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Influence of single nucleotide polymorphisms in the *MMP1* promoter region on cutaneous melanoma progression

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Abstract

Recently, we reported on the associations of seven single-nucleotide polymorphisms (SNPs) in the promoter region of *MMP1* gene with susceptibility to cutaneous melanoma (CM). Considering the reported correlation between *MMP1* expression and melanoma progression, we hypothesized that these promoter SNPs might affect CM progression and prognosis. In this study, we examined the associations of the seven SNPs with overall survival as well as six clinicopathologic factors in 754 patients with CM. After adjustment for 11 covariates, we observed significant association of the SNP -422A > T (rs475007) with ulceration status ($P = 0.012$), primary tumor thickness ($P = 0.040$), and anatomic site ($P = 0.030$). We also observed significant association of the SNP -755T > G (rs498186) with ulceration status ($P = 0.038$) and anatomic site ($P = 0.003$). Two SNPs -839G > A and -519A > G were marginally associated with primary tumor thickness, ulceration status, and anatomic site. Furthermore, the frequency of haplotype 2G-G-G-A-A-G-T was higher in patients with ulceration (odds ratio [OR] = 2.18, 95% confidence interval [CI] 1.08–4.40, $P = 0.030$) than that in those without ulceration. However, we did not find significant associations of these SNPs with overall survival and other clinical factors. Since primary tumor thickness and ulceration status are two important indicators of tumor progression and have significant associations with melanoma prognosis, our results suggested that these promoter SNPs in *MMP1* might have potential effects on melanoma progression and prognosis by influencing related clinical factors.

Keywords

Genotypes; Melanoma; Survival; Tumor characters; Molecular epidemiology

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Conflict of interest statement

None declared.

Introduction

Melanoma is the most aggressive form of skin cancer, and its incidence is increasing [1]. Melanoma progresses in two phases: the radial and vertical growth phases. The cure rate for melanoma in the radial growth phase is nearly 100% after surgery [2]. However, melanoma in the vertical growth phase is more aggressive and able to metastasize. Because few treatments are effective against advanced melanoma, the prognosis for it is poor. The one year survival rates were 33%–62% in patients with distant metastatic melanoma [3]. Therefore, identification of predictive markers that distinguish high risk melanoma cases is of great clinical importance.

Melanoma progression is a multiple-step process that involves many genes. Degradation of the extracellular matrix (ECM) and basement membrane barriers is a key step in this process. Researchers have shown that the matrix metalloproteinases (MMPs), a family of calcium- and zinc-dependent endopeptidases, are critical for ECM degradation [4]. To date, studies have identified at least 24 MMPs in humans [5]. They are classified into four groups based on their substrate specificity and cellular location: collagenases, gelatinases, stromelysins, and membrane-associated MMPs. MMP1, one of the most widely expressed interstitial collagenases, plays a significant role in ECM degradation. The *MMP1* gene is located on 11q22 and expressed in various cells [6]. Previous studies have shown that increased expression of *MMP1* modulates melanoma development, progression, and metastasis [7–10]. Specifically, patients with melanoma having increased expression of MMP1 have exhibited worse outcomes than patients having lower expression of it [11].

It has been reported that the promoter region of the *MMP1* gene contains binding sites for various transcription factors, such as AP-1, AP-2, and Ets/PEA-3, and response elements to glucocorticoids, retinoic acid, and cyclic adenosine monophosphate [12, 13]. Polymorphisms in this region may regulate *MMP1* expression by influencing the binding of these transcription factors. The polymorphism of insertion/deletion of guanine at position –1607 has been found to have a role in the regulation of *MMP1* expression by creating an Ets binding site [14]. Groups have widely studied the association of this variation with risk of development or progression of various cancers [15–18]. Specifically, –1607 1G/2G and six other SNPs (–839G > A, –755T > G, –519A > G, –422A > T, –340A > G, and –320T > C) in the *MMP1* promoter region exhibited haplotype effects on *MMP1* promoter activity [19]. We recently reported the significant associations of several of these SNPs in the *MMP1* promoter region with cutaneous melanoma (CM) susceptibility [20]. However, until now, little is known about the correlation between these SNPs and melanoma progression and prognosis [21]. In this study, we investigated the association of these seven promoter SNPs with CM progression and prognosis in 754 CM patients with available genotyping and clinical data.

