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Promoter polymorphisms in *matrix metallopeptidase 1* and risk of cutaneous melanoma

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ABSTRACT

Matrix metallopeptidase 1 (MMP1) is one of the interstitial collagens in the extracellular matrix metalloproteinase family and involved in tumour behaviours. However, there is no report on the role of genetic variation in *MMP1* in risk of cutaneous melanoma (CM). We investigated the association between genotypes and haplotypes of seven reported *MMP1* promoter polymorphisms [–1607G ins/del (2G/1G), –839G > A, –755T > G, –519A > G, –422A > T, –340A > G and –320T > C, genotyped by the TaqMan assay] and CM risk in 872 patients and 873 cancer-free controls. These seven polymorphisms were not in linkage disequilibrium among each other ($r^2 < 0.63$). Compared to their common homozygous genotypes, the variant –519GG was associated with significantly decreased CM risk [adjusted odds ratio (OR) = 0.71, 95% confidence interval (CI) = 0.52–0.99], whereas variants –422TT and –320CC were associated with significantly increased CM risk (OR = 1.50, 95% CI = 1.11–2.03 and OR = 1.72, 95% CI = 1.05–2.81, respectively) after adjustment for age, sex, family history and sun-exposure-related risk factors. The number of risk alleles of these three polymorphisms was associated with CM risk in a dose–response manner ($P_{trend} = 0.0002$). In the stratification analysis, we found that the associations of these polymorphisms with CM risk were modified by some of the risk factors. Furthermore, the haplotypes Gdel–A–G–A–T–G–T and G–G–G–A–T–A–T were associated with significantly increased CM risk (ORs = 1.56 and 2.13, 95% CIs = 1.02–2.38 and 1.22–3.70, respectively). These findings suggest that *MMP1* promoter polymorphisms may individually or jointly play roles in the development of CM.

1. Introduction

Cutaneous melanoma (CM) is the most aggressive form of malignant skin cancers. In the United States (US), CM accounts for approximately 65% of all skin cancer-related deaths.[1] The incidence of CM has increased considerably in Caucasians in US and Europe for the past several decades.[2–5] According to Cancer Statistics of American Cancer Society, the estimated new cases and deaths of CM in 2009 were 68,720 and 8650, respectively.[6]

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Epidemiological studies have reported that ultraviolet radiation (UV) exposure[7–9] and family history[2,10] are the major risk factors for CM. However, only a proportion of individuals who had been exposed to excessive sunlight develops CM, suggesting that genetic susceptibility may also play a substantial role in the aetiology of CM. Studies have identified cyclin-dependent kinase inhibitor 2A (CDKN2A/p16) and cyclin-dependent kinase 4 (CDK4) as high-penetrance CM susceptibility genes and their germline mutations contribute to the 20–40% of familial CM.[11–13] Mutations and genetic polymorphisms in potential low-penetrance genes have also been reported to be associated with risk of CM, such as genes encoding melanocortin-1 receptor, glutathione s-transferase M1 and vitamin D receptor.[12,14–18]

The matrix metallopeptidase 1 gene (*MMP1*) is located on chromosome 11q22.3 and it belongs to the MMP family that is responsible for the degradation of extracellular matrix components. There is considerable evidence indicating that MMP1 is involved in various cell and tumour behaviours, including cancer-cell development, growth, proliferation, apoptosis, invasion and metastasis, as well as angiogenesis and immune surveillance.[19–22] Recent studies have demonstrated an elevated expression and activation of MMP1 in several kinds of human tumour tissues including melanoma.[23–26] Also, cancer patients with tumours expressing higher levels of MMP1 have poor prognosis compared with those with lower expression.[23,25,26]

It has been reported that a single nucleotide polymorphism (SNP) –1607G ins/del (designated as 2G/1G) in the *MMP1* promoter can affect transcriptional regulation of the *MMP1* gene by creating an Ets-binding site that increases promoter transcription and activity in both normal fibroblasts and melanoma cells.[27,28] A number of genetic epidemiological studies have confirmed an association between this SNP and susceptibility to various cancer types in different ethnic populations.[24,29–32] Ye and colleagues have also reported that this SNP was associated with deep invasiveness of CM in 139 Caucasian patients in a British population.[33]

