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Genotypes and haplotypes of matrix metalloproteinase 1, 3 and 12 genes and the risk of lung cancer

Li Su[1,†], Wei Zhou[1,†], Kofi Asomaning[1], Xihong Lin[2], John C.Wain[3], Thomas J.Lynch[4], Geoffrey Liu[1,4] and David C.Christiani[1,5,*]

[1]Department of Environmental Health, [2]Department of Biostatistics, Harvard School of Public Health, [3]Thoracic Surgery Unit, Department of Surgery, [4]Department of Hematology-Oncology, and [5]Pulmonary and Critical Care Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[*]To whom correspondence should be addressed at: Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA. Tel: +1 617 432 3323; Fax: +1 617 432 6981; Email: dchristi@hsph.harvard.edu

The MMPs (matrix metalloproteinases) are a family of secreted zinc metalloproteases that degrade the collagens of the extracellular matrix important in tissue remodeling and repair during development and inflammation. We investigated the associations between polymorphisms of *MMP-1* (*-1607 1G/2G*, rs1799750), *MMP-3* (*-1171 5A/6A*, rs3025058), and *MMP-12* (*-82AG*, rs2276109, and *1082A/G*, rs652438) and the risk of lung cancer in 2014 Caucasian lung cancer patients and 1323 healthy controls. The results were analyzed using logistic regression models, adjusting for covariates. The four polymorphisms were in Hardy-Weinberg disequilibrium. Except for the *1G-1082A*, the other linkage disequilibrium tests between the four *MMP* polymorphisms were statistically significant ($P < 0.001$). There was no overall association between individual *MMP* polymorphism and the risk of lung cancer. The *MMP* polymorphisms jointly were associated with a non-statistically significant higher risk of lung cancer, with the adjusted odds ratio (AOR) of subjects with 5+ variant alleles versus zero variant allele of 1.31 [95% confidence interval (CI), 0.92–1.88]. Stronger associations were observed in never-smokers and males, with the corresponding AORs of 2.44 (95%CI, 1.10–5.43, $P_{trend} = 0.04$) in never smokers and 1.35 (95%CI, 0.79–2.30, $P_{trend} = 0.04$) in men. In haplotype analysis, the *1G-6A-82A-1082G* haplotype was associated with higher risk of lung cancer among never smokers, with the AOR of 3.65 (95%CI, 1.62–8.20) when compared with the most common *1G-5A-82A-1082A* haplotype. In conclusion, the combined *MMP* genotypes and associated haplotypes may be associated with higher risk of lung cancer, particularly among never smokers and men.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; MMP, matrix metalloproteinases.

[†]These authors contributed equally to this work.

Introduction

Matrix metalloproteinases (MMPs) are a pivotal family of zinc enzymes responsible for degradation of the extracellular matrix components including basement membrane collagen, interstitial collagen, fibronectin and various proteoglycans, during normal remodeling and repair processes. MMPs play a central role in the enhancement of tumor-induced angiogenesis, cell migration, proliferation, apoptosis and connective tissue degradation (1). Among the 20+ MMPs, MMP-1 (collagenase) may degrade the interstitial types I, II and III collagens and contribute to tumor initiation and development by altering the cellular microenvironment that facilitates tumor formation. MMP-3 (stromelysin-1, also known as STR1, STMY1) is capable of degrading proteoglycan, fibronectin, laminin, type IV collagen, and may activate other MMPs including MMP-1 (2). MMP-12 (macrophage metalloelastase, MME) shares the highly conserved exon size and intron-exon borders with other MMPs (3), and participates in aortic elastin degradation (4).

MMP-1, *MMP-3* and *MMP-12* are located in the same chromosome region (*11q22-q23*), with functional polymorphisms suggested in *in vitro* studies. The *2G* allele of the *MMP-1-1607 1G/2G* polymorphism (rs1799750) has been associated with higher MMP-1 expression levels (5) and higher risk of lung cancer (6,7). The *6A* allele of the *MMP-3-1171 5A/6A* polymorphism (rs3025058) is in linkage disequilibrium with the *MMP-1 2G* allele (8), while it was associated with lower promoter activity in a transient expression experiment (9), and lower risk of lung cancer among smokers in a Chinese study (8). Moreover, the *MMP 2G/6A* haplotype was associated with lower risk of lymphatic metastasis of lung cancer when compared with the *1G/5A* haplotype (8). The *A* allele of the *MMP-12 -82AG* polymorphism (rs2276109) shows a higher affinity for the transcription factor activator protein-1 (AP-1) and higher gene expression in reporter gene assays (10). The *MMP-12 1082A/G* (*357Asn/Ser*, rs652438) polymorphism is located in the coding region of the hemopexin domain that is responsible for MMP-12 activity, while the function of this polymorphism remains unknown. MMP-12 expression levels were found to be upregulated in recurrent versus non-recurrent stage IB lung cancer (11) and correlated with local recurrence and metastasis (12). Currently, there are no reports on the *MMP-12* polymorphisms in the risk of lung cancer, although haplotyes of the *1G/1082A* polymorphism have been associated with higher rate of decline of lung function (13).