Materials and methods

Study subjects

Recruitment of the study subjects was described previously [20]. Briefly, 754 patients with newly diagnosed, histologically confirmed, and untreated CM were recruited consecutively at The University of Texas MD Anderson Cancer Center from April 1994 to April 2008. A onetime blood sample (30 ml) was drawn from each study participant. Informed consent to participate in the study was obtained from the patients, and the study was approved by the MD Anderson Institutional Review Board.

Patients' clinicopathological information, including disease stage at diagnosis (based on the 2001 American Joint Committee on Cancer [AJCC] staging system), disease progression,

and survival duration, was obtained. The main clinical factors used in this study included primary tumor thickness (Breslow), ulceration status, Clark level, and anatomic site, as well as sentinel lymph node status and stage [22]. In the analysis, tumor thicknesses were divided into three categories: thin (≤ 1 mm), intermediate (1.01–4.00 mm), and thick (> 4.00 mm) [3]. The five Clark levels of invasion were grouped into three categories: I, II–III, and IV–V. Considering melanomas on the extremities have better prognosis than those on the head, neck, or trunk (axial) [23], we treated the primary CM anatomic site as a dichotomous variable: extremity (e.g., arm, hand, foot, leg) and axial (e.g., face, forehead, ear, cheek, nose, neck, eye, scalp, trunk, buttock, groin).

SNP selection and genotyping

The SNP selection method we used was described previously [20]. Briefly, seven reported common (minor allele frequency $\geq 5\%$) SNPs in the *MMP1* promoter region (–1607 1G/2G [rs1799750], –839 G > A [rs473509], –755 T > G [rs498186], –519 A > G [rs1144393], –422 A > T [rs475007], –340 A > G [rs514921], and –320 T > C [rs494379]) were selected for genotyping. These SNPs were not in linkage disequilibrium (LD) as reported previously [15, 20].

The TaqMan genotyping method (Applied Biosystems, Foster City, CA, USA) was used as described previously [20] with genomic DNA extracted from whole blood. Each 96-well plate had one positive control, one negative control and 5 duplicate samples.

Statistical analysis

The chi-square test and unconditional logistic regression analysis were used to evaluate the correlations between SNPs and the six clinicopathological factors for CM. The raw and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated without/with adjustment for the 11 covariates including age, sex, family history of any cancer in first-degree relatives, and the sunlight exposure-related factors including moles, dysplastic nevi, hair color, eye color, skin color, tanning ability, freckling in the sun as a child and history of sunburn. In the survival analysis, deaths from any causes were treated as events, and living subjects were censored at the time of last contact. The overall survival was calculated from the date of diagnosis of CM till death or censorship. The association between each SNP and overall survival duration was estimated using the Kaplan-Meier method and log-rank test. The hazard ratio (HR) and 95% CI were assessed in a Cox regression analysis without/with adjustment for sex, age, stage, primary tumor thickness, ulceration status, Clark level, and anatomic site. Because sentinel lymph node biopsy was not performed in 248 patients (32.9%) with thin melanoma or in situ melanoma, this clinical variant was not included in the Cox model. Haplotype analysis was carried out using SAS (Statistical Analysis System)/Genetics Module.

All statistical analyses were performed using the SAS software (version 9.2; SAS Institute Inc., Cary, NC). Two-sided *P* values less than 0.05 were considered statistically significant.

Results

The clinical and histological characteristics of the study population are shown in Table 1. All of the subjects were non-Hispanic white individuals. The final study population consisted of 490 male and 264 female patients. The age at diagnosis ranged from 21 to 84 years old (median = 54 years). At diagnosis, 79 patients had melanoma in situ, 567 had stage I–II CM (415 at stage I, 43 at stage I/II, and 109 at stage II), and 108 had stage III–IV CM (106 at stage III and 2 at stage IV).