In addition to the –1607 2G/1G, it is likely that other promoter polymorphisms of the *MMP1* gene may also regulate transcription activity. Recently, it has been reported that there are haplotype effects on the *MMP1* promoter activity in melanoma, breast cancer, lung cancer and colorectal cancer-cell lines.[34] To further evaluate the possible functional relevance of genotypes and haplotypes of the known *MMP1* promoter polymorphisms, we conducted a molecular epidemiological case–control study to investigate the association between genotypes and haplotypes of the seven common promoter SNPs in the *MMP1* gene and risk of CM.

2. Materials and methods

2.1. Study subjects

Recruitment of the study population was described previously.[16] Briefly, in this hospital-based case–control study, 872 non-Hispanic patients with newly diagnosed, histologically confirmed, untreated CM were consecutively recruited at the University of Texas M.D. Anderson Cancer Center in Houston, Texas, between April 1994 and April 2008. An additional 873 cancer-free controls matched to the cases with age (±5 years), sex and ethnicity were recruited during the same period from among visitors to M.D. Anderson Cancer Center, who were accompanying patients to outpatient clinics but not seeking medical care nor being biologically related to the patients included in this study. After an informed consent was obtained, each participant was asked to complete a questionnaire to provide information about their demographic and known risk factors for CM. A one-time blood sample (30 mL) was drawn from each study participant. The research protocol was approved by the Institutional Review Board at M.D. Anderson Cancer Center.

2.2. Genotyping

The seven selected SNPs in the *MMP1* promoter region (i.e. –1607 2G/1G [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 the known common (a minor allele frequency [MAF] $\geqslant$ 5%) SNPs in Caucasians reported in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and National Institute of Environmental Health Sciences (NIEHS) Environmental Genome Project SNP database (http://egp.gs.washington.edu/). These seven SNPs have been also confirmed by re-sequencing in healthy British Caucasians.[34]

Genomic DNA was extracted from leucocyte cell pellet isolated from whole blood samples of 872 cases and 873 controls. Genotyping was carried out using the TaqMan assays (Applied Biosystems, Foster City, CA, USA), the widely used genotyping platform. The call rate was 98.5%. All genotype results were incorporated into Microsoft Excel 2003 for further statistical analysis.

2.3. Statistical analysis

First, a univariate analysis was performed to evaluate the distribution of selected variables in the cases and controls. Pearson's chi-square test was computed to compare the differences in the frequency distributions of demographic characteristics, the known risk factors and genotypes between cases and controls. We compared the observed genotype frequencies with those calculated from the Hardy–Weinberg equilibrium theory and evaluated the linkage disequilibrium (LD) for each of the SNP pairs in this study population.

Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional univariate and multivariate logistic regression analysis, respectively, for estimating the association between genotypes/haplotypes and risk of CM without and with adjustment for the covariates including age, sex, family history in first-degree relatives with any cancer and sunlight exposure-related variables including moles, dysplastic nevi, hair colour, eye colour, skin colour, tanning ability, freckling and sunburn. We performed Proc Haplotype procedure in the SAS/Genetics programme to reconstruct haplotypes in the promoter region on the basis of the observed genotypes. Gene–environment interaction analyses between genotypes in the *MMP1* promoter and

sunlight exposure-related variables were performed in the stratification analysis using logistic regression models as well. All data were analysed by using Statistical Analysis System/Genetics software (version 9.1.3; SAS Institute Inc., Cary, NC). All statistical analyses were two-sided with a statistical significance level of a *P* value less than 0.05.

