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RESEARCH

Association and Prognostic Significance of the Functional −1562C/T Polymorphism in the Promoter Region of MMP-9 in Turkish Patients with Gastric Cancer

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Abstract Matrix metalloproteinases (MMPs) are a group of zinc-dependent peptidases that participate in matrix turnover in solid malignancies. The aim of this study was twofold. First, we sought to investigate under a case–control design the association between the functional −1562C/T polymorphism in the promoter region of MMP-9 and gastric cancer (GC) in a Turkish sample. Second, we examined its prognostic significance in GC patients. A total of 144 subjects were enrolled in the case–control study (79 GC cases and 65 controls). Overall survival (OS) and progression-free survival (PFS) served as the main outcome measures in the longitudinal study. The MMP-9 −1562C/T polymorphism was genotyped using a polymerase chain reaction-restriction fragment length polymorphism method. The odds ratio (OR) of GC for the CC genotype relative to the CT+TT genotypes was not significant (OR=0.89, 95 % confidence interval [CI]=0.44–1.82, P=0.75). These results did not change after allowance for age and sex in multivariable regression analysis (OR=0.81, 95 % CI=0.40–1.94, P=0.84). When the MMP-9 −1562C/T polymorphism was analyzed among GC patients in relation to OS and PFS, we found no significant differences between subjects with the CC and CT+TT genotypes. In conclusion, the results of our study did not point toward a major role of the MMP-9 −1562C/T polymorphism in the pathogenesis and clinical course of GC in Turkish subjects.

Keywords Gastric cancer · Matrix metalloproteinases · Polymorphism · Association study · Prognosis

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Introduction

Although steady declines in gastric cancer (GC) incidence rates have been observed worldwide in the last few decades, this malignancy continues to remain one of the leading causes of cancer-related mortality worldwide [1–3]. The incidence of GC shows marked geographical variation, with over 50 % of all new cases occurring in developing countries [3]. The main high-risk areas for GC include Eastern Europe, Central and South America, and East Asia (China, Japan) [4]. From a pathophysiological standpoint, a complex combination of environmental factors and genetic alterations is deemed to play a role in GC, although the interactions of such events may vary from one individual to another [5, 6].

Matrix metalloproteinases (MMPs) are proteolytic enzymes that contribute to tissue growth and remodeling [7, 8]. Importantly, a growing amount of evidence has suggested that MMPs may contribute to tumor spread by degrading components of the extracellular matrix (ECM), including collagens, elastins, and matrix glicoproteins [9, 10]. MMP-9, a member of the MMPs family, plays a key role in the breakdown of ECM occurring both in local tumor invasion and distant metastasis [11]. Importantly, recent meta-analyses reported that an increased MMP-9 protein expression might be a factor for a poor prognosis in patients with gastric cancer [11, 12]. Interestingly, MMP-9 expression may be influenced in an allele-specific manner by a functional −1562C/T single-nucleotide polymorphism (rs3918242) located in the MMP9 gene promoter [13–15]. Owing to its functional effect, numerous studies have recently investigated the potential association between the MMP-9 −1562C/T polymorphism and the risk of GC. However, a recent meta-analysis focusing on 14 reports covering 8146 patients (2980 GC cases and 5166 controls) failed to identify a significant association between this polymorphism in the promoter region of MMP-9 with the risk of GC under both dominant or recessive models [16]. Because the association between specific polymorphisms and disease states may vary in different populations [17], we designed the current study to investigate under a case–control design the association between the functional −1562C/T polymorphism in the promoter region of MMP-9 and GC in a Turkish sample. We also examined its prognostic significance in our GC cohort.

Materials and Methods

Study Participants

The study sample for this case–control study consisted of 144 unrelated subjects of Turkish descent, including patients with GC (CC cases, n=79) and subjects without GC (controls, n=65). All of the patients in the GC group underwent surgery and had a pathologically-confirmed diagnosis. The general characteristics of the study participants were collected from clinical charts. The control subjects were apparently healthy subjects, without a personal or family history of GC and no history of gastrointestinal symptoms. The local Ethics Committees approved the study, and written informed consent was obtained from all participants. The study was conducted in compliance with the tenets of the Declaration of Helsinki.

