Genetics and Molecular Research

Tumor necrosis factor alpha gene -308G>A polymorphism association with the risk of esophageal cancer in a Han Chinese population

H. Zhao[1], H.W. Zhang[1], T. Zhang[2] and X.M. Gu[1]

[1]Department of Thoracic Surgery, Qilu Hospital of Shandong University, Jinan, Shandong Province, China
[2]Department of Radiology, Jinan Central Hospital, Jinan, Shandong Province, China

Corresponding author: X.M. Gu
E-mail: ql_guxiaomeng@126.com

Genet. Mol. Res. 15 (2): gmr.15025866
Received July 24, 2015
Accepted November 2, 2015
Published April 27, 2016
DOI http://dx.doi.org/10.4238/gmr.15025866

ABSTRACT. In the present study, we aimed to investigate the association between the *TNF-α* -308G>A polymorphism and the risk of esophageal cancer in a Han Chinese population. The case group included 342 patients with esophageal cancer and the control group comprised 300 healthy individuals. The *TNF-α* -308G>A polymorphism was genotyped using polymerase chain reaction-restriction fragment length polymorphism. Conditional logistic regression was performed to analyze the associations between *TNF-α* -308 G>A polymorphism variation and the risk of esophageal cancer, which were estimated by ORs and their 95%CIs. The results indicated that the genotypic frequencies in the patients were not similar to those of the controls, and that the differences were statistically significant (P = 0.014). Using the GG genotype as the reference genotype, the AA genotype was found to be significantly associated with an increased risk of esophageal cancer (adjusted OR = 8.91, 95%CI = 4.72-17.89, P = 0.007). Similarly, the AG+AA genotypes showed 7.82-fold increased esophageal cancer

risk in a dominant model. Furthermore, we found that the A allele was significantly associated with a higher risk of esophageal cancer than the G allele (OR = 6.26, 95%CI = 2.73-10.35, P = 0.013). The results of this study therefore suggested that the presence of the high producer -308A allele in the *TNF-α* gene appeared to be associated with an increased risk for the development of esophageal cancer in the Han Chinese population.

Key words: Tumor necrosis factor-alpha; Esophageal cancer; Polymorphism; Genetic variant

INTRODUCTION

Esophageal cancer is the sixth most common cancer and the sixth leading cause of cancer deaths worldwide. The incidence of esophageal cancer, however, is higher in men than in women (Enzinger and Mayer, 2003). The occurrence of esophageal cancer has been associated with defects in many genes, including those involving ethanol metabolism, folate metabolism, cell cycle regulation, and DNA repair, as well as with known oncogenes (Hiyama et al., 2007).

Tumor necrosis factor-alpha (TNF-α) is a mediator of the inflammatory process that is secreted by monocytes, macrophages, neutrophils, T cells, and NK cells after stimulation. TNF-α is a pro-inflammatory molecule that is thought to play an important role in the development of the immune response (Watts, 2005). The *TNF-α* gene is located in the major histocompatibility complex III locus on chromosome 6p21.3. In recent years, several common single nucleotide polymorphisms have been identified in the *TNF-α* promoter regions; these, including *TNF-α* -308G>A (rs1800629), can regulate the expression level of *TNF-α* (Fargion et al., 2001). Previously, multiple case-control studies have been conducted to investigate the association between the *TNF-α* -308G>A polymorphism and cancer risk (Fei et al., 2004; García-González et al., 2009; Akkiz et al., 2009; Kesarwani et al., 2009; Karakus et al., 2011). However, only two studies have investigated the effects of the *TNF-α* -308G>A polymorphism on esophageal cancer risk (El-Omar et al., 2003; Guo et al., 2005). To provide more evidence regarding the association between the *TNF-α* -308G>A polymorphism and esophageal cancer risk, we conducted the present case-control study.

