Esophageal carcinoma is characterized by a widely ranged incidence variation among the different geographic regions. Anyang is a county in Henan Province of North China with the highest prevalence of esophageal carcinoma. Human papillomavirus (HPV) infection has been linked to the etiology of esophageal cancer in this area. In this study, we investigated correlations of the polymorphisms at low molecular weight polypeptide (LMP) and transporters with antigen processing (TAP) genes, with the risk of esophageal carcinoma. DNA extracted from either tumor specimens or esophageal epithelial cells was used to test HPV infection. Peripheral blood lymphocyte DNA was used for LMP/TAP genotyping. Polymerase chain reaction was performed to analyze HPV infection and LMP/TAP gene polymorphisms. The combined effect of LMP/TAP polymorphism; TAP, transporters with antigen processing.

Introduction
Esophageal carcinoma is one of the major cancers worldwide. More than 90% of esophageal cancers are either squamous cell carcinomas or adenocarcinomas. The prevalence of esophageal cancer and the risk factors show marked geographic variation worldwide (1). Epidemiological studies indicate that in the low incidence area, substantial alcohol intake in combination with smoking greatly increase the risk of developing squamous cell carcinoma (2). In contrast, diets deficient in vitamins and intake of carcinogens have been speculated as the risk factors of esophageal cancer in China and other central Asian countries (3). In addition, infection with high-risk human papillomaviruses (HPVs) has been reported to be linked with the risk of esophageal squamous cell carcinoma. However, unlike HPV infection in cervical carcinoma, the incidence of infection among esophageal carcinoma patients differs between the high incidence and low incidence areas (4) implicating that the role of HPV infection in esophageal epithelium carcinogenesis is more complicated. Host genetic backgrounds may account, at least in part, for the outcome of HPV infection associated esophageal cancer.

Major histocompatibility complex (MHC) class I molecules are cell surface glycoprotein, which bind the intracellularly processed peptides and present them on the cell surface to cytotoxic T lymphocytes. Class I molecules, therefore, play a key role in immune recognition of virally infected and transformed cells (5). Two groups of proteins that participate in the antigen processing are low molecular weight polypeptides (LMPs) and transporters with antigen processing (TAP). LMP2 and LMP7 are proteasome components, which enhance the proteolytic production of certain peptides (6,7) while TAP1 and TAP2 form heterodimers and pump the antigenic peptides into the lumen of the endoplasmic reticulum (8,9). Downregulation of TAP1, TAP2, LMP2 and LMP7 was found to suppress MHC class I molecule surface expression (10–13). Deficits in expression of LMP and TAP have been detected in a variety of cancer cell lines and tissues (13).

Esophageal carcinoma is the second most common cancer in China. Anyang County is located in Henan Province, North of China and has the highest incidence and mortality of esophageal cancer in China. A high incidence of HPV infection as found in the esophageal carcinoma specimens from this area (4). We have previously reported a high basal HPV infection in normal Anyang controls (14). In the present study, we analyzed the polymorphisms of LMP and TAP in the patients with esophageal squamous cell carcinoma and controls. We observed the association of single nucleotide polymorphisms (SNPs) at TAP2 and LMP7 with the risk of esophageal carcinoma. One haplotype covering these SNPs is linked to esophageal carcinoma development. Moreover, individuals carrying the risk haplotype and infected with HPV have a higher probability of developing esophageal cancer.

Abbreviations: CI, confidence interval; HPV, human papillomavirus; LMP, low molecular weight polypeptide; MHC, major histocompatibility complex; OR, odds ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; TAP, transporters with antigen processing.
Materials and methods

Detection of HPV DNA

DNA was extracted from the paraffin sections as described (15). The HPV infection was first determined by PCR using degenerate primer set, GP1+–GP6+. 5′-TTTTGGTCTACTGTTGATACATA-3′ and 5′-CCTATATAC- TAAATGTCAAATAAAAAG-3′, which produce a 150 bp fragment of the LI gene in a wide range of HPV types as described (16). For HPV 16 DNA detection, E7 was amplified with the forward primer 5′-GATGGAATTGGTACATC-3′ and the reverse primer 5′-CCTGTTGAGCGCACAAC-3′. Similarly, E7 of HPV 18 DNA was amplified with the forward primer 5′-AACATTTCTGTTCAATACCTGAC-3′. In the PCR reaction, β-actin PCR analysis was used to validate the DNA quality. The mouse liver tissue was used in the sample process and PCR analysis to monitor the contamination.

