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Role of Matrix Metallopeptidase-2 Genotypes in Taiwanese Patients With Colorectal Cancer

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Abstract. *Background/Aim: Matrix metalloproteinase-2 (MMP2) has been reported to plays a critical role in the metastatic behaviors of cancer via regulation of the extracellular matrix. However, its genotypes have seldom been examined in colorectal cancer (CRC). We examined the role of MMP2 promoter -1306 (rs243865) and -735 (rs2285053) genotypes in colorectal cancer (CRC). Materials and Methods: Genotypes of MMP2 were determined by typical polymerase chain reaction-restriction fragment length polymorphism methodology in 362 CRC cases and 362 age-, sex- and behavior-matched controls. Results: The genotypic analysis showed that MMP2 -1306 CT and TT genotypes were significantly associated with an increased CRC risk (odds ratios=1.41 and 3.55, 95% confidence intervals=1.02-1.96 and 1.75-7.19, and p=0.0482 and p=0.0004, respectively). The allelic frequency analysis showed that the T allele for MMP2 -1306 increased CRC risk (odds ratio=1.71, 95% confidence interval=1.32-2.23, p=4.89×10^5). Stratification analysis showed that MMP2 -1306 genotypes were specifically associated with alcohol drinking, and metastatic status among patients with CRC. There was no association with MMP2 -735. Conclusion: The MMP2 -1306 genotype serves as a novel predictive marker for CRC risk among Taiwanese, and patients who have a tendency to undergo metastasis.*

Colorectal cancer (CRC) is the third most common cancer all over the world (1-3). Globally, the patterns of CRC incidence and mortality greatly differ by as much as 10-fold among different countries (1, 2, 4). Epidemiologically, many factors have been proposed for this variation, such as red meat, smoking, and carcinogen exposure (5, 6). In Taiwan, the incidence and mortality of CRC have both ranked in top three among all types of cancer for many years. Since up to one-fifth of patients with CRC have a familial cancer history (7, 8), inherited factors are believed to play a critical role in CRC etiology. However, discovery of genetic biomarkers of CRC is still one of the main missions for translational scientists. Although some genetic biomarkers for CRC have been established during recent years (9-14), genetic–environmental and genetic–behavioral interactions are still largely unknown. A further understanding about the genetic factors, and their interactions with environmental and behavioral factors may help to achieve the goal of precise prediction and therapy of cancer.

The extracellular matrix (ECM) controls cell attachment to and communication with neighboring cells, playing an important role in cell proliferation, movement and other cell functions. The ECM regulates homeostasis of the tumor micro-environment and any imbalance of the ECM may be associated with cancer initiation and development (15, 16).

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Key Words: Alcohol drinking, colorectal cancer, genotype, *MMP2*, metastasis, polymorphism, Taiwan.

