# No association of *MDM2* T309G polymorphism with susceptibility to Korean gastric cancer patients

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Mouse double minute 2 (Mdm2) acts as a negative regulator of p53 by binding to the amino-terminus of p53. The common T309G polymorphism of *Mdm2* has been the most frequently investigated, which can influence in cancer susceptibility and disease outcome. The specific aim of this study is to investigate whether the T309G polymorphism of *Mdm2* was associated with individual susceptibility to gastric cancer in Korea. The frequency of the polymorphism was examined in 239 gastric cancer patients and 299 healthy controls. Polymorphism analysis was performed by amplifying the first intron of the *Mdm2* and digesting with restriction enzyme and sequencing the products. The frequencies of genotypes: T/T, T/G and G/G were 26.8% (64/239), 46.0% (110/239) and 27.2% (65/239), respectively, in gastric cancer cases and 20.4% (61/299), 50.8% (152/299) and 28.8% (86/299), respectively, in healthy controls. Statistically, there was no significant difference in the frequency of genotype and allele between healthy control and gastric cancer patients. Finally, the polymorphism was not associated with increased risk of gastric cancer in this population. When stratified by histological subtype of gastric cancer, the risk was also not statistically significant. Our findings suggested that the T309G polymorphism of *Mdm2* was not associated with an increased risk for gastric cancer in Korean population.

*Key words: Mdm2*, *p53*, Gastric cancer, Polymorphism, Susceptibility

The p53 protein plays an important role in the negative regulation of cell growth and inactivation of *p53* gene is frequently associated with human cancers [1–3]. In addition, p53 is known to be an important factor of DNA damage induced apoptosis and inactivation of p53 in tumor could influence overall survival in patients with many forms of cancer [4]. Interestingly, *p53* mutations have also been demonstrated in areas of *Helicobacter pylori* (*H. pylori)* associated gastritis [5] and the frequency of *p53* mutations in early and advanced differentiated gastric carcinoma is consistent at around 40% each [6], suggesting that genetic alteration of *p53* gene is an early event in the development of gastric cancer. In addition to *p53* mutations, it is possible that another factor potentially associates with inactivation of *p53* gene during the development of gastric cancers.

The *mouse double minute 2 (Mdm2)* is a known human oncogene that is amplified and overexpressed in various human malignancies [7, 8]. The *Mdm2* gene promotes cell survival and cell cycle progression by inhibiting the p53's transactivating function in the nucleus or by targeting p53 degradation in the cytoplasm [9–11]. Recently, it has been shown that a common polymorphism in the *Mdm2* promoter region, a T to G change at nucleotide 309 in the first intron leads to increased expression of Mdm2 and accelerated tumor formation in hereditary and sporadic cancers [12]. Overproduction of Mdm2, resulting from a naturally occurring single nucleotide polymorphism (SNP), inhibits chromatin-bound p53 from activating the transcription of its target genes, suggesting an oncogenic function of the variant SNP309.

Because of the significance of Mdm2 in human cancer, a large number of epidemiological studies have evaluated the influence of this particular polymorphism in cancer susceptibility and disease outcome. Previous studies have shown that the T309G polymorphism of *Mdm2* influences susceptibility to breast, gastric, liver, colorectal, ovarian, and nasopharyngeal cancers [6, 13–18]. In Japanese population, it has been reported that the polymorphism is associated with increased