Table I. Polymorphisms selection of LMP/TAP genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Code</th>
<th>Substitution for the nucleotide</th>
<th>Amino acid position</th>
<th>Substitution for the amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP2</td>
<td>CGC→TGC</td>
<td>60</td>
<td>Arg→Cys</td>
<td></td>
</tr>
<tr>
<td>LMP7</td>
<td>CAG→AAG</td>
<td>145</td>
<td>Gin→Lys</td>
<td></td>
</tr>
<tr>
<td>TAP1</td>
<td>ATC→GTC</td>
<td>333</td>
<td>Ile→Val</td>
<td></td>
</tr>
<tr>
<td>TAP1-2</td>
<td>GAC→GGC</td>
<td>637</td>
<td>Asp→Gly</td>
<td></td>
</tr>
<tr>
<td>TAP2-1</td>
<td>GTA→ATA</td>
<td>379</td>
<td>Val→Ile</td>
<td></td>
</tr>
<tr>
<td>TAP2</td>
<td>ACA→GCA</td>
<td>665</td>
<td>Thr→Ala</td>
<td></td>
</tr>
<tr>
<td>TAP2-3</td>
<td>TAG→CAG</td>
<td>687</td>
<td>Stop→Gin</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Primer sets used for amplification and sequencing* of LMP/TAP genes

<table>
<thead>
<tr>
<th>Fragment</th>
<th>PCR primers (sense/antisense)</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP2</td>
<td>5′-CTCCTACCTTACAGATGCAGA-3′, 5′-ACCTTTGTACGCTTGACCT-3′</td>
<td>330</td>
</tr>
<tr>
<td>LMP7</td>
<td>5′-TCATGCGCTACTAGTGATTGTG-3′, 5′-AACCTTTCTGTTCAATACCTGAC-3′</td>
<td>351</td>
</tr>
<tr>
<td>TAP1-1</td>
<td>5′-GCAGGATCACTACATGGCTCG-3′, 5′-GACAACATTTGAGGAGAACG-3′</td>
<td>430</td>
</tr>
<tr>
<td>TAP1-2</td>
<td>5′-CAGTATCTGTTGGCTTACTTC-3′, 5′-ATGATGCTGCTTACCCTGATAC-3′</td>
<td>405</td>
</tr>
<tr>
<td>TAP2-1</td>
<td>5′-CTCTCGGGAGATCCAGGATGC-3′, 5′-CCACGCTTCTTGGTATGAC-3′</td>
<td>472</td>
</tr>
<tr>
<td>TAP2-2</td>
<td>5′-AGTCAGCGCGGAGAAGACGG-3′, or TAP2-3 5′-CTCAGGAAACGCTTTGTC-3′</td>
<td>449</td>
</tr>
</tbody>
</table>

*Every sequencing primer used the sense sequence.

Results

The esophageal carcinomas and controls used in this study were matched for gender and age.

There were no significant differences between cases and controls in cigarette smoking and alcohol consumption.

The presence of HPV DNA was detected by amplification of LI gene. HPV16 and 18 were identified by PCR. HPV infection was found in 207 cases (78.11%) and 203 controls (56.86%). Among the 207 HPV positive patients, HPV16 and/or 18 DNA was found in 186 individuals including 175 infected by HPV16 alone, 4 infected by HPV18 alone and 7 infected by both types. In the control group, among the 203 HPV positive individuals, 181 were infected by HPV16 and/or 18 including 178 infected by HPV16, 4 infected by HPV18 and 1 infected by both HPV16 and 18. In both case and control groups, the HPV16 and 18 infections represent >89% of the total HPV infections (90% for cases and 89% for controls).

Since only the high-risk HPVs, especially types 16 and 18, have been identified as the risk factor for cervical cancer as well as other cancers, we used HPV16 and 18 infected individuals as the infected subjects and thereafter, the association of esophageal cancer risk and HPV16 and 18 infection was assessed. The infection of HPV types 16 and/or 18 significantly
increases the risk for developing esophageal carcinoma (OR = 2.33, 95% CI = 1.66–3.27, P < 0.0001) in this area.

Seven polymorphisms in the coding regions of LMP2, LMP7, TAP1 and TAP2 genes reported previously (19–21) were tested. The genotype distributions of the LMP and TAP in the cases and controls are summarized in Table III.