As shown in Table 2, even though none of the seven SNPs was significantly associated with primary tumor thickness in the univariate analysis, after adjustment for the 11 covariates, SNP -422A > T showed significant association with primary tumor thickness under the dominant genetic model ($P = 0.04$); and another SNP -839 G > A showed marginally association with primary tumor thickness under the codominant model ($P = 0.062$) (Table 2). In addition, more patients with thin tumors than with thick tumors were variant allele carriers of -839 G > A (GA or AA genotype) and -422A > T (AT or TT genotype).

As shown in Table 3, SNP -422A > T showed significant association with ulceration status ($P = 0.023$ for codominant) in the univariate analysis. After adjustment for the 11 covariates, two SNPs (-755T > G and -422A > T) had significant association with ulceration status under the dominant genetic model. Compared with wild-type homozygotes (TT genotype), variant carriers (TG or GG genotypes) of -755T > G had an OR of 1.85 (95% CI: 1.04–3.33, $P = 0.038$) for ulceration, whereas variant carriers (AT or TT genotypes) of -422A > T had an OR of 0.52 (95% CI: 0.31–0.86, $P = 0.012$) compared with the homozygotes (AA genotype). SNP -839 G > A exhibited a marginally significant association with ulceration status ($P = 0.055$) under the dominant model.

Table 4 showed the correlation results between *MMP1* promoter SNPs and anatomic site. In the univariate analysis, significant association was found between SNP -755T > G and primary anatomic site (Crude OR = 1.52, 95% CI: 1.11–2.07, $P = 0.009$ under the dominant model). After adjustment for the 11 covariates, two SNPs were significantly associated with anatomic site ($P = 0.003$ for -755T > G under the dominant genetic model and $P = 0.030$ for -422A > T under the codominant genetic model). More patients with axial than with extremity CM were variant allele carriers of -755T > G (TG or GG genotype) (70.6% vs. 61.4%, adjusted OR = 1.67, 95% CI: 1.120–2.34). Also, the SNP -519A > G was marginally associated with anatomic site (adjusted OR = 0.71, 95% CI: 0.51–0.99, $P = 0.044$ in the dominant model).

We also performed haplotype analysis to evaluate the joint effect of the seven SNPs on CM overall survival and six clinical factors. There were seven haplotypes with frequencies greater than 0.05, and we combined the rest haplotypes into one group in this analysis (Table 5). Compared with the most frequent haplotype G-A-T-G-T-A-T (the SNP order was -1607 2G/1G, -839G > A, -755T > G, -519A > G, -422A > T, -340A > G, and -320T > C), the frequency of haplotype 2G-G-G-A-A-G-T was significantly higher in CM patients with ulceration (adjusted OR = 2.18, 95% CI: 1.08–4.40, $P = 0.030$) than that in those without ulceration. No other significant associations were observed between haplotypes and other clinical phenotypes (data were not shown).

We also examined the association of these seven SNPs with overall survival. The median follow-up time among all subjects was 32.9 months (range: 0.8 – 364.1 months). Forty-six patients died during follow-up because of melanoma or other reasons. However, no SNPs showed significant effect on melanoma survival in the univariate and multivariate Cox regression analyses. We did not find any SNPs in the *MMP1* promoter region were significantly associated with Clark level, sentinel lymph node status, and melanoma stages either (data not shown).

Discussion

In this study, for the first time we investigated the associations of seven promoter SNPs in *MMP1* with six melanoma clinicopathologic factors and overall survival. Our results showed that four SNPs (-839G > A, -755T > G, -519A > G and -422A > T) were associated with three clinical factors: primary tumor thickness, ulceration status, and

anatomic site, even though no significant association was observed between these SNPs and melanoma overall survival with only 46 deaths out of 754 CM patients. Therefore, our results partially supported the hypothesis that *MMP1* promoter SNPs might have potential effects on melanoma progression and prognosis by influencing these clinical factors.

