Association of Caveolin-1 Genotypes with Nasopharyngeal Carcinoma Susceptibility in Taiwan

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Abstract. Background: Caveolin-1 (Cav-1), which has been proposed as a candidate tumor suppressor, plays a regulatory role in several signaling pathways. High expression of Cav-1 in nasopharyngeal carcinoma (NPC) may enhance tumor cell migration and correlate with poor prognosis of the patients, while the genetic alterations of Cav-1 during nasopharyngeal carcinogenesis are still largely unknown. The aim of this study was to evaluate the association between NPC susceptibility and Cav-1 genotypes. Patients and Methods: One hundred and seventy-six patients with NPC and 176 age- and gender-matched healthy controls recruited in Taiwan were genotyped and analyzed by PCR-restriction fragment length polymorphism. Results: There were significant differences between the NPC and control groups in the distributions of the genotypic (p=0.0019) and allelic frequencies (p=2.5*10^{-4}) in the Cav-1 T29107A (rs7804372) polymorphism. Conclusion: In this first report of Cav-1 involvement in NPC the A allele of Cav-1 T29107A is found to be protective against the development of NPC and may be a novel useful genomic marker for early screening and prediction of NPC.

Nasopharyngeal carcinoma (NPC) occurs sporadically in the West (with an age-standardized incidence rate (ASR) <1/100,000), but is a leading form of tumor in Southern China (ASR=30-50/100,000) Southeast Asia (ASR=9-12/100,000) and Taiwan (ASR=8.2-8.4/100,000) (1-3). The geographical pattern of NPC incidence suggests a unique interaction of environmental and genetic factors. Although the etiology of NPC remains to be elucidated, Epstein-Barr virus (EBV) infection (4, 5), environmental risk factors (6), certain dietary factors (7) and genetic differences such as single nucleotide polymorphisms (SNPs) may all contribute to NPC carcinogenesis (8, 9).

Three caveolin proteins, caveolin-1, -2 and -3, serve as the structural components of the caveolae and also function as scaffolding proteins, which are capable of recruiting numerous signaling molecules to the caveolae and regulating their activity. It has been reported in a caveolin-deficient animal model that caveolins play a role in human disease processes, including diabetes, cancer, cardiovascular diseases, atherosclerosis, pulmonary fibrosis and a variety of degenerative muscular dystrophies (10). Caveolin-1 (Cav-1), a protein of 178 amino acids, initially was identified as a tumor suppressor gene (11). It has been demonstrated that Cav-1 is down-regulated in sarcoma, lung carcinoma and ovarian carcinoma (12-14). However, elevated expression of Cav-1 has also been reported to be associated with the metastasis of esophageal squamous cell carcinoma and prostate cancer and negatively correlated with patient survival (15, 16). These findings indicate that the role of Cav-1 may vary considerably, depending on the tissue involved. However little data are available which consider Cav-1 for genetic predisposition to carcinogenesis (17, 18, 19).

In 2009, it was reported that highly expression of caveolin-1 in NPC, together with its downstream protein CD147, enhanced tumor cell migration and correlated with poor prognosis of the NPC patients (20). Up to now, the association of Cav-1 polymorphism with NPC has not been reported. Thus, the objectives of the current study were to determine the genotypic frequency of six polymorphisms of the Cav-1 gene at C521A (rs1997623), G14713A (rs3807987), G21985A (12672038), T28608A (rs3757733), T29107A (rs7804372), and G32124A (rs3807992). To the best of our knowledge, this is the first study carried out to evaluate the contribution of Cav-1 polymorphisms in NPC.
Patients and Methods

Patient population and sample collection. One hundred and seventy six patients diagnosed with NPC were recruited at the outpatient clinics of general surgery between 2003-2009 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. All the patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. The questionnaire included questions on history and frequency of alcohol consumption, betel quid chewing and smoking habits and “ever” was defined as more than twice a week for years. Self-reported alcohol consumption, betel quid chewing and smoking habits were evaluated and classified as categorical variables. One hundred and seventy six non-NPC or other types of cancer, healthy people as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all the participants.