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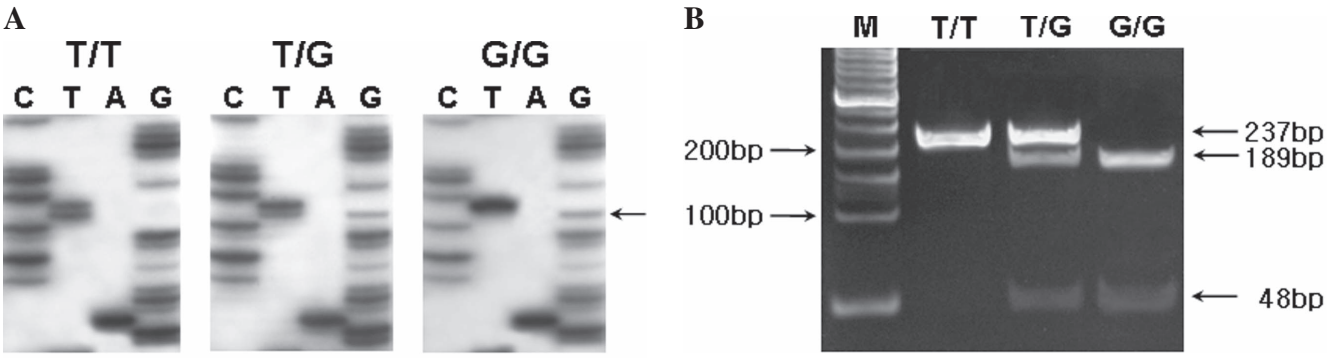


**Figure 1. Genotypes analysis of T309G polymorphism of the *Mdm2* gene: Sequencing (A) and RFLP patterns (B) of homozygote T/T and G/G, and heterozygote with T/G (arrow).**

susceptibility to gastric carcinoma and poor prognosis [14]. Although the majority of studies link the G-allele to an oncogenic function and an increased cancer risk, some studies have implicated the G-allele in increased cancer risk or found no association at all [19, 20].

In the present study, we investigated the association of the T309G polymorphism of *Mdm2* with individual susceptibility to gastric cancer in Korea. In addition, we studied whether the polymorphism was associated with the histological classification of intestinal- and diffuse-type of gastric cancer.

## Materials and methods

*Tissue samples.* Archival normal gastric mucosa specimens from 239 gastric cancer patients who had undergone surgery at the College of Medicine, The Catholic University of Korea in Seoul, between 2000 and 2003 were included in this study. All neoplasms were stomach adenocarcinomas. The 239 cases included 154 men (64.4%) and 85 women (35.6%) with a median age of 61 (22–85) years at initial diagnosis. Histologically, the cancers consisted of 126 intestinal-type (52.7%) and 113 diffuse-type (47.3%) gastric cancers. The healthy control population consisted of 164 males and 135 females with a mean age of 45. To exclude ethnic differences, only Korean population was included in this study. Informed consents were obtained according to the Declaration of Helsinki. This study was approved by the institutional review board of the Catholic University of Korea, College of Medicine.

*DNA extraction.* In Gastric cancer patients, normal cells were obtained from the cancer-free gastric mucosa. The DNA extraction was performed by a modified single-step DNA extraction method, as was described previously [20]. For the control population, a leukocyte cell pellet from each blood sample was obtained from the buffy coat by centrifugation of 2 ml of whole blood. The cell pellet was used for DNA extraction. The Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) was used according to the manufacturer's instructions to obtain genomic DNA. The DNA purity and concentration were determined by Nanodrop® ND-1000 spectrophotometer (Nanodrop technologies, Wilmingon, DE, USA).

*RFLP and DNA sequencing.* Genomic DNAs from gastric mucosal tissues and blood were amplified with primers covering the *Mdm2* polymorphism in intron 1 as previously [14]. Each polymerase chain reaction (PCR) procedure was performed under standard conditions in a 10 μl reaction mixture containing 1 μl of template DNA, 0.5 μM of each primer, 0.2 μM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 0.4 unit of Ampli Taq gold polymerase (Perkin-Elmer, Foster City, CA, USA), and 1 μl of 10X buffer. The reaction mixture was denatured for 12 min at 95°C and then incubated for 35 cycles (denaturing for 30 s at 95°C, annealing for 30 s at 62°C and extension for 30 s at 72°C). A final extension step was performed for 5 min at 72°C. After amplification, the PCR products were digested with 5 U of restriction enzyme *MSPAI*1 at 37°C for 16 hr. DNA fragments were then electrophoresized on a 12% polyacrylamide gel (Fig. 1A). To ensure the reliability of the results of restriction fragment length polymorphism, sequencing of the PCR products was carried out using the cyclic sequencing kit (Perkin-Elmer, Foster City, CA, USA) according to the manufacturer's recommendation (Fig. 1B).

