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Research Paper

Impact of matrix metalloproteinase-11 gene polymorphisms on development and clinicopathologcial variables of uterine cervical cancer in Taiwanese women

Soo-Cheen Ng[1,2], Po-Hui Wang[1,2,3], Yueh-Chun Lee[1,3,4], Chung-Yuan Lee[3,5,6], Shun-Fa Yang[3,7], Huang-Pin Shen[1,2,3], Yi-Hsuan Hsiao[1,8,✉]

1. School of Medicine, Chung Shan Medical University, Taichung, Taiwan
2. Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan
3. Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
4. Radiation Oncology Department, Chung Shan Medical University Hospital, Taichung, Taiwan
5. Department of Obstetrics and Gynecology, Chiayi Chang Gung Memorial Hospital, Chiayi, Taiwan
6. Department of Nursing, Chang Gung University of Science and Technology, Chiayi Campus, Chiayi, Taiwan
7. Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
8. Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan

✉ Corresponding author: Yi-Hsuan Hsiao, MD., Ph.D., Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan; E-mail: 54315@cch.org.tw

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Abstract

The purposes of this study were to examine whether there were associations among matrix metalloproteinase-11 (MMP-11) gene polymorphisms, development and clinicopathological characteristics of uterine cervical cancer as well as patient survival or not. Five single-nucleotide polymorphisms (SNPs) of the MMP-11 gene rs738791, rs738792, rs2267029, rs28382575, and rs131451 from one hundred and thirty patients with invasive cancer, 99 patients with high-grade cervical intraepithelial neoplasia (CIN) of uterine and 335 normal controls were analyzed using real-time polymerase chain reaction. Our results revealed that genotypic frequencies of CT/TT in MMP-11 SNP rs738791, with CC as a reference, tended to exhibit significantly different distributions (p=0.044, AOR: 0.63, 95% CI: 0.41-0.99) between patients with cervical invasive cancer and normal control women when controlling age. After multiple significance adjustment, the tendency becomes insignificant (Holm's adjusted p 0.176). Although CT/TT genotype of MMP-11 gene rs738791 tended to increase the risk of developing stage II disease at least (p=0.035; OR: 2.16, 95% CI: 1.05-4.44) and deep stromal invasion more than 10 mm (p=0.043; OR: 2.08, 95% CI: 1.02-4.26) with CC as a reference in patients with uterine cervical cancer. They became insignificant after multiple significance adjustment and the Holm's adjusted p values would become as 0.245 and 0.258, respectively. However, lymph node metastasis exhibited significant worse recurrence-free survival (p=0.033; HR: 2.83, 95% CI: 1.09-7.35), and overall survival (p=0.001; HR: 4.80, 95% CI: 1.82-12.62) compared to those without pelvic lymph node metastasis. In conclusion, it indicates no impact of the MMP-11 SNPs on uterine cervical cancer in Taiwanese women.

Key words: matrix metalloproteinase-11; gene polymorphisms; uterine cervical cancer; single nucleotide polymorphism

Introduction

Uterine cervical cancer is one of the leading causes of cancer mortality and presents the fourth most frequent malignancy in women worldwide [1]. It is also a serious health problem in Taiwan. The 2013 annual cancer registry report revealed that uterine cervical cancer was the third leading cancer in Taiwanese women. High preoperative primary tumor size and metastatic pelvic lymph node are vital prognoses of adverse events or death [2]. Uterine cervical cancer is usually developed by a pre-invasive phase cervical intraepithelial neoplasia (CIN) [3]. CIN can be categorized into three grades, including CIN1 (mild dysplasia; low-grade CIN), CIN2 (moderate dysplasia) and CIN3 (severe dysplasia); furthermore,

CIN 2 and CIN 3 have been regarded as high-grade CIN. Carcinogenesis of uterine cervix is considered as a continuum of neoplastic transition from dysplasia to invasive cancer. Most low-grade CIN lesions regress; however, high-grade CIN lesions significantly progress to invasive cancer [3].

Matrix metalloproteinases (MMPs) play an important role for biological behaviors of solid malignant tumors. MMPs comprise a family of endopeptidases, with the capacity of degrading the extracellular matrix (ECM) proteins and remodeling the ECM, which play essential roles for the progression of invasion and metastasis of malignant solid tumors [4]. MMP-11, also named stromelysin-3, was first identified in breast carcinoma [5]. The expression of MMP-11 is associated with embryonic development and tissue remodeling processes in normal physiologic conditions [6]; MMP-11 degrades the ECM and promotes the tumorigenesis and progress of cancer [4]. Increased expression of MMP-11 has been identified in many types of cancer, such as nonsmall cell lung cancer [7], gastric carcinoma [8], oral cancer [9], de novo colorectal cancer [10], esophageal adenocarcinoma [11] and pancreatic carcinoma [12]. Furthermore, overexpression of MMP-11 in cervical cancer [13] and a relative increased expression of MMP-11 from LSIL and HSIL samples [14] have been demonstrated.

