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Lack of Association between Matrix Metalloproteinase-1 (MMP-1) Promoter Polymorphism and Risk of Renal Cell Carcinoma

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ABSTRACT

*Objective: I*nvestigate the possible association of insertion/deletion (2G/G) polymorphism at nucleotide -1607 of the MMP-1 promoter with the development and progression of renal cancer.
Materials and Methods: In this study, we genotyped 217 individuals, 99 patients with renal cell carcinoma (RCC) and 118 controls without cancer. DNA specimens were extracted from epithelial buccal cells and paraffin-embedded tissue of RCC patients and from epithelial buccal cells and blood cells of healthy controls.
Results: The difference in frequency of 2G/2G genotype between controls (22.9%) and RCC patients (28.6%) was not statistically significant (p = 0.461). We also did not find correlation between 2G/2G and histological type of RCC. The comparison of genotype distribution and frequency of 2G allele in different populations showed a strong variability of 2G allele frequency among the different ethnic groups. This fact may influence on the collaboration of this 2G allele in RCC or others diseases.
Conclusion: Our data suggest that the matrix metalloproteinase-1 (MMP-1) promoter polymorphism may not play a significant role in renal cell carcinoma patients in Brazil.

***Key words**: MMP-1; polymorphism; renal cell carcinoma*
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INTRODUCTION

The development of cancer is a complex, multistage process during which a normal cell undergoes genetic changes that result in phenotypic alterations and the acquisition of the ability to invade and colonize distant sites (1,2). Although many factors are involved in tumor development, interactions between neoplastic cells and the surrounding microenvironment are crucial to each step of tumorigenesis. The MMP family comprises over 20 enzymes that are associated with degradation of the ECM, including the basement membrane, as their name implies (3). Among the MMPs, matrix metalloproteinase-1 (MMP-1, col-

lagenase-1), is the most highly expressed interstitial collagenase degrading fibrillar collagen, the most abundant protein in the human body (4). MMP-1 expression level is increased in several diseases such as arthritis (5), periodontitis (6) arteriosclerosis (7) and cancer (8,9).

Recently, an insertion/deletion (2G/G) polymorphism was reported at nucleotide -1607 relative to the transcription site of the MMP-1 gene (9). The 2G polymorphism creates a binding site (5'-GGA-'3) for the ETS transcription factor, influencing its transcriptional activity (10,11) Promoter containing 2G allele displays a significantly higher transcriptional activity than 1G promoters (8).

The 2G allele of the MMP-1 promoter polymorphism is relatively common and has a frequency of a little less than 50% of the general population (12). Association studies have been done to determine whether the MMP-1 genotype affects the risk of different types of cancers (5,8,9,11-16).

Renal cell carcinoma accounts for 2 percent of all cancers. Renal cell carcinoma originates in the cortex and accounts for 80 to 85 percent of malignant kidney tumors (17). This carcinoma occurs nearly twice as often in men than in women. Patients are generally over 40 years old at diagnosis, and the disease occurs predominantly in the seventh and eighth decades of life (18). However, small, localized tumors rarely produce symptoms, and for this reason, the diagnosis is often delayed until after the disease is advanced. To improve the prognosis of this disease it is important to clarify the molecular mechanism of invasion and metastasis of renal cell carcinoma.

The aim of this study was to investigate possible correlations between MMP-1 promoter and renal cell carcinoma (RCC) in a Brazilian group.

MATERIALS AND METHODS

Subject selection - This case-control study consisted of 99 patients with renal cell carcinoma (56 men, 43 women mean age 59.97 years) and 118 population-derived, age-matched controls (62 men, 56 women; mean age 60.5 years), all being ethnic Brazilian. This study protocol was approved by the institutional review board. At recruitment, written informed consent was obtained from each subject.

All of the RCC patients were diagnosed histologically and tumors were staged according to the 1997 TNM classification system (19) and graded according to the Fuhrman classification system (20).

Sample acquisition - DNA samples of the patients were obtained from formalin-fixed paraffin-embedded tissue (23 patients), epithelial buccal cells (60 patients) and blood cells (16 patients). Patients were recruited between 2004 and 2006, at the São Paulo Hospital and Public Servant Hospital of São Paulo, Brazil (São Paulo, Brazil), and histopathologically confirmed as a renal cell carcinoma. All DNA samples of the control group were extracted from epithelial buccal cells.