Researchers have shown that -1607 1G/2G can regulate *MMP1* expression [14]. The association of this SNP with cancer progression and prognosis were not consistent among previous studies. For example, -1607 1G/2G was significantly associated with disease-free or overall survivals in patients with epithelial ovarian cancer [24], colorectal cancer [13]. However, the significant association was not found in breast cancer [18], esophageal adenocarcinoma [17] and lung cancer [25]. In the present study, we did not observe significant association of this variant with melanoma overall survival and the six clinicopathologic factors. This was consistent with one previous study[21], which observed that this variant had no significant effect on melanoma disease free survival, as well as primary tumor thickness and stage. These results may indicate a multilevel regulation of *MMP1* expression during tumor progression. In addition to the promoter SNPs, it is known that other factors are involved in the regulation of *MMP1* transcription, such as external stimuli and promoter methylation [26–29].

Except for -1607 1G/2G, the effects of other promoter SNPs in *MMP1* on cancer progression are little known. In our study, we observed -422A > T was associated with thinner melanoma and absence of ulceration, whereas -755 T > G was associated with presence of ulceration. Although there are little functional studies for the two SNPs, *in silico* prediction using TFSEARCH (<http://www.cbrc.jp/research/db/TFSEARCH.html>) shows variant allele T in the position of -422 would abolish the binding site of human transcript factor USF, whereas the variant allele G in -755 could create a new binding site for p300 [30]. Further functional analysis may be warranted for the validation of these predictions.

A previous study demonstrated that the seven promoter SNPs exerted a haplotype effect on the *MMP1* promoter activity in cancer cells [19]. In the present study, we observed that the haplotype 2G-G-G-A-A-G-T was more frequent in CM patients with ulceration than patients without ulceration. This result was partially supported by the results of the aforementioned study[19], in which this haplotype (reported as GG-G-G-A-T-C-T) and the haplotype 2G-G-GA-T-A-T (identical to the reported GG-G-G-A-T-T-T) had significantly higher promoter activity than that of other haplotypes in the A2058 melanoma cell line. In our recent study, two *MMP1* haplotypes (1G-A-G-A-T-G-T and 2G-G-G-A-T-A-T) were also significantly associated with increased risk of CM [20]. Thus, both of our results are evidence that these seven promoter SNPs of *MMP1* may jointly affect CM development and progression.

The correlations between abnormal expression of MMPs and melanoma progression have been evidenced in a number of studies [31]. However, previous studies on functional polymorphisms in *MMP2*, *MMP3* and *MMP9* did not provide strong evidence for the roles of these genes in melanoma progression [32, 33]. In this study, we also did not observe direct association between promoter SNPs in *MMP1* and melanoma progression and clinical outcome, which indicated that these SNPs might just have moderate effects on melanoma progression. Future investigations on more other *MMPs* genes and their interactions may be helpful to better understand the genetic mechanism underlying melanoma progression and reveal new progression predictive biomarkers. For example, recently one systematic mutation analysis of MMPs family found the mutation of *MMP8* could facilitate melanoma progression [34].

In conclusion, we observed significant associations of SNPs in the *MMP1* promoter with three clinicopathologic factors, and one common haplotype composed of the seven promoter

SNPs was over-represented among patients with ulceration. These findings provide evidence that potential functional SNPs in the *MMP1* promoter may individually or jointly influence CM progression. Certainly, there are limitations in this study. First, because we have less patients with advanced stages, the number of outcome events was small ($n = 46$), which limited the power of our survival analysis. Second, we do not have the tumor tissues for *MMP1* gene expression analysis to correlate with the SNP data. However, the analysis of the available SNPs in the *MMP1* promoter region and its mRNA expression from the 90 HapMap CEU cell lines showed a significant correlation for SNP $-755T > G$ (rs498186) [35]. Although the expression data were obtained from human peripheral blood cells not tissue samples, the result might provide some functional evidence for our findings. Definitely, further validation of these results in a larger patient population, the functional significance of each of the promoter SNPs, and their correlation with gene expression levels in tissue samples are warranted to confirm the roles of these SNPs in melanoma progression. Recently available genome-wide association studies may also provide great opportunities for us to further examine the effect of tagging SNPs in the entire *MMP1* and other *MMPs* genes on CM risk and progression.