The *MMP-1 2G* allele has been associated with higher risk of lung cancer in never smokers and males in our previous analysis (7). We hypothesized that the *6A* allele of *MMP-3* polymorphism, the *G* allele of the *MMP-12-82AG* polymorphism, and the *G* allele of the *MMP-12 1082A/G* polymorphism, which have been shown to be in linkage disequilibrium with the *MMP-1 2G* allele (8,13), are also associated with higher risk of lung cancer, specially among never smokers and men.

In addition, we hypothesized that the joint effects or haplotypes of these polymorphisms are stronger than the individual effect of each polymorphism. In this ongoing study with an expanded sample size, we investigated the associations between these *MMP* polymorphisms and the risk of lung cancer, using gene–gene joint effects as well as haplotype analyses.

Materials and methods

Study population

This is a hospital based case–control study with details described previously (14–16). Briefly, all eligible cases (patients with histologically confirmed lung cancers) at Massachusetts General Hospital were recruited between December 1992 and December 2004. Controls were recruited among healthy friends and non-blood-related family members (usually spouses) of several groups of hospital patients: (i) patients with cancer, whether related or not related to a case; or (ii) patients with a cardiothoracic condition undergoing surgery. No matching was performed. Importantly, none of the controls were themselves patients. Potential controls who carried a previous diagnosis of any cancer (other than non-melanoma skin cancer) were excluded from participation. Over 85% eligible cases, and over 90% controls participated in this study and provided blood samples. Interviewer-administered questionnaires collected information on demographic and detailed smoking histories from each subject. The study was approved by the Human Subjects Committees of Massachusetts General Hospital and the Harvard School of Public Health, Boston, MA.

MMPs genotyping

DNA was extracted from peripheral blood samples using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). The *MMP-1*, *MMP-3* and *MMP-12* polymorphisms were genotyped by the 5′ nuclease assay (TaqMan) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster city, CA). The primers, probes and reaction conditions are available upon request. Genotyping was performed by laboratory personnel blinded to case–control status, and a random 5% of the samples were repeated to validate genotyping procedures. Two authors reviewed independently all genotyping results.

Statistical analysis

Although individuals of all races were recruited for this study, we restricted our analyses to Caucasians (97%) to minimize confounding due to allele frequency variation by race. We analyzed all Caucasians with complete information on age, gender, smoking status (never smokers, ex-smokers and current smokers), pack-years of smoking, and years since smoking cessation (for ex-smokers).

Hardy–Weinberg Disequilibrium of each polymorphism in cases and controls were tested using chi-square test; detection of linkage disequlibrium between the four polymorphisms was based on Lewontin's D′ in controls. Haplotype frequencies and individual haplotypes were generated using the Expectation Maximization (EM) algorithm, which reconstruct individual probabilities for individual phasing accuracy based on unphased genotype data, as well as estimates on the overall haplotype frequencies and their standard errors (17–20).

Analyses of all genotype and haplotype associations with lung cancer risk were performed using logistic regression models. A total of three types of models were fit to examine the relationship between the log odds of lung cancer and each covariate, after adjusting for possible confounding factors such as age, gender, smoking status, pack-years of smoking and years since smoking cessation (if ex-smoker). First, we investigated the associations between individual *MMP* polymorphism and the risk of lung cancer in separate logistic regression models, overall and in different strata of gender and smoking status (Model type I). Second, we investigated the joint effects of all of the four *MMP* polymorphisms, based on the results of linkage disquilibrium and the number of variant alleles, where the wild genotype (*1G*/*1G* of *MMP-1*, *5A*/*5A* of *MMP-3*, *A*/*A* of *MMP-12 -82A*/*G*, and *A*/*A* of the *MMP-12 1082A*/*G* polymorphism) has zero variant allele, heterozygous genotype has one variant allele, and homozygous variant genotype has two variant alleles (Model type II). Last, we investigated the associations between *MMP* haplotypes and the risk of lung cancer using the 'expectation substitution' approach in SAS macro of 'HAPPY' by Dr Peter Kraft (17,18,20), which treats expected haplotype scores (calculated under additive model) as observed covariates in a standard unconditional logistic analysis, instead of assigning each subject with the most likely haplotype pair (Model type III). A lack of fit test, as described in Hosmer and Lemeshow (21), was performed to summarize the goodness-of-fit for each logistic regression model. Where appropriate, the odds ratios (OR) and 95% confidence intervals (CI) for the risk of lung cancer were calculated from these models. All reported *P*-values are from two-sided tests. *P*-values <0.05 were considered statistically significant. All analyses were performed using SAS software version 9 (SAS Institute, Cary, NC).

Results

Population characteristics

There were no significant demographic differences (age and gender) between enrolled and unenrolled eligible cases and controls. Genotyping success rate was 98% for all of the four *MMP* polymorphisms. We restricted our analysis to the 3337 Caucasians with complete data (excluding 116 non-Caucasian subjects). Of these, there were 2014 lung cancer cases and 1323 controls. There was 100% concordance of the randomly repeated samples.