Paraffin-embedded tissue - 10-µm sections were obtained from paraffin-embedded tissue. The sections were deparaffinized by immersing twice in xylene for 2 min, followed by twice 99.5% ethanol for 2 min, and further two times 70% ethanol. Thereafter the samples were digested with 1,000µL 0.1M Tris-HCl (pH 8.0), 0.5 M NaCl, 0.05 M EDTA, 1% sodium dodecyl sulfate (SDS), and 1 unit of proteinase K at 55°C overnight. Then the samples were mixed well, and centrifuged at 10,000 X g for 15 min. The DNA was extracted with phenol/chloroform (1:1). DNA was precipitated by adding cold ethanol, centrifuged and resuspended in 50µL water (21,22).

Epithelial buccal cells - The ephitelial buccal cells were extracted with 100 ng/mL proteinase K (Sigma Chemical Co.) at 37°C for 1 hour. DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation. DNA was dissolved in 70µL TE buffer (10 mm Tris (pH 7.8), 1mm EDTA), and its concentration was determined by measurements of OD 260 (6).

Statistical analysis - Differences in the genotypes distribution from those expected by the Hardy-Weinberg equilibrium and the significance of differences in the observed frequencies of SNP in both groups were assessed by χ^2 test. We also used the χ^2 test to compare the distribution of genotype and frequency of G2 allele in different populations. T Stu-

Table 1 – *Characteristics of cases and controls.*

	Cases (N = 99)	**Controls** (N = 118)	**p Value**
Male/Female	58/46	62/56	0.726 (a)
Age (mean ± SD)	60.54 ± 13.68	59.90 ± 12.50	0.623 (b)
Smokers (N/%)	30 (28.85%)	29 (24.58%)	0.345 (a)

a = chi square test, b = student t test.

dent test and Fisher test were employed to evaluate the homogeneity of control and case populations. For all tests, the p-values of 0.05 were regarded significant.

RESULTS

We studied a total of 217 individuals: 99 renal cancer patients and 118 non-cancer controls. The baseline characteristics of the patients and controls are summarized in Table-1.

The genotypes of all subjects were clearly determined by PCR-RFLP. Figure-1 shows the RFLP pattern after digestion with BGL II and after digestion with Alw I.

Polymorphism distribution in the control and case population was according to the Hardy-Weinberg principle ($\chi^2 = 0.00$, p = 1.000 and $\chi^2 = 0.04$, p = 0.981 respectively).

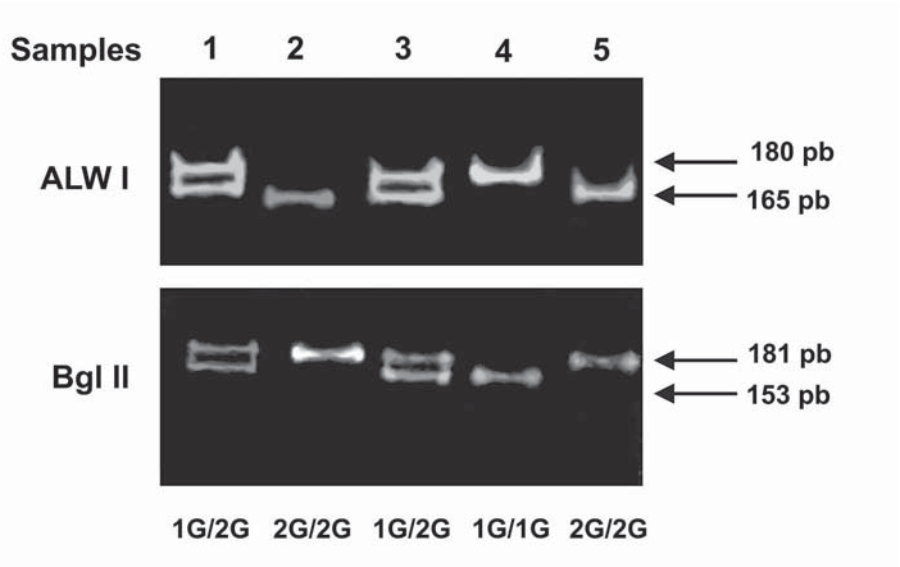

Figure 1 – *RFLP analyses of MMP-1 promoter polymorphism. ALWI digests the 181 bp 2G amplicon (166 bp + 15 bp), leaving the 1G amplicon (180 pb) intact. Bgl II digests the 180 pb 1G (153 bp + 27 bp), leaving the 2G amplicon (181 bp) intact. Samples 1 and 3 are 1G/2G heterozygotes. Samples 2 and 5 are 2G/2G homozygotes. Sample 4 is 1G/1G homozygote.*