MATERIAL AND METHODS

Study subjects

The subjects included a patient and a control group. The patient group included 342 subjects with esophageal cancer that had been histopathologically confirmed in the Department of Thoracic Surgery, Qilu Hospital of Shandong University between March 2007 and August 2013; selection was not limited by gender, age, or histological type, and participants did not undergo preoperative treatment involving anticancer drugs or radiotherapy. The group comprised 189 men and 153 women (mean age 63.2 ± 6.8 years). The control group comprised 300 healthy individuals who underwent physical examinations in our hospital in the same period, and who were not blood relatives of the patients. The group comprised 198 men and

102 women (mean age 66.7 ± 8.2 years). The patient records included age, sex, history of smoking and alcohol consumption, and family history. The present study was approved by the Medical Ethics Committee of Qilu Hospital affiliated to Shandong University, and informed consent was obtained from all participants.

DNA extraction

A 5-mL venous blood sample was collected from each subject into a test tube containing EDTA as an anticoagulant. Genomic DNA was extracted from 2 mL peripheral blood by standard methods with proteinase K digestion (Invitrogen Company, USA), followed by phenol-chloroform extraction. After ethanol precipitation, the DNA was dissolved in double distilled water and frozen at -20°C until use.

Polymorphism genotyping

The -308 *TNF-α* promoter polymorphism was determined by as previously described (Wilson et al., 1992). Amplification was performed using a GeneAmp polymerase chain reaction (PCR) System 9700 thermocycler (Applied Biosystems, Singapore) with 100 ng genomic DNA, 25 pmol each primer, 200 µM total dNTPs, 1.5 mM MgCl, 1X PCR buffer, and 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA). The following cycling conditions were used: 95°C for 5 min, followed by 35 cycles of 94°C for 60 s, 63°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 10 min. Restriction enzyme digestion of the PCR product with *Nco*I (Promega) was carried out overnight and analyzed on a 3% agarose gel. DNA products were visualized by ethidium bromide staining. The -308G allele yielded two fragments (presence of these exclusively indicated homozygosity for the -308G allele), while its homologue -308A, which lacked the *Nco*I site, remained undigested and resulted in a single band (the exclusive presence of which indicated homozygosity for the -308A allele). The presence of all three fragments defined heterozygotic individuals.

Statistical analysis

Continuous variables are reported as means ± standard deviation, and categorical variables are reported as frequencies (N) and percentages (%). The χ^2 test was used to assess differences between patients and controls with regard to clinical characteristics. A goodness-of-fit χ^2 test was used to evaluate the Hardy-Weinberg equilibriums in controls. Conditional logistic regression was performed to analyze the associations between the *TNF-α* -308G>A polymorphism variants and the risk of esophageal cancer, which were estimated using ORs and their 95%CIs. The significance levels of all tests were set at $P < 0.05$. All statistical analyses were performed using the Statistical Package for Social Science software version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic characteristics

Demographic characteristics are presented in Table 1. In the present case-control study,

a total of 642 Han Chinese subjects were enrolled, consisting of 342 patients with esophageal cancer and 300 cancer-free controls. There were no significant differences between the patients with esophageal cancer and cancer-free controls with regard to gender and age distributions. In addition, for other general characteristics such as alcohol consumption and tobacco smoking, no significant differences were observed between the patients with esophageal cancer and cancer-free controls (all P > 0.05).

Table 1. Distribution of selected characteristics among esophageal cancer cases and healthy controls.

Characteristics	Cases (N)	%	Controls (N)	%	P value
N	342		300		
Age (years)					
<55	145	42.40	153	51.00	0.572
≥55	197	57.60	147	49.00	
Gender					
Male	201	58.77	187	62.33	0.498
Female	141	41.23	123	41.00	
Smoking status					
Yes	278	81.29	254	84.67	0.456
No	64	18.71	46	[illegible]	
Drinking status					
Yes	245	71.64	[illegible]	77.3	0.387
No	97	28.36	[illegible]	[illegible]	

Genotypic frequencies of the *TNF-α* -308 G>A polymorphism in patients and controls

The observed genotype distribution in the controls did not differ from those expected from Hardy-Weinberg equilibrium (P > 0.05). Compared to healthy controls, patients with esophageal cancer had a lower frequency of the GG genotype (81.29 *vs* 95.33%) and a higher frequency of AG (11.99 *vs* 3.67%). The homozygous AA genotype was found in 23 patients with esophageal cancer and in 3 controls. Thus, the genotypic frequencies in the patients were not similar to those of the controls, with the differences being statistically significant (P = 0.014; Table 2).