3. Results

The final analysis included 872 CM patients and 873 cancer-free controls. All subjects were non-Hispanic whites. The age and sex were frequency-matched between two groups ($P = 0.407$ and 0.640, respectively; Table 1). The age ranges (mean ± standard deviation) were 21–84 (53.0 ± 12.1) years for CM patients and 21–87 (53.3 ± 12.1) years for cancer-free controls. The proportion of men was higher in both cases (64.0%) and control (65.2%) than that of women. There were significantly more cases than controls who reported to have moles, dysplastic nevi, light hair colour, blue eyes, fair complexion, poor tanning ability, freckling, sunburns and family history of cancer ($P < 0.001$ for all categories, Table 1). These variables were adjusted for their possible confounding on the main effect of the selected *MMP1* polymorphisms on risk of CM in multivariate logistic regression models.

The genotype and allele distributions of the seven promoter SNPs of *MMP1* are summarised in Table 2. The observed genotype frequencies of these SNPs were all in agreement with the Hardy–Weinberg equilibrium in the control subjects

Table 1 – Frequency distributions of demographic characteristics and sun-exposure factors in cutaneous melanoma patients and cancer-free controls.

Variables[a]	Cases (no. = 872) no. (%)	Controls (no. = 873) no. (%)	P-value[b]
Age (years)			0.407
Range	21–84	21–87	
⩽45	234 (26.8)	212 (24.3)	
46–60	392 (45.0)	416 (47.6)	
>60	246 (28.2)	245 (28.1)	
Sex			0.640
Males	559 (64.1)	569 (65.2)	
Females	313 (35.9)	304 (34.8)	
Moles			<0.001
No	222 (25.5)	461 (54.3)	
Yes	650 (74.5)	388 (45.7)	
Dysplastic nevi			<0.001
No	796 (92.0)	819 (98.3)	
Yes	69 (8.0)	14 (1.7)	
Hair colour			<0.001
Black or brown	557 (65.3)	673 (78.1)	
Blond or red	296 (34.7)	189 (21.9)	
Eye colour			<0.001
Not blue	508 (58.3)	598 (68.9)	
Blue	363 (41.7)	270 (31.1)	
Skin colour			<0.001
Dark or brown	368 (42.2)	511 (58.9)	
Fair	503 (57.8)	356 (41.1)	
Tanning ability			<0.001
Good (high)	528 (60.6)	641 (73.8)	
Poor (low)	343 (39.4)	228 (26.2)	
Freckling in the sun as a child			<0.001
No	386 (44.3)	519 (59.9)	
Yes	485 (55.7)	348 (40.1)	
Sunburns with blistering			<0.001
No	242 (27.8)	351 (40.5)	
Yes	628 (72.2)	516 (59.5)	
First-degree relatives with any cancer			
No	314 (36.1)	356 (41.0)	<0.001
Yes	556 (63.9)	513 (59.0)	

[a] With missing values in some strata: 24 in moles, 47 in dysplastic Nevi, 30 in hair colour, 6 in eye colour, 7 in skin colour, 5 in tanning ability, 7 in freckling, 8 in sunburn, 6 in family history.

[b] Two-sided χ^2 test.

Table 2 – Genotype frequencies of *MMP1* promoter polymorphisms in CM patients and cancer-free controls and their associations with risk of CM.