Single nucleotide polymorphism (SNP) is a form of DNA variation among individuals [15]. In the shared sequence of a gene, a single nucleotide develops that achieves a frequency of more than 5 % of the population for a species is referred as a SNP [15]. Genetic polymorphisms of MMP-11 have been reported in several different types of cancer including oral squamous cell carcinoma [16], hepatocellular carcinoma [17], and breast cancer [18]. However, the impact of MMP-11 gene polymorphisms on the risk and prognosis of cervical cancer remains poorly investigated. Therefore, we conducted a case-control study to examine whether there were associations among MMP-11 gene polymorphisms, development and clinicopathological characteristics of uterine cervical cancer as well as patient survival or not.

Materials and Methods

Data source and studied subjects

The retrospective study was conducted by enrolling individuals consecutively. One hundred and thirty patients with invasive cancer, 99 patients with high-grade CIN of uterine cervix, and 335 normal controls were enrolled at the Department of Obstetrics and Gynecology in Chung Shan Medical University Hospital in Taichung, Taiwan from February 1994 to October 2014. One hundred and seven cervical cancer patients had squamous cell carcinoma (SCC) and 21 adenocarcinoma; 2 were unknown. Seventy five cervical cancer patients were staged I, 34 staged II, 11 staged III and 7 staged IV according to the 2009 International Federation of Gynecology and Obstetrics system. Three patients could not be staged. They received standard treatment protocols in this hospital modified from National Comprehensive Cancer Network guidelines. They underwent radical abdominal hysterectomy and pelvic lymph node dissection or plus para-aortic lymph node sampling and/or concurrent chemoradiotherapy or chemotherapy. Patients with high-grade CIN were known as precancerous lesions and underwent abdominal or vaginal total hysterectomy, large loop excision of transformation zone or simple trachelectomy. Their prognosis was well. None of them had recurrence or developed invasive cancer in the follow-up. The diagnoses of all patients with cervical invasive cancer or precancerous lesions were further verified according to the pathologic report of colposcopy-directed punch biopsy of cervix before the treatment began. Normal controls received Papanicolaou smears at outpatient department for general examination and the normal cytologic diagnosis was further verified with normal colposcopic findings in Chung Shan Medical University Hospital. All studied subjects were Taiwanese women who resided in central Taiwan. This study was approved by the institutional review board of Chung Shan Medical University Hospital (CSMUH No: CS14014).

Selection of matrix metalloproteinase-11 polymorphisms

A total of 5 SNPs in MMP-11 were selected from the International HapMap Project data for this study. The synonymous SNPs rs28382575 (Pro475Pro) and nonsynonymous SNPs rs738792 (Ala38Val) in the coding sequences of the gene were included. We examined rs2267029 with minor allelic frequencies of >5% in order to obtain adequate power to evaluate the potential association. The rs738791 and rs131451 were selected in this study as the previous cancer research [17].

Determination of genotypes

Total genomic DNA was isolated from whole blood specimens using QIAamp DNA blood mini kits (Qiagen, Valencia, CA), in accord with the manufacturer's instructions. The MMP-11 SNPs were determined by using the TaqMan SNP genotyping assay (Applied Biosystems, Warrington, UK), according to the manufacturer's protocols [19].

Statistical analysis

Analysis of variance (ANOVA) was used to compare the age difference among patients with invasive cancer and those with precancerous lesions of uterine cervix, and control women. Tukey HSD test was used for post hoc analysis. Chi-square or Fisher's exact tests were used to associate genotypic distributions of MMP-11 gene single SNPs with the incidence of cervical neoplasias. The adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) were applied to check the associations among genotypic distributions of MMP-11 SNPs and the incidence of cervical neoplasias (including precancerous lesions and invasive cancer) using the logistic and multinomial logistic regression models after controlling for age. Chi-square or Fisher's exact tests were applied to associate MMP-11 SNP and clinicopathological parameters factors with recurrence or death events. Holm's test was applied for multiple significance adjustment (multiple comparisons) and Holm's adjust p values could be calculated in the associations of MMP-11 SNPs with the development of cervical cancer or with clinicopathological variables. Kaplan-Meier curve model (univariate analysis over time) was used to identify factors significantly associated with recurrence-free survival rate and overall survival rate between initial standard treatment and the subsequent appearance of recurrence, death or closing date of the study (December 4, 2018). Cox proportional hazard model (multivariate analysis over time) was used to relate MMP-11 SNP with recurrence-free survival and overall survival of patients with cervical cancer, with adjusting for various clinicopathological parameters, relative to recurrence and survival time or until closing date of the study (December 4, 2018). The SPSS, version 18.0 and WinPepi Software, version 10.0 were used for statistical analysis. P <0.05 was considered as statistically significant difference.