Genotyping

Genomic DNA was purified from peripheral blood samples using a commercially available DNA isolation kit (Dr. Zeydanlı Life Sciences Ltd, Ankara, Turkey) according to the manufacturer's recommendations. The procedure for detecting the the MMP-9 −1562C/T polymorphism was based on PCR amplification (forward primer: 5′- GCCTGGCACATAGTAGGCCC-3′; reverse primer: 5′-CTTCCTAGCCAGCCGGCATC-3′) and restriction cleavage with *SphI* (New England Biolabs, Ipswich, MA, USA) as previously described [18]. PCR amplification conditions were as follows: initial denaturation at 95 °C for 4 min followed by 35 cycles of 60 s denaturation at 94 °C, 60 s annealing at 64.5 °C, and 60 s extension at 72 °C, with a final extension at 72 °C for 7 min. PCR products were analyzed by electrophoresis in a 2 % agarose gel alongside with a DNA ladder. The gel was then stained with ethidum bromide for 20 min and finally visualized in a gel documentation system. The separation of the CC genotype resulted in a single undigested fragment of 436 bp, whereas the homozygous TT genotype resulted in two fragments of 242 and 194 bp. Finally, the digestion of the heterozygous CT genotype produced three fragments of 436, 242, and 194 bp (Fig. 1). For quality control, genotyping analyses were done blind with respect to case/control status, and a random 25 % of the samples were repeated. The concordance rate for duplicate genotyping was 100 %. Two investigators independently reviewed all results.

Data Analysis

Continuous data were expressed as mean±SD and compared using the Student's t-test or analysis of variance (ANOVA), as appropriate. Genotypes were obtained by direct counting followed by allele frequency calculations. We tested the Hardy–Weinberg equilibrium using online resources (http://www.husdyr.kvl.dk/htm/kc/popgen/genetik/applets/kitest.htm) [19, 20]. Genotype frequencies and categorical variables were compared using the χ^2 test. We used multivariable logistic regression analysis to analyze the association between the MMP-9 −1562C/T polymorphism and GC after adjusting

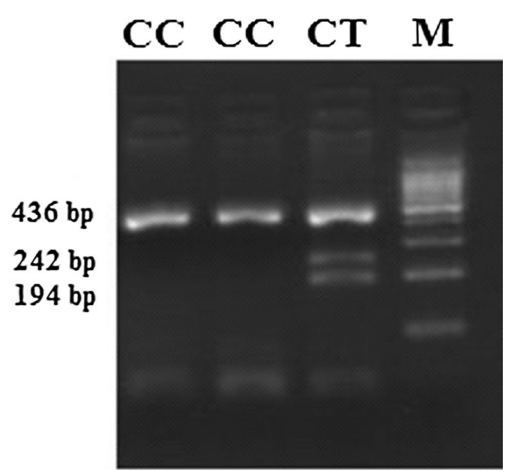

Fig. 1 PCR-RFLP of the −1562C/T polymorphism in the promoter region of MMP-9 gene using restriction cleavage with *SphI*. The separation of the CC genotype resulted in a single undigested fragment of 436 bp, whereas the heterozygous CT genotype produced three fragments of 436, 242, and 194 bp. M = molecular ladder

for age and sex. Results were expressed as odds ratios (OR) with their 95 % confidence intervals (CI). Overall survival (OS) was defined as the time from study entry to death, irrespective of cause. Progression-free survival (PFS) was measured from the date of randomization to the date of study entry to the first documented disease progression or the date of death due to any cause, if death occurred progressive disease. Survival curves were plotted using the Kaplan-Meier method and compared with the log-rank test. Statistical calculations were performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The statistical power was calculated using with the StatMate software, version 2.0 (GraphPad, San Diego, CA, USA). Two-tailed P values<0.05 were considered statistically significant.

Results

General Characteristics of the Study Participants

The mean age did not differ significantly between GC patients (57.9±10.7 years) and controls (56.0±11.6 years, P=0.30). Moreover, the prevalence of male sex did not differ significantly between GC cases (60/19) and controls (43/22, P=0.20). Among the 79 GC patients, 16 (20.2 %) underwent total gastrectomy, whereas the remaining 83 (79.8 %) received partial gastrectomy. Tumor localization was as follows: gastroesophageal junction (n=24, 30.4 %), corpus/fundus (n=27, 34.2 %), antrum/pylorus (n=19, 24.0 %), and unknown (n=9, 2.4 %). Histological subtype was adenocarcinoma in 53 patients (67.1 %), signet ring cell carcinoma in 25 patients (31.6 %), and unkwown in 1 patient (1.3 %). GC was well-differentiated in 52 patients (65.8 %), moderately differentiated in 18 patients (22.8 %), and poorly differentiated in 9 patients (11.4 %). Vascular invasion was noted in 14 study participants (17.7 %), whereas lymph invasion was noted in 27 patients (34.2 %). The TNM stage at diagnosis was as follows: 11 patients (13.9 %) had stage 1, 11 (13.9 %) had stage II, 22 (27.8 %) had stage III, and 35 (44.4 %) had stage IV disease.