Gene/single nucleotide polymorphism (SNP)[a]	Cases (no. = 872) no. (%)	Controls (no. = 873) no. (%)	*P*-value[b]	Crude OR (95% CI)	Adjusted OR (95% CI)[c]
MMP1 –16072G/1G, rs1799750			0.879		
1G/1G (del/del)	238 (27.5)	240 (28.3)		1.00	1.00
1G/2G (del/ins)	436 (50.5)	418 (49.2)		1.12 (0.90–1.39)	1.09 (0.85–1.39)
2G/2G (ins/ins)	190 (22.0)	191 (22.5)		1.07 (0.82–1.39)	1.09 (0.81–1.47)
2G allele frequency	0.472	0.471			
MMP1 –839G > A, rs473509			0.363		
GG	299 (34.4)	275 (32.2)		1.00	1.00
GA	426 (49.1)	419 (49.0)		0.98 (0.80–1.21)	0.97 (0.77–1.23)
AA	143 (16.5)	161 (18.8)		0.86 (0.65–1.13)	0.83 (0.61–1.13)
A allele frequency	0.410	0.433			
MMP1 –755T > G, rs498186			0.633		
TT	276 (32.0)	288 (33.8)		1.00	1.00
TG	421 (48.8)	397 (46.6)		1.15 (0.93–1.41)	1.18 (0.93–1.50)
GG	165 (19.2)	167 (19.6)		1.07 (0.82–1.40)	1.13 (0.84–1.52)
G allele frequency	0.436	0.429			
MMP1 –519A > G, rs1144393			0.154		
AA	336 (38.9)	310 (36.3)		1.00	1.00
AG	412 (47.7)	403 (47.2)		0.98 (0.80–1.20)	0.93 (0.74–1.17)
GG	115 (13.4)	141 (16.5)		0.78 (0.58–1.04)	**0.71 (0.52–0.99)**
G allele frequency	0.372	0.401			
MMP1 –422A > T, rs475007			0.485		
AA	253 (29.2)	267 (31.3)		1.00	1.00
AT	424 (48.8)	415 (48.7)		1.14 (0.92–1.42)	1.19 (0.93–1.52)
TT	191 (22.0)	171 (20.0)		1.25 (0.96–1.63)	**1.50 (1.11–2.03)**
T allele frequency	0.464	0.444			
MMP1 –340A > G, rs514921			0.401		
AA	440 (50.7)	451 (52.8)		1.00	1.00
AG	344 (39.6)	335 (39.2)		1.09 (0.89–1.33)	1.12 (0.90–1.40)
GG	84 (9.7)	68 (8.0)		1.31 (0.93–1.85)	1.34 (0.90–1.98)
G allele frequency	0.295	0.276			
MMP1 –320T > C, rs494379			0.142		
TT	525 (60.6)	545 (63.7)		1.00	1.00
TC	288 (33.3)	274 (32.1)		1.11 (0.91–1.37)	1.09 (0.87–1.37)
CC	53 (6.1)	36 (4.2)		**1.56 (1.01–2.42)**	**1.72 (1.05–2.81)**
C allele frequency	0.227	0.202			

MMP1: *matrix metallopeptidase 1*; CM: cutaneous melanoma.

[a] With missing values in each SNP: 32 in –16072G/1G, 22 in –839G > A, 31 in –755T > G, 28 in 519A > G, 24 in –422A > T, 23 in 340A > G, 24 in –320T > C.

[b] Two-sided χ^2 tests.

[c] Adjusted for age (in years), sex, family history and eight sun-exposure risk factors as defined in Table 1.

(*P* = 0.726 for –1607 2G/1G, 0.950 for –839 G > A, 0.154 for –755 T > G, 0.604 for –519 A > G, 0.672 for –422 A > T, 0.600 for –340 A > G and 0.833 for –320 T > C, respectively). The LD analyses with Haploview software in controls showed that the –340 A > G and –320 T > C were in the same block because of their close distance. However, r^2 values for each pair of SNPs were less than 63, suggesting that all of these SNPs may be relatively independent (Fig. 1).

As shown in Table 2, the chi-square test did not find significant differences in the genotype distributions of any of the seven SNPs. However, in the multivariate analysis, compared with the *MMP1* –519AA genotype, –519GG was associated with significantly reduced risk of CM (OR = 0.71, 95% CI = 0.52–0.99) after adjustment for age, sex, moles, dysplastic nevi, hair colour, eye colour, skin colour, tanning ability, freckling, sunburns and family history of cancer; –422TT was associated with significantly increased risk of CM (adjusted OR = 1.50, 95% CI = 1.11–2.03) compared with the –422AA genotype. Furthermore, compared with the –320TT genotype, –320CC was associated with significantly increased risk of CM both before and after adjustment in the logistic regression models (crude OR = 1.56, 95% CI = 1.01–2.42 and adjusted OR = 1.72, 95% CI = 1.05–2.81, respectively).