PCR-restriction fragment length polymorphism genotyping conditions. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous papers (17, 21-27). Briefly, the following primers were used for Genomic DNA was prepared from peripheral blood leucocytes using PCR-restriction fragment length polymorphism genotyping conditions.

**Table I. Characteristics of nasopharyngeal carcinoma patients and controls.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n=176)</th>
<th>Patients (n=176)</th>
<th>( P_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>49.3 (9.4)</td>
<td>48.2 (11.1)</td>
<td>0.6851</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>128</td>
<td>72.7%</td>
<td>128</td>
</tr>
<tr>
<td>Female</td>
<td>48</td>
<td>27.3%</td>
<td>48</td>
</tr>
<tr>
<td>Indulgence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smokers</td>
<td>73</td>
<td>43.8%</td>
<td>77</td>
</tr>
<tr>
<td>Betel quid chewers</td>
<td>54</td>
<td>31.3%</td>
<td>55</td>
</tr>
<tr>
<td>Alcohol drinkers</td>
<td>72</td>
<td>45.5%</td>
<td>80</td>
</tr>
</tbody>
</table>

\( aP\)-value based on Chi-square test.

There were no significant differences between the two groups in their age, sex and or individual behavior factors (Table I). The frequencies of the alleles for Cav-1 C521A, G14713A, G21985A, T28608A, T29107A and G32124A in the controls and NPC patients is shown in Table II. The genotype distribution of the various genetic polymorphisms of Cav-1 SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s Chi-square test or Fisher’s exact test (where number in any cell was less than five) was used to compare the distribution of the Cav-1 genotypes between the cases and controls. Data was recognized as significant when the statistical \( p\)-value was less than 0.05.

Results

There were no significant differences between the two groups in their age, sex and or individual behavior factors (Table I). The frequencies of the alleles for Cav-1 C521A, G14713A, G21985A, T28608A, T29107A and G32124A in the controls and NPC patients is shown in Table II. The genotype distribution of the various genetic polymorphisms of Cav-1 SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s Chi-square test or Fisher’s exact test (where number in any cell was less than five) was used to compare the distribution of the Cav-1 genotypes between the cases and controls. Data was recognized as significant when the statistical \( p\)-value was less than 0.05.

The frequencies of the alleles for Cav-1 C521A, G14713A, G21985A, T28608A, T29107A and G32124A in the controls and NPC patients is shown in Table III. The T29107A genotype of Cav-1 SNP in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s Chi-square test or Fisher’s exact test (where number in any cell was less than five) was used to compare the distribution of the Cav-1 genotypes between the cases and controls. Data was recognized as significant when the statistical \( p\)-value was less than 0.05.
The present study revealed that Cav-1 T29107A (rs7804372) polymorphisms were associated with the susceptibility to NPC (Table II and III), while the other five polymorphisms investigated were not. Although these genetic variations do not directly result in amino acid coding change, it is plausible to suspect modifications such as alternative splicing, may happen during carcinogenesis via influencing the expression level or stability of the Cav-1 protein.

The sample sizes of NPC investigations are often not as large as other types of cancer and as many as possible were enrolled in our hospital during these years. The similar trends of significant data after age- and behavior-adjustments strengthen the accuracy and reliability of the present findings, and the frequencies of the Cav-1 polymorphisms variant alleles were similar to those reported in the National Center Biotechnology Information (NCBI) website in other Asian population studies. For instance, the minor A allele frequencies of Cav-1 T29107A were 34.9% in the present control group, close to those of 31.1–31.8% for the Tokyo population in the NCBI, which strongly suggested no selection bias for subject enrolments in terms of genotypes.

Using a candidate gene approach, this present study provided evidence supporting the NPC tumorigenic contribution of Cav-1, of which the polymorphisms of T29107A were the most significantly associated. Additional functional analyses of the gene and polymorphisms would be useful for exploring the mechanisms by which Cav-1 and its regulated proteins affect NPC risk.

In conclusion, Cav-1 T29107A, but not C521A, G14713A, G21985A, T28608A or G32124A, was associated with higher susceptibility to NPC. The A allele of Cav-1 T29107A might become a novel biomarker for NPC oncology early screening and prediction.

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References