Results

Age distributions and Weinberg equilibrium

There was significant difference in age distribution between patients with cervical neoplasia and normal control women (50.5 ± 13.7 vs. 43.9 ± 10.4, p<0.001). Based on ANOVA with Tukey HSD test for post hoc analysis, the age distribution was significantly different between patients with cervical invasive cancer and those with precancerous lesion (55.3 ± 12.3 vs. 44.1 ± 12.9, p<0.001), and between those with cervical cancer and control women (55.3 ± 12.3 vs. 43.9 ± 10.4, p<0.001) but not significantly different between those with precancerous lesions and control women (44.1 ± 12.9 vs. 43.9 ± 10.4, p= 0.992).

Relationship of MMP-11 genetic polymorphisms distributions with uterine cervical carcinogenesis

A total of 564 individuals participated in this study. Of these, 130 patients with invasive cancer, 99 patients with high-grade CIN of uterine, and 335 normal controls were included. Genetic polymorphism distributions of MMP-11 gene in Taiwanese women with neoplasias of the uterine cervix and normal controls were shown in Table 1. There were no significantly different genotypic distributions of MMP-11 SNPs rs738791, rs2267029, rs28382575 and rs131451 between patients with cervical neoplasias and normal control women. With TT/TC as a reference, genotypic frequencies of TT/TC and CC of MMP-11 SNP rs738792 were significantly different distributions between patients with cervical neoplasias and normal control women; however, there was no significant difference after controlling age.

After dividing the cervical neoplasias to precancerous lesions and invasive cancer, genotypic distributions of MMP-11 gene in Taiwanese women with uterine cervical invasive cancer, precancerous lesions and normal controls were shown in Table 2. There were no significantly different distributions of MMP-11 SNPs rs738791, rs738792, rs2267029, rs28382575 and rs131451 among patients with cervical invasive cancer, precancerous lesions or normal control women. However, genotypic frequencies of CT/TT in MMP-11 SNP rs738791, with CC as a reference, tended to exhibit significantly different distributions (p=0.044, AOR: 0.63, 95%CI: 0.41-0.99) between patients with cervical invasive cancer and normal control women when controlling age. After multiple significance adjustment for the associations of rs738791 with development of cervical cancer using Holm's test, the 4 p values 0.076, 0.144, 0.044 and 0.319 were adjusted as 0.228, 0.288, 0.176 and 0.319. Previous tendency became insignificant and the Holm's adjusted p value was 0.176.

Association of MMP-11 gene variant rs738791 with clinicopathological variables

The associations of genotypic distribution of MMP-11 gene variant rs738791 with clinicopathological characteristics of the patients with invasive cancer of uterine cervix were shown in Table 3. Although genotypes CT/TT of MMP-11 SNP rs738791 tended to increase the risk of developing stage II disease at least (p=0.035; OR: 2.16, 95% CI: 1.05-4.44) and deeply progressed stromal invasion

more than 10 mm (p=0.043; OR: 2.08, 95% CI: 1.02-4.26) with CC as a reference in patients with uterine cervical cancer (Table 3), they were not significantly associated with stage and stromal invasion after adjusting multiple comparisons for 7 clinicopathological variables including clinical stage, pathologic type, stromal invasion depth, tumor diameter, parametrium invasion, vagina invasion and pelvic lymph node metastasis. When applying Holm's test for multiple significance adjustment, the Holm's adjusted p values for the associations of rs738792 with stage as well as rs738792 with stromal invasion became obviously insignificant as 0.245 and 0.258, respectively (Table 3). There were also no significant differences in pathologic type, tumor diameter, parametrium invasion, vaginal invasion or pelvic lymph node metastasis of invasive cervical cancer in comparing CC and CT/TT carriers of MMP-11 gene variant rs738791.

Influence of MMP-11 genetic polymorphism and clinicopathological characteristics on recurrence and survival as well as recurrence-free survival and overall survival of the patients with uterine cervical cancer

While correlating MMP-11 and clinicopathological variables with recurrence event in patients with cervical cancer, MMP SNPs were not found to be significantly related to recurrence event (Table 5, for rs738791 CT/TT vs CC, p=0.142, other SNPs results not showed). When they were correlated with the death event, MMP-11 SNPs were also not found to be significantly associated with survival (Table 5, for rs738791 CT/TT vs CC, p=0.160, other SNPs results not showed).

Table 1. Genetic polymorphism distributions of matrix metalloproteinase-11 gene in Taiwanese women with neoplasias of the uterine cervix and normal controls.