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Table 1

Primary clinical and histological characteristics of the 754 study patients.

Variable	# Patients (%)
<i>Age (years)</i>	
Median	54
Range	21–84
<i>Primary tumor thickness</i>	
Thin (≤ 1 mm)	317 (42.0)
Intermediate (1.01–4.00 mm)	271 (35.9)
Thick (> 4 mm)	37 (4.9)
In situ only	79 (10.5)
Unknown	50 (6.6)
<i>Presence of ulceration</i>	
No	503 (66.7)
Yes	80 (10.6)
In situ only	79 (10.5)
Unknown	92 (12.2)
<i>Sentinel lymph node status</i>	
Negative	433 (57.4)
Positive	73 (9.7)
No SLN biopsy performed ^a	248 (32.9)
<i>Sex</i>	
Female	264 (35.0)
Male	490 (65.0)
<i>Clark level</i>	
I	78 (10.3)
II–III	339 (45.0)
IV–V	285 (37.8)
Unknown	52 (6.9)
<i>Primary CM anatomic site</i>	
Axial	447 (59.3)
Extremity	300 (39.8)
Unknown	7 (0.9)
<i>Stage at diagnosis</i>	
In situ only	79 (10.5)
I–II	567 (75.2)
III–IV	108 (14.3)

^a Sentinel lymph node (SLN) biopsy was not performed for most patients with in situ or thin melanoma.

Table 2

Associations between SNPs in the *MMP1* promoter region and primary tumor thickness.

SNP	# Patients (%)			P-value ^a	P-value ^b
	Thin (≤1 mm)	Intermediate (1.01–4.00 mm)	Thick (>4 mm)		
-1607 G/G (rs1799750)					
1G	81 (25.8)	79 (29.5)	10 (27.8)	0.398	0.761
1G/2G	160 (51.0)	134 (50.0)	14 (38.9)		
2G/2G	73 (23.2)	55 (20.5)	12 (33.3)		
1G/2G + 2G/2G	233 (74.2)	189 (70.5)	26 (72.2)	0.611	0.482
-839 G > A (rs473509)					
GG	103 (32.5)	94 (35.1)	18 (50.0)	0.127	0.062
GA	170 (53.6)	126 (47.0)	13 (36.1)		
AA	44 (13.9)	48 (17.9)	5 (1.9)		
GA + AA	214 (67.5)	174 (64.9)	18 (50.0)	0.110	0.068
-755 T > G (rs498186)					
TT	106 (33.8)	82 (30.8)	12 (33.3)	0.423	0.721
TG	146 (46.5)	133 (50.0)	13 (36.1)		
GG	62 (19.8)	51 (19.2)	11 (30.6)		
TG + GG	208 (66.3)	184 (69.2)	24 (66.7)	0.749	0.532
-519 A > G (rs1144393)					
AA	122 (38.9)	107 (40.1)	19 (52.8)	0.598	0.457
AG	154 (49.0)	126 (47.2)	14 (38.9)		
GG	38 (12.1)	34 (12.7)	3 (8.3)		
AG + GG	192 (61.1)	160 (59.9)	17 (47.2)	0.272	0.211
-422 A > T (rs475007)					
AA	82 (26.0)	76 (28.2)	16 (43.2)	0.157	0.078
AT	167 (52.9)	132 (49.1)	12 (32.4)		
TT	67 (21.2)	61 (22.7)	9 (24.3)		
AT + TT	234 (74.1)	193 (71.8)	21 (56.7)	0.085	0.040
-340 A > G (rs514921)					
AA	165 (52.2)	129 (48.1)	20 (54.1)	0.876	0.995
AG	121 (38.3)	111 (41.4)	14 (37.8)		