To further analyse the combined effects of these three apparently important SNPs, we grouped these SNPs by the number (0–6) of putative risk alleles (i.e. –519A, –422T and –320C) as listed in Table 3. Even though we found that the distribution of putative risk alleles was significantly different between cases and controls (*P* = 0.016) compared with the group having none of these risk alleles (only 14 cases and 17

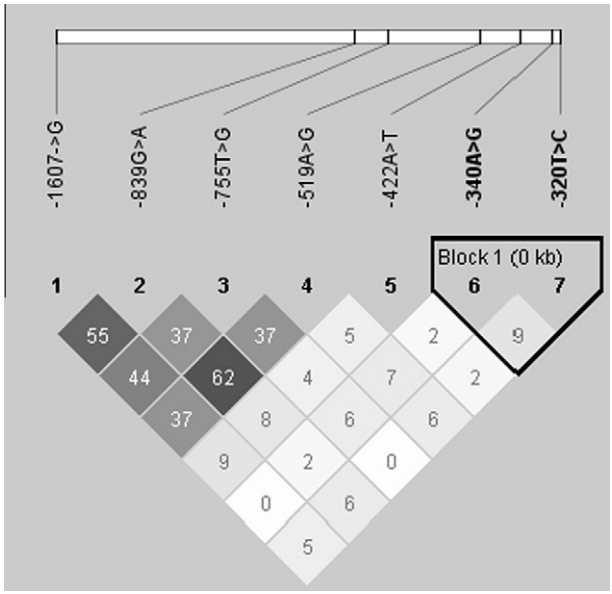

Fig. 1 – Linkage disequilibrium (LD) for the seven single nucleotide polymorphisms (SNPs) of matrix metallopeptidase 1 (*MMP1*) in r^2 values.

controls), we did not find a significant association between any other group and CM risk. However, there was a significant trend among the risk allele groups as the putative risk allele number increased. Therefore, we combined the risk allele numbers into three groups as 0–1 (with 96 cases and 138 controls), 2–3 and 4–6 with more samples in each group to increase the statistical power for subgroup analysis. As shown in Table 3, the difference in the distribution of putative risk alleles between cases and controls remained significant. When the group with 0–1 risk alleles of three SNPs was used as the reference, the other two groups were associated with significantly increased risk (adjusted OR = 1.68, 95% CI = 1.24–2.27 for 2–3 and 1.95 = 1.35–2.83 for 4–6 risk alleles) of CM without or with adjustment for the confounders (Table 3), having statistically significant allele effects in a dose–response manner (adjusted $P_{trend} = 0.0005$).

We then evaluated gene–environment interactions by stratifying the combined risk allele groups of these three SNPs and the known risk factors listed in Table 1. We found that the distributions of risk allele groups of *MMP1* between cases and controls were significantly different in following groups: age >45 years ($P = 0.003$ versus 0.415 for ⩽45 years); with moles ($P = 0.012$ versus 0.186 without moles); with black or brown hair ($P = 0.005$ versus 0.201 for blond or red hair); without blue eyes ($P = 0.037$ versus borderline 0.090 with blue eyes); with poor tanning ability ($P = 0.031$ versus borderline 0.059 with good tanning ability); without freckling in the sun as a child ($P = 0.004$ versus 0.121 with freckling); without sunburns with blistering ($P = 0.006$ versus borderline 0.076 with sunburns).

In further logistic regression analyses, all of the groups with 2–3 or 4–6 risk alleles of *MMP1* –519A + –422T + –320C in those strata of the known risk factors having significantly different distributions of *MMP1* risk alleles remained statistically significant and were associated with a 1.64- to 2.99-fold increased risk of CM after adjusting for known risk factors. Trend tests were significant for these strata as well. In particular, in those strata with borderline different distributions in risk alleles, the presence of 4–6 risk alleles in the groups with blue eyes, good tanning ability or sunburns and 2–3 risk alleles in the groups with good tanning ability, freckling in the sun as a child exhibited significantly increased risk of CM as well (Table 4), suggesting that these risk factors modified the *MMP1* genetic effects on risk of CM.