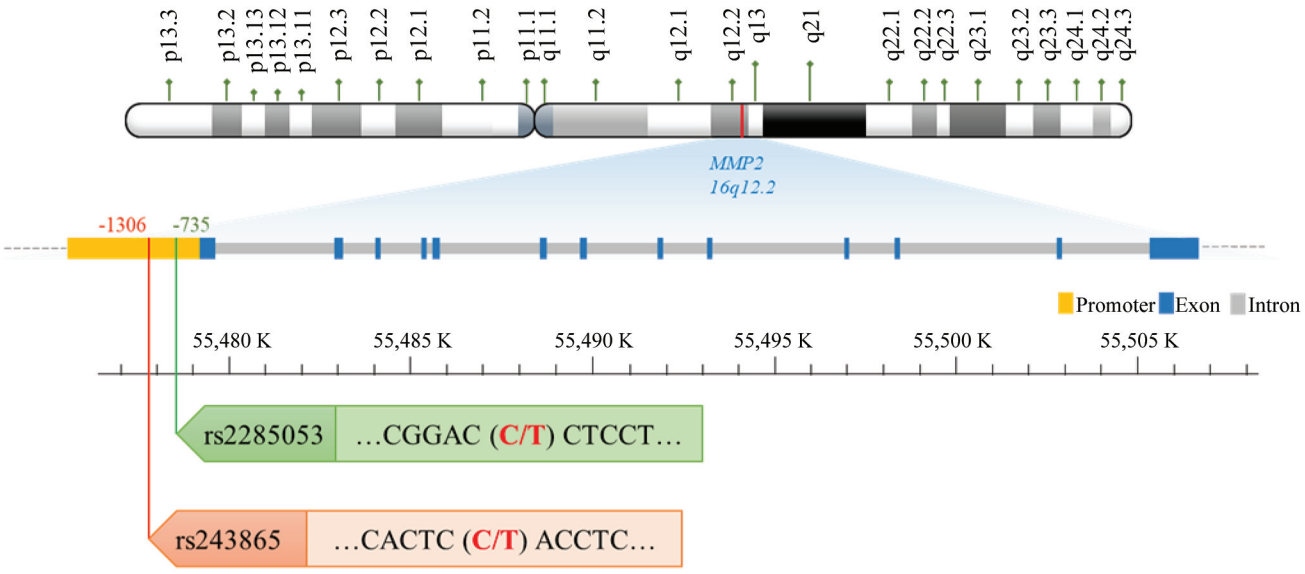

Figure 1. *Map of matrix metalloproteinase-2 (MMP2) promoter -1306 and -735 polymorphic sites.*

Notably, matrix metalloproteinases (MMPs) are usually found to be overexpressed in tumor sites and are associated with metastatic behaviors *via* degradation of ECM components (16-18). In literature, MMP2 expression has been proven to be closely related to metastatic behaviors of a variety of cancer types, including glioma, ovarian, pancreatic, gastric and CRC (19-24). In 2017, the levels of MMP2 in serum from 470 patients with CRC were found to be up-regulated compared to adjacent non-cancerous tissues (25). In addition, MMP2 expression was significantly associated with more invasive cancer stages and death, that is to say, patients with CRC who had a stronger expression of MMP2 tended to have shorter survival (26). Furthermore, MMP2 is a practical target against CRC (27, 28).

MMP2 is located on chromosome 16q21 of human genome, coding for an endopeptidase that is expressed in a variety of tissues (29-31). In literature, two genotypes of *MMP2* -1306 and -735 are reported to affect *MMP2* mRNA and protein expression levels, and eventually increase the risk of several types of cancer, including breast, oral, esophageal, leukemia, and most of all, colorectal (32-36). In 2004, Xu *et al*. were the first to report that *MMP2* -1306 CT and TT genotypes were associated with higher CRC risk in Chinese patients with CRC (37). In 2014, Shalaby *et al*. also reported that *MMP2* -1306 genotypes CT and TT genotypes were associated with higher CRC risk in Saudis (38). In 2016, Banday *et al*. announced that *MMP2* -1306 CT genotype was associated with reduced CRC risk in Kashmiris, compared with the CC genotype (39). There were also two studies reporting a negative association (40, 41). According to the above literature, the role of *MMP2* genotypes in determining CRC risk is inconsistent in different populations. Therefore, we aimed to examine the contributions of *MMP2* promoter -1306 (rs243865) and -735 (rs2285053) polymorphisms to the risk of CRC in Taiwanese, and investigated their interactions with smoking, alcohol drinking, and clinical indices. We have also summarized the related literature and provide a tentative conclusion about the role of MMP2 in CRC risk. The map of *MMP2* promoter -1306 and -735 polymorphisms is shown in Figure 1.

Materials and Methods

Collection of 362 CRC cases and 362 controls. The CRC cases and healthy controls were collected as described as in our previously published articles (14, 42). In brief, the CRC cases were recruited, pathological statuses were defined, graded and well recorded. Next, each of the cases were well matched by age, sex, cigarette smoking and alcohol drinking habits one by one. The research protocols were approved by the Institutional Review Board of the China Medical University Hospital (coding number: DMR99-IRB-108). Some demographic characteristics are shown in Table I and analyzed in other Tables.

