RESEARCH COMMUNICATION

# Role of the Metalloproteinase-7 (181A>G) Polymorphism in Gastric Cancer Susceptibility: A Case Control Study in Kashmir Valley

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## Abstract

**Matrix metalloproteinase-7 (MMP-7) is a small secreted proteolytic enzyme with broad substrate specificity against extracellular matrix (ECM) and non-ECM components. A promoter polymorphism MMP-7 181A>G is known to modify the gene transcription activity of the proteinase gene and influence susceptibility to various cancers. The present case-control study comprising 108 gastric cancer (GC) patients and 195 healthy controls was carried out to determine any association of this polymorphism in the Kashmir valley where the GC incidence is very high. Genotypic data were statistically analyzed by logistic regression models. In combined analysis, homozygous variant GG genotype of MMP-7 (-181A>G) polymorphism was associated with a more than two fold increased risk of GC (OR=2.13; 95% CI =1.13-4.01; p=0.020; P-trend=0.01) compared with the common AA genotype and the data fitted a recessive model. After sub-grouping based on tumor histology, the risk was more pronounced with squamous cell histology (OR=9.34; 95% CI =1.97-44.33; p=0.005) as compared to adenocarcinoma. The cancer risk due to smoking or high intake of salted tea was not influenced by the MMP-7 polymorphism. In conclusion, results from present study suggest that common MMP-7 (181A>G) genetic polymorphism may contribute to squamous cell gastric cancer susceptibility in the Kashmir valley.**

**Keywords:** Kashmir valley - gastric cancer - MMP-7 (-181A>G) polymorphism - PCR/RFLP

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## Introduction

Matrix metalloproteinase-7 (MMP-7) is an important member of the MMP family that has broad substrate specificity against both extracellular matrix (ECM) and non-ECM components (Wilson et al., 1996). Best known as a contributor to tumor invasion and metastasis, a growing body of evidence also implicates MMP-7 in earlier stages of tumorigenesis, including cellular transformation, cell survival, tumor growth, angiogenesis, and evasion of immune surveillance (Nishizuka et al., 2001; Egeblad et al., 2002; Beeghly-Fadiel et al., 2008). The gene encoding MMP-7 is localized on chromosome 11q21-q22. Two polymorphisms exist in the MMP-7 promoter region, -181A>G and -153 C>T which are known to modify the gene transcription activity (Jormsjö et al., 2001; Jones et al., 2004). Association of MMP-7 (-181A>G) (rs11568818) polymorphism has also been reported for a variety of cancers, including gastric cancer (Murray et al., 1998; Zhang et al., 2005; Peng et al., 2010; Yeh et al., 2010).

Gastric cancer is the most aggressive malignant tumors of gastrointestinal tract with diverse risk factors and incidence patterns. Within the Indian subcontinent, the valley of Kashmir presents a strikingly different picture where incidence of gastric cancer has been reported to exceed 40% of all cancers and incidence is 3-6 times higher than various metropolis cancer registries in India (Khuroo et al., 1992). The people of the Kashmir valley have many unique dietary features which are different from rest of world. Salted tea used by people is prepared by using baking soda (sodium bicarbonate) along with common salt (sodium chloride) and boiled for few hours before consuming. Some of the genetic and environment factors have been reported to be associated with an increased risk of gastric cancer in Kashmir valley (Siddiqui et al., 1992; Malik et al 2009a; 2009b). However, till date, no study has been carried out to evaluate the influence of MMP7 polymorphisms in relation to higher prevalence of GC in Kashmir valley. Therefore, the present case-control study was designed to investigate the role of MMP-7 (-181A>G) (rs11568818) in conferring genetic susceptibility to gastric cancer in the Kashmir Valley.

## Materials and Methods

The present study comprised untreated histopathologically confirmed cases with GC (108) and healthy controls (195). The sample size of present study was adequate to provide 80% power. The mean age of

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healthy subjects (controls) and patients with GC was 58.0yrs± 12.7 and 55.9yrs±9.73 respectively (t-test P value=ns). All subjects were unrelated permanent residents of Kashmir and were referred from the Department 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. Excluded were those non-malignant conditions like Barrett's esophagus, gastro-esophageal reflux disease (GERD) and non-ulcer dyspepsia. Controls were also recruited from Sher-i-Kashmir Institute of Medical Sciences, Srinagar. Healthy controls were the individuals who came for their routine health checkups or minor illness like fever, common headache or minor surgery etc. They were ethnicity matched with cases, and free from any chronic disease, unrelated to patients and having similar socio-economic background. 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 (Miller et al., 1988). The quality and quantity of DNA was checked by gel electrophoresis and spectrophotometry using Nanodrop Analyser (ND-1000) spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA). The ratio of absorbance at 260 and 280 nm of DNA was around 1.7-1.9. The isolated DNA was stored at -70$^0$C.