*Statistical analysis.* The chi-square test for association was used to test differences of genotype frequencies between normal controls and gastric cancer patients, and between the two histological types. The genotype specific risks were estimated as odds ratios and 95% confidence intervals (CI).

## Results

The genotype frequencies of T309G polymorphism of *Mdm2* in Korean gastric cancer cases and controls are summarized in table 1. The frequencies of genotype T/T, T/G and G/G in normal healthy individuals were 20.4%, 50.8% and 28.8%, respectively. The frequencies of T and G alleles were 45.8% and 54.2% in healthy control individuals, respectively. Genotype frequencies of all groups were in accordance with those previously reported in the Japanese population [13].

**Table 1. Distribution of *MDM2* genotype and allele frequency in gastric cancer patients and controls**

| *MDM2* genotype | Cases (n=239) | | Controls (n=299) | | Crude OR (95% CI) | Adjusted[a] OR(95% CI) |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | | |
| TT | 64 | 26.8 | 61 | 20.4 | 1.00 | 1.00 |
| TG | 110 | 46.0 | 152 | 50.8 | 0.690 (0.450-1.058) | 0.836 (0.490-1.427) |
| GG | 65 | 27.2 | 86 | 28.8 | 0.720 (0.447-1.160) | 0.814 (0.451-1.470) |
| T:G allele frequency[b] | 238:240 | | 274:324 | | | |
| Trend test [c] | | | | | 0.856(0.674-1.086) | 0.906(0.674-1.216) |

[a] Adjusted for age (in year) and sex

[b] Two-sided $\chi^2$– test: for allele frequencies, $P = 0.1949$ ; for genotype distribution, $P = 0.2152$

[c] Calculated in the logistical regression model using the number of T alleles in the genotypes as a continuous variable.

**Table 2. Stratification analysis of *MDM 2* genotype frequency in gastric cancer.**

| Variable | *MDM2* genotype | | | | | | Adjusted OR[a] (95% CI) | |
|---|---|---|---|---|---|---|---|---|
| | No. of cases | | | No. of controls | | | TT versus TG | TT versus GG |
| | TT | TG | GG | TT | TG | GG | | |
| Age (in years) | | | | | | | | |
| ≤50 | 11 | 25 | 13 | 45 | 129 | 70 | 0.779 (0.354-1.715) | 0.749 (0.308-1.819) |
| >50 | 53 | 85 | 52 | 16 | 23 | 16 | 1.120 (0.541-2.321) | 1.023 (0.461-2.271) |
| Sex | | | | | | | | |
| Male | 42 | 74 | 38 | 38 | 79 | 47 | 1.211 (0.592-2.476) | 0.902 (0.408-1.994)[b] |
| Female | 22 | 36 | 27 | 23 | 73 | 39 | 0.511(0.224-1.165) | 0.711 (0.292-1.734) |
| Lauren's | | | | | | | | |
| Diffuse | 30 | 54 | 29 | | | | | |
| Intestinal | 34 | 56 | 36 | $P = 0.8462$ ($\chi^2$– test) | | | | |

[a] Adjusted for the other covariates [age (in years) as a continuous variable] presented in this table in a logistic regression model for each stratum.

[b] Unadjusted OR (95% CI) adding 0.5 to all response categories due to zero cell.

Statistically, there were no significant differences in genotype and T/G allele frequencies of the polymorphism in comparisons between male and female in healthy controls (Chi-Square test, $P = 0.3918$ & 0.437) (Table 2).

