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

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Tsou *et al*: Cav-1 Polymorphisms in NPC

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. Materials 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.

Materials 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 leucocytes 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 *Cav-1* C521A (rs1997623): 5'-GTGTCCGCTTCTGC TATCTG-3' and 5'-GCCAAGATGCAGAAGGAG TT-3';

for *Cav-1* G14713A (rs3807987): 5'-CCTTCCAGTAAGCAAGCTGT-3' and 5'-CCTCTCAATCTTGCCATAGT-3';

for *Cav-1* G21985A (12672038): 5'-GGTGTCAGCAAGGCTATGCT-3' and 5'-CCAGACACTCAGAATGTGAC-3';

for *Cav-1* T28608A (rs3757733): 5'-GCTCAACCTCATCTGAGGCA-3' and 5'-GGCCTATTGTTGAGTGGATG-3';

for *Cav-1* T29107A (rs7804372): 5'-GCCTGAATTGCAATCCTGTG-3' and 5'-ACGGTGTGAACACGGACATT-3'

and for *Cav-1* G32124A (rs3807992): 5'-GGTGTCTTGCAGTTGAATG-3' and 5'-ACGGAGCTACTCAGTGCCAA-3'. The following cycling conditions were performed: one cycle at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 30 sec, 55^{o}C for 30 sec, and 72^{o}C for 30 sec; and a final extension at 72^{o}C for 10 min. The PCR products were studied after digestion with Avr II, Bfa I, Hae III, Tsp509 I, Sau3AI and Nla III, restriction enzymes for *Cav-1* C521A (cut from 485 bp C type into 170+315 bp A type), *Cav-1* G14713A (cut from 268 bp A type into 66+202 bp G type), *Cav-1* G21985A (cut from 251+43 bp A type into 153+98+43 bp G type), *Cav-1* T28608A (cut from 298 bp T type into 100+198 bp A type), *Cav-1* T29107A (cut from 336 bp

A type into 172+164 bp T type) and *Cav-1* G32124A (cut from 213+142+67 bp A type into 142+118+95+67 bp G type), respectively.

Statistical analyses. Only those matches with all SNP data (case/control =176/176) were selected into final analyzing. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies 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.

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 genotypes for *Cav-1* C521A, G14713A, G21985A, T28608A, T29107A and G32124A between the controls and NPC patients is shown in Table II. The genotype distribution of the various genetic polymorphisms of *Cav-1* T29107A were significantly different between the NPC and control groups (P=0.0019), while those for *Cav-1* C521A, G14713A, G21985A, T28608A and G32124A were not significant (P>0.05) (Table II).

The frequencies of the alleles for *Cav-1* C521A, G14713A, G21985A, T28608A, T29107A and G32124A between the controls and NPC patients are shown in Table III. The T29107A genotype of *Cav-1* found to be associated with NPC cancer in Table II was also found to be associated with higher NPC susceptibility in the allele frequency analysis ($P=2.5*10^{-4}$). As for other five SNPs, the distributions of their allele frequencies were not significantly different in the controls and NPC patients (Table III).

Discussion

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 spicing, 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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Table I. Characteristics of nasopharyngeal carcinoma patients and controls.

Characteristics	Controls (n = 176)			Patients (n = 176)			*P*[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (y)			49.3 (9.4)			48.2 (11.1)	0.6851
Gender							1.0000
Male	128	72.7%		128	72.7%		
Female	48	27.3%		48	27.3%		
Indulgence							
Cigarette smokers	73	43.8%		77	43.8%		0.7465
Betel quid chewers	54	31.3%		55	31.3%		1.0000
Alcohol drinkers	72	45.5%		80	45.5%		0.5414

[a]*P* based on Chi-square test.

Table II. Distribution of *Cav-1* genotypes among nasopharyngeal carcinoma patients and controls.

Genotype	Controls		Patients		P^a
C521A rs1997623					1.0000
CC	171	97.2%	171	97.2%	
AC	5	2.8%	5	2.8%	
AA	0	0.0%	0	0.0%	
G14713A rs3807987					0.9441
GG	116	65.9%	113	64.2%	
AG	45	25.6%	47	26.7%	
AA	15	8.5%	16	9.1%	
G21985A rs12672038					0.9090
GG	106	60.2%	102	58.0%	
AG	57	32.4%	60	34.1%	
AA	13	7.4%	14	8.0%	
T28608A rs3757733					0.7621
TT	100	56.8%	106	60.2%	
AT	59	33.5%	56	31.8%	
AA	17	9.7%	14	8.0%	
T29107A rs7804372					**0.0019**
TT	86	48.9%	109	61.9%	
AT	57	32.4%	55	31.3%	
AA	33	18.7%	12	6.8%	
G32124A rs3807992					0.6834
GG	88	50.0%	82	46.6%	
AG	68	38.6%	76	43.2%	
AA	20	11.4%	18	10.2%	

[a]*P* based on Chi-square test.

Table III. Distribution of *Cav-1* alleles among nasopharyngeal carcinoma patients and controls

Allele	Controls		Patients		P^a
C521A rs1997623					1.0000
Allele C	347	98.6%	347	98.6%	
Allele A	5	1.4%	5	1.4%	
G14713A rs3807987					0.7154
Allele G	277	78.7%	273	77.6%	
Allele A	75	21.3%	79	22.4%	
G21985A rs12672038					0.6603
Allele G	269	76.4%	264	75.0%	
Allele A	83	23.6%	88	25.0%	
T28608A rs3757733					0.4343
Allele T	259	73.6%	268	76.1%	
Allele A	93	26.4%	84	23.9%	
T29107A rs7804372					**$2.5*10^{-4}$**
Allele T	229	65.1%	273	77.6%	
Allele A	123	34.9%	79	22.4%	
G32124A rs3807992					0.7450
Allele G	244	69.3%	240	68.2%	
Allele A	108	30.7%	112	31.8%	

[a]*P* based on Chi-square test.