Table 2. Genotypic frequencies of TNF-α -308 G> A in esophageal cancer cases and healthy controls.

TNF-α -308 G> A	Cases	%	Controls	%	P value
GG	278	81.29	286	95.33	0.014
AG	41	11.99	11	3.67	
AA	23	6.73	3	1.00	

Interaction between the *TNF-α* -308G>A polymorphism and the risk of esophageal cancer

We further analyzed the effects of the tested genotypes under different genetic models (Table 3). Using the GG genotype as the reference genotype, the AA genotype was significantly associated with an increased risk of esophageal cancer (adjusted OR = 8.91, 95%CI = 4.72-17.89, P = 0.007). Similarly, the AG+AA genotypes showed a 7.82-fold increased esophageal cancer risk in the dominant model. Furthermore, we found that the A allele was significantly associated with a higher risk of esophageal cancer than the G allele (OR = 6.26, 95%CI = 2.73-10.35, P = 0.013).

Table 3. Association of *TNF-α* -308G>A polymorphism with esophageal cancer risk.

TNF-α -308 G>A polymorphism	Esophageal cancer patients	Controls	OR (95%CI)[1]	P value
General genotype				
GG	278	286	1.00 (Reference)	
AG	41	11	3.34 (1.57-5.98)	0.035
AA	23	3	8.91 (4.72-17.89)	0.007
Dominant genotype				
GG	278	286	1.00 (Reference)	
AG+AA	64	14	7.82 (3.73-19.27)	0.003
Recessive genotype				
AG+GG	319	297	1.00 (Reference)	
AA	23	3	1.32 (0.67-4.74)	0.08
Allele frequency				
G	597	583	1.00 (Reference)	0.013
A	87	17	6.26 (2.73-10.35)	

[1]Adjusted for gender, age, smoking status, and drinking status.

DISCUSSION

Esophageal cancer is a common digestive system m[illegible]gnancy. [illegible]nsidered a multifactorial disease that appears to result from a combin[illegible]tion of abnor[illegible]al levels of certain proteins and their interaction with environmental fac[illegible]rs. In addit[illegible]n, se[illegible]eral low penetrance susceptibility genes combined with environment[illegible] factors h[illegible]ve be[illegible]n sugge[illegible]ed to be important in the growth and development of cancer (O'Brie[illegible], 2000).

One gene with variants kno[illegible] to inhibit cance[illegible] devel[illegible]pment and growth is *TNF-α*. *TNF-α* encodes a pro-inflamma[illegible]y cytok[illegible]e that is secre[illegible]ed primarily by macrophages and plays critical roles in the p[illegible]thoge[illegible]esis of i[illegible]flammat[illegible]y autoimmune and malignant diseases. The TNF-α protein i[illegible]duce[illegible]e e[illegible]pre[illegible]sion of [illegible]dhesion molecules, facilitating the invasion of metastatic tu[illegible] cells, high level[illegible] of e[illegible]dogenous TNF-α have been observed in the blood of some ca[illegible]er pati[illegible]ts (Cham[illegible] et al., 2012). TNF-α expression is regulated primarily at the transcriptio[illegible]l le[illegible]el, a[illegible]d p[illegible]ymo[illegible]phisms within the *TNF-α* promoter have been related to the level of *TNF-* exp[illegible]ssion (Majeer and Hutchinson, 2000; Kirkpatrick et al., 2004). Therefore, *TNF-α* promo[illegible] pol[illegible]morphisms have been intensively studied as potential determinants of disease suscepti[illegible]ity in numerous disorders where TNF-α levels have been thought to be important. Of these, the *TNF-α* -308G>A polymorphism is the best studied. It involves the substitution of a guanine (G) by an adenine (A) and is associated with an increase in TNF-α expression levels (Louis et al., 1998; Kroeger et al., 2000).

Previously, numerous case-control studies have been conducted to investigate the association between the *TNF-α* -308G>A polymorphism and cancer risk (Fei et al., 2004; García-González et al., 2007; Akkiz et al., 2009; Kesarwani et al., 2009; Karakus et al., 2011). Furthermore, meta-analyses of these studies have also been conducted. For example, Jin et al. (2014) carried out a meta-analysis of 20 published case-control studies with 12,360 patients with breast cancer and 15,110 controls. Their results indicated that the *TNF-α* -308G>A polymorphism was not associated with breast cancer risk in the overall population but that the A allele might be a protective factor for breast cancer in postmenopausal women, and the AA genotype might be a breast cancer risk factor in premenopausal women. Ma et al. (2014) conducted a meta-analysis of all available studies investigating the effects of the *TNF-α* -308G>A polymorphism on prostate cancer risk, and they found that the *TNF-α* -308G>A polymorphism might significantly contribute to prostate cancer susceptibility in

healthy volunteers. The meta-analysis by Guo et al. (2005) indicated that the *TNF-α* -308 gene polymorphism might be significantly associated with the risk of gastric and hepatocellular carcinomas, but not of colorectal or pancreatic cancer in the Asian population. However, until now, only two studies have been conducted to investigate the effects of the *TNF-α* -308G>A polymorphism on esophageal cancer risk (El-Omar et al., 2003; Guo et al., 2005). To provide more evidence about the association between the *TNF-α* -308G>A polymorphism and esophageal cancer risk, we conducted the present case-control study. In this study, we found that the genotypic frequencies in the patients were not similar to those of the controls, with the differences being statistically significant. We further analyzed the effects of the tested genotypes under different genetic models. Using the GG genotype as the reference genotype, the AA genotype was found to be significantly associated with an increased risk of esophageal cancer. Similarly, the AG+AA genotypes showed a 7.82-fold increased esophageal cancer risk in a dominant model. Furthermore, we found that the A allele was significantly associated with a higher risk of esophageal cancer than the G allele.

In conclusion, the results of our study suggested that the presence of the high producer -308A allele in the *TNF-α* gene appeared to be associated with an increased risk for the development of esophageal cancer in the Han Chinese population.

Conflicts of interest

The authors declare that they have no competing interest.

REFERENCES

Akkiz H, Bayram S, Bekar A, Akdil B, et al. (2009). G-308A TNF-alpha polymorphism is associated with an increased risk of hepatocellular carcinoma in the Turkish population: case-control study. *Cancer Epidemiol.* 33: 261-264. http://dx.doi.org/10.1016/j.canep.2009.06.001

Champ CE, Volz LJS, Sigl J, Li L, et al. (2012). Weight gain, metabolic syndrome, and breast cancer recurrence: are dietary recommendations supported by the data? *Int. J. Breast Cancer* 2012: 506868. http://dx.doi.org/10.1155/2012/506868

El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, et al. (2003). Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 124: 1193-1201. http://dx.doi.org/10.1016/S0016-5085(03)00157-4

Enzinger PC and Mayer RJ (2003). Esophageal cancer. *N. Engl. J. Med.* 349: 2241-2252. http://dx.doi.org/10.1056/NEJMra035010

Fargion S, Valenti L, Dongiovanni P, Scaccabarozzi A, et al. (2001). Tumor necrosis factor alpha promoter polymorphisms influence the phenotypic expression of hereditary hemochromatosis. *Blood* 97: 3707-3712. http://dx.doi.org/10.1182/blood.V97.12.3707

Fei BY, Xia B, Deng CS, Xia XQ, et al. (2004). Association of tumor necrosis factor genetic polymorphism with chronic atrophic gastritis and gastric adenocarcinoma in Chinese Han population. *World J. Gastroenterol.* 10: 1256-1261.