The distributions of the genotype among cases and controls were in Hardy–Weinberg equilibrium. There was no significant difference in the distributions of the polymorphisms at LMP2 codons 60 TAP1 codons 333 and 637, TAP2 codons 665 and 687 among cases and controls. The frequencies of LMP7 codon145 lysine homozygote and heterozygote genotypes in cases differed significantly from those in controls. In addition, TAP2 codon 379 isoleucine homozygote and heterozygote frequencies are significantly higher in cases than in controls. Since TAP2 and LMP7 are located next to each other, we construct the haplotypes covering SNPs in the TAP2 codon 375 and LMP7 codon 145 using PHASE software.

A total of four haplotypes (A, B, C and D) were constructed. All four haplotypes were found in both case and control groups. The distribution of different haplotypes in each group is shown in Table IV.

The A, B and C types represented the major haplotypes in the tested subjects. The sum of the A, B and C haplotype frequency corresponded to >98.0% genotypes in both case and control groups. The A, B and C haplotypes were then selected to assess the susceptibility to esophageal carcinoma.

The association analysis and logistic regression analysis was performed to estimate the risk of LMP7/TAP2 haplotypes on esophageal carcinoma development. The result showed that only haplotype C was found to be associated with the cancer risk. Haplotype C represents the minority genotype in all haplotypes. Its homozygote (C/C) has higher frequency in the cases than in the controls and increases the risk of esophageal carcinoma, while the heterozygote (C/-) slightly increases the risk of cancer development. The results are summarized in Table V.

We next investigated whether the LMP7/TAP2 haplotype C affects the HPV infection related esophageal carcinoma risk. In the presence of HPV infection, LMP7/TAP2 haplotype C (C/C + C/-) showed significantly increased risk to the development of esophageal cancer compared with those that carried only LMP7/TAP2 haplotype C without HPV infection or those HPV positive with null LMP7/TAP2 haplotype C. The data are summarized in Table VI.

<p>| Table III. Genotype frequencies of LMP/TAP gene polymorphisms in cases and controls |</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case n (%)</th>
<th>Control n (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP2 Arg/Arg</td>
<td>167 (63.02)</td>
<td>239 (66.95)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>LMP2 Arg/Cys</td>
<td>83 (31.32)</td>
<td>102 (28.57)</td>
<td>0.376</td>
<td>0.35 (0.59–1.22)</td>
</tr>
<tr>
<td>LMP2 Cys/Cys</td>
<td>15 (5.66)</td>
<td>16 (4.48)</td>
<td>0.622</td>
<td>0.83 (0.39–1.76)</td>
</tr>
<tr>
<td>LMP7 Gln/Gln</td>
<td>130 (49.06)</td>
<td>210 (58.82)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>LMP7 Gln/Lys</td>
<td>114 (43.02)</td>
<td>130 (36.41)</td>
<td>0.034</td>
<td>1.45 (1.03–2.03)</td>
</tr>
<tr>
<td>LMP7 Lys/Lys</td>
<td>21 (7.92)</td>
<td>17 (4.77)</td>
<td>0.027</td>
<td>2.19 (1.09–4.37)</td>
</tr>
<tr>
<td>TAP1-1 Ile/Ile</td>
<td>167 (63.02)</td>
<td>215 (60.22)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TAP1-1 Ile/Val</td>
<td>86 (32.45)</td>
<td>125 (35.02)</td>
<td>0.326</td>
<td>0.84 (0.59–1.19)</td>
</tr>
<tr>
<td>TAP1-1 Val/Val</td>
<td>12 (4.53)</td>
<td>17 (4.76)</td>
<td>0.715</td>
<td>0.86 (0.40–1.89)</td>
</tr>
<tr>
<td>TAP1-2 Asp/Asp</td>
<td>184 (69.43)</td>
<td>244 (68.35)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TAP1-2 Asp/Gly</td>
<td>73 (27.55)</td>
<td>101 (28.29)</td>
<td>0.632</td>
<td>0.91 (0.63–1.32)</td>
</tr>
<tr>
<td>TAP1-2 Gly/Gly</td>
<td>8 (3.02)</td>
<td>12 (3.36)</td>
<td>0.900</td>
<td>0.94 (0.37–2.39)</td>
</tr>
<tr>
<td>TAP2-1 Val/Val</td>
<td>175 (66.04)</td>
<td>282 (78.99)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TAP2-1 Val/Ile</td>
<td>75 (28.30)</td>
<td>66 (18.49)</td>
<td>0.001</td>
<td>1.94 (1.31–2.87)</td>
</tr>
<tr>
<td>TAP2-1 Ile/Ile</td>
<td>15 (5.66)</td>
<td>9 (2.52)</td>
<td>0.023</td>
<td>2.74 (1.15–6.49)</td>
</tr>
<tr>
<td>TAP2-2 Thr/Thr</td>
<td>151 (56.87)</td>
<td>159 (44.54)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TAP2-2 Thr/Ala</td>
<td>97 (36.60)</td>
<td>151 (42.30)</td>
<td>0.075</td>
<td>1.38 (0.97–1.97)</td>
</tr>
<tr>
<td>TAP2-2 Ala/Ala</td>
<td>32 (12.08)</td>
<td>47 (13.16)</td>
<td>0.347</td>
<td>1.28 (0.76–2.15)</td>
</tr>
<tr>
<td>TAP2-3 Ile/Ile</td>
<td>9 (1.70)</td>
<td>3 (0.42)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Logistic regression model, adjusted by gender, age, smoking, drinking and infection of HPV.