Table 2 – *Pathological characteristics of 99 RCC patients.*

		N (%)
Histological type	Clear cells	75 (75.8)
	Chromophobe	15 (15.1)
	Papillary	7 (7.0)
	transitional cell carcinoma	2 (2.0)
TNM stage (a)	T_1	36 (36.4)
	T_2	20 (20.2)
	T_3	27 (27.3)
	T_4	16 (16.2)
Fuhrman grade (b)	G1	9 (9.1)
	G2	55 (55.6)
	G3	17 (17.2)
	G4	3 (3.0)

a = tumor stage according to TNM (20); b = Tumor stage according to the 1997 grading system (19).

We analyzed the correlation between tobacco smoking and RCC and no association was found (p = 0.345) (Table-1).

In the renal cancer group, allele frequency of 2G was 51%, in comparison with 49.0% in control group, did not show any significant statistic difference (p = 0.48). The genotype distribution in RCC patients and controls are shown in Table-2. The frequency of the 2G/2G genotype showed in Table-3 in patients (29.3%) did not significantly differ from the values for normal controls (22.9%) (p = 0.461). Further, we analyzed the a possible correlation between pathological data and genotype frequency of MMP-1 polymorphism in RCC patients and we also found no correlation (Table-4).

In Table-5 we compared the distribution of the MMP-1 genotype in our controls with the data previously reported for other study populations (9,15,16,23-26). Chi-square analysis indicated significant differences in genotype distributions of MMP-1 -1607 promoter between Brazilians and reported data on Japanese (p = 0.003 and p < 0.001), Tai-

Table 3 *– Distribution of MMP-1 polymorphism in case and controls.*

	Cases		Controls		
Genotype	**N**	**%**	**N**	**%**	**p Value ***
2G/2G	29	28.6	27	22.9	
1G/2G	42	48.8	59	50.0	0.461
1G/1G	28	22.6	32	27.1	
Total	99	100	118	100	

** = p Value obtained by chi-square test.*

Table 4 *– Correlation between pathological data and genotype frequency of MMP-1 polymorphism in RCC patients.*

		Genotype			
		2G/2G N (%)	**1G/2G N (%)**	**1G/1G N (%)**	**p Value**
Histological type	Clear cells	22 (73.4%)	29 (70.7%)	24 (85.7%)	0.618 (c)
	Chromophobe	4 (13.4%)	8 (20%)	3 (10.7%)	
	Papillary	2 (6.7%)	4 (10%)	1 (3.6%)	
	Transitional cell carcinoma	2 (6.7%)	0 (0.0%)	0 (0.0%)	
TNM stage (a)	T_1	10 (34.5%)	14 (33.3%)	3 (12.5%)	0.273 (d)
	T_2	5 (17.2%)	8 (19.1%)	16 (66.7%)	
	T_3	8 (27.6%)	16 (38.1%)	4 (16.7%)	
	T_4	6 (20.7%)	4 (9.5%)	6 (21.4%)	
Fuhrman grade (b)	G1	2 (8.3%)	4 (11.1%)	3 (12.5%)	0.803 (c)
	G2	15 (62.5%)	24 (66.7%)	16 (66.7%)	
	G3	5 (20.8%)	8 (22.2%)	4 (16.7%)	
	G4	2 (8.3%)	0 (0.0%)	1 (4.2%)	

a = tumor stage according to TNM (20); b = tumor stage according to the 1997 grading system (19); c = p Value obtained by Fisher exact test; d = p Value obtained by chi-square test.