SNP	# Patients (%)			P-value ^a	P-value ^b
	Thin (≤1 mm)	Intermediate (1.01–4.00 mm)	Thick (>4 mm)		
GG	30 (9.5)	28 (10.5)	3 (8.1)		
AG + GG	151 (47.8)	139 (51.9)	17 (45.9)	0.561	0.957
-320T > C (rs494379)					
TT	185 (58.5)	161 (60.3)	22 (59.5)	0.732	0.438
TC	108 (34.2)	94 (35.2)	13 (35.1)		
CC	23 (7.3)	12 (4.5)	2 (5.4)		
TC + CC	131 (41.5)	106 (39.7)	15 (40.5)	0.912	0.879

SNP, single nucleotide polymorphism.

^aTwo-sided chi-square test for genotype distribution.

^bAdjusted for age, sex, skin color, hair color, eye color, freckling in the sun as a child, sunburns, tanning ability, number of moles, dysplastic nevi, and first-degree relatives with any cancer.

Table 3

Associations between SNPs in the *MMP1* promoter region and ulceration status.

SNP	# Patients (%)		P-value ^d	OR (95% CI)		P-value ^b
	Ulceration absent	Ulceration present		Crude	Adjusted ^b	
-1607 1G/2G (rs1799750)						
1G	138 (37.6)	20 (25.3)	0.417	1.00 (Ref)	1.00 (Ref)	0.425
1G/2G	256 (51.2)	37 (46.8)		1.00 (0.55–1.79)	1.21 (0.66–2.21)	
2G/2G	106 (21.2)	22 (27.9)		1.43 (0.74–2.76)	1.58 (0.79–3.14)	
1G/2G + 2G/2G	362 (72.4)	59 (74.7)	0.672	1.13 (0.65–1.94)	1.32 (0.75–2.33)	0.342
-839 G > A (rs473509)						
GG	163 (32.6)	35 (43.8)	0.148	1.00 (Ref)	1.00 (Ref)	0.159
GA	256 (51.2)	34 (42.5)		0.62 (0.37–1.03)	0.61 (0.36–1.04)	
AA	81 (16.2)	11 (13.8)		0.63 (0.31–1.31)	0.62 (0.29–1.30)	
GA + AA	337 (67.4)	45 (56.3)	0.051	0.62 (0.39–1.01)	0.61 (0.37–1.01)	0.055
-755 T > G (rs498186)						
TT	165 (33.2)	18 (23.5)	0.108	1.00 (Ref)	1.00 (Ref)	0.059
TG	234 (47.1)	47 (58.0)		1.84 (1.03–3.28)	2.04 (1.12–3.73)	
GG	98 (19.7)	15 (18.5)		1.40 (0.68–2.91)	1.40 (0.65–3.04)	
TG + GG	332 (66.8)	62 (76.5)	0.056	1.71 (0.98–2.99)	1.85 (1.04–3.33)	0.038
-519 A > G (rs1144393)						
AA	192 (38.6)	38 (47.5)	0.268	1.00 (Ref)	1.00 (Ref)	0.361
AG	241 (48.5)	35 (43.8)		0.74 (0.45–1.21)	0.76 (0.45–1.28)	
GG	64 (12.9)	7 (8.8)		0.55 (0.24–1.30)	0.57 (0.24–1.37)	
AG + GG	305 (61.4)	42 (52.6)	0.134	0.70 (0.43–1.12)	0.72 (0.44–1.18)	0.195
-422 A > T (rs475007)						
AA	128 (25.5)	31 (39.5)	0.023	1.00 (Ref)	1.00 (Ref)	0.022
AT	263 (52.5)	30 (37.0)		0.47 (0.27–0.81)	0.45 (0.26–0.79)	
TT	110 (22.0)	19 (23.5)		0.71 (0.38–1.33)	0.67 (0.35–1.28)	
AT + TT	373 (74.5)	49 (60.5)	0.014	0.54 (0.33–0.89)	0.52 (0.31–0.86)	0.012
-340 A > G (rs514921)						
AA	261 (52.0)	37 (46.3)	0.322	1.00 (Ref)	1.00 (Ref)	0.278