The distributions of demographic characteristics for cases and controls are summarized in Table I. Compared to the controls, cases were older, had a higher proportion of males, more likely to be current smokers or heavy smokers, and had a shorter time since smoking cessation (if an ex-smoker) and larger pack-years of smoking. The distribution of smoking variables in our controls was similar to the general Massachusetts population over the age of 45 years (15,22). Adenocarcinoma, squamous cell carcinoma, large cell carcinoma,

Table I. Demographic characteristics among lung cancer cases and controls

Characteristics	Cases ($n = 2014$) *n* (%)	Controls ($n = 1323$) *n* (%)	*P*-value
Age[a]	67 (26–91)	59 (19–96)	<0.001
Gender[b]			
Male	1041 (52%)	584 (44%)	
Female	973 (48%)	739 (56%)	<0.001
Smoking status[b]			
Never	166 (8%)	463 (35%)	
Ex-smoker	1048 (52%)	600 (45%)	
Current smoker	800 (40%)	260 (20%)	<0.001
Pack-years[a,c]	51 (0.1–231)	25 (0.1–218)	<0.001
Cigarettes/day[a,c]	30 (0.1–120)	20 (0.1–100)	<0.001
Smoking duration[a,c]	40 (0.5–73)	26 (0.5–62)	<0.001
Years since quitting smoking[a,d]	13 (1–59)	18 (1–65)	<0.001
MMP-1 1G/2G polymorphism[b]			
1G/1G	541 (27%)	367 (28%)	
1G/2G	1015 (50%)	642 (48%)	
2G/2G	458 (23%)	314 (24%)	0.57
MMP-3 5A/6A polymorphism[b]			
5A/5A	485 (24%)	325 (24%)	
5A/6A	1012 (50%)	648 (50%)	
6A/6A	517 (26%)	350 (26%)	0.77
MMP-12 -82A/G polymorphism[b]			
A/A	1535 (76%)	1008 (76%)	
A/G	449 (22%)	289 (22%)	
G/G	30 (2%)	26 (2%)	0.56
MMP-12 1082A/G polymorphism[b]			
A/A	1767 (88%)	1180 (89%)	
A/G	235 (12%)	137 (10%)	
G/G	12 (1%)	6 (1%)	0.42

[a]Median (range), tested by non-parametric Wilcoxon's rank sum test.
[b]Cases and controls were compared using Pearson chi-square tests.
[c]Excludes individuals who have never smoked.
[d]Ex-smokers only.

Table II. Adjusted odds ratios (AORs, 95%CI) of different *MMP* polymorphisms in lung cancer risk

	MMP-1 -1607 1G/2G		*MMP-3 -1171 5A/6A*		*MMP-12 -82AG*	*MMP-12 1082AG*
	1G/2G versus *1G/1G*	*2G/2G* versus *1G/1G*	*5A/6A* versus *5A/5A*	*6A/6A* versus *5A/5A*	*AG + G/G* versus *A/A*	*AG + G/G* versus *A/A*
Overall, crude	1.07 (0.91–1.27)	0.99 (0.81–1.20)	1.05 (0.88–1.24)	0.99 (0.81–1.20)	1.00 (0.85–1.18)	1.15 (0.93–1.44)
Adjusted[a]	1.12 (0.92–1.36)	1.11 (0.88–1.40)	1.14 (0.94–1.40)	1.02 (0.82–1.29)	1.09 (0.90–1.32)	1.20 (0.93–1.54)
Smoking status						
Never, n[b]	86/210 versus 38/130	42/123 versus 38/130	84/227 versus 31/122	51/114 versus 31/122	47/114 versus 119/349	29/52 versus 137/411
AOR[c]	1.51 (0.96–2.36)	1.24 (0.74–2.07)	1.46 (0.91–2.34)	1.76 (1.04–2.97)	1.18 (0.79–1.77)	1.55 (0.93–2.57)
Ex-, n[b]	534/291 versus 293/174	221/135 versus 293/174	533/300 versus 253/138	262/162 versus 253/138	252/136 versus 796/464	111/67 versus 937/533
AOR[d]	1.16 (0.89–1.51)	1.07 (0.77–1.48)	1.10 (0.83–1.46)	0.91 (0.66–1.26)	1.12 (0.86–1.47)	0.95 (0.66–1.36)
Current, n[b]	395/141 versus 210/63	195/56 versus 210/63	395/121 versus 201/65	204/74 versus 201/65	180/65 versus 620/195	107/24 versus 693/236
AOR[e]	0.88 (0.61–1.29)	1.12 (0.71–1.75)	1.09 (0.74–1.59)	0.89 (0.58–1.36)	1.00 (0.70–1.44)	1.45 (0.87–2.43)
Female, n[b]	483/371 versus 262/188	228/180 versus 262/188	486/356 versus 231/174	256/209 versus 231/174	234/185 versus 739/554	106/85 versus 867/654
AOR[f]	0.99 (0.75–1.30)	1.02 (0.74–1.40)	1.08 (0.82–1.44)	0.86 (0.62–1.18)	1.00 (0.77–1.30)	0.92 (0.64–1.32)
Male, n[b]	532/271 versus 279/179	230/134 versus 279/179	526/292 versus 254/151	261/141 versus 254/151	245/130 versus 796/454	141/58 versus 900/526
AOR[f]	1.28 (0.97–1.68)	1.20 (0.86–1.67)	1.20 (0.90–1.60)	1.23 (0.88–1.71)	1.20 (0.91–1.59)	1.51 (1.04–2.09)

