wide scan for familial nasopharyngeal carcinoma reveals evidence of linkage to chromosome 4. *Nat Genet* 2002;31:395–9. [PubMed: 12118254]
15. Pickard A, Chen CJ, Diehl SR, Liu MY, Cheng YJ, Hsu WL, et al. Epstein-Barr virus seroreactivity among unaffected individuals within high-risk nasopharyngeal carcinoma families in Taiwan. *Int J Cancer* 2004;111:117–23. [PubMed: 15185352]
16. Yang XR, Diehl S, Pfeiffer R, Chen CJ, Hsu WL, Dosemeci M, et al. Evaluation of risk factors for nasopharyngeal carcinoma in high-risk nasopharyngeal carcinoma families in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2005;14:900–5. [PubMed: 15826929]
17. Hildesheim A, Anderson LM, Chen CJ, Cheng YJ, Brinton LA, Daly AK, et al. CYP2E1 genetic polymorphisms and risk of nasopharyngeal carcinoma in Taiwan. *J Natl Cancer Inst* 1997;89:1207–12. [PubMed: 9274915]
18. Cheng YJ, Hildesheim A, Hsu MM, Chen IH, Brinton LA, Levine PH, et al. Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. *Cancer Causes Control* 1999;10:201–7. [PubMed: 10454065]
19. International Histocompatibility Working Group. 13th IHWG HLA Class I Reverse Line Strip Typing Protocol. <http://www.ihwg.org/protocols/protocol.htm>. Accessed October 23, 2008
20. Henle G, Henle W. Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. *Int J Cancer* 1976;17:1–7. [PubMed: 175020]
21. Chen JY, Liu MY, Chen CJ, Hsu MM, Tu SM, Lee HH, et al. Antibody to Epstein-Barr virus-specific DNase as a marker for the early detection of nasopharyngeal carcinoma. *J Med Virol* 1985;17(1): 47–9. [PubMed: 2995572]
22. Chen MR, Yang JF, Hsu TY, Liu MY, Chen JY, Yang CS. Use of bacterially expressed GST/EBNA-1 fusion proteins for detection of antibodies in sera from patients with nasopharyngeal carcinoma and healthy donors. *Chin J Microbiol Immunol* 1996;29(2):65–79.
23. Cheng YC, Chen JY, Glaser R, Henle W. Frequency and levels of antibodies to Epstein-Barr virus-specific DNase are elevated in patients with nasopharyngeal carcinoma. *Proc Natl Acad Sci USA* 1980;77:6162–5. [PubMed: 6255477]
24. Zeng Y, Zhang LG, Wu YC, Huang YS, Huang NQ, Li JY, et al. Prospective studies on nasopharyngeal carcinoma in Epstein-Barr virus IgA/VCA antibody-positive persons in Wuzhou City, China. *Int J Cancer* 1985;36:545–7. [PubMed: 4055129]
25. Fisher RA. On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society* 1922;85:87–94.
26. Diggle, J.; Liang, KY.; Zeger, SL. *Analysis of Longitudinal Data*. Clarendon Press; Oxford: 1994.
27. Hosmer, D.; Lemeshow, S. *Applied logistic regression*. John Wiley & Sons; New York: 1989.
28. Haseman JK, Elston RC. The investigation of linkage between a quantitative trait and a marker locus. *Behav Genet* 1972;2:3–19. [PubMed: 4157472]

29. Pharoah PD, Dunning AM, Ponder BA, Easton DF. Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Cancer* 2004;4(11):850–60. [PubMed: 15516958]
30. Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. *Science* 2008;322(5903):881–8. [PubMed: 18988837]

Table 1
HLA Class I Allele Frequencies for NPC Affected and Unaffected Groups from the NPC Family Study in Taiwan