SNP	# Patients (%)		OR (95% CI)		P-value ^a	Adjusted ^b	P-value ^b
	Ulceration absent	Ulceration present	Crude				
AG	197 (39.2)	32 (40.0)	1.15 (0.69–1.91)			1.20 (0.71–2.04)	
GG	44 (8.8)	11 (13.8)	1.76 (0.84–3.72)			1.56 (0.70–3.48)	
AG + GG	241 (48.0)	43 (53.8)	1.26 (0.78–2.02)		0.341	1.27 (0.77–2.09)	0.344
–320T > C (rs494379)							
TT	299 (59.8)	42 (53.2)	1.00 (Ref)		0.531	1.00 (Ref)	0.635
TC	172 (34.4)	32 (40.5)	1.33 (0.81–2.18)			1.28 (0.76–2.16)	
CC	29 (5.8)	5 (6.3)	1.23 (0.45–3.35)			1.23 (0.44–3.45)	
TC + CC	201 (40.2)	37 (46.8)	1.31 (0.81–2.11)		0.266	1.27 (0.77–2.10)	0.343

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

^aTwo-sided chi-square test for genotype distribution.

^bAdjusted for age, sex, skin color, hair color, eye color, freckling in the sun as a child, sunburns, tanning ability, number of moles, dysplastic nevi, and first-degree relatives with any cancer.

Table 4

Associations between SNPs in the *MMP1* promoter region and primary CM anatomic site.

SNP	# Patients (%)		P-value ^a	OR (95% CI)		P-value ^b
	Extremity	Axial		Crude	Adjusted ^b	
-1607 1G/2G (rs1799750)						
1G	88 (29.8)	121 (27.2)	0.335	1.00 (Ref)	1.00 (Ref)	0.177
1G/2G	150 (50.9)	218 (49.0)		1.06 (0.75–1.49)	1.20 (0.83–1.75)	
2G/2G	57 (19.3)	106 (23.8)		1.35 (0.89–2.07)	1.55 (0.98–2.45)	
1G/2G + 2G/2G	207 (70.2)	324 (72.8)	0.435	1.14 (0.82–1.58)	1.30 (0.91–1.84)	0.146
-839 G > A (rs473509)						
GG	93 (31.4)	160 (35.8)	0.465	1.00 (Ref)	1.00 (Ref)	0.412
GA	153 (51.7)	215 (48.1)		0.82 (0.59–1.14)	0.80 (0.56–1.15)	
AA	50 (16.9)	72 (16.1)		0.84 (0.54–1.30)	0.78 (0.48–1.25)	
GA + AA	203 (68.6)	287 (64.2)	0.218	0.82 (0.60–1.12)	0.80 (0.57–1.12)	0.185
-755 T > G (rs498186)						
TT	114 (38.6)	130 (29.4)	0.031	1.00 (Ref)	1.00 (Ref)	0.011
TG	130 (44.1)	226 (51.0)		1.52 (1.10–2.12)	1.71 (1.19–2.44)	
GG	51 (17.3)	87 (19.6)		1.50 (0.98–2.29)	1.58 (1.00–2.52)	
TG + GG	181 (61.4)	313 (70.6)	0.009	1.52 (1.11–2.07)	1.67 (1.20–2.34)	0.003
-519 A > G (rs1144393)						
AA	102 (34.8)	187 (42.0)	0.144			0.113
AG	150 (51.2)	204 (45.8)		0.74 (0.54–1.02)	0.73 (0.52–1.04)	
GG	41 (14.0)	54 (12.1)		0.72 (0.45–1.15)	0.64 (0.38–1.07)	
AG + GG	191 (65.2)	258 (57.9)	0.050	0.74 (0.54–1.00)	0.71 (0.51–0.99)	0.044
-422 A > T (rs475007)						
AA	89 (29.9)	125 (28.0)	0.057	1.00 (Ref)	1.00 (Ref)	0.030
AT	133 (44.6)	235 (52.7)		1.26 (0.89–1.78)	1.27 (0.87–1.85)	
TT	76 (25.5)	86 (19.3)		0.81 (0.53–1.22)	0.74 (0.48–1.15)	
AT + TT	309 (70.1)	321 (72.0)	0.587	1.09 (0.79–1.51)	1.07 (0.75–1.51)	0.723
-340 A > G (rs514921)						
AA	159 (53.5)	224 (50.2)	0.565	1.00 (Ref)	1.00 (Ref)	0.717