*Genotyping*

Genotyping was done by PCR-RFLP methods for MMP-7 (-181A>G) polymorphisms by using published primer sequences (Jormsjö S, et al, 2001). The PCR products 172-bp were subsequently digested with 10 units of EcoR1 for 12 hrs at 37$^o$C and separated on a 15% polyacrylamide gel. The MMP-7 AA genotype showed a 150-bp PCR product resistant to enzyme digestion, whereas the MMP-7 GG genotype showed 120bp and 30bp bands. When 10% of samples were randomly selected for confirmation, the results were 100% concordant.

*Statistical Analysis*

Demography of patients and controls were presented as means and standard deviations (SD) or frequencies and percentages. The chi-square ($\chi^2$) goodness of fit test was used for any deviation from Hardy Weinberg Equilibrium. Binary logistic regression was used for all analysis variables to estimate risk as odds ratio (OR) with 95% confidence intervals (CIs) using age and sex as covariates. Risk estimates were calculated for codominant, dominant and recessive genetic models using the most common homozygous genotype as reference. Gene-environment interactions were examined between genotypes and environmental risk factors in all gastric cancer cases as well as in controls. All statistical analyses were performed using SPSS software version 15.0 (SPSS, Chicago, Illinois, USA) and tests of statistical significance were two-sided and differences were taken as significant when P-value was less than 0.05. Bonferoni correction was applied in multiple subgroup comparisons.

## Results

The mean age of healthy subjects (controls) and patients with GC was 57.98yrs± 12.67; 55.91yrs±9.73 respectively (t-test P value=ns). Cancers were highly prevalent in males (83.3% in GC) than in females. In GC patients most of the cases were with adenocarcinoma (ADC, 79.6%). Smoking habit (Hukka) showed significantly higher risk in GC (8.98; 95%CI=5.16-15.63; P=0.0001) patients. Individuals consumed salted-tea in a range of 2-8 cups per day; and median consumption of tea was 4 cups per day. So, we grouped individuals in to ≤4 cups or >4 cups per day and individuals consumed 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 GC (OR=14.78; 95%CI=8.03-27.24; P-value=0.0001). None of patients or controls reported consumption of alcohol, so interaction of alcohol intake with genetic variations could not be analyzed.

*Association of genetic variants of MMP-7 (-181A>G) polymorphism with susceptibility to GC*

The MMP-7 (-181A>G) GG genotype frequency

**Table 1. Frequency Distribution of Genotypes and of MMP-7 (181A>G) Genotypes with Risk of Gastric Cancer**

| Genotypes | Healthy Controls | | Gastric cancer | | |
|---|---|---|---|---|---|
| | N=195 | (%) | N=108 | (%) | OR*(95%CI)P |
| AA | 63 | 32.3% | 29 | 26.9% | 1 (Reference) |
| AG | 92 | 47.2% | 39 | 36.1% | 0.88 (0.49-1.58) 0.67 |
| GG | 40 | 20.5% | 40 | 37.0% | 2.13 (1.13-4.01) 0.02 |
| Dominant model | | | | | |
| AA | 63 | 32.3% | 29 | 26.9% | 1 (Reference) |
| AG+GG | 132 | 67.7% | 79 | 73.1% | 1.25 (0.74-2.12) 0.42 |
| Recessive model | | | | | |
| AA+AG | 155 | 79.5% | 68 | 63.0% | 1 (Reference) |
| GG | 40 | 20.5% | 40 | 37.0% | 2.30 (1.35-3.92) 0.002 |
| P-trend | | | | | 0.01 |

*Age and gender adjusted odds ratio

**Table 2. Association of MMP-7 (-181A>G) Genotypes with Tumor Histopathology and Risk of Gastric Cancer**

| Genotypes | Controls N(%) | GSCC[1] N(%) | OR*(95%CI) ☼P-value | GADC[2] N(%) | OR*(95%CI) P-value |
|---|---|---|---|---|---|
| AA | 63(32.3%) | 2 (9.1%) | 1 (Reference) | 27 (31.4%) | 1 (Reference) |
| AG | 92(47.2%) | 8 (36.4%) | 2.69 (0.55-13.17) 0.23 | 31 (36.0%) | 0.74 (0.39-1.37) 0.34 |
| GG | 40(20.5%) | 12 (54.5%) | 9.34 (1.97-44.33) 0.005 | 28 (32.6%) | 1.58 (0.81-0.81) 0.19 |

[1]Gastric squamous cell carcinoma; [2]Gastric adenocarcinoma; *Age and gender adjusted odds ratio ☼Bonforeni corrected P-values

**Table 3. Interaction of MMP-7 (-181A>G) Genotypes with Salted Tea**

| Genotypes | Controls | | Gastric cancer# | | |
|---|---|---|---|---|---|
| | Tea(Cups)/day<4 | Tea(Cups)/day>4 | Tea(Cups)/day<4 | Tea(Cups)/day>4 | OR*(95%CI) ☼P-Value |
| AA | 51 (32.1%) | 8 (30.8%) | 10 (32.3%) | 17 (24.3%) | 1 (Reference) |
| AG | 74 (46.5%) | 13 (50.0%) | 13 (41.9%) | 25 (35.7%) | 0.89 (0.33-2.29) 0.81 |
| GG | 34 (21.4%) | 5 (19.2%) | 8 (25.8%) | 28 (40.0%) | 3.23 (1.01-10.39) 0.15 |