MMP2 genotyping methodology. Genomic DNA was extracted from the leukocytes of peripheral blood within 24 h after sampling at their diagnosis as we previously reported (43-45). The *MMP2* -1306 and -735 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism methodology with the help of a BioRad Mycycler (BioRad, Hercules, CA, USA). After the polymerase chain reaction processes, the DNA adducts were subjected to endonuclease digestions (*Xsp* I for *MMP2* -1306 and *Hinf* I for *MMP2* -735, respectively) and then subjected to 3% electrophoresis and UVC visualization.

Table I. *Summary of demographic data from 362 patients with colorectal cancer and 362 non-cancer healthy controls.*

		Controls		Cancer		
Characteristic	Subgroup	n	%	n	%	*p*-Value[a]
Age	≤60 Years	95	26.2%	95	26.2%	>0.99
	>60 Years	267	73.8%	267	73.8%	
Sex	Male	203	56.1%	203	56.1%	>0.99
	Female	159	43.6%	159	43.9%	
Smoking	Yes	84	23.2%	91	25.1%	0.5434
	No	278	76.8%	271	74.9%	
Alcohol drinking	Yes	51	14.1%	44	12.2%	0.4410
	No	311	85.9%	318	87.8%	
BMI	<24 kg/m^2	175	48.3%	193	53.3%	0.1809
	≥24 kg/m^2	187	51.7%	169	46.7%	
Tumor size	<5 cm			195	53.9%	
	≥5 cm			167	46.1%	
Tumor location	Colon			257	71.0%	
	Rectum			105	29.0%	
Lymph node involvement	Negative			210	58.0%	
	Positive			152	42.0%	

BMI: Body mass index. [a]Based on chi-square test without Yates' correction.

Statistical analyzing methodology. Typical unpaired Student's *t*-test was applied to test the distributions of ages between case and control groups. Pearson's chi-square test was applied to compare the distributions of the *MMP2* genotypes among subgroups (when n≥5, we used chi-square without Yates' correction, while when n<5, we used Fisher's exact test). The associations between the *MMP2* genotypes and colorectal cancer risk were examined by odds ratios (ORs) and 95% confidence intervals (CIs). Any *p*-value less than 0.05 was considered statistically significant.

Results

Comparison of demographic characteristics. There was no difference in age sex, the percentage of smokers or alcohol drinkers, and body mass index between the control and CRC case groups (all $p>0.05$) (Table I). One hundred and ninety-five CRC patients were suffering from tumors less than 5 cm, while one hundred and sixty-seven CRC patients were suffering from tumor of 5 cm or larger (Table I). Two hundred and fifty-seven patients were suffering from colonic cancer, while 105 were suffering from rectal cancer (Table I). A total of 152 patients were suffering from metastatic cancer (Table I).

Genotypic comparison for MMP2 -1306 and -735. Firstly, the frequency distributions of both *MMP2* -1306 and -735 genotypes in the control group fitted well with the Hardy–Weinberg equilibrium ($p=0.4628$ and 0.3319, respectively). Secondly, the genetic frequencies of *MMP2* -1306 were found to be differentially distributed between the CRC case and control groups (p for trend=0.0034). The frequency of the CT genotype of *MMP2* -1306 was significantly higher in the CRC group than in the control group (OR=1.41, 95% CI=1.02-1.96, $p=0.0482$). More significantly, the genotypic frequency of *MMP2* -1306 TT was higher in the CRC group than in the control group (OR=3.55, 95% CI=1.75-7.19, $p=0.0004$). The differential distributions remained statistically significant when the CT and TT genotypes were combined (OR=1.64, 95% CI=1.20-2.23, $p=0.0023$) (Table II). On the contrary, the distributions of *MMP2* -735 genotypes were not significantly different between the CRC case and control groups in any models (Table II).

Comparison of MMP2 -1306 and -735 allelic frequencies. Supporting the findings in Table II, the frequency of the T allele at *MMP2* -1306 was 24.4% in the CRC case group, significantly higher than that in the control group (OR=1.71, 95% CI=1.32-2.23, $p=4.89\times10^{-5}$) (Table III). On the contrary, the T allelic frequencies at *MMP2* -735 were 22.1% and 19.1% in the CRC case and control groups, respectively, showing no statistical significance (OR=1.20, 95% CI=0.93-1.56) (Table III).