HLA-A alleles	Case (n=602) %	Sibling Controls (n=962) %	pvalue [*]	Spouse Controls (n=424) %	pvalue [*]
101	0.2	0.4		1.2	0.04
201	8.8	8.1		9.9	
203	7.6	8.0		5.7	
206	2.0	2.2		2.4	
207	16.1	15.3		11.6	0.04
224	0.0	0.0		0.2	
1101	17.4	21.6	0.04	29.3	<0.0001
1102	3.3	4.0		3.8	
2402	23.1	19.0		17.9	
2403	0.2	0.3		0.2	
2407	0.0	0.5		0.5	
2410	0.0	0.5		0.2	
2601	3.7	5.4		3.5	
2901	0.3	0.3		0.0	
3001	0.7	0.6		0.9	
3101	1.0	0.5		1.4	
3201	0.2	0.0		0.0	
3303	14.6	12.6		10.9	
HLA-B alleles	Case (n=590)	Sibling Controls (n=946)		Spouse Controls (n=416)	
1301	4.4	5.4		6.7	
1302	1.7	1.2		1.0	
1301/1302	6.1	6.6		7.7	
1501	4.9	4.2		3.6	
1502	3.4	3.7		6.0	
1505	0.0	0.1		0.2	
1511	0.3	0.7		1.0	
1512	0.2	0.3		0.0	
1518	1.0	0.4		0.2	
1525	0.2	0.2		1.0	
1527	0.3	0.1		0.2	
1802	0.2	1.0		0.2	
2704	0.9	2.4		3.1	0.01
2706	0.2	0.2	0.02	0.0	
3501	2.0	2.0		3.1	
3505	0.2	0.2		0.2	
3701	0.2	0.3		0.0	
3802	6.1	5.9		2.6	0.01
3901	1.2	2.2		2.6	
4001	20.5	21.7		20.7	
4002	2.7	1.7		1.7	
4006	2.4	1.4		0.7	
4403	0.3	0.0		0.5	
4601	18.3	16.5		16.8	
4801	1.5	1.2		1.4	
5101	4.8	4.9		3.9	
5102	0.2	0.3		1.0	
5201	0.5	0.0	0.03	0.5	
5401	2.2	2.8		2.6	
5502	1.0	2.5	0.04	2.9	0.03
5504	0.0	0.7	0.04	0.5	
5603	0.5	0.1		0.7	

	Case (n=602)	Sibling Controls (n=962)	Spouse Controls (n=424)	
	%	%	%	pvalue *
5701	0.0	0.1	0.7	0.04
5801	14.9	12.8	10.1	0.02
6701	0.9	0.2	0.0	
8101	0.0	0.0	0.2	

Bolded rows indicate an allelic freq ≥ 5%

Highlighted rows indicate alleles with allelic frequency ≥ 5% and p-value <0.05.

* Only p-values<0.05 from Pearson's X² or Fisher's exact tests (n/cell <5) are shown. All statistical tests are two-sided.

Table 2
Association between Selective HLA Alleles and NPC

HLA-A alleles	Sibling Controls (n=481)			Spouse Controls (n=212)			Community Controls (n=317)		
	%	OR ¹	95% CI	OR ²	95% CI	%	OR ³	95% CI	95% CI
0207	30.6	28.5	1.1	(0.86-1.4)	1.0	(0.78-1.3)	22.2	1.5	(1.0-2.3)
1101	32.2	39.7	0.7	(0.55-0.95)	0.7	(0.52-0.92)	51.9	0.4	(0.31-0.62)
3303	27.9	23.5	1.3	(0.96-1.7)	1.3	(0.97-1.7)	19.8	1.6	(1.0-2.4)
HLA-B alleles	Sibling Controls (n=473)			Spouse Controls (n=208)			Community Controls (n=221)		
3802	11.9	11.2	1.1	(0.72-1.6)	1.1	(0.71-1.6)	5.3	2.4	(1.2-5.0)
4601	33.6	30.4	1.2	(0.89-1.5)	1.0	(0.77-1.4)	29.8	1.2	(0.82-1.7)
5801	28.5	24.1	1.3	(0.94-1.7)	1.3	(0.98-1.7)	18.8	1.7	(1.2-2.6)

¹ ORs controlled for family

² ORs controlled for family, sex, and age

³ Crude ORs

⁴ ORs adjusted for sex and age

Table 3
Joint Effect of HLA A*0207 and B*4601 on NPC Risk

	%	% OR ¹	95% CI	OR ²	95% CI	%	% OR ¹	95% CI	OR ²	95% CI	%	% OR ³	95% CI	OR ⁴	95% CI
0207-4601 Case (n=289)															
0207 only	7.3	6.8	1.1 (0.70-1.8)	1.1	(0.66-1.7)	3.5	2.4	(0.90-6.3)	1.9	(0.61-6.0)	3.2	2.5	(1.0-6.0)	2.1	(0.83-5.3)
4601 only	10.7	8.6	1.3 (0.82-2.1)	1.2	(0.74-2.0)	10.0	1.2	(0.67-2.2)	1.3	(0.68-2.6)	20.4	0.6	(0.34-0.95)	0.5	(0.27-0.93)
Both	22.8	21.9	1.1 (0.83-1.5)	1.0	(0.73-1.3)	19.4	1.3	(0.85-2.1)	1.5	(0.84-2.6)	12.7	1.9	(1.2-3.2)	1.9	(1.0-3.4)

¹ ORs controlled for family
² ORs controlled for family, sex, and age
³ Crude ORs
⁴ ORs adjusted for sex and age