[a]Adjusted for age, gender, smoking status, pack-years of smoking and years since smoking cessation. Each polymorphism was analyzed in separate logistic regression models.
[b]The frequencies are case–controls for each genotype.
[c]Adjusted for age and gender.
[d]Adjusted for age, gender, pack-years of smoking and years since smoking cessation.
[e]Adjusted for age, gender and pack-years of smoking.
[f]Adjusted for age, smoking status, pack-years of smoking and years since smoking cessation.

and small-cell carcinoma represented 51, 21, 8 and 9% of cases, respectively. Eleven percent of patients were of mixed histological subtype or had more than one primary tumor. Clinical AJCC stage data were available for 1987 cases, 45% were early stage (I or II).

Distribution of MMP polymorphisms among cases and controls

All *MMP* polymorphisms in the control and case populations were consistent with Hardy–Weinberg equilibrium ($P > 0.05$, chi-squared goodness-of-fit). Genotype frequencies of *MMP* polymorphisms (Table I) were comparable with previous studies (6,13). Except for the linkage of *1G-1082A* ($D' = 0.16$), the other linkages disequilibrium tests between the four *MMP* polymorphisms were statistically significant ($P < 0.001$), with the D' *of 1G-5A* of 0.46, *1G-82A* of 0.80, *5A-82A* of 0.81, *5A-1082A* of 0.98 and *82A-1082G* of 1.00.

Associations between MMP genotypes and lung cancer risk

There were no overall crude or adjusted associations between individual *MMP* polymorphism and the risk of lung cancer (Table II, Model type I). In the subgroup analysis, although the variant genotypes of all of the *MMP* polymorphisms were associated with higher risk of lung cancer in never smokers than in ever smokers, in men than in women, the majority of the results were not statistically significant (Table II). Specifically, the *6A/6A* genotype of the *MMP-3* polymorphism was associated with higher risk of lung cancer in never smokers [adjusted odds ratio (AOR), 1.76, 95%CI, 1.04–2.97; *6A/6A* versus *5A/5A*], and the *G* allele of the *MMP-12 1082 A/G* polymorphism was associated with higher risk of lung cancer in men (AOR, 1.51, 95%CI, 1.04–2.09; *A/G + G/G* versus *A/A*).

In Model type II, the combined *MMP* polymorphisms were dichotomized into six groups based on the number of variant alleles and sample sizes of cases and controls. The joint *MMP* genotypes were associated with a non-statistically significant higher risk of lung cancer (Table III): the AOR of subjects with 4 and 5+ variant alleles versus zero variant allele were 1.23 (95%CI, 0.92–1.68) and 1.31 (95%CI, 0.92–1.88), respectively ($P_{trend} = 0.29$). By different strata of smoking status and gender, the joint *MMP* polymorphisms were associated with higher risk of lung cancer in never smokers and in men: the AORs of subjects with 4 and 5+ variant alleles versus zero variant allele were 2.37 (95%CI, 1.15–4.90) and 2.44 (95%CI, 1.10–5.43), respectively ($P_{trend} = 0.04$), in never smokers; and 1.61 (95%CI, 1.02–2.54) and 1.35 (95%CI, 0.79–2.30), respectively ($P_{trend} = 0.04$), in males.

Associations between MMP haplotypes and lung cancer risk

There are total of seven common haplotypes (>2%) among both cases and control. The distributions of different haplotypes were similar between cases and controls (Table IV). The most common haplotype was the *1G-5A-82A-1082A* haplotype, with the frequencies of 36% in both cases and controls. In the haplotype analysis where the expected haplotype scores were fitted as observed covariates in the logistic regression model (Model type III), the most common *1G-5A-82A-1082A* haplotype was treated as reference group. In the overall analysis, although the *1G-6A-82A-1082G* haplotype was associated with borderline significantly higher risk of lung cancer with the AOR of 1.52 (95%CI, 1.00–2.30), the global test of haplotype association was not statistically significant ($P = 0.18$ in likelihood test). Stratified by smoking status, the higher risk of the *1G-6A-82A-1082G* haplotype was observed in never smokers (AOR of 3.65, 95%CI, 1.62–8.20), while not in ex-smokers (AOR of 0.91, 95%CI, 0.53–1.58) or current smokers

Table III. Adjusted odds ratios (AORs, 95%CI) of combined *MMP* polymorphisms and lung cancer risk

