

## Original Article

# Association between TAP1 gene polymorphism and esophageal cancer in a Han Gansu population

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**Abstract:** This study aims to investigate the association between transporter associated with antigen processing 1 gene (TAP1) polymorphism and esophagus cancer in a Han Gansu population. TAP1 637A/G polymorphism was analyzed in 200 Han Gansu patients with esophagus cancer and 100 ethnically matched healthy Gansu population by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The adjusted odds ratios (OR) and 95% confidence intervals (CI) were performed by logistic regression model. The results indicated that significant difference was observed in genotype frequencies of TAP1 polymorphisms (AA, AG and GG) between esophagus cancer patients and normal subjects ( $P=0.045$ ). However, only AG genotype was significantly elevated in esophagus cancer compared with controls (35 vs. 23%;  $P=0.035$ ;  $OR=2.8$ ), suggesting a significant positive association with esophagus cancer susceptibility. Meanwhile, AG genotype was found to be associated with gender, age, lymph node metastasis and distant metastasis ( $P<0.05$ ) but rather than with smoke history and tumor size in esophagus cancer. In conclusion, TAP1 637-Asp/Gly polymorphism correlates with the risk of esophagus cancer in a Han Gansu population, which might be served as a genetic susceptibility marker in esophagus cancer patients.

**Keywords:** TAP1, polymorphisms, esophagus cancer, genotype, risk factor

## Introduction

Esophagus cancer is one of most common malignant tumor in the world with a poor prognosis. The 5-year survival rate of patients with localized esophageal cancer is less than 37.4% [1]. The highest incidence of esophagus cancer patients is recorded in Eastern Asian compared to Western countries [2]. However, the etiology of esophagus cancer remains unknown.

Recently, obesity and diet deficiencies are identified as risk factors of esophagus cancer [3-5]. Importantly, genetic susceptibility is also reported to be a risk factor for the disease. Studies have demonstrated the associations between esophagus cancer susceptibility and gene polymorphisms. For example, Zhang et al. have reported that 3'-UTR rs8126 genetic polymorphism in TNFAIP2 is contributed to the risk of esophageal squamous cell carcinoma [6]. Guo et al. [7] have revealed that PLCE1 rs2274223 polymorphism is significantly corre-

lated with esophageal cancer in yellow race populations. Therefore, study on gene polymorphism that might be associated with esophagus cancer would be helpful to elaborate genetic susceptibility.

Transporter associated with antigen processing (TAP) genes including TAP1 and TAP2 are located in human major histocompatibility complex (MHC) class II region between DQB1 and HLA-DPB1 loci and involved in endogenous antigen processing [8]. TAP1 is an ATP-dependent peptide transporter and participates in antigenic peptides transportation from the cytoplasm into the lumen of endoplasmic reticulum [9, 10]. Present studies have demonstrated that TAP1 gene has two polymorphic sites (Ile/Val-333 and Asp/Gly-637) and four alleles (TAP1\*A, TAP1\*B, TAP1\*C, and TAP1\*D), which is associated with disease susceptibility [11, 12]. Meanwhile, TAP1 polymorphism is found to be associated with several malignancies [13-15]. However, whether TAP1 polymorphism cor-

# TAP1 gene polymorphism associates with esophagus cancer

**Table 1.** Alleles and genotypes distributions between esophagus cancer patients and healthy controls

Gene types	Patients (2N=400), no. (%)	Controls (2N=400), no. (%)	OR (95% CI)	P value
<b>Alleles</b>				
A allele	301 (75.3)	306 (76.5)	0.98 (0.91-1.06)	0.741
G allele	99 (24.7)	94 (23.5)	1.05 (0.82-1.35)	
<b>Gene types</b>				
AA	107 (53.5)	126 (63.0)	0.68 (0.45-1.01)	0.054
AG	87 (43.5)	54 (27.0)	2.01 (1.37-3.17)	0.001
GG	6 (3.0)	20 (10.0)	0.28 (0.11-0.71)	0.005

relates with esophagus cancer in Chinese population is unknown. Thus, we investigate the association between the 637-Asp/Gly polymorphism of TAP1 and esophagus cancer in this study.

## Materials and methods

### Patients and controls

A total of 200 Han patients with primary esophagus cancer and 100 ethnically matched healthy population from Gansu province, China were enrolled in this study. All samples were obtained from the Department of Gastroenterology, the First hospital of Lanzhou university, Gansu Province, China between January 2013 and December 2014. All esophagus cancer patients were diagnosed by biopsy. The healthy controls were from unrelated blood donors with no personal or family history of cancer. 5 mL peripheral blood was obtained from esophagus cancer patients before radiotherapy or chemotherapy and healthy controls. Samples were stored at -20°C until analysis. Esophagus cancer patients ranged from 25 to 78 years (Mean 45 years) consisted of 126 males and 74 females. Healthy controls ranged from 23 to 75 years (Mean 42 years) consisted of 50 males and 50 females. Tumor stages were classified according to American Joint Committee on Cancer (AJCC)/Union International Control Cancer (UICC) criteria. Clinicalpathological features including smoke history, tumor size, histopathological type, lymph node metastasis and distant metastasis were obtained by hospital records. Patients with a history of other malignant neoplasm were excluded. All patients signed informed consent and permitted to use samples. This study was

reviewed and approved by Ethics Committee of the First hospital of Lanzhou university, Gansu Province, China.