*Age and gender adjusted odds ratio.# Data missing in some subjects. P-value was calculated only with Tea (Cups)/day>4. ☼Bonforeni corrected P-values

**Table 4. Interaction of MMP-7 (-181A>G) Genotypes with Smoking**

| Genotypes | Controls | | Gastric cancer# | | |
|---|---|---|---|---|---|
| | Non-smokers | Smokers | Non-smokers | Smokers | OR*(95%CI)P-Value |
| AA | 51 (34.7%) | 10 (26.3%) | 13 (40.6%) | 16 (21.3%) | 1 (Reference) |
| AG | 69 (46.9%) | 17 (44.7%) | 6 (18.8%) | 33 (44.0%) | 1.064(0 .419- 2.701)0.896 |
| GG | 27 (18.4%) | 11 (28.9%) | 13 (40.6%) | 26 (34.7%) | 1.731(0.616- 4.865)0.298 |

*Age and gender adjusted odds ratio; # Data missing in some subjects. P-value was calculated only with smokers

was higher in patients as compared to control group. In the present study, when we used the MMP-7 (181A>G) AA genotype as the reference, we found that persons with MMP-7 (181A>G) GG genotype were significantly associated with more than two fold increased risk of GC (OR=2.13; 95% CI =1.13-4.01; p=0.020; P-trend=0.01. Moreover, in recessive model, our results showed that MMP-7 (-181A>G) "GG" allele was conferring significant increased risk for GC (OR=2.30; 95% CI =1.35-3.92; p=0.002) (Table 1).

*Association of MMP-7 (181A>G) genotypes with tumor histopathology*

When tumor histopathologies were analyzed, MMP-7 (-181A>G) GG genotype was found to be significantly associated with increased risk for gastric squamous cell carcinoma (GSCC) (OR=9.34; 95%CI=1.97-44.33; P-value=0.005) (Table 2). However, we did not find significant association in gastric adenocarcinoma.

*Interaction of MMP-7 (181A>G) genotypes with environmental factors*

On analyzing gene environment interaction, we did not find any significant modulation of cancer risk by MMP-7 (-181A>G) genotypes with smoking or excessive consumption of salted tea (Tables 3 and 4).

## Discussion

The present study shows that the -181A>G polymorphism in the MMP-7 promoter significantly increase susceptibility to gastric cancer. Promoter polymorphisms in the matrix metalloproteinase MMP-7 genes have been associated with altered susceptibility to various cancers including gastric cancer in human populations (Murray et al., 1998; Etoh et al., 2000; Ghilardi et al., 2003; Zhang et al., 2005; Hellmig et al., 2006; Vairaktaris et al., 2007; Peng et al., 2010; Yeh et al., 2010). Functional analysis has shown that nuclear proteins derived from differentiated U937 cells bind with higher affinity to the -181G allele than to the -181A allele. This binding difference has been supposed to be related to a putative binding site (NGAAN) for a heat shock transcription factor (HSTF), which exists in the -181G allele but is absent in the -181A allele (Jormsjo et al., 2001). The higher promoter activity of the -181G allele may induce elevation of the MMP-7 mRNA and subsequently increase protein expression. Individuals with excess MMP-7 activity by harboring the -181G allele may predispose to malignant transformation through the 'sheddase' activity of MMP-7 protein, via recently described substrates such as tumor necrosis factor a, E-cadherin and Fas ligand. These substrates have been known to play important roles in signal transduction, cell-cell adhesion and apoptosis (Fingleton et al., 2001; Noe et al., 2001; Vargo-Gogola et al., 2002; Carneiro et al., 2004; Pecina-SLaus et al 2004).

In present study we found that MMP7 -181A>G GG genotype was significantly associated with gastric squamous cell carcinoma. Wu et al., (2006) reported that MMP-7 is highly expressed in metastatic cervical squamous cell carcinoma, and may serve as a marker in estimating the invasive and metastatic potential of cervical squamous cell carcinoma. It may however be added that in the present study, number of samples in squamous cell carcinoma were lower than adenocarcinoma, and it would be desirable to reconfirm this observation in larger sample size of squamous cell carcinoma.

As previously reported, the etiology and incidence of various gastrointestinal tract cancers in Kashmir

population has been attributed to a probable exposure to nitroso compounds, amines and nitrates reported to be present in local food stuffs (Siddiqi et al., 1988; Malik et al., 2010a; 2010b). It has also been suspected that the high concentration of salts present in hot salted tea might cause thermal injury to esophageal and gastric epithelium (Khuroo et al., 1992). In the present study, high consumption of salted tea (>4 cups a day) was independently associated with increased risk for GC (OR=14.78; P=0.0001) but the risk was not modulated by the MMP-7 polymorphism. Similarly, higher risk in smokers also was not further enhanced due to MMP-7 -181 genotypes.

In summary, the present study provided evidence that the MMP-7 (-181A>G) gene polymorphism might be associated with susceptibility to gastric cancer in the Kashmir valley.

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