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Single-Nucleotide Polymorphisms and Haplotypes of *Intercellular Adhesion Molecule-1* in Uterine Cervical Carcinogenesis in Taiwanese Women

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Abstract

The association of *intercellular adhesion molecule 1* (*ICAM-1*) genetic polymorphisms with uterine cervical carcinogenesis has seldom been reported. Therefore, the aim of this study was to investigate the association of single-nucleotide polymorphisms (SNPs) and haplotypes of *ICAM-1* with cervical tumorigenesis in Taiwanese women. Four hundred forty four women, including 91 with cervical invasive cancer, 63 with precancerous lesions, and 290 normal controls, were recruited. The genotypic distribution of 4 SNPs of *ICAM-1*, rs5498 (A1548G), rs5491 (K56M), rs281432 (C8823G), and rs3093030 (C-286T) was determined using real-time polymerase chain reactions and genotyping. Compared to homozygous wild CC, heterozygous CG, homozygous mutant GG, or genotypes with CG/GG display increased risks or a tendency of precancerous lesions or invasive cancer with strong power in rs281432. The homozygotic mutant alleles TT in rs3093030 and homozygotic mutant alleles GG in rs5498 were associated with a higher risk of invasive cancer and precancerous lesions, respectively, but with lower power. The CG/TA/TG haplotypes of *ICAM-1* SNPs rs3093030 and rs5498 exhibited a tendency to increase susceptibility to precancerous lesions and invasive cancer. In conclusion, Taiwanese women with *ICAM-1* SNP rs281432 and haplotypes CG/TA/TG of rs3093030 and rs5498 are associated with uterine cervical carcinogenesis.

Keywords

intercellular adhesion molecule 1, single-nucleotide polymorphisms, haplotypes, uterