

# A six-nucleotide deletion polymorphism in the casp8 promoter is associated with reduced risk of esophageal and gastric cancers in Kashmir valley

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**BACKGROUND:** Caspase-8 (CASP8) is a key regulator of apoptosis or programmed cell death, an essential defense mechanism against hyperproliferation and malignancy. To evaluate the role of CASP8 polymorphisms in esophageal (EC) and gastric cancers (GC) in the Kashmir valley, we examined the risk due to -652 6N ins/del polymorphism (rs3834129) in the promoter of CASP8 in a case-control study.

**MATERIALS AND METHODS:** Genotypes of the CASP8 polymorphisms (-652 6N ins/del; rs3834129) were determined for 315 patients (135 EC and 108 GC) and 195 healthy controls by polymerase chain reaction. Data was statistically analyzed using Chi-square test and logistic regression model by using the SPSS software.

**RESULTS:** Carriers for the “del” allele of rs3834129 single nucleotide polymorphism were associated with decreased risk for both EC (odds ratio [OR] = 0.278; 95% confidence interval [95% CI] = 0.090–0.853; *P* = 0.025) and GC (OR = 0.397; 95% CI = 0.164–0.962; *P* = 0.041). Also, in a recessive model, our results showed that CASP8 -652 6N ins/del “del/del” allele was conferring significant low risk for both EC (OR = 0.380; 95% CI = 0.161–0.896; *P* = 0.027) and GC (OR = 0.293; 95% CI = 0.098–0.879; *P* = 0.029). However, interaction of CASP8 -652 6N ins/del genotypes with smoking and high consumption of salted tea did not further modulate the risk of EC and GC.

**CONCLUSIONS:** Polymorphism in CASP8 -652 6N ins/del polymorphism modulates the risk of EC and GC in Kashmir valley.

**Key words:** Caspase 8, esophageal and gastric cancer, Kashmir valley, polymorphism

## Introduction

Among human cancers, esophageal and gastric carcinogenesis also appear to be a complex multistep processes with multi-functional etiologies, where environmental, geographical and genetic factors have been attributed to play major roles in the causation of the cancers. Esophageal cancer (EC) is the eighth and gastric cancer (GC) is the second most commonly occurring cancer in the world.<sup>[1]</sup> In India, EC and GC are the leading sites of tobacco-related cancers. Within the Indian subcontinent, the Valley of Kashmir presents a strikingly different picture, where the incidence of EC and GC have been reported to exceed 40% of all cancers, and the incidence is three- to six-times higher than that at various metropolis cancer registries in India.<sup>[2,3]</sup> Some of the environment factors have been reported to be associated with an increased risk of EC and GC in Kashmir valley.<sup>[4]</sup> However, very few reports have associated this malignancy with specific risk factors prevalent in the area.

Apoptosis is an essential genetic program necessary for the proper development of an organism.<sup>[5]</sup> Thus far, two major apoptosis-signaling pathways have been described: extrinsic and intrinsic pathways. In both pathways, the initiator caspase, caspase-8 encoded by CASP8 (MIM: 601763) located at 2q33, plays an important role in transducing the death signal to more downstream death effectors such as caspase-3 and caspase-7.<sup>[6]</sup> In the extrinsic apoptosis pathway, caspase-8 triggers

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apoptosis caused mainly by death receptor-induced apoptotic signaling and mediated by Fas and Fas ligand.<sup>[7,8]</sup> There are at least 353 single-nucleotide polymorphisms (SNP) of the CASP8 reported in the dbSNP database ([http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?chooseRs=allandgo=GoandlocusId=841](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?chooseRs=allandgo=GoandlocusId=841)); however, only two common polymorphic variations, D302H (rs1045485) and 6N ins/del (rs3834129), have been reported to influence the risk of cancer development.<sup>[8,9]</sup> The D302H polymorphism with unknown functionality seems extremely rare (minor allele frequency <1%) in Asian populations (based on the HapMap Project and the Environmental Genome Project databases). Therefore, in the current study, we investigated the roles of CASP8 -652 6N ins/del (rs3834129) polymorphisms in conferring genetic susceptibility to EC and GC in Kashmir valley.

## Materials and Methods

The present case-control study comprised untreated histopathologically confirmed cases with EC (135), GC (108) and healthy controls (195). All subjects were unrelated permanent residents of Kashmir and were referred from the Departments of Gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, from May 2006 to December 2008. Patients and controls were matched by ethnicity, mean age and gender. Patients were excluded if they had nonmalignant conditions like corrosive esophageal injury, achalasia injury, Barrett's esophagus, gastro-esophageal reflux disease (GERD) and nonulcer dyspepsia. Controls were also recruited from Sher-i-Kashmir Institute of Medical Sciences, Srinagar, which included medical staff as well as individuals who came for their routine checkup for conditions not related to cancer and were diagnosed as no severe ailments. All individuals were personally interviewed about their age, occupational history, medical history of other diseases, demographic features, family history of cancer, use of hot noon chai (salted tea), drinking alcohol and smoking habits. Tobacco use included smoking cigarettes or "hukka" (water pipe). Written informed consent was obtained from all study participants. The research protocol was approved by the ethics committee of Sanjay Gandhi Postgraduate Institute

of Medical Sciences, Lucknow (project number: 5/13/48/2002-NCDIII). Sample collection, storage and transport were in compliance with committee guidelines. Blood samples were collected in EDTA and genomic DNA was extracted from peripheral blood leukocyte pellet using the standard salting-out method.<sup>[10]</sup> The quality and quantity of DNA was checked by agarose gel electrophoresis and spectrophotometry using a Nanodrop Analyser (ND-1000) (Nano Drop Technologies Inc., Wilmington, DE, USA).

### Genotyping by polymerase chain reaction (PCR) and restriction fragment analysis

The CASP8 -652 6N ins/del polymorphism was determined using PCR. The details of the genotyping method used were same as that described previously.<sup>[11,12]</sup> More than 15% of the samples were randomly selected for confirmation, and the results were 100% concordant.

### Statistical analysis

Demographic characteristics of patients and controls were described as frequencies and percentages, whereas descriptive statistics of patients and controls were presented as mean and standard deviations for continuous measures. Statistical significance of frequency differences between patients and control groups was evaluated using the  $\chi^2$  test. Deviation from the Hardy-Weinberg equilibrium in controls was assessed using the  $\chi^2$  test; *P*-value was considered significant at the <0.05 level. Risk estimates were calculated for codominant, dominant and recessive genetic models using the most common homozygous genotype as reference. Observed genotype frequencies for CASP8 -652 6N ins/del polymorphism in controls were examined for deviation from the Hardy-Weinberg equilibrium (HWE) using a goodness-of-fit  $\chi^2$ -test with one degree of freedom. The same controls were used for analyzing two sets of cancer cases. Binary logistic regression analysis was used to fit statistical models to predict the association of CASP8 -652 6N ins/del genotypes with susceptibility to EC and GC. Association was expressed as odds ratios (OR) for risk estimation with 95% confidence intervals (95% CI). All statistical analyses were performed using SPSS software version 15.0 (SPSS, Chicago, IL, USA).

**Results**

Population characteristics: The mean age of healthy subjects (controls) and patients with EC and GC was 57.98 ± 12.664 years, 60.38 ± 8.402 years and 55.91 ± 9.728 years, respectively (t-test; P-value = ns). Both cancers were highly prevalent in males (68.1% in EC and 83.3% in GC) than in females. In patients with EC, squamous cell carcinoma (SCC) histopathology was common (76.3%), but in GC most of the cases were with adenocarcinoma (ADC, 79.6%). Smoking habit (Hukka) showed significantly higher risk both in EC (OR = 21.443; 95% CI = 11.628–39.543; P = 0.0001) and in

GC (8.975; 95% CI = 5.156–15.622; P = 0.0001) patients. Individuals consumed salted-tea in a range of two to eight cups per day, and median consumption of tea was four cups per day. Therefore, we grouped individuals into ≤4 cups or >4 cups per day, and individuals consuming salted tea >4 cups per day were regarded as high salted tea consumers. Higher consumption of salted tea was also found to be associated with increased risk of EC (OR = 14.856; 95% CI = 8.411–26.241; P-value = 0.0001) and GC (OR = 14.778; 95% CI = 8.020–27.231; P-value = 0.0001) [Table 1]. None of the patients or controls reported consumption of alcohol and, therefore, interaction of alcohol intake with genetic variations could not be analyzed. Other dietary factors like consumption of “Haak” and “Wur” were not found to be associated with EC or GC development (data not shown).

**Table 1: Characteristics of esophageal, gastric cancer patients and healthy individuals of the Kashmir valley**

Variables	Esophageal cancer patients n = 135	Gastric cancer patients n = 108	Healthy individuals n = 195
Mean age ± SD (years)	60.38 ± 8.402	55.91 ± 9.728	57.98 ± 12.664
Sex			
Male	92 (68.1%)	90 (83.3%)	139 (71.3%)
Female	43 (31.9%)	18 (16.7%)	56 (28.7%)
Histology			
Adenocarcinoma	32 (23.7%)	86 (79.6%)	
Squamous cell carcinoma	103 (76.3%)	22 (20.4%)	
Smoking <sup>#</sup>			
Smokers (Hukka)	106 (84.1%) <sup>a</sup>	48 (67.6%) <sup>b</sup>	38 (20.5%)
Salted tea intake <sup>#</sup>			
(≤4 cups daily)	36 (28.6%)	31 (30.7%)	159 (85.9%)
(>4 cups daily)	90 (71.4%) <sup>c</sup>	70 (69.3%) <sup>d</sup>	26 (14.1%)
Alcohol consumption	Nil	Nil	Nil