Variables	Normal controls (n=335)	Cervical neoplasias[a] (n=229)	ORs (95% CIs)[a]	p values	AORs (95% CIs)[b]	Adjusted p values
rs738791						
CC[c]	148	112	1.00	0.536	1.00	0.530
CT	144	91	0.84 (0.58-1.20)	0.326	0.82 (0.56-1.19)	0.288
TT	43	26	0.80 (0.46-1.38)	0.420	0.82 (0.47-1.45)	0.495
CC[c]	148	112	1.00	0.269	1.00	0.260
CT/TT	187	117	0.83 (0.59-1.16)		0.82 (0.58-1.16)	
CC/CT[c]	292	203	1.00	0.598	1.00	0.711
TT	43	26	0.87 (0.52-1.46)		0.90 (0.53-1.55)	
rs738792						
TT[c]	180	125	1.00	0.077	1.00	0.175
TC	137	81	0.85 (0.60-1.22)	0.377	0.86 (0.59-1.24)	0.418
CC	17	22	1.86 (0.95-3.65)	0.070	1.69 (0.84-3.40)	0.142
TT[c]	180	125	1.00	0.828	1.00	0.796
TC/CC	154	103	0.96 (0.69-1.35)		0.96 (0.67-1.36)	
TT/TC[c]	317	206	1.00	0.037	1.00	0.092
CC	17	22	1.99 (1.03-3.84)		1.80 (0.91-3.56)	
rs2267029						
GG[c]	186	128	1.00	0.226	1.00	0.329
GA	136	85	0.91 (0.64-1.29)	0.592	0.93 (0.64-1.34)	0.681
AA	13	16	1.79 (0.82-3.85)	0.137	1.71 (0.78-3.75)	0.183
GG[c]	186	128	1.00	0.930	1.00	0.994
GA/AA	149	101	0.99 (0.70-1.38)		1.00 (0.70-1.42)	
GG/GA[c]	322	213	1.00	0.101	1.00	0.103
AA	13	16	1.86 (0.88-3.95)		1.87 (0.88-3.97)	
rs28382575						
TT[c]	324	216	1.00	0.349	1.00	0.376
TC	11	11	1.50 (0.64-3.52)	0.352	1.49 (0.62-3.61)	0.376
CC	0	0	u.a.	u.a.	u.a.	u.a.
TT[c]	324	216	1.00	0.349	1.00	0.376
TC/CC	11	11	1.51 (0.64-3.52)		1.49 (0.62-3.61)	
TT/TC[c]	335	227	1.00	u.a.	1.00	u.a.
CC	0	0	u.a.		u.a.	
rs131451						
TT[c]	124	80	1.00	0.636	1.00	0.778
TC	153	102	1.03 (0.71-1.51)	0.864	1.05 (0.71-1.55)	0.815
CC	57	46	1.25 (0.77-2.02)	0.360	1.20 (0.73-1.97)	0.482
TT[c]	124	80	1.00	0.622	1.00	0.648
TC/CC	210	148	1.09 (0.77-1.55)		1.09 (0.76-1.57)	
TT/TC[c]	277	182	1.00	0.349	1.00	0.504
CC	57	46	1.23 (0.80-.89)		1.17 (0.74-1.83)	

Statistical analysis: logistic regression model or chi-square test

[a]Cervical neoplasias included precancerous lesions and invasive cancer of the uterine cervix.

[b]The adjusted p values as well as adjusted odds ratios and their 95% confident intervals were determined by logistic regression model after controlling age.

[c]Used as a reference for comparison to determine the odds ratios of other genotypes.

ORs, odds ratios; 95% CIs, 95% confidence intervals; AOR; adjusted odds ratio; u.a., unavailable.

Table 2. Genotypic distributions of matrix metalloproteinase-11 gene in Taiwanese women with uterine cervical invasive cancer or precancerous lesions and normal controls.