SNP	# Patients (%)		OR (95% CI)		P-value ^a	Adjusted ^b	P-value ^b
	Extremity	Axial	Crude				
AG	113 (38.1)	176 (39.5)	1.11 (0.81–1.51)	1.03 (0.74–1.44)			
GG	25 (8.4)	46 (10.3)	1.31 (0.77–2.21)	1.27 (0.72–2.26)			
AG + GG	138 (46.5)	222 (48.8)	1.14 (0.85–1.53)	1.07 (0.78–1.47)	0.376		0.666
-320T > C (rs494379)							
TT	173 (58.3)	269 (60.5)	1.00 (Ref)	1.00 (Ref)	0.702		0.880
TC	106 (35.7)	146 (32.8)	0.89 (0.65–1.21)	0.92 (0.66–1.30)			
CC	18 (6.1)	30 (6.7)	1.07 (0.58–1.98)	1.03 (0.54–1.99)			
TC + CC	124 (41.8)	176 (39.5)	0.92 (0.68–1.23)	0.94 (0.68–1.30)	0.550		0.703

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; Ref, reference.

^aTwo-sided chi-square test for genotype distribution.

^bAdjusted for age, sex, skin color, hair color, eye color, freckling in the sun as a child, sunburns, tanning ability, number of moles, dysplastic nevi, and first-degree relatives with any cancer.

Table 5
Haplotype distribution of the seven SNPs in the study patients with and without ulceration.

Haplotype ^a	# Patients (%)		OR (95% CI)		
	Ulceration absent	Ulceration present	P-value ^b	Raw	Adjusted ^c P-value ^c
G-A-T-G-T-A-T	205 (21.2)	24 (16.3)	---	1.00 (Ref)	1.00 (Ref) ---
2G-G-G-A-A-A-T	103 (10.6)	18 (12.2)	0.231	1.49 (0.78–2.88)	1.56 (0.78–3.01) 0.205
G-A-T-G-A-A-T	84 (8.7)	9 (6.1)	0.830	0.92 (0.41–2.05)	1.03 (0.45–2.34) 0.954
2G-G-G-A-A-G-T	76 (7.9)	18 (12.2)	0.038	2.02 (1.04–3.94)	2.18 (1.08–4.40) 0.030
2G-G-T-A-A-A-C	73 (7.5)	12 (8.1)	0.370	1.40 (0.67–2.95)	1.48 (0.69–3.18) 0.313
2G-G-G-A-A-A-C	52 (5.4)	9 (6.1)	0.353	1.48 (0.65–3.37)	1.58 (0.68–3.67) 0.291
2G-G-G-A-T-G-T	53 (5.5)	8 (5.4)	0.560	1.29 (0.55–3.03)	1.44 (0.60–3.46) 0.413
Other ^d	322 (33.3)	50 (33.8)	0.285	1.33 (0.79–2.23)	1.33 (0.77–2.28) 0.305

OR, odds ratio; CI, confidence interval; Ref, reference.

^aThe SNP order was –1607 2G/1G, –839G > A, –755T > G, –519A > G, –422A > T, –340A > G, and –320T > C.

^bTwo-sided chi-square test for haplotype distribution.

^cAdjusted for age, sex, skin color, hair color, eye color, freckling in the sun as a child, sunburns, tanning ability, number of moles, dysplastic nevi, and first-degree relatives with any cancer.

^dRare haplotypes with frequencies less than 0.05 were combined in one group.