Distribution of the MMP-9 −1562C/T Polymorphism in GC Cases and Controls

Table 1 displays the distribution of the functional −1562C/T polymorphism in the promoter region of MMP-9 in GC cases and controls. Genotype frequencies in this study were in Hardy-Weinberg equilibrium both in GC patients (χ^2=0.87; P=0.35) and controls (χ^2=0.26; P=0.61). The odds ratio of GC for the CC genotype relative to the CT+TT genotypes was not significant (OR=0.89, 95 % CI=0.44–1.82, P=0.75). These results did not change after allowance for age and sex in multivariable regression analysis (OR=0.81, 95 % CI=0.40–1.94, P=0.84).

Prognostic Significance of the MMP-9 −1562C/T Polymorphism in GC Cases

In the entire cohort of GC patients, the mean OS was 1.4 years (interquartile range: 0.6–1.6 years), whereas the mean PFS was 0.8 years (interquartile range: 0.4–0.9 years). When the MMP-9 −1562C/T polymorphism was analyzed among GC patients in relation to OS, we found no significant differences (log-rank test, P=0.41), between subjects with the CC genotype (1.6±1.9 years) and those with the CT+TT genotypes (2.0±1.5 years, Fig. 2). Similarly, no significant differences (log-rank test, P=0.38) were found in terms of PFS in subjects with the CC genotype (1.3±1.0 years) compared with those bearing the CT+TT genotypes (1.7±1.1 years, Fig. 3)

Discussion

Growing evidence suggests that MMP-9, a major member of MMPs, may contribute to cancer invasiveness [9–12]. However, our current findings indicate that the functional −1562C/T polymorphism in the MMP-9 gene was associated with neither susceptibility to GC nor with its prognosis in Turkish subjects. For the purpose of this study, we selected this well-defined single nucleotide polymorphism because it has been previously shown to have functional consequences [13–15].

Table 1 Distribution of the MMP-9 −1562C/T polymorphism in patients with gastric cancer and healthy controls

	GC cases (n=79)	Controls (n=65)
CC	54 (68.3 %)	46 (70.8 %)
CT	24 (30.4 %)	18 (27.7 %)
TT	1 (1.3 %)	1 (1.5 %)
Odds ratio (95 % confidence interval) [CC vs. CT+TT]	0.89 [0.44–1.82], P=0.75	

Data are expressed as counts and percentages

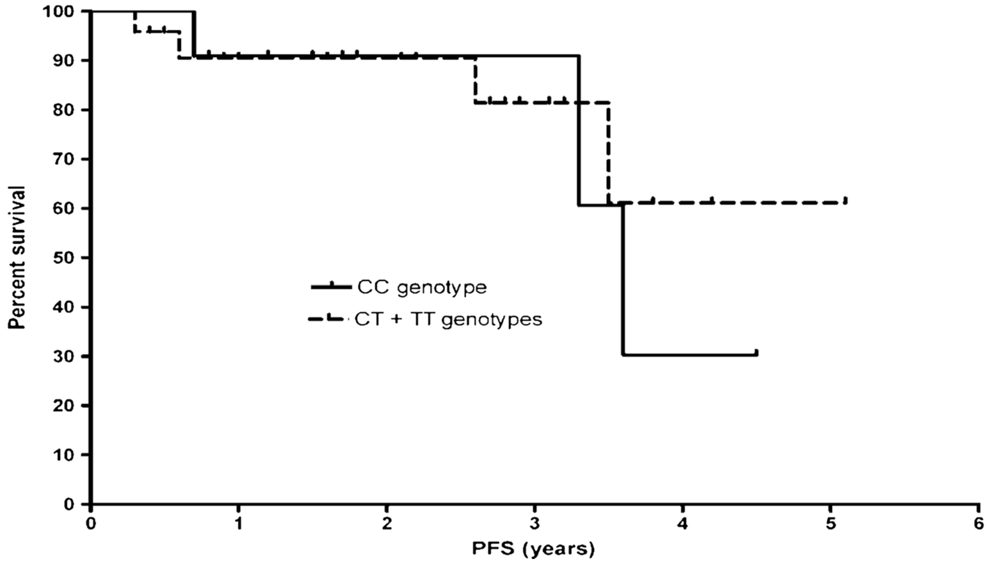

Fig. 2 Kaplan-Meier curve of progression-free survival in gastric cancer patients according to the functional −1562C/T polymorphism in the promoter region of MMP-9 gene. No significant difference in overall survival could be observed for patients with the CC genotype compared with those bearing the CT+TT genotypes (P=0.38). Survival time on the x-axis is depicted in years

Based on the observed prevalence of the minor T allele, our study had 80 % power to detect a relative risk of 1.85 with a significance level (alpha) of 0.05 (two-tailed) between CC cases and controls.