### Genomic DNA extraction

Genomic DNA was extracted from peripheral blood by TIANamp Blood DNA Kit (Generay biotechnology, Shanghai, China) according to the manufacturer's instructions. Briefly,

blood samples were mixed with TBM Solution, incubated with RNase A and Proteinase K for 10 min at 55°C. The supernatant was collected, centrifuged and transferred to GenClean Column. After using wash solution and Elution Buffer, DNA fluid was obtained by centrifugation and stored at -20°C until analysis.

### TAP1 genotyping

The TAP1 637A/G polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR-RFLP was performed as described by Dai et al. [16]. The PCR primers were designed as described previously [12]. 25 µL PCR mixture including genomic DNA samples, 0.2 mmol/L dNTPs, 0.2 mmol/L of each primer, and 0.5 units of Taq DNA polymerase (Invitrogen, Shanghai, China) were performed by the ABI 9600 (Applied Biosystems, Foster City, USA). Reaction conditions were 95°C for 4 min, 32 cycles of 95°C for 45 s, followed by annealing for 45 s and 72°C for 10 min. Reaction products were digested with 5 units of restriction enzyme (New England Biolabs, Shanghai, China) at 37°C overnight and analyzed on 6% nondenaturing polyacrylamide gel electrophoresis. 20% of samples were selected randomly to perform DNA sequencing analysis.

### Statistical analysis

Data analysis was performed by SPSS19.0 (SPSS, Inc., Chicago, IL, USA). The Hardy-Weinberg equilibrium of TAP1 gene allele frequencies in both esophagus cancer patients and healthy controls was tested. The association of TAP1 637-Asp/Gly polymorphisms with esophagus cancer risk and clinical characteristics was evaluated by Pearson's  $\chi^2$  test. Odds ratio (OR)

**Table 2.** The correlation between TAP1 AG genotypes and clinicalpathological features in esophagus cancer

Clinicalpathological features	N	AG genotype (%)	OR (95% CI)	P value
Gender				
Male	126	65 (51.6)	1.74 (1.18-2.56)	0.003
Female	74	22 (29.7)	0.69 (0.55-0.87)	
Age				
≤40 years	85	25 (29.4)	0.55 (0.38-0.79)	0.001
>40 years	115	62 (53.9)	1.53 (1.20-1.95)	
Smoke history				
Negative	95	38 (40.0)	0.86 (0.62-1.18)	0.392
Positive	105	49 (46.7)	1.13 (0.88-1.43)	
T size				
≤1 cm	80	37 (46.3)	1.11 (0.81-1.52)	0.562
>1 cm	120	50 (41.7)	0.92 (0.72-1.19)	
Histopathological type				
Esophageal squamous cell carcinoma	125	55 (44.0)	1.03 (0.74-1.43)	0.884
Esophageal adenocarcinoma	75	32 (42.7)	0.98 (0.76-1.25)	
Lymph node metastasis				
Negative	134	48 (35.8)	0.61 (0.45-0.82)	0.002
Positive	66	39 (59.1)	1.57 (1.14-2.15)	
Distant metastasis				
Negative	165	59 (35.8)	0.45 (0.34-0.58)	<0.001
Positive	35	28 (80.0)	3.21 (1.64-6.29)	

with 95% confidence interval (95% CI) were calculated with an unconditional logistic regression model.  $P < 0.05$  was considered as significant.

## Results

The correlation of TAP1 637-Asp/Gly polymorphism with esophagus cancer patients and healthy controls was shown in **Table 1**. The genotype distributions of TAP1 gene in both esophagus cancer patients and healthy controls groups were accorded with Hardy-Weinberg equilibrium ( $P > 0.05$ ). Then, we compared the distribution of alleles and gene types between esophagus cancer patients and healthy controls. The results revealed that no significant difference was found in alleles between the two groups ( $P > 0.05$ ). However, the frequency of gene types in esophagus cancer patients was markedly different compared with those in healthy controls. AG genotype was significantly elevated in esophagus cancer compared with controls (43.5% vs. 23%,  $OR = 2.01$ ,  $P = 0.001$ ). Moreover, the frequency of GG genotype was significantly decreased in esophagus cancer patients compared with healthy con-

trols (3.0% vs. 10%,  $OR = 0.28$ ,  $P = 0.005$ ). However, no significant difference was found in AA genotype in the two groups (53.5% vs. 63.0%,  $OR = 0.68$ ,  $P = 0.054$ ).