Interaction of MMP2 genotypes with cigarette consumption. We investigated the possible combined influence of *MMP2* -1306 genotype and cigarette consumption behavior on the risk of CRC (Table IV). Among non-smokers, *MMP2* -1306 CC carriers had no difference in the risk of CRC compared with *MMP2* -1306 CT or TT carriers (OR=0.91, 95% CI=0.63-1.29, $p=0.6490$). For smokers, *MMP2* -1306 CT or

Table II. *Distributions of genotypic frequencies of matrix metalloproteinase-2 (MMP2) polymorphisms among patients with colorectal cancer and healthy controls.*

MMP2 genotype		Cases (n=362)	Controls (n=362)	Odds ratio (95% CI)	*p*-Value[a]
-1306 (rs243865)	CC	218 (60.2)	258 (71.3)	1.00 (Reference)	
	CT	111 (30.7)	93 (25.7)	1.41 (1.02-1.96)	**0.0482**
	TT	33 (9.1)	11 (3.0)	3.55 (1.75-7.19)	**0.0004**
	CT+TT	144 (39.8)	104 (28.7)	1.64 (1.20-2.23)	**0.0023**
p-Value for trend					**0.0034**
P_{HWE}					0.4628
-735 (rs2285053)	CC	225 (62.1)	240 (66.3)	1.00 (Reference)	
	CT	114 (31.5)	106 (28.3)	1.15 (0.83-1.58)	0.4492
	TT	23 (6.4)	16 (4.4)	1.53 (0.79-2.98)	0.2698
	CT+TT	137 (37.9)	122 (33.7)	1.20 (0.88-1.62)	0.2777
p-Value for trend					0.3622
P_{HWE}					0.3319

CI: Confidence interval; PHWE: *p*-value from Hardy-Weinberg equilibrium test. [a]Based on chi-square test. Bold values indicate statistical significance.

Table III. *Allelic frequencies of matrix metalloproteinase-2 (MMP2) polymorphisms among patients with colorectal cancer and healthy controls.*

MMP2 allele	Allele	Cases (n=362)	Controls (n=362)	Odds ratio (95% CI)	*p*-Value[a]
-1306 (rs243865)	C	547 (75.6)	609 (84.1)	1.00 (Reference)	
	T	177 (24.4)	115 (15.9)	1.71 (1.32-2.23)	**4.89×10^{-5}**
-735 (rs2285053)	C	564 (77.9)	586 (80.9)	1.00 (Reference)	
	T	160 (22.1)	138 (19.1)	1.20 (0.93-1.56)	0.1527

CI: Confidence interval. [a]Based on chi-square test. Bold value indicates statistical significance.

Table IV. *Interaction of matrix metalloproteinase-2 (MMP2) -1306 genotype and cigarette consumption for colorectal cancer risk.*

MMP2 -1306 CC carrier	Cigarette smoker	Controls/Cases, n	Odds ratio (95% CI)	*p*-Value[a]
No	No	79/104	1.00 (Reference)	
Yes	No	167/199	0.91 (0.63-1.29)	0.6490
No	Yes	25/40	1.22 (0.68-2.17)	0.6069
Yes	Yes	59/51	0.66 (0.41-1.06)	0.1058

CI: Confidence interval. [a]Based on chi-square test.

TT carriers were at 1.22-fold risk of CRC, while CC carriers were at 0.66-fold risk of CRC (95% CI=0.68-2.17 and 0.41-1.06, *p*=0.6069 and 0.1058, respectively). Overall, no additive or synergistic effects were found for cigarette consumption in association with *MMP2* -1306 genotype regarding CRC risk (Table IV).

Interaction of MMP2 genotype with alcohol drinking. We also analyzed the interaction of *MMP2* -1306 and alcohol drinking in relation to the risk of CRC (Table V). Among non-drinkers, the *MMP2* -1306 CC carriers were found to have significantly reduced risk of CRC compared with CT or TT carriers (OR=0.62, 95% CI=0.44-0.86, *p*=0.0057). For alcohol drinkers, *MMP2* -1306 CT or TT carriers were at 0.87-fold risk of CRC, while CC carriers were at 0.48-fold risk of CRC (95% CI=0.44-1.75 and 0.26-0.87, *p*=0.8402 and 0.0214, respectively). Overall, a protective effect was found for alcohol drinking in association with *MMP2* -1306 CC genotype regarding CRC risk (Table V).