<p>| Table IV. Distribution of the estimated haplotype frequencies for LMP7/TAP2 genes in cases and controls |</p>
<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Loci</th>
<th>Case n (%)</th>
<th>Control n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of chromosome Location</td>
<td>LMP7-TAP2-1</td>
<td>530 (100)</td>
<td>714 (100)</td>
</tr>
<tr>
<td>A Gln-Val</td>
<td>278 (52.45)</td>
<td>469 (65.69)</td>
<td></td>
</tr>
<tr>
<td>B Lys-Val</td>
<td>147 (27.74)</td>
<td>161 (22.55)</td>
<td></td>
</tr>
<tr>
<td>C Gln-Ile</td>
<td>96 (18.11)</td>
<td>81 (11.34)</td>
<td></td>
</tr>
<tr>
<td>D Lys-Ile</td>
<td>9 (1.70)</td>
<td>3 (0.42)</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table V. Distribution of haplotype frequencies in LMP7 and TAP2-1 among cases and controls |</p>
<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Case n (%)</th>
<th>Control n (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gin-Val/HPV</td>
<td>64 (24.15)</td>
<td>42 (11.76)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Gin-Val/C</td>
<td>124 (46.79)</td>
<td>161 (45.10)</td>
<td>0.002</td>
<td>0.48 (0.30–0.78)</td>
</tr>
<tr>
<td>A/HPV</td>
<td>77 (29.06)</td>
<td>154 (43.14)</td>
<td>0.001</td>
<td>0.30 (0.18–0.48)</td>
</tr>
<tr>
<td>Gin/Ile-HPV</td>
<td>132 (49.81)</td>
<td>212 (59.38)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Gin/Ile/C</td>
<td>119 (44.91)</td>
<td>129 (36.14)</td>
<td>0.012</td>
<td>1.55 (1.10–2.18)</td>
</tr>
<tr>
<td>B/HPV</td>
<td>14 (5.28)</td>
<td>16 (4.48)</td>
<td>0.252</td>
<td>1.57 (0.73–3.40)</td>
</tr>
<tr>
<td>B/C</td>
<td>70 (26.42)</td>
<td>67 (18.77)</td>
<td>0.009</td>
<td>1.69 (1.14–2.51)</td>
</tr>
<tr>
<td>C/C</td>
<td>13 (4.90)</td>
<td>7 (1.96)</td>
<td>0.027</td>
<td>2.96 (1.13–7.81)</td>
</tr>
</tbody>
</table>

* Logistic regression model, adjusted by gender, age, smoking, drinking and infection of HPV.

<p>| Table VI. The additive effect of HPV infection and LMP7/TAP2 haplotype C on the risk of esophageal cancer |</p>
<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Infection</th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gin-Val/HPV</td>
<td>51 (64.56)</td>
<td>136 (77.27)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gin-Val/C</td>
<td>28 (35.44)</td>
<td>40 (22.73)</td>
<td>0.0452</td>
<td>1.82 (1.01–3.28)</td>
<td></td>
</tr>
<tr>
<td>A/HPV</td>
<td>131 (70.43)</td>
<td>147 (81.22)</td>
<td>&lt;0.0001</td>
<td>2.40 (1.60–3.59)</td>
<td></td>
</tr>
<tr>
<td>A/C</td>
<td>55 (29.57)</td>
<td>34 (18.78)</td>
<td>&lt;0.0001</td>
<td>4.33 (2.53–7.42)</td>
<td></td>
</tr>
</tbody>
</table>

* Logistic regression model, adjusted by gender, age, smoking and drinking.
Discussion