Lastly, we constructed the haplotypes with the seven observed promoter SNPs. There were a total of 128 haplotypes estimated, of which 19 haplotypes had a frequency >1% and those with a frequency <1% were grouped into 'others' (data not shown). Overall, we did not find a difference in the distribution of these 20 haplotypes between the CM cases and controls ($P = 0.140$). However, in the logistic regression analysis, using the most frequent haplotype 1G–A–T–G–T–A–T (the

Table 3 – Distribution of combined *MMP1* promoter genotypes in CM patients and cancer-free controls and their associations with risk of CM.

No. of risk alleles	Cases (no. = 857) no. (%)	Controls (no. = 835) no. (%)	*P*-value[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
MMP1 –519A + –422T + –320C					
0	14 (1.6)	17 (2.0)	**0.016**	1.00	1.00
1	96 (11.2)	138 (16.5)		0.85 (0.40–1.80)	0.80 (0.35–1.85)
2	278 (32.4)	272 (32.6)		1.24 (0.60–2.57)	1.28 (0.57–2.87)
3	294 (34.3)	268 (32.1)		1.33 (0.64–2.75)	1.48 (0.66–3.32)
4	150 (17.5)	118 (14.1)		1.54 (0.73–3.26)	1.49 (0.65–3.41)
5	22 (2.6)	22 (2.6)		1.21 (0.48–3.05)	2.28 (0.81–6.42)
6	3 (0.4)	0 (0)		–	–
				$P_{trend} = 0.002$	**$P_{trend} = 0.0002$**
Combined groups					
0–1	110 (12.9)	155 (18.5)	**0.002**	1.00	1.00
2–3	572 (66.7)	540 (64.7)		**1.49 (1.14–1.96)**	**1.68 (1.24–2.27)**
4–6	175 (20.4)	140 (16.8)		**1.76 (1.27–2.45)**	**1.95 (1.35–2.83)**
				$P_{trend} = 0.001$	**$P_{trend} = 0.0005$**

[a] Two-sided χ^2 tests.
[b] Adjusted for age and sex, family history and eight sun-exposure risk factors as defined in Table 1 in logistic regression models.

Table 4 – Stratification analysis of *MMP1* promoter SNPs by risk factors in CM cases and cancer-free controls.