Several studies have previously investigated the potential association between the −1562C/T polymorphism in the MMP-9 gene and different types of solid malignancies. For example, this common genetic variant has been associated with an increased risk of prostate cancer [21], breast cancer [22], and hepatocellular carcinoma [23]. However, the results of our study did not point toward a major role of the MMP-9 −1562C/T polymorphism in the pathogenesis and clinical course of GC in Turkish subjects. Our current findings are in line with those of a recent meta-analysis that failed to identify a significant association between this polymorphism and the risk of GC under both dominant or recessive models [16]. However, we cannot exclude that our negative findings might be due to the small sample size. Notably, the minimum detectable difference in our cohort of 144 subjects was a relative risk of 1.85 (power=0.80; alpha level=0.05), making potentially smaller differences undetactable. In any case, our longitudinal study confirmed that the MMP-9 −1562C/T polymorphism is unlikely to be a major prognostic factor in GC, although we cannot exclude that this gene could have minor effects on the clinical course. In our study, the distribution of the selected polymorphism was in Hardy-Weinberg equilibrium, suggesting the absence of genotyping errors and/or selection bias. As is the case for any malignancies, other factors may be involved

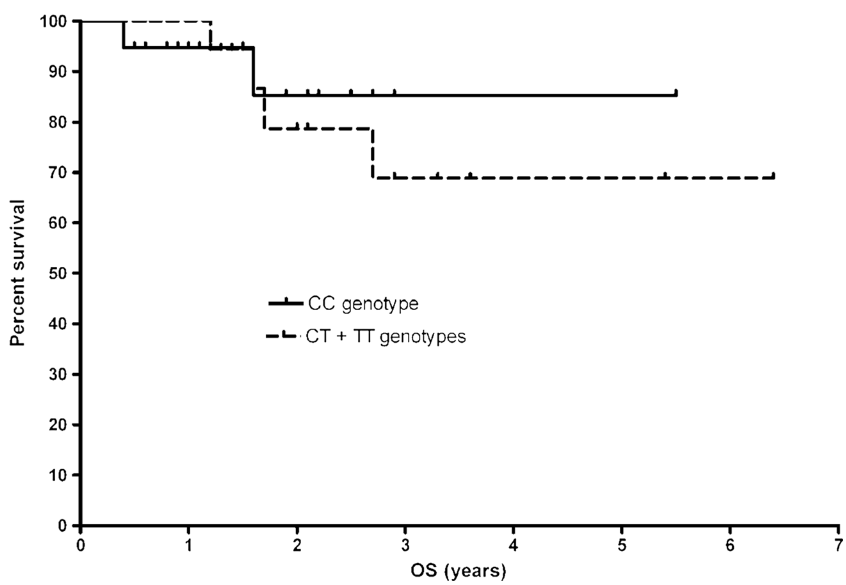

Fig. 3 Kaplan-Meier curve of overall survival in gastric cancer patients according to the functional −1562C/T polymorphism in the promoter region of MMP-9 gene. No significant difference in overall survival could be observed for patients with the CC genotype compared with those bearing the CT+TT genotypes (P=0.41). Survival time on the x-axis is depicted in years

in the pathogenesis of GC besides genetic predisposition, including well-known environmental and host factors [1–3].

Several caveats inherent in this study merit consideration. First, the number of participants was relatively small. We thus believe that our findings should be considered explorative, and need to be confirmed in larger sample sizes. Second, as our study cohort consisted only of Turkish subjects without ethnical diversity, these results require replication in different ancestry populations.

Third, it should be noted that our study shares the limitations of case–control investigations with regard of population stratification risk. Moreover, because we did not have information on environmental factors such as *Helicobacter pylori* infection, we could not assess the interactions between such variables and the MMP-9 −1562C/T polymorphism. Fourth, Genetic and non-genetic factors may cause intratumoral heterogeneity. This heterogeneity will effect cell growth, differentiation, migration, angiogenesis on tumor, so it can change the prognosis. This study should be supported by case studies showing intratumoral homogeneity. Finally, we are aware that the use of only one single nucleotide polymorphism in this report negates the exploration of significant interactions among distinct polymorphisms in a given haplotype. As several polymorphisms have been identified in the MMP-9 gene [16], a larger number of genetic markers should be examined to shed more light on the putative association of this gene with GC.

In summary, the results of our study did not point toward a major role of the MMP-9 −1562C/T polymorphism in the pathogenesis of GC. Indeed, we failed to identify a significant association of this common genetic variant with the risk of GC. Moreover, this polymorphism was not found to have prognostic significance among our GC patients. We conclude that role of the gene encoding MMP-9 in predisposing to GC is not definitely established.

Competing Interests The authors declare that they have no competing interests.

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