Variables	Normal controls (n =335)	Precancerous lesions (n = 99)	Invasive cancer (n = 130)	p	AORs (95% CIs)[a]	Ad. p	AORs (95% CIs)[b]	Ad. p
rs738791								
CC[c]	148	42	70	0.340	1.00		1.00	
CT	144	43	48		1.05 (0.65-1.70)	0.841	0.65 (0.41-1.05)	0.076
TT	43	14	12		1.15 (0.57-2.30)	0.698	0.57 (0.26-1.22)	0.144
CC[c]	148	42	70	0.124	1.00	0.761	1.00	0.044
CT/TT	187	57	60		1.07 (0.68-1.69)		0.63 (0.41-0.99)	
CC/CT[c]	292	85	118	0.463	1.00	0.734	1.00	0.319
TT	43	14	12		1.12 (0.58-2.14)		0.69 (0.33-1.43)	
rs738792								
TT[c]	180	60	65	0.070	1.00		1.00	
TC	137	28	53		0.61 (0.37-1.01)	0.056	1.17 (0.73-1.86)	0.521
CC	17	10	12		1.76 (0.77-4.07)	0.183	1.64 (0.69-3.87)	0.263
TT[c]	180	60	65	0.236	1.00	0.201	1.00	0.384
TC/CC	154	38	65		0.74 (0.47-1.17)		1.22 (0.78-1.90)	
TT/TC[c]	317	88	118	0.108	1.00	0.073	1.00	0.316
CC	17	10	12		2.12 (0.93-4.79)		1.53 (0.66-3.54)	
rs2267029								
GG[c]	186	59	69	0.171	1.00		1.00	
GA	136	31	54		0.72 (0.44-1.17)	0.184	1.16 (0.73-1.84)	0.523
AA	13	9	7		2.19 (0.89-5.38)	0.089	1.34 (0.49-3.68)	0.574
GG[c]	186	59	69	0.614	1.00	0.475	1.00	0.483
GA/AA	149	40	61		0.85 (0.54-1.34)		1.17 (0.75-1.83)	
GG/GA[c]	322	90	123	0.118	1.00	0.044	1.00	0.651
AA	13	9	7		2.48 (1.02-5.99)		1.26 (0.47-3.39)	
rs28382575								
TT[c]	324	96	120	0.343	1.00		1.00	
TC	11	3	8		0.92 (0.25-3.37)	0.901	2.08 (0.75-5.78)	0.160
CC	0	0	0		u.a	u.a.	u.a.	
TT[c]	324	96	120	0343	1.00	0.901	1.00	0.160
TC/CC	11	3	8		0.92 (0.25-3.37)		2.08 (.075-5.78)	
TT/TC[c]	335	99	128	u.a.	1.00		1.00	u.a.
CC	0	0	0		u.a.		u.a	
rs131451								
TT[c]	124	40	40	0.416	1.00			
TC	153	38	64		0.77 (0.47-1.27)	0.310	1.42 (0.86-2.35)	0.168
CC	57	21	25		1.14 (0.62-2.11)	0.672	1.27 (0.66-2.42)	0.477
TT[c]	124	40	40	0.304	1.00	0.556	1.00	0.186
TC/CC	210	59	89		0.87 (0.55-1.38)		1.38 (0.86-2.210	
TT/TC[c]	277	78	104	0.606	1.00	0.348	1.00	0.917
CC	57	21	25		1.31 (0.75-2.29)		1.03 (0.58-1.83)	

[a]Adjusted p values and adjusted odds ratios with their 95% CIs were calculated using multinomial logistic regression models after controlling age between patients with cervical precancerous lesions and control women.

[b]Adjusted p values and adjusted odds ratios with their 95% CIs were calculated using multinomial logistic regression models after controlling age between patients with cervical invasive cancer and control women.

[c]Used as a reference for comparison to calculate the odds ratios of other genotypes.

AORs, adjusted odds ratios; 95% CIs, 95% confidence intervals; Ad. p, adjusted p value; u.a., unavailable.

The associations of MMP-11 genetic polymorphism rs738791 and various clinicopatholgical parameters with the recurrence-free survival and overall survival of the patients with uterine cervical cancer were shown in Table 5 (univariate analysis, Kaplan-Meier curves model) and 6 (multivariate analysis, Cox proportional hazard model). Pelvic lymph node metastasis was demonstrated to be an only predictor for poorer recurrence-free survival [p=0.033, hazard ration (HR): 2.83, 95% CI: 1.09-7.35] and overall survival (p=0.001, HR: 4.80, 95% CI: 1.82-12.62) of the patients with uterine cervical cancer using Cox proportional model analysis. MMP-11 genetic polymorphism rs738791 (CT/TT vs CC) and other clinicopathological characteristics, including stage, pathologic type, stromal invasion depth, tumor diameter, parametrium invasion or vaginal invasion showed no influence on recurrence-free survival or overall survival of the patients with uterine cervical cancer.

Discussion

Our study revealed that genotypes CT/TT tended to reduce the susceptibility of Taiwanese women to invasive cancer with CC as a reference in MMP-11 SNP rs738791 (p= 0.044, Table 2). Furthermore, our finding showed that, for MMP-11 gene, carriers of the genotypes CT/ TT of rs738791 tended to have a higher risk than CC carriers of developing ≥ stage II disease at least (p=0.035) and stromal invasion more than 10 mm (p=0.043) for patients with invasive cancer of uterine cervix (Table 3). However, above tendency would become insignificant after multiple significance adjustment.