According to these observations, we considered that TAP1 637 genotypes might be correlated with the esophagus cancer risk. Thus, we analyzed the correlation between TAP1 637 AG and GG genotypes and clinicalpathological features in esophagus cancer patients. As showed in **Table 2**, TAP1 637 AG genotype was correlated with gender, age, lymph node metastasis and distant metastasis ( $P < 0.05$ ). Older patients (>40 years) exhibited higher frequency of 637 AG genotype compared with younger patients (≤40 years) (29.4 vs. 53.9%,  $P = 0.001$ ). Also, higher frequency of TAP1 637 AG genotype was observed in male patients in comparison with female patients (51.6 vs. 29.7%,  $P = 0.003$ ). Moreover, a significantly greater prevalence of 637 AG genotype was found in patients with lymph node metastasis and distant metastasis ( $OR = 1.57$ ,  $P = 0.002$ ;  $OR = 3.21$ ,  $P < 0.001$ , respectively). However, no significant associations of 637 AG genotype with smoke history, tumor size or histo-pathological types

were observed. Furthermore, we didn't find significant correlation between GG genotype and clinical-pathological features in esophagus cancer patients ( $P>0.05$ , data not shown).

## Discussion

TAP1 is an ATP-dependent peptide transporter and plays key role in antigen presentation mediated by major histocompatibility complex (MHC) class I, which is crucial for immune surveillance against intracellular pathogens and tumor [9, 10]. Studies have demonstrated that TAP1 gene polymorphism is associated with several MHC-associated diseases, such as allergic rhinitis, systemic sclerosis, Grave's disease and insulin-dependent diabetes mellitus [12, 17-19]. Meanwhile, TAP1 gene polymorphism has been reported to be associated with childhood cystic echinococcosis and persistent HBV infection [20, 21]. Moreover, the association between TAP1 gene polymorphism and tumor susceptibility has been frequently reported. Hassen et al. reported that the genotypes of TAP1 A allele were highly present in NPC patients compared with controls (98.5% vs. 93.9%;  $P=0.032$ ;  $OR=4.43$ ), suggesting TAP1 A allele was significantly associated with NPC risk in Tunisian population [13]. Ozbas-Gerceker et al. [14] reported that TAP1-333 polymorphism was associated with multiple myeloma-MM. Gostout et al. reported that TAP1 C/C was correlated with the occurrence of cervical cancer [15], while Kordi et al. [22] considered that no association of TAP1 gene polymorphism with risk of cervical cancer was found in north Indian population. However, it is unclear that whether TAP1 gene polymorphism correlates with esophagus cancer susceptibility in Chinese population.

In present study, we investigated the association of 637-Asp/Gly polymorphism of TAP1 with esophagus cancer susceptibility in Chinese population. Results exhibited that the frequencies of A and G alleles in esophagus cancer patients were not significantly different from those in healthy controls, indicating that A and G alleles might be not associated with esophagus cancer risk. However, TAP1-637 AG genotype was found to be markedly elevated in esophagus cancer patients compared with controls (43.5% vs. 23%,  $OR=2.01$ ,  $P=0.001$ ), suggesting that AG genotype positively correlated

with the increase of esophagus cancer susceptibility. Moreover, the frequency of TAP1-637 GG genotype was found to be significantly decreased in esophagus cancer patients compared with healthy controls (3.0% vs. 10%,  $OR=0.28$ ,  $P=0.005$ ), suggesting GG genotype might play a protective role in esophagus cancer. Thus, these data indicated that TAP1 637 genotype was associated with esophagus cancer risk, which was in line with the observations in nasopharyngeal carcinoma and multiple myeloma-MM [13, 14]. Then, we analyzed the potential correlation of TAP1 637 AG and GG genotypes with clinicalpathological features in esophagus cancer. Results revealed that TAP1 637 AG genotype rather than GG genotype was correlated with clinicalpathological features. TAP1 637 AG genotype was highly present in older patients ( $>40$  years). Meanwhile, Hassen et al. observed that TAP1\*B allele was significantly decreased in older patients ( $>30$  years) compared with age-matched controls in nasopharyngeal carcinoma [13]. In addition, our data indicated that higher frequency of 637 AG genotype was observed in male patients with esophagus cancer, and positively correlated with lymph node metastasis and distant metastasis but not with smoke history, histopathological type and tumor size. However, no publications for reference, present results need to be further validated in another study.

Until now, the mechanism of TAP1 gene polymorphism in esophagus cancer remains elusive. But it is clear that human leukocyte antigen (HLA) haplotypes and genes are associated with esophageal cancer in Chinese population [23, 24]. Meanwhile, TAP1 is located in the HLA class II region and plays a key role in antigen presentation and immune surveillance against intracellular pathogens and tumors [9, 10]. Base on our observation, we consider that TAP1 637-Asp/Gly polymorphism may enhance the genetic susceptibility of esophagus cancer by regulating HLA haplotypes and genes in Chinese population. Of course, further analysis is needed.

In conclusion, our observations demonstrate that TAP1 637-Asp/Gly polymorphism correlates with the risk of esophagus cancer in Chinese Han Gansu population, which might be served as a genetic susceptibility marker in esophagus cancer patients.

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## Disclosure of conflict of interest

None.

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