Table 5 – *Distribution of genotype and frequency of G2 allele in different populations.*

	Genotypes					
Population	**1G/1G (%)**	**1G/2G (%)**	**2G/2G (%)**	**G2 Allele**	**p Value** (*)	**Reference**
Brazilian (n = 118)	32 (27.1)	59 (50.0)	27 (22.9)	0.45		This study
Brazilian (n = 37)	10 (27)	18 (48.7)	9 (24.3)	0.49	0.982	De Souza et al. [6]
Norwegian (n = 364)	87 (25)	197 (52)	80 (23)	0.49	0.949	Wiencke et al. [23]
Caucasian US American (n = 451)	111 (24.61)	196 (43.46)	144 (31.93)	0.53	0.260	Young et al. [24]
Japanese (n = 210)	25 (11.9)	96 (45.7)	89 (42.4)	0.65	< 0.001	Hirata et al. [9]
Japanese (n = 150)	30 (20.0)	56 (37.3)	64 (42.7)	0.62	0.003	Nishioka et al. [25]
Japanese (n = 140)	17 (12.1)	48 (34.3)	75 (53.6)	0.70	< 0.001	Hashimoto et al. [15]
Taiwanese (n = 135)	17 (13)	57 (42)	61 (45)	0.66	< 0.001	Lai et al. [16]
Korean (n = 332)	33 (9.9)	154 (46.4)	145 (43.7)	0.67	< 0.001	W. Ju et al [26]

* = *p Value obtained by chi-square test.*

wanese ($p < 0.001$) and Koreans ($p < 0.001$) (9,15,25). However, there was no difference between Brazilians, Norwegians and Caucasian US Americans (23,24).

COMMENTS

Renal cell carcinoma (RCC) accounts for 3% of adult human cancers and it is becoming more com-

mon (27). Major risk factors include cigarette smoking, obesity and hypertension (28). RCC represents just about 85% of newly diagnosed kidney malignancies, occurring at an estimated rate of 4.4 to 11.1 cases per 100,000 person-years with a steady rise in the rates of RCC of 2.3% to 4.3% annually (29). There is a lack of an effective systemic therapy for RCC, which is necessary for approximately 30% of initially localized disease and 30% of patients presenting RCC with metastases identified at the time of diagnosis, with a 1-year survival rate of 26% (30). Renal tumors are classified as different histopathological subtypes with diverse clinical behavior and genetic mutations that are not completely understood.

A better understanding of the tumor gene activity and its relationships may help on prognosis prediction and on molecular therapies development.

Proteolytic enzymes play a fundamental role in cancer progression providing an access for tumor cells to the vascular and lymphatic systems. Among all proteolytic enzymes, the MMP family has reached an outstanding importance due to their ability to cleave virtually any component of the extracellular matrix (31). Matrix metalloproteinase-1 is a member of this family that is expressed by most normal cell types and there is evidence suggesting that in pathological conditions like cancers the expression is up regulated (5,8,9,11-16).

Rutter et al. (10) reported that a common single nucleotide polymorphism (SNP) in the promoter of MMP-1 is associated with enhanced transcription of this gene. A correlation between the transcription-enhancing insertion of a single G nucleotide in the MMP-1 promoter has been associated with different types of tumors (8,9,11,12). In a recent study Hirata and collaborators reported that distribution of 2G/2G in RCC patients was statistically different from the control group in the Japanese population (9).

The present population-based, control-case in São Paulo, Brazil, did not confirm the relationship between MMP-1 promoter polymorphism and risk of renal cell carcinoma. Using a total of 217 individuals, 99 RCC patients and 118 control individuals we found a lack of association between MMP-1 polymorphism and renal cell carcinoma. The discrepancy among our results and the others (8,9,11,12) may be caused by the relatively small numbers of patients and multi-ethnic composition of the Brazilian group.

Comparing the distribution MMP-1 promoter -1607 genotype in our controls with the data reported previously for other study populations (Table-5), It is clear that the 2G variant genotype is associated to ethnicity. Chi-square analysis showed a significant difference in the genotype distribution between our Brazilian group and data reported for Japanese ($p \leq 0.001$ and $p = 0.003$), Taiwanese ($p \leq 0.001$) and Korean ($p \leq 0.001$) populations (9,25,26). This fact suggests that this polymorphism may be associated with ethnicity and shows the importance of the molecular epidemiological studies in different populations.

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CONFLICT OF INTEREST

None declared.

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