There were no significant differences between CC or CT/ TT carriers of MMP-11 rs739791 in recurrence-free survival or overall survival. All above p values seemed close to 0.05 in this study for MMP-11 SNP rs738791 which was associated with clinical stage and stromal deep invasion and exerted different distribution between patients with cervical invasive cancer and control women, the ORs and 95% CIs were 2.16 (1.05-4.44), 2.08 (1.02-4.26) and AOR 0.63 (0.41-0.99), respectively. They became obviously insignificant after multiple significance adjustment (p=0.245, 0.258 and 0.176, respectively). It implies that even when the number of patients could increase, the role of SNPs in this gene do not impact on clinicopathological variables and development of cervical cancer. These findings suggest that MMP-11 SNPs may show poor impacts of the SNPs in MMP-11 genes on the development of cervical cancer and clinicopathological variables and patient survival.

Table 3. Associations of genotypic distribution of matrix metalloproteinase-11 gene variant rs738791 with clinicopathological characteristics of the patients with invasive cancer of uterine cervix.

	MMP-11 (rs738791)				
Variables[a]	CC[b]	CT/ TT	*p* value	ORs (95% CIs)	Holm's adjusted *p* value
Clinical stage			0.035		0.245
stage I[b]	46	29		1.00	
≥ stage II	22	30		2.16 (1.05-4.44)	
Pathologic type			0.527		1.000
squamous cell carcinoma[b]	59	48		1.00	
adenocarcinoma	10	11		1.35 (0.53-3.45)	
Stromal invasion depth			0.043		0.258
≤10 mm[b]	41	25		1.00	
>10 mm	26	33		2.08 (1.02-4.26)	
Tumor diameter			0.633		1.000
≤ 4cm[b]	38	30		1.00	
>4cm	31	29		1.18 (0.59-2.38)	
Parametrium			0.747		1.000
no invasion[b]	46	37		1.00	
invasion	22	20		1.13 (0.54-2.38)	
Vagina			0.169		0.845
no invasion[b]	50	36		1.00	
invasion	19	23		1.68 (0.80-3.53)	
Pelviclymph node			0.433		1.000
no metastasis[b]	53	41		1.00	
metastasis	16	17		1.37 (0.62-3.04)	

Statistical analyses: chi-square or Fisher's exact tests and Holm's test

[a]Some clinicopathological data could not be obtained from the patients with cervical invasive cancer due to incomplete records of medical chart.

[b]As a reference. ORs, odds ratios; 95% CIs, 95% confidence intervals.

Table 4. Analysis of matrix metalloproteinase-11 gene variant rs738791 and clinicopathological variables for recurrence or survival events in cervical cancer patients

	Recurrence				Survival			
Variables[a]	+	−	*p* value	ORs (95% CIs)	+	−	*p* value	ORs (95% CIs)
MMP-11 rs738791			0.142				0.160	
CC[b]	11	55		1.00	54	12		1.00
CT/TT	16	42		1.90 (0.74-5.03)	42	17		1.82 (0.73-4.66)
Clinical stage			0.004				<0.001	
stage I[b]	9	62		1.00	65	6		1.00
≥ stage II	18	34		3.65 (1.36-10.18)	29	23		8.59 (2.94-28.09)
Pathologic type			0.041				0.246	
squamous cell carcinoma[b]	19	85		1.00	82	22		1.00
adenocarcinoma	8	12		2.98 (0.91-9.19)	13	7		2.01 (0.60-6.19)
Stromal invasion depth			0.003				<0.001	
≤10 mm[b]	7	55		1.00	56	6		1.00
>10 mm	20	39		4.03 (1.44-12.28)	37	22		5.55 (1.92-18.110)
Tumor diameter			0.003				<0.001	
≤ 4cm[b]	7	57		1.00	58	6		1.00
>4cm	20	40		4.07 (1.46-12.38)	37	23		6.01 (2.09-19.51)
Parametrium			0.065				<0.001	
no invasion[b]	13	66		1.00	70	9		1.00
invasion	13	29		2.28 (0.85-6.04)	23	19		6.43 (2.34-18.24)
Vagina			0.026				0.061	
no invasion[b]	13	69		1.00	67	15	1.00	1.00
invasion	14	28		2.65 (1.01-6.96)	28	14		2.23 (0.87-5.69)

	Recurrence				Survival			
Variables[a]	+	−	*p* value	ORs (95% CIs)	+	−	*p* value	ORs (95% CIs)
Pelvic lymph node			0.001				<0.001	
no metastasis[b]	13	77		1.00	80	10		1.00
metastasis	14	19		4.36 (1.59-11.88)	14	19		10.86 (3.79-31.55)

Statistical analysis: Chi-square or Fisher's exact tests

[a]Some clinicopathological data could not be obtained from the patients with cervical invasive cancer due to incomplete records of medical chart.

[b]As a reference. MMP-11, matrix metalloproteinase-11. Recurrence; +, recurrence; −, no recurrence. Survival; +, survival, −, dead.