	No. of risk alleles of *MMP1* –519A + –422T + –320C						P[a]	Adjusted OR 95% CI[b]			P_{trend}[b]
	Cases			Controls							
	0–1	2–3	4–6	0–1	2–3	4–6		0–1	2–3	4–6	
Age (years)											
⩽45	36 (15.6)	142 (61.5)	53 (22.9)	39 (19.1)	127 (62.3)	38 (18.6)	0.415	1.00	1.53 (0.84–2.77)	1.53 (0.75–3.13)	0.260
>45	74 (11.8)	430 (68.7)	122 (19.5)	116 (18.4)	413 (65.4)	102 (16.2)	**0.003**	1.00	**1.75 (1.23–2.50)**	**2.10 (1.36–3.25)**	**0.001**
Moles											
No	28 (12.7)	148 (67.0)	45 (20.3)	78 (17.5)	294 (65.9)	74 (16.6)	0.186	1.00	1.45 (0.87–2.41)	1.78 (0.97–3.28)	0.067
Yes	82 (12.9)	424 (66.7)	130 (20.4)	73 (19.7)	235 (63.3)	63 (17.0)	**0.012**	1.00	**1.81 (1.24–2.64)**	**2.05 (1.29–3.26)**	**0.003**
Hair colour											
Black or brown	68 (12.4)	366 (66.9)	113 (20.7)	123 (19.0)	418 (64.4)	108 (16.6)	**0.005**	1.00	**1.80 (1.26–2.58)**	**2.09 (1.36–3.23)**	**0.001**
Blond or red	37 (12.7)	194 (66.7)	60 (20.6)	32 (18.1)	116 (65.5)	29 (16.4)	0.201	1.00	1.44 (0.80–2.59)	1.76 (0.86–3.62)	0.129
Eye colour											
Not blue	70 (14.0)	334 (66.8)	96 (19.2)	113 (19.8)	364 (63.6)	95 (16.6)	**0.037**	1.00	**1.74 (1.20–2.52)**	**1.87 (1.19–2.95)**	**0.008**
Blue	40 (11.2)	237 (66.6)	79 (22.2)	42 (16.2)	173 (66.8)	44 (17.0)	0.090	1.00	1.63 (0.95–2.80)	**2.20 (1.15–4.21)**	**0.019**
Tanning ability											
Good (high)	68 (13.1)	351 (67.6)	100 (19.3)	111 (18.0)	404 (65.5)	102 (16.5)	0.059	1.00	**1.64 (1.13–2.37)**	**1.85 (1.17–2.92)**	**0.010**
Poor (low)	42 (12.5)	220 (65.3)	75 (22.2)	44 (20.5)	133 (61.8)	38 (17.7)	**0.031**	1.00	**1.77 (1.04–3.00)**	**2.37 (1.26–4.49)**	**0.009**
Freckling in the sun as a child											
No	43 (11.4)	255 (67.5)	80 (21.1)	90 (18.2)	330 (66.7)	75 (15.1)	**0.004**	1.00	**1.86 (1.20–2.89)**	**2.81 (1.65–4.79)**	**0.0002**
Yes	67 (14.0)	316 (66.1)	95 (19.9)	65 (19.4)	206 (61.5)	64 (19.1)	0.121	1.00	**1.53 (1.00–2.35)**	1.42 (0.85–2.37)	0.229
Sunburns with blistering											
No	25 (10.4)	167 (69.9)	47 (19.7)	68 (20.5)	206 (62.3)	57 (17.2)	**0.006**	1.00	**2.70 (1.54–4.73)**	**2.99 (1.51–5.90)**	**0.002**
Yes	85 (13.8)	403 (65.4)	128 (20.8)	87 (17.4)	330 (66.1)	82 (16.5)	0.076	1.00	1.39 (0.96–2.02)	**1.68 (1.07–2.63)**	**0.026**

[a] Two-sided χ^2 tests.
[b] Adjusted for age and sex, family history and eight sun-exposure risk factors as defined in Table 1 in logistic regression models.

orders were –1607 2G/1G, –839G > A, –755T > G, –519A > G, –422A > T, –340A > G and –320T > C) as the reference, we found that two haplotypes, 1G–A–G–A–T–G–T and G–G–G–A–T–A–T, each with a frequency less than 5%, were associated with significantly increased risk of CM (adjusted ORs = 1.56 and 2.13, 95% CI = 1.02–2.38 and 1.22–3.70, respectively).

4. Discussion

In this study, we comprehensively investigated the association between *MMP1* promoter polymorphisms and CM risk. We provided statistical evidence that polymorphisms of *MMP1* –519A > G, –422A > T and –320T > C might independently contribute to CM risk, with possible interactions with other known environmental risk factors as well. A dose-dependent influence of these genetic variants was also observed, as patients carrying an increased number of risk alleles exhibited a greater risk of CM.

Melanoma is a multifactorial disease originated from both environmental and genetic factors and their interactions. Previous studies have demonstrated an association of increased UV exposure[7–9] or reduced DNA repair capacity with an elevated risk of CM.[35] In addition, individual characteristics or host factors, including the presence and number of moles and dysplastic nevi, blond hair, blue eye colour, fair skin colour, poor tanning ability, freckling in the sun as a child, sunburns with blistering and first-degree relative with cancer, were also identified as risk factors of CM, especially in Caucasians, as previously reported.[9] Recent genome-wide association studies have identified a few SNPs in *MC1R*, *TRP*, adjacent to *MTAP* and flanking *CDKN2A* associated with melanoma risk,[36] suggesting that other SNPs may have only

a moderate or weak effect on melanoma risk. Studies have found that some particular genes, such as those encoding *MMP*, *RhoC* and *fibronectin*, are involved in the progression (invasiveness and metastasis) of CM,[37,38] probably through degradation or interaction with the extracellular matrix proteins. However, it remains unknown if these genes could also influence CM tumourigenesis as observed for other types of cancer.[31,32,39,40]