Correlation between MMP2 -1306 genotypes and clinicopathological properties. The last part of the analysis is the correlation between *MMP2 -1306* genotypes and clinicopathological properties. There was no significance in

Table V. *Interaction of matrix metalloproteinase-2 (MMP2) -1306 genotype and alcohol consumption for colorectal cancer risk.*

MMP2 -1306 CC carrier	Alcohol drinker	Controls/Cases, n	Odds ratio (95% CI)	*p*-Value[a]
No	No	87/123	1.00 (Reference)	
Yes	No	224/195	0.62 (0.44-0.86)	**0.0057**
No	Yes	17/21	0.87 (0.44-1.75)	0.8402
Yes	Yes	34/23	0.48 (0.26-0.87)	**0.0214**

CI: Confidence interval. [a]Based on chi-square test. Bold values indicate statistical significance.

Table VI. *Summary of studies on matrix metalloproteinase-2 (MMP2) -1306 genotypes in colorectal cancer risk.*

First author (Ref)	Published year	Study ethnicity	CC: CT:TT Genotypic frequency, n		Highlighted findings
			Controls	Cases	
Xu *et al*. (37)	2004	Chinese	92:32:2	106:19:1	CT+TT Genotypes contributed to higher risk
Elander *et al*. (40)	2006	Swedish	109:89:10	69:49:9	No specific association
Saeed *et al*. (41)	2013	Saudi	92:23:1	66:24:5	No specific association
Shalaby *et al*. (38)	2014	Saudi	103:20:2	92:21:12	CT+TT Genotypes contributed to higher risk
Banday *et al*. (39)	2016	Kashmiri	125:59:0	110:32:0	CT Genotype contributed to lower risk
Yueh *et al*.	Current	Taiwanese	258:93:11	218:111:33	CT and TT Genotypes contributed to higher risk

Table VII. *Correlation between matrix metalloproteinase-2 (MMP2) -1306 genotypes and clinicopathological properties of 362 patients with colorectal cancer.*

Factor	Subgroup	Cases, n	MMP2 -1306 genotype, n (%)			*p*-Value[a]
			CC	CT	TT	
Age	≤60 Years	95	59 (62.1)	31 (32.6)	5 (5.3)	
	>60 Years	267	159 (59.5)	80 (30.0)	28 (10.5)	0.3110
Gender	Male	203	123 (60.6)	65 (32.0)	15 (7.4)	0.4066
	Female	159	95 (59.8)	46 (28.9)	18 (11.3)	
BMI	<24 kg/m^2	193	119 (61.7)	58 (30.1)	16 (8.2)	
	≥24 kg/m^2	169	99 (58.6)	53 (31.4)	17 (10.0)	0.7782
Tumor size	<5 cm	195	118 (60.5)	62 (31.8)	15 (7.7)	
	≥5 cm	167	100 (59.9)	49 (29.3)	18 (10.8)	0.5705
Location	Colon	257	153 (59.5)	81 (31.5)	23 (9.0)	
	Rectum	105	65 (61.9)	30 (28.6)	10 (9.5)	0.8574
Lymph node involvement	Negative	210	141 (67.1)	59 (28.1)	10 (4.8)	
	Positive	152	77 (50.7)	52 (34.2)	23 (15.1)	**0.0004**

BMI: Body mass index. [a]Based on chi-square test without Yates' correction. Bold value indicates statistical significance.

the distribution of *MMP2 -1306* genotypes between younger (≤60 years) and older (>60 years), male and female, lower (<24 kg/m^2) and higher (<24 kg/m^2) body mass index, smaller (<5 cm) and larger (<5 cm) tumor, colonic and rectal tumor groups (all $p>0.05$). Only by lymph node involvement were genotypes differentially distributed. The frequencies of *MMP2 -1306* CT and TT carriers were higher in patients with positive lymph node involvement than in those without ($p=0.0004$).