Table 5. Univariate analysis for the associations of matrix metalloproteinase-11 genetic polymorphism rs738791 and various clinicopatholgical parameters with the recurrence-free survival and overall survival of the patients with uterine cervical cancer

	Recurrence-free survival		Overall survival	
Variables	*p* value	HR & 95% CI[b]	*p* value	HR & 95% CI[b]
matrix metalloproteinase-11 genetic polymorphism				
rs738791 CT/TT vs CC[a]	0.122	1.82 (0.84-3.94)	0.161	1.69 (0.80-3.50)
Clinicopathological characteristics				
Stage				
≥ stage II vs stage I[a]	0.002	3.29 (1.47-7.35)	<0.001	5.39 (2.18-13.32)
Pathologic type				
squamous cell carcinoma[a] adenocarcinoma	0.030	2.43 (1.06-5.58)	0.120	1.95 (0.83-4.57)
Stromal invasion depth				
>10 mm vs ≤10 mm[a]	0.001	3.81 (1.61-9.04)	0.001	4.25 (1.72-10.49)
Tumor diameter				
>4 cm vs ≤ 4cm[a]	0.001	3.91 (1.65-9.28)	<0.001	4.78 (1.94-11.75)
Parametrium				
invasion vs no invasion[a]	0.031	2.29 (1.06-4.99)	<0.001	4.59 (2.06-10.19)
Vagina				
invasion vs no invasion[a]	0.013	2.52 (1.18-5.37)	0.066	1.96 (0.94-4.07)
Pelvic lymph node				
metastasis vs no metastasis[a]	<0.001	4.27 (1.99-9.16)	<0.001	6.95 (3.22-15.03)

Statistical analyses: Kaplan-Meier curves model

[a]As a comparison reference

[b]HR, hazard ratio and 95% CI, 95% confidence interval for matrix metalloproteinase-11 gene variant rs738791 and clinicopathological variables, compared to their respective controls.

Table 6. Multivariate analysis for the associations of matrix metalloproteinase-11 genetic polymorphism rs738791 and various clinicopatholgical parameters with the recurrence-free survival and overall survival of the patients with uterine cervical cancer.

	Recurrence-free survival		Overall survival	
Variables	*p* value	HR & 95% CI[b]	*p* value	HR & 95% CI[b]
matrix metalloproteinase-11 genetic polymorphism				
rs738791 CT/TT vs CC[a]	0.355	1.49 (0.64-3.48)	0.130	1.94 (0.82-4.58)
Clinicopathological characteristics				
Stage				
≥ stage II vs stage I[a]	0.601	1.35 (0.44-4.17)	0.329	1.91 (0.52-6.97)
Pathologic type				
squamous cell carcinoma[a] adenocarcinoma	0.059	2.39 (0.97-5.90)	0.468	1.48 (0.52-4.21)
Stromal invasion depth				
>10 mm vs ≤10 mm[a]	0.348	1.71 (0.56-5.24)	0.483	1.56 (0.45-5.38)
Tumor diameter				
>4 cm vs ≤ 4cm[a]	0.324	1.92 (0.53-6.99)	0.657	1.41 (0.31-6.30)
Parametrium				
invasion vs no invasion[a]	0.349	0.59 (0.19-1.78)	0.425	1.71 (0.46-6.33)
Vagina				
invasion vs no invasion[a]	0.623	1.33 (0.43-4.10)	0.063	0.39 (0.14-1.05)
Pelvic lymph node				
metastasis vs no metastasis[a]	0.033	2.83 (1.09-7.35)	0.001	4.80 (1.82-12.62)

Statistical analyses: Cox proportional hazard model

[a]As a comparison reference

[b]HR, hazard ratio and 95% CI, 95% confidence interval for matrix metalloproteinase-11 gene variant rs738791 genetic polymorphism and clinicopathological variables, compared to their respective controls.

To the best of our knowledge, this is the first study to investigate the association between MMP-11 genetic polymorphisms and clinicopathologic characteristics, as well as survival, in patients with uterine cervical cancer. The influence of the MMP-11 gene on the metastatic phenotype of uterine cervical cancer was examined. There was no significant difference between patients carrying the rs738791 CT/TT genotype and CC carriers in pelvic lymph node metastasis, stromal invasion depth and stage. Furthermore, MMP-11 SNPs were not found to be significantly associated with cervical cancer patient survival in this study. Considering clinicopathological characteristics, only pelvic lymph node metastasis displayed a critical predictor of recurrence-free survival and overall survival in this limited sample study. This corroborates the finding of other studies that lymph node metastasis is the most pivotal prognostic variable for cervical cancer patient survival [2, 20-22]. The 5-year survival rate was significantly declined from 85%-90% in cervical cancer patients with negative pelvic lymph nodes to 30%-50% in those with positive pelvic lymph nodes [23].