	Number of variant alleles of combined *MMP* polymorphisms						P_{trend}
	Zero	One	Two	Three	Four	Five and above	
Overall, *n*[a]	253/183	372/217	488/311	428/318	291/182	182/112	0.35
Crude OR	1	1.24 (0.96–1.60)	1.14 (0.90–1.44)	0.97 (0.77–1.24)	1.16 (0.89–1.51)	1.18 (0.87–1.59)	0.90
AOR[b]	1	1.27 (0.94–1.71)	1.12 (0.85–1.49)	1.15 (0.87–1.53)	1.23 (0.90–1.68)	1.31 (0.92–1.88)	0.29
Smoking status							
Never, *n*[a]	14/74	25/67	39/102	38/115	31/63	19/42	0.21
AOR[c]	1	1.84 (0.87–3.87)	1.95 (0.98–3.90)	1.75 (0.88–3.48)	2.37 (1.15–4.90)	2.44 (1.10–5.43)	0.04
Ex-, *n*[a]	130/74	213/109	241/147	233/137	148/87	83/46	0.96
AOR[d]	1	1.20 (0.79–1.81)	0.97 (0.65–1.44)	1.20 (0.80–1.79)	1.06 (0.69–1.65)	1.05 (0.62–1.77)	0.94
Current, *n*[a]	109/35	134/41	208/62	157/66	112/32	80/24	0.58
AOR[e]	1	1.13 (0.64–1.99)	1.04 (0.62–1.75)	0.86 (0.51–1.45)	1.09 (0.60–1.99)	1.22 (0.64–2.33)	0.86
Females, *n*[a]	125/102	176/107	236/160	213/196	123/108	100/66	0.08
AOR[f]	1	1.42 (0.93–2.17)	1.23 (0.83–1.82)	1.05 (0.72–1.55)	0.97 (0.63–1.50)	1.23 (0.76–1.99)	0.58
Males, *n*[a]	128/81	196/110	252/151	215/122	168/74	82/46	0.64
AOR[f]	1	1.16 (0.76–1.77)	1.04 (0.70–1.55)	1.31 (0.87–1.98)	1.61 (1.02–2.54)	1.35 (0.79–2.30)	0.04

[a]The frequencies are case–controls, with the *P*-values for Pearson's chi-square test.
[b]Adjusted for age, gender, smoking status, pack-years of smoking and years since smoking cessation.
[c]Adjusted for age and gender.
[d]Adjusted for age, gender, pack-years of smoking and years since smoking cessation.
[e]Adjusted for age, gender, and pack-years of smoking.
[f]Adjusted for age, smoking status, pack-years of smoking and years since smoking cessation.

Table IV. Inferred haplotype frequencies and adjusted odds ratios (AORs, 95%CI) of *MMP* haplotypes in lung cancer risk

	Haplotypes							
	0000, *1G-5A-82A-1082A*	1100, *2G-6A-82A-1082A*	1000, *2G-5A-82A-1082A*	0100, *1G-6A-82A-1082A*	1110, *2G-6A-82G-1082A*	1101, *2G-6A-82A-1082G*	0101, *1G-6A-82A-1082G*	Others
Overall, *n*[a]	1459/953	860/566	486/317	456/321	440/292	131/82	117/65	79/52
AOR[b]	1	1.03 (0.88–1.21)	1.02 (0.83–1.27)	0.82 (0.66–1.02)	1.10 (0.90–1.35)	0.96 (0.66–1.39)	1.52 (1.00–2.30)	0.85 (0.52–1.39)
Smoking status								
Never, *n*[a]	102/347	70/197	42/115	35/90	45/109	13/30	19/21	7/16
AOR[c]	1	1.30 (0.91–1.85)	1.25 (0.79–2.00)	1.22 (0.73–2.01)	1.40 (0.91–2.16)	1.14 (0.50–2.60)	3.65 (1.62–8.20)	1.29 (0.40–4.15)
Ex-, *n*[a]	777/423	440/260	243/137	253/163	229/122	59/35	55/35	41/24
AOR[d]	1	0.95 (0.76–1.19)	0.98 (0.71–1.34)	0.81 (0.60–1.08)	1.11 (0.82–1.49)	0.87 (0.52–1.47)	0.91 (0.53–1.58)	0.74 (0.39–1.43)
Current, *n*[a]	580/183	350/108	201/64	168/67	166/61	60/17	43/8	31/11
AOR[e]	1	1.00 (0.73–1.37)	0.99 (0.66–1.47)	0.66 (0.43–1.01)	0.98 (0.68–1.43)	0.95 (0.47–1.92)	2.21 (0.80–6.10)	0.70 (0.27–1.83)
Females, *n*[a]	705/517	426/321	230/171	221/178	223/180	55/51	53/35	33/26
AOR[f]	1	0.98 (0.78–1.22)	1.12 (0.82–1.51)	0.80 (0.59–1.09)	0.98 (0.75–1.29)	0.62 (0.37–1.04)	1.49 (0.81–2.74)	0.69 (0.32–1.49)
Males, *n*[a]	754/436	434/244	256/145	235/143	217/112	76/31	64/30	46/25
AOR[f]	1	1.09 (0.86–1.37)	0.95 (0.70–1.30)	0.84 (0.62–1.15)	1.28 (0.94–1.75)	1.42 (0.82–2.44)	1.53 (0.86–2.71)	0.95 (0.50–1.84)

[a]Inferred haplotype frequencies for case–controls, where each subject has two haplotypes.
[b]Adjusted for age, gender, smoking status, pack-years of smoking and years since smoking cessation.
[c]Adjusted for age and gender.
[d]Adjusted for age, gender, pack-years of smoking and years since smoking cessation.
[e]Adjusted for age, gender and pack-years of smoking.
[f]Adjusted for age, smoking status, pack-years of smoking and years since smoking cessation.

(AOR of 2.21, 95%CI, 0.80–6.10). Similar associations between *MMP* haplotypes and the risk of lung cancer were found among women and men, with the AORs (*1G-6A-82A-1082G* versus *1G-5A-82A-1082A*) of 1.49 (95%CI, 0.81–2.74) for women and 1.53 (95%CI, 0.86–2.71) for men.

Discussion

MMPs are secreted zinc metalloproteases that degrade the collagens of the extracellular matrix important in tissue remodeling and repair during development and inflammation. MMPs may alter cell cycle checkpoint controls, promote genomic instability conceivably by affecting cell adhesion (23), and contribute to tumor initiation and development by altering the cellular microenvironment that facilitates tumor formation (6). Excessive or inappropriate expression of MMP may contribute to the pathogenesis of tissue destructive processes in a wide variety of diseases including lung cancer (24). There are currently more than 20 MMPs reported that can be categorized by substrate specificity, with functional polymorphisms reported for *MMP-1*, *MMP-2*, *MMP-3*, *MMP-9* and *MMP-12*. In this study, we investigated the associations between genotypes and haplotypes of functional polymorphisms of *MMP-1* (*-1607 1G*/*2G*), *MMP-3* (*-1171 5A*/*6A*) and *MMP-12* (*-82AG* and *1082A*/*G*), which are located in the same chromosome region and in linkage disequilibrium with each other, and the risk of

lung cancer. Although we did not observe the overall associations between individual polymorphism and lung cancer risk, we found that the joint *MMP* polymorphisms and specific *MMP* haplotype (*1G-6A-82A-1082G*) may be associated with higher risk of lung cancer, specifically among never smokers or among men, consistent with our previous findings for the *MMP-1 -1607 1G/2G* polymorphism (7).

In the genotype analysis where each *MMP* polymorphism was analyzed individually, the *MMP-3 6A/6A* genotype was associated with higher risk of lung cancer among never smokers (Table II), which is similar to the findings of coronary heart disease (25). The *6A/6A* genotype was associated with lower promoter activity in *in vitro* assay (9), and this lower level of proteolytic activity would favor extracellular matrix deposition in lung lesions, since MMP-3 is capable of degrading proteoglycan, fibronectin, laminin and type IV collagen. We did not observe the effect of *MMP-3* polymorphism (or other *MMP* genotypes) in ever smokers. One possible explanation is that the effect of *MMP* polymorphisms on lung cancer risk may be overwhelmed by the effect of cigarette smoking among smokers. Alternatively, cigarettes smoking is a major source of extracellular matrix and may induce mRNA levels of *MMPs* and tissue inhibitors of metalloproteases (26). Therefore, the effect of *MMP* polymorphisms in smokers may depend upon the balance between MMPs and tissue inhibitors of metalloprotease. Our results are not consistent with the Chinese study, where the *MMP-3 6A/6A* genotype was associated with a lower risk of lung cancer among smokers (8).

We did not observe an association between the *MMP-12 -82A/G* polymorphism and risk of lung cancer overall, or in different strata of smoking or gender. Instead, the *G* allele (*A/G* + *G/G*) of the *MMP-12 1082A/G* polymorphism was associated with higher risk of lung cancer among men, and not among women (Table II). Another study suggested that the *MMP-12* polymorphisms were not associated with breast cancer risk in women (27). Although the function of the *MMP-12 1082A/G* polymorphism remains unknown, the *MMP-12 1082A/G* polymorphism is located in the coding region of the hemopexin domain and is in high linkage disequilibrium with the *MMP-12 -82A/G* polymorphism, which has been shown to affect *MMP-12* gene expression levels (10). Although there is no report of an association between *MMP-12* polymorphisms and lung cancer risk, studies have shown that MMP-12 expression levels are upregulated in lung cancer tissues (11,12,28).

In the joint effect analysis of *MMP* polymorphisms, where the four polymorphisms were combined based on the number of variant alleles, subjects with 4 or 5+ variant alleles had higher risk of lung cancer, especially among never smokers and men (Table III). Carcinogenesis is a multicellular and multistage process, and different genes that metabolize different types of collagens and stromelysins may be involved in different stages of carcinogenesis. Therefore, gene–gene joint effects may provide more complete and reliable information than the single polymorphism analysis, which may only contribute partially to the MMPs pathway.

In the haplotype analysis, the *1G-6A-82A-1082G* haplotype was associated with higher risk of lung cancer among never smokers (when compared with the *1G-5A-82A-1082A* haplotype). The results of haplotype analysis were consistent with the genotype analysis, where the associations were driven by the *MMP-3 6A* and *MMP-12 1082 G* alleles. Previous studies have suggested that the *MMP 1G/5A* haplotype was associated with increased risks of lung cancer lymphatic metastasis (8) and head and neck squamous cell carcinoma (29), when compared with the *2G/6A* haplotype. In our analysis, we did not observe a significant difference between the *2G-6A-82A-1082A* haplotype and the *1G-5A-82A-1082A* haplotype (Table IV).

We acknowledge several limitations to our study. First, this is a hospital-based case–control study. Second, we only evaluated the four functional polymorphisms of *MMPs* located in the same chromosome region of 11q22–q23, in order to perform haplotype analysis. It is possible that functional polymorphisms of other *MMP* genes such as *MMP-2* and *MMP-9* may affect the association between these four polymorphisms and lung cancer risk. In addition, the potent proteolytic activities of MMPs are mainly regulated by the balance with specific tissue inhibitors of MMPs (24). However, adding other polymorphisms into the analysis will require a larger sample size, especially in the subgroup analysis by smoking status. Third, although we adjusted for various smoking variables in our analysis, second hand smoke exposure, alcohol consumption, diet, and environmental and occupational exposure data were not adjusted in our logistic regression models because of incomplete and missing information. Given the consistent results between gene–gene joint effects and haplotype analysis, these confounders will only probably have had mild effects on the results, if any. Fouth, the inferred haplotype frequencies were based on the selected functional polymorphisms instead of haplotype tagging SNPs, which may introduce inherited error in the analysis. Lastly, the significant associations between *MMP* polymorphisms and lung cancer risk were observed only among never smokers. Although the sample size of this case–control population is large, results from stratified analysis are based on relatively small sample sizes. We cannot exclude the possibility of 'false positive' results, especially in light of the multiple comparisons performed. However, we did observe consistent results in genotype and haplotype analyses.

In conclusion, this is the first study on the associations between the joint polymorphisms and haplotypes of *MMP-1*, *MMP-3*, and *MMP-12* and the risk of lung cancer. Our results suggested that the joint *MMP* polymorphisms are associated with higher risk of lung cancer, specifically among never smokers and men. In addition, the *1G-6A-82A-1082G* haplotype is associated with higher risk of lung cancer among never smokers. These results need to be confirmed by other independent studies, and further studies are needed to investigate the gene–environment interactions between *MMP* polymorphisms and cigarettes smoking.