The infection of the high-risk type HPV has been considered as the necessary factor for the development of cervical cancer based on the fact that HPV DNA can be detected in >90% cervical cancer specimens (22). In addition to cervical cancer, high-risk HPV infection has also been found in the other squamous cell carcinomas including anal, perianal, vulvar, oropharyngeal and esophageal cancers (23). Studies focused on HPV infection in esophageal cancer generated controversial results. The samples collected from the high esophageal cancer incidence areas tended to be HPV DNA positive while the subjects from the low incidence area were less likely to have HPV infection (4). Despite the discrepancy, the majority of studies using specimens from China found HPV infection in proportion with the esophageal carcinomas. In the present study, we found that 70.19% esophageal cancer specimens were HPV16 or 18 positive. The prevalence is among the highest HPV infection found in esophageal cancer to date. Although the normal controls also had HPV infection as high as 50.70%, comparison between cases and controls indicated that HPV infection is associated with esophageal cancer. Anyang is one of the highest esophageal carcinoma incidence areas worldwide. The significant link between esophageal cancer and HPV infection as well as the high basal level infection in the general population suggests that HPV infection may play a role in the development of esophageal cancer in this area. A population based epidemiological study should be carried out to address this issue.

We tested seven previously reported polymorphisms in the coding regions of LMP2, LMP7, TAP1 and TAP2 genes. We observed all seven polymorphisms in subjects from Anyang area. The polymorphism at LMP2 codon 60 was reported as the substitution of arginine to histidine. We did not find thisidine at LMP2 codon 60 in any of the cases or controls. Instead, a cysteine replacement was observed in this population, suggesting that LMP and TAP polymorphisms may differ among populations as reported by other authors (24,25).

MHC class I molecules play a critical role in reorganization of viral infection and malignant transformation. LMP and TAP are the key players in MHC class I molecules function. The defects of MHC class I surface expression caused by down-regulation of LMP and TAP in a variety of cancer cells have been detected and are considered to result in the evasion of immune surveillance (26). The impairment of LMP and TAP functions may therefore enhance the tumorigenesis. In addition, high frequency of polymorphisms of LMP and TAP were detected in human tumors (27). Moreover, the polymorphisms of TAP1 and TAP2 were found to be linked to the risk of cervical cancer leading to the argument that genetic makeup on host immune response gene may contribute to the biologic variability to HPV infection (28). However, other studies point to the opposite conclusions. In the TAP1 and LMP2 knockout mice, the tumor incidence does not differ from the controls (29). In the present study, we analyzed polymorphisms of LMP and TAP in the esophageal cancer patients and controls from the same ethnic group and found that the glutamine allele at LMP7 codon 145 and the isoleucine allele at TAP2 codon 379 are associated with the risk of esophageal cancer. We also observed a significant association between LMP7/TAP2 haplotype C tagging the above two polymorphisms and esophageal carcinoma development. Our results support the notion that genetic makeup in the immune response genes may play roles in esophageal tumorigenesis. The biological function of naturally occurring TAP polymorphisms is inconclusive (30).

The subjects carrying the LMP7/TAP2 haplotype C and infected with HPV showed an additive risk to develop esophageal carcinoma. There was no increased LMP7/TAP2 haplotype C frequency observed among the HPV infected individuals (Table VI) suggesting that LMP7/TAP2 haplotype C does not affect HPV infection. The additive effect is probably owing to the other mechanisms. First, the differences in the host antigen presenting genes may influence the clearance of HPV infection leading to cell transformation. Alternatively, HPV infection and LMP/TAP polymorphism may function independently to increase the risk of esophageal cancer.

Since the regulation of immune response is a complicated process involved in numerous genes, it would be expected that individual genes might have only limited effect to disease susceptibility. In order to assess the accurate association between LMP and TAP polymorphism and esophageal carcinoma risk, the case–control studies with a large number of subjects should be performed. Our current report is a preliminary one, even though the results are unlikely to be attributable to selection bias since the subjects were from the same ethnic population. Further research with a larger population in multiple areas is suggested to confirm this result.

Acknowledgements

This study is supported by State Key Basic Research Program Grant G1998051203, ‘863’ key project of National Ministry of Science and Technology Grant 2002BA711A06 and Beijing Municipal Science and Technology Commission (to Y.K.).

Conflict of Interest Statement: None declared.
B. Cao et al.


Received December 30, 2004; revised March 2, 2005; accepted March 8, 2005.