Discussion

In the current study, the association of *MMP2* -1306 and -735 genotypes with Taiwanese CRC risk was firstly investigated among 362 CRC cases and 362 controls. We found that the variant CT and TT genotypes of *MMP2* -1306 were more frequent in the case group than in the control group (Table II). The allelic frequency analysis also showed that the T allele of *MMP2* -1306 was more prevalent in the case group

than in the control group (Table III). The above findings indicating that *MMP2* -1306 genotype may serve as a marker for prediction of CRC risk are consistent with those of Xu *et al*. in Chinese (37) and Shalaby *et al*. in Saudi patients (38), while they are different from those of Banday *et al*. in Kashmiris (39). Xu and Shalaby both reported that *MMP2* -1306 CT and TT genotypes were associated with higher CRC risk. However, their sample sizes (controls:cases=126:126 for Xu and 125:125 for Shalaby) were restricted; Xu *et al*. even found that TT alone contributed to lower CRC risk due to the bias of small sample size. Banday *et al*. reported that *MMP2* -1306 CT genotype was associated with reduced CRC risk in Kashmiris compared with the CC genotype (39). There were also two studies with negative association (40, 41). The tentative conclusion that *MMP2* -1306 genotype can serve as a tool for early detection of CRC risk needs further investigation. To date, we have summarized all the literature concisely in Table VI for a comprehensive understanding of the role of *MMP2* -1306 genotype in CRC risk determination.

The inconsistency noted above may have at least two explanations. The first is that different populations were investigated, and the second is that the influence of *MMP2* on CRC risk may be somehow affected by other risk factors such as personal behaviors. Although the International Agency for Research on Cancer concluded that alcoholic beverages are causally related to several types of cancer, including CRC (46), solid evidence for any gene–behavior interaction is lacking. In our analysis of gene–behavior interaction, neither the CT nor the TT genotype at *MMP2* -1306 appear to interact with smoking behavior (Table IV) to influence the risk of CRC. Instead, alcohol drinking appears to enhance the protective effects of carrying the CC genotype of *MMP2* -1306 (Table V). To the best of our knowledge, this is the first epidemiological study based on molecular genetics to evaluate the contribution of *MMP2* -1306 genotypes and susceptibility to CRC in association with cigarette smoking and alcohol drinking status. The interaction between alcohol consumption and *MMP2* genotype remains unresolved.

In literature, the *MMP2* -1306 variants have been reported to undermine the binding site of transcription factor Sp1, resulting in the suppression of its transcription (47). In 2017, the levels of MMP2 in serum from 470 patients with CRC were found to be up-regulated compared to adjacent non-cancerous tissues (25). In addition, MMP2 expression was significantly associated with more invasive cancer stages and death, that is to say, patients with CRC with a stronger expression of MMP2 tended to have shorter survival (26). Although we have no phenotype data showing the expression levels of *MMP2* mRNA or protein among various *MMP2* -1306 genotypes, we did find that the CT and TT genotypes at *MMP2* -1306 contribute to positive lymph node status of CRC (Table VII). It is very possible that CRC cells with CT/TT genotypes at *MMP2* -1306 may have higher *MMP2* expression levels than cells with CC genotype.

In recent years, our laboratory has reported that *MMP2* -1306 CC genotype contributes to higher risk of gastric cancer (44), while having no significant association with childhood leukemia (48) in Taiwanese. This difference is found in other ethnicities (37-41). Thus, the role of the *MMP2* -1306 polymorphism in carcinogenesis seems to be complicated and might differ among various types of cancer.

In conclusion, our findings provide solid evidence for T-bearing genotypes of *MMP2* -1306 being associated with a higher risk of CRC, and the genotype of *MMP2* might serve as a prognostic marker for metastatic status of CRC.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Research design: Yueh TC, Hung YC, Lee HT and Bau DT; questionnaire summarization: Yang MD, Ke TW and Yueh TC; Experiment Performance: Yang YC, Wang ZH, Chang WS and Tsai CW; statistical analysis and confirmation: Pei JS, DT Bau and Chang WS; article writing: Yueh TC, Hung YC, Lee HT and Bau DT; article polishing and correction: Chang WS, Wang ZH and Bau DT.

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