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References

1. Nagase,H. and Woessner,J.F.,Jr. (1999) Matrix metalloproteinases. *J. Biol. Chem.*, **274**, 21491–21494.

2. Johansson,N., Ahonen,M. and Kahari,V.M. (2000) Matrix metalloproteinases in tumor invasion. *Cell Mol. Life Sci.*, **57**, 5–15.
3. Belaaouaj,A., Shipley,J.M., Kobayashi,D.K., Zimonjic,D.B., Popescu,N., Silverman,G.A. and Shapiro,S.D. (1995) Human macrophage metalloelastase. Genomic organization, chromosomal location, gene linkage, and tissue-specific expression. *J. Biol. Chem.*, **270**, 14568–14575.
4. Curci,J.A., Liao,S., Huffman,M.D., Shapiro,S.D. and Thompson,R.W. (1998) Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. *J. Clin. Invest.*, **102**, 1900–1910.
5. Rutter,J.L., Mitchell,T.I., Buttice,G., Meyers,J., Gusella,J.F., Ozelius,L.J. and Brinckerhoff,C.E. (1998) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res.*, **58**, 5321–5325.
6. Zhu,Y., Spitz,M.R., Lei,L., Mills,G.B. and Wu,X. (2001) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. *Cancer Res.*, **61**, 7825–7829.
7. Su,L., Zhou,W., Park,S., Wain,J.C., Lynch,T.J., Liu,G. and Christiani,D.C. (2005) Matrix metalloproteinase-1 promoter polymorphism and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 567–570.
8. Fang,S., Jin,X., Wang,R., Li,Y., Guo,W., Wang,N., Wang,Y., Wen,D., Wei,L. and Zhang,J. (2005) Polymorphisms in the MMP1 and MMP3 promoter and non-small cell lung carcinoma in North China. *Carcinogenesis*, **26**, 481–486.
9. Ye,S., Eriksson,P., Hamsten,A., Kurkinen,M., Humphries,S.E. and Henney,A.M. (1996) Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J. Biol. Chem.*, **271**, 13055–13060.
10. Jormsjo,S., Ye,S., Moritz,J., Walter,D.H., Dimmeler,S., Zeiher,A.M., Henney,A., Hamsten,A. and Eriksson,P. (2000) Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. *Circ. Res.*, **86**, 998–1003.
11. Cho,N.H., Hong,K.P., Hong,S.H., Kang,S., Chung,K.Y. and Cho,S.H. (2004) MMP expression profiling in recurred stage IB lung cancer. *Oncogene*, **23**, 845–851.
12. Hofmann,H.S., Hansen,G., Richter,G., Taege,C., Simm,A., Silber,R.E. and Burdach,S. (2005) Matrix metalloproteinase-12 expression correlates with local recurrence and metastatic disease in non-small cell lung cancer patients. *Clin. Cancer Res.*, **11**, 1086–1092.
13. Joos,L., He,J.Q., Shepherdson,M.B., Connett,J.E., Anthonisen,N.R., Pare,P.D. and Sandford,A.J. (2002) The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum. Mol. Genet.*, **11**, 569–576.
14. Xu,L.L.,Wain,J.C., Miller,D.P., Thurston,S.W., Su,L., Lynch,T.J. and Christiani,D.C. (2001) The NAD(P)H:quinone oxidoreductase 1 gene polymorphism and lung cancer: differential susceptibility based on smoking behavior. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 303–309.
15. Miller,D.P., Liu,G., De Vivo,I., Lynch,T.J., Wain,J.C., Su,L. and Christiani,D.C. (2002) Combinations of the variant genotypes of GSTP1, GSTM1, and p53 are associated with an increased lung cancer risk. *Cancer Res.*, **62**, 2819–2823.
16. Zhou,W., Liu,G., Miller,D.P., Thurston,S.W., Xu,L.L., Wain,J.C., Lynch,T.J., Su,L. and Christiani,D.C. (2003) Polymorphisms in the DNA repair genes XRCC1 and ERCC2, smoking, and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 359–365.
17. Schaid,D.J. (1996) General score tests for associations of genetic markers with disease using cases and their parents. *Genet. Epidemiol.*, **13**, 423–449.
18. Zaykin,D.V., Westfall,P.H., Young,S.S., Karnoub,M.A., Wagner,M.J. and Ehm,M.G. (2002) Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Hum. Hered.*, **53**, 79–91.
19. Stram,D.O., Leigh Pearce,C., Bretsky,P., Freedman,M., Hirschhorn,J.N., Altshuler,D., Kolonel,L.N., Henderson,B.E. and Thomas,D.C. (2003) Modeling and E-M estimation of haplotype-specific relative risks from genotype data for a case–control study of unrelated individuals. *Hum. Hered.*, **55**, 179–190.
20. Kraft,P., Cox,D.G., Paynter,R.A., Hunter,D. and De Vivo,I. (2005) Accounting for haplotype uncertainty in matched association studies: a comparison of simple and flexible techniques. *Genet Epidemiol.*, **28**, 261–272.
21. Hosmer,D.W. and Lemeshow,S. (1989) *Applied Logistic Regression*. John Wiley and Sons, New York.
22. Zhou,W., Liu,G., Park,S., Wang,Z., Wain,J.C., Lynch,T.J., Su,L. and Christiani,D.C. (2005) Gene-smoking interaction associations for the ERCC1 polymorphisms in the risk of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 491–496.
23. Tlsty,T.D. (1998) Cell-adhesion-dependent influences on genomic instability and carcinogenesis. *Curr. Opin. Cell. Biol.*, **10**, 647–653.
24. Ohbayashi,H. (2002) Matrix metalloproteinases in lung diseases. *Curr. Protein Pept. Sci.*, **3**, 409–421.
25. Humphries,S.E., Martin,S., Cooper,J. and Miller,G. (2002) Interaction between smoking and the stromelysin-1 (MMP3) gene 5A/6A promoter polymorphism and risk of coronary heart disease in healthy men. *Ann. Hum. Genet.*, **66**, 343–352.
26. Yin,L., Morita,A. and Tsuji,T. (2000) Alterations of extracellular matrix induced by tobacco smoke extract. *Arch. Dermatol. Res.*, **292**, 188–194.
27. Shin,A., Cai,Q., Shu,X.O., Gao,Y.T. and Zheng,W. (2005) Genetic polymorphisms in the matrix metalloproteinase 12 gene (MMP12) and breast cancer risk and survival: the Shanghai Breast Cancer Study. *Breast Cancer Res.*, **7**, R506–12.
28. Kettunen,E., Anttila,S., Seppanen,J.K. *et al.* (2004) Differentially expressed genes in nonsmall cell lung cancer: expression profiling of cancer-related genes in squamous cell lung cancer. *Cancer Genet Cytogenet.*, **149**, 98–106.
29. Zinzindohoue,F., Blons,H., Hans,S., Loriot,M.A., Houllier,A.M., Brasnu,D., Laccourreye,O., Tregouet,D.A., Stucker,I. and Laurent-Puig,P. (2004) Single nucleotide polymorphisms in MMP1 and MMP3 gene promoters as risk factor in head and neck squamous cell carcinoma. *Anticancer Res.*, **24**, 2021–2026.

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