Only 21 cases of adenocarcinoma were involved in this study. The sample size was too small to analyze the associations among MMP-11 SNPs, the development of cervical cancer, and clinicopathological variables. Therefore, it seems unreasonable to categorize our cases into adenocarcinoma group for statistical analysis. However, we cannot find any significant association between MMP-11 SNPs and the development of cervical adenocarcinoma in the limited samples (the data not showed). No significant difference was also found between MMP-1 SNPs and clinicopathological variables of cervical adenocarcinoma (the data not showed).

MMP-11 is secreted into microenvironment, degrades the ECM and promotes the tumorigenesis and progress of cancer [4]. Several potential physiological substrates of MMP-11 have been identified, including insulin-like growth factor-binding protein-1 (IGFBP-1), proteinase inhibitor, laminin receptor and collagen VI [4, 24-27].

The role of MMP-11 might play activator or repressor functions in the metastatic process, probably depending on various spatiotemporal occasions [4]. The complexity of MMP-11 function was shown by an animal study, including favoring implantation and growth of lung metastasis during metastasis process but limiting lung foci number and repressing dissemination to other organs [28]. In the cervical cancer, increased transcription of gene MMP-11 was observed [13]. Expressions of MMP-11 in LSIL, HSIL and cervical cancer have been investigated [14]. A relative increased expression of MMP-11 from LSIL and HSIL samples was demonstrated by real time RT-PCR or immunohistochemistry assay [14]. Positive samples for MMP-11 expression were also increased according to disease progression [14].

SNPs may associate with diseases by influencing promoter activity, messenger RNA conformation, subcellular localization of messenger RNAs and/or proteins [15]. Identifying variations in genes and analyzing their effects on diseases may lead to understanding the impacts on health of an individual [15]. Furthermore, Genetic polymorphisms, in exons or promoter regions, may affect gene expression. When a SNP occurs in a coding sequence, the encoded amino acids may be changed in the related protein and is referred to nonsynonymous. If the same amino acid is produced, it is known as synonymous. A SNP occuring in the 3'-untranslated region of a gene may exert an impact on biological processes [15]. SNPs, in introns, can play important biological roles and may have a strong influence on disease progressing [29]. In this study, MMP-11 SNPs rs28382575 (Pro475Pro) is synonymous and SNPs rs738792 (Ala38Val) is nonsynonymous. However, rs738791, rs2267029 and rs131451 are all located in the intron regions. Until now, the mechanisms that MMP-11 rs738791 exerts the influence on its gene expressions have not be delineated. Rs738791 may present as a functional intronic polymorphism which can affect the expression of this gene that hosts it [30-32]. Genetic variants in intron can induce aberrant splicing that is readily distinguishable from normal splicing by RNA analysis. Introns probably represent a variety of mutational targets because of their containing a multiplicity of functional elements including intron splicing enhancers, silencers which can regulate alternative slicing and other regulatory elements [33-36]. By functional intronic polymorphisms, the susceptibility to disease may be conferred. This also modulates genotype-biological phenotype relationship and may affect biological phenotypes depending on various spatiotemporal occasions [30-32]. Because there were no significant associations after multiple significance test, the role of SNPs in this gene do not impact on uterine cervical cancer based on the current study. The molecular mechanism of functional intronic polymorphisms actually could not be related to MMP-11 SNPs. MMP-11 SNP rs738791, intron variant, could play a role for development, progression or protection in different type of cancer. Carriers of MMP-11 rs738791 CT+TT were at higher risk of Hepatocellular carcinoma (HCC) than carriers of wild type (CC) [17]. Individuals with CT/CC genotype at the MMP-11 SNP rs28382575 were at greater risk of developing large tumors, stage III/IV disease or lymph node metastasis. The authors claimed that genetic variations in the MMP-11 gene may be helpful in predicting early-stage HCC [17]. In a transgenic animal model study found that dual MMP-11 function, including tumor enhancing or repressing in processes, led to local or distal invasion [37]. A previous research demonstrated no significant difference in the incident of oral squamous cell carcinoma (OSCC) amongst individuals with MMP-11 genetic polymorphisms in rs738791, rs2267029, rs738792 or rs28382575; however, the effect of MMP-11 gene polymorphisms might be collaborated with environmental carcinogens (betel nut chewing or tobacco use) to increase risk of developing OSCC [16]. These findings demonstrate different MMP-11 polymorphisms play different functions on cancer development.

In conclusion, our study demonstrates no impact of the MMP-11 gene polymorphisms on the development and clinicopathological variables of cervical cancer as well as patient survival in Taiwan. Further studies are needed to examine the impact of MMP-11 on the pathogenesis and pathway.

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Competing Interests

The authors have declared that no competing interest exists.

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