Smoking <sup>a</sup>OR\* = 21.443; 95% CI = 11.628–39.543; P-value = 0.0001 (esophageal cancer vs controls) <sup>b</sup>OR\* = 8.975; 95% CI = 5.156–15.622; P-value = 0.0001 (gastric cancer vs controls) Salted tea <sup>c</sup>OR\* = 14.856; 95% CI = 8.411–26.241; P-value = 0.0001 (esophageal cancer vs controls) <sup>d</sup>OR\* = 14.778; 95% CI = 8.020–27.231; P-value = 0.0001 (gastric cancer vs controls) <sup>#</sup>Age- and gender-adjusted OR. <sup>#</sup>Data missing.

**Association between CASP8 -652 6N ins/del polymorphism and the EC and GC risk:** The genotype and allele frequencies of the CASP8 polymorphisms among cases and controls are shown in Table 2. The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium (P = 0.127; χ<sup>2</sup> = 2.332). In the present study, when we used the CASP8 -652 6N ins/ins genotype as the reference, we found that the CASP8 -652 6N del/del genotype was significantly associated with low risk in EC (OR = 0.278; 95% CI = 0.090–0.853; P-value = 0.025) as well as with GC (OR = 0.397; 95% CI = 0.164–0.962; P-value = 0.041), respectively. Furthermore, a significant decreased risk of EC and GC was found with the CASP8 -652 (del/del genotype) compared with the -652 6N ins/del + del/del genotypes, suggesting a recessive protective effect of this polymorphism on both EC

**Table 2: Frequency distribution of CASP8 -652 6N ins/del and risk assessment in esophageal and gastric cancer patients and controls**

Genotypes	Controls (195)		Esophageal cancer (135)		Gastric cancer (108)	
	n (%)	n (%)	OR* (95% CI)	P	n (%)	OR* (95% CI) P
CASP8 -652 6N ins/del (rs3834129)						
Ins/ins	96 (49.2)	68 (50.4)	Reference		59 (54.6)	Reference
Ins/del	75 (38.5)	59 (43.7)	0.889 (0.537–1.471)	0.647	44 (40.7)	1.102 (0.690–1.758) 0.685
del/del	24 (12.3)	8 (5.9)	0.278 (0.090–0.853)	0.025	5 (4.6)	0.397 (0.164–0.962) 0.041
Dominant model						
Ins/ins	96 (49.2)	68 (50.4)	Reference		60 (55.6)	Reference
ins/del+del/del	99 (50.8)	67 (49.6)	0.926 (0.593–1.445)	0.735	48 (44.4)	0.751 (0.463–1.220) 0.248
Recessive model						
Ins/ins+ins/del	171 (87.7)	127 (94.1)	Reference		104 (96.3)	Reference
del/del	24 (12.3)	8 (5.9)	0.380 (0.161–0.896)	0.027	4 (3.7)	0.293 (0.098–0.879) 0.029

<sup>#</sup>Age- and gender-adjusted OR Significant values shown in bold



make malignant cells less likely escape from CTL killing, ultimately protecting against EC and GC.<sup>[18]</sup>

The people of the valley have many unique dietary features that are different from the rest of the world. Salted tea used by people is prepared by using baking soda (sodium bicarbonate) along with common salt (sodium chloride) and boiled for a few hours before consumption. It has been suspected that the salts might cause thermal injury to the esophageal and gastric epithelium.<sup>[2]</sup> In the present study, high consumption of salted tea (>4 cups a day) was independently associated with increased risk for EC (OR = 14.856; 95% CI = 8.411–26.241; *P*-value = 0.0001) and GC (OR = 14.778; 95% CI = 8.020–27.231; *P*-value = 0.0001). Our results also show a significant association of smoking (Hukka) with EC (OR = 21.443; *P*-value = 0.0001) and GC (OR = 8.975; *P*-value = 0.0001). However, based on our gene environmental interactions, we did not find any significant association of CASP8 -652 6N ins/del genotypes with smoking or high salted tea consumption.

The limitation in our study was smaller sample size. As it is the first report of genetic susceptibility of EC and GC due to CASP8 -652 6N ins/del polymorphisms in the Kashmiri valley, there is a definite need to perform a similar study in a larger sample size before its clinical application.

In conclusion, the present case–control study found that CASP8 -652 6N ins/del polymorphism was associated with reduced risk for EC and GC risk in the Kashmir valley. High salted tea intake and smoking itself are risks for developing EC and GC, but the risk was not further enhanced due to interaction of genetic variants analyzed in the present study.

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