For the gastric cancer patients, the T/T, T/G and G/G genotypes had a prevalence of: 26.8% (64/239), 46.0% (110/239) and 27.2% (65/239), respectively, and the frequencies of T and G alleles were 49.8% and 50.2%. Finally, we did not observe a significant difference in the genotype and allele frequencies between the gastric cancer patients and healthy controls (Table 1). There was also no statistically significant difference in the genotype and allele frequencies in comparisons between male and female gastric cancer patients (Chi-Square test, $P = 0.4872$ & 0.375). In addition, elevated risk for gastric cancer was not statistically significant even in patients having already lymph node metastases. When stratified by histological subtype and age at initial diagnosis of gastric cancer, the risk was not statistically significant (Chi-Square test, $P = 0.8462$ & 0.8145) (Table 2).

## Discussion

Single positions of variation in DNA, called SNP, are feasible to identify genetic predisposition to disease and to give insight into the gene involved in the disease process. Genetic factors, including polymorphisms of genes involved in tumorigenesis, may partly explain the difference in individual susceptibility to cancer.

The *p53* tumor suppressor is found to be mutated and abundant in a wide variety of tumors. It negatively regulates the cell cycle, and requires loss of function mutations for tumor formation [21]. Loss of p53 function could result from mutation, allelic loss, and genetic polymorphism. The incidence of *p53* mutations ranges from a low of 0% to a high of 76.9% in invasive gastric carcinomas, suggesting that there are conflicting results with respect to the prevalence of *p53* mutations [22]. At least 10 different polymorphisms have been detected in the human genomic *p53* [23] and a polymorphism at codon 72 is closely associated with a prolonged survival of patients with gastric adenocarcinoma [24]. Interestingly, the *Mdm2* oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation [10]. Recent studies have shown that a SNP (T309G) in the intronic promoter region of *Mdm2* can significantly result in an eight-fold increase in the expression of *Mdm2* mRNA and thereby suppress the p53 pathway [12]. In addition, it has been shown that the Mdm2 protein encoded by the homozygous variant SNP309 genotype (G/G) may more efficiently target the p53 protein for degradation [6] and accelerated tumor formation in hereditary and sporadic cancers [12]. Despite the fact that the G allele

of SNP309 is a highly plausible cancer predisposing allele in gastric cancers and an independent marker of poor overall survival in gastric cancer [13], no significant difference in genotype and allele frequency of the T309G polymorphism of *Mdm2* was found in this population. For the *Mdm2* gene, the polymorphism frequency in the Korean population is approximately 45.8% for T and 54.2% for G; the frequencies of each allele were similar to those reported in the Japanese population and no significant increase in gastric carcinoma risk was observed among the three genotypes [13]. These findings suggest that the SNP309 may not contribute to individual risk for the development and/or progression of gastric cancer in Korean population (Table 1). Further studies for the different genetic background which varies from one geographic region or population to another may be very important for elucidate the susceptibility to gastric cancer.

*H. pylori* infection has been considered a major factor in gastric carcinogenesis [25]. Molecular, histological and epidemiological studies have provided evidence that gastric adenocarcinoma is a heterogeneous disease with two main histological types: the intestinal- and the diffuse-type [26, 27]. Although both types seem to start from *H. pylori*-related chronic gastritis [25], epidemiologic and histopathological evidence have shown that gastric carcinoma may be influenced by genetic polymorphisms, age of cancer onset and gender. A significant increase of Mdm2 protein expression was found *H. pylori*-infected gastric mucosa; but successful eradication of *H. pylori* dramatically reduced the Mdm2 protein level [28]. When the patients were stratified by histological type, there were no significant differences in the genotype and allele frequencies of the polymorphism. Thus, our findings suggest that the SNP309 polymorphism of *Mdm2* may not affect the histologic type of gastric cancer in this population. Further studies in a large population with other risk factors, including SNP in inflammation-related genes, should be performed to confirm this initial observation.

In conclusion, the present study demonstrated that gastric cancer does not appear to be associated with the *SNP309* of *Mdm2* gene in Korean population. In addition, we also found no association of gastric cancer with the polymorphism studied when patients were sorted by histological type. Further molecular genetic studies should be performed in a large population to identify the mechanisms associated with Mdm2 activation.

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