MMP1 is a major proteinase of the MMP family and is widely expressed in a variety of normal cells, including stromal fibroblasts and epithelial cells. Its expression is low in physiological conditions but increased substantially in pathological conditions, such as in cancers including CM, in both cancer cells and the surrounding stromal cells.[41] Considering the critical importance of the interaction between cancer cells and the surrounding microenvironment,[42] up-regulated expression of *MMP1* may contribute to CM tumourigenesis by creating and maintaining a microenvironment that facilitates tumour growth. Indeed, *MMP1* overexpression was found to promote melanoma cell growth by both degrading extracellular matrix proteins and generating active tumour growth factors, such as TGF-β *in vivo*.[43]

Our large epidemiological study supports this hypothesis by demonstrating a significant association between the *MMP1* promoter polymorphisms and CM risk. Although biological functions of the *MMP1* –519A > G, –422A > T and –320T > C polymorphisms have not been fully understood, they are located in the proximity to the transcriptional start site and may therefore interfere with the binding affinity of transcription factors, hence influencing the *MMP1* expression. Unlike most previous studies[24,29–32] that have shown an association between the functional *MMP1* –1607 2G/1G polymorphism and cancer susceptibility, our study did not identify the evidence for its association with CM; such a null association was also observed in prostate, kidney and upper digestive tract cancers.[44–46] It is possible that tumours from different origins might have different mechanisms to regulate the *MMP1* expression. Future studies should include concurrent examination of *MMP1* messenger RNA levels that can be correlated to those *MMP1* promoter polymorphisms.

In the present study, patients carrying an increased number of *MMP1* –519A, –422T or –320C alleles showed a tendency of a greater CM risk, which was almost doubled in individuals carrying all three homozygous variants (–519AA/–422TT/–320CC) compared to individuals carrying no risk alleles (–519GG/–422AA/–320TT), an observation further confirmed by a statistically significant OR, after combining several risk allele groups together to improve statistical power (Table 3). The interactive influence of these three *MMP1* polymorphisms on CM risk cannot be simply described as the sum of their separate effects. Their close physical distance in the promoter region implies that they may participate and potentiate one another in the interaction of the transcriptional factor. Finally, in the stratified analyses by the aforementioned demographic characteristics, we found that the influence of the combined risk alleles seemed to be modified by some known epidemiological risk factors, such as age, hair colour and freckling in the sun as a child. It is not clear what the underlying mechanism for these modification effects is or it is just a chance finding. However, these known epidemiological risk factors, to a large extent, also reflect an individual's genetic predisposition. For example, the hair, eye or skin colour was genetically determined. The poor tanning ability, freckling in the sun as a child or sunburns with blistering may suggest an increased photosensitivity trait. Therefore, it is highly possible that the observed modification effects may result from gene–gene interactions. Future mechanistic studies are required to elucidate our findings.

It has been previously shown that promoter polymorphisms can cooperate or interact to exert a joint effect on gene expression and influence cancer susceptibility.[34,47,48] Through haplotype analysis, we identified that two haplotypes, Gdel–A–G–A–T–G–T and G–G–G–A–T–A–T, were associated with significantly increased risk of CM in our study population. This result was partly supported by the study of Pearce and colleagues, in which the G–G–G–A–T–A–T (same as reported GG–G–G–A–T–T–T) were found to have highest promoter activities in both A2058 and A375 melanoma cell lines.[34]

To the best of our knowledge, this is the first comprehensive epidemiological study to investigate the association between all known, common *MMP1* promoter polymorphisms and CM risk and provides important information on the role of *MMP1* genetic variation in the development of CM. These data will allow us to conduct a large-scale case-only analysis to elucidate the contribution of genotypes and haplotypes of the *MMP1* promoter to CM progression and prognosis in the future.

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Conflict of interest statement

None declared.

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