García-González MA, Lanas A, Quintero E, Nicolás D, et al.; Spanish Gastroenterological Association AEG (2007). Gastric cancer susceptibility is not linked to pro-and anti-inflammatory cytokine gene polymorphisms in whites: a Nationwide Multicenter Study in Spain. *Am. J. Gastroenterol.* 102: 1878-1892. http://dx.doi.org/10.1111/j.1572-0241.2007.01423.x

Guo W, Wang N, Li Y and Zhang JH (2005). Polymorphisms in tumor necrosis factor genes and susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a population of high incidence region of North China. *Chin. Med. J. (Engl.)* 118: 1870-1878.

Hajeer AH and Hutchinson IV (2000). TNF-alpha gene polymorphism: clinical and biological implications. *Microsc. Res. Tech.* 50: 216-228. http://dx.doi.org/10.1002/1097-0029(20000801)50:3<216::AID-JEMT5>3.0.CO;2-Q

Hiyama T, Yoshihara M, Tanaka S and Chayama K (2007). Genetic polymorphisms and esophageal cancer risk. *Int. J. Cancer* 121: 1643-1658. http://dx.doi.org/10.1002/ijc.23044

Jin G, Zhao Y, Sun S and Kang H (2014). Association between the tumor necrosis factor alpha gene -308G> A polymorphism and the risk of breast cancer: a meta-analysis. *Tumour Biol.* 35: 12091-12098. http://dx.doi.org/10.1007/s13277-014-2510-z

Karakus N, Kara N, Ulusoy AN, Ozaslan C, et al. (2011). Tumor necrosis factor alpha and beta and interferon gamma gene polymorphisms in Turkish breast cancer patients. *DNA Cell Biol.* 30: 371-377. http://dx.doi.org/10.1089/dna.2010.1113

Kesarwani P, Mandhani A and Mittal RD (2009). Polymorphisms in tumor necrosis factor-A gene and prostate cancer risk in North Indian cohort. *J. Urol.* 182: 2938-2943. http://dx.doi.org/10.1016/j.juro.2009.08.016

Kirkpatrick A, Bidwell J, van den Brule AJ, Meijer CJ, et al. (2004). TNFalpha polymorphism frequencies in HPV-associated cervical dysplasia. *Gynecol. Oncol.* 92: 675-679. http://dx.doi.org/10.1016/j.ygyno.2003.11.025

Kroeger KM, Steer JH, Joyce DA and Abraham LJ (2000). Effects of stimulus and cell type on the expression of the -308 tumour necrosis factor promoter polymorphism. *Cytokine* 12: 110-119. http://dx.doi.org/10.1006/cyto.1999.0529

Louis E, Franchimont D, Piron A, Gevaert Y, et al. (1998). Tumour necrosis factor (*TNF*) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin. Exp. Immunol.* 113: 401-406. http://dx.doi.org/10.1046/j.1365-2249.1998.00662.x

Ma L, Zhao J, Li T, He Y, et al. (2014). Association between tumor necrosis factor-alpha gene polymorphisms and prostate cancer risk: a meta-analysis. *Diagn. Pathol.* 9: 74. http://dx.doi.org/10.1186/1746-1596-9-74

O'Brien JM (2000). Environmental and heritable factors in the causation of cancer: analyses of cohorts of twins from Sweden, Denmark, and Finland. by P. Lichtenstein, N.V. Holm, P.K. Verkasalo, A. Iliadou, J. Kaprio, M. Koskenvuo, E. Pukkala, A. Skytthe, and K. Hemminki. *N. Engl. J. Med.* 343:78-84. *Surv. Ophthalmol.* 45: 167-168. http://dx.doi.org/10.1016/S0039-6257(00)00165-X

Watts TH (2005). TNF/TNFR family members in costimulation of T cell responses. *Annu. Rev. Immunol.* 23: 23-68. http://dx.doi.org/10.1146/annurev.immunol.23.021704.115839

Wilson AG, di Giovine FS, Blakemore AI and Duff GW (1992). Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by *Nco*I restriction of PCR product. *Hum. Mol. Genet.* 1: 353. http://dx.doi.org/10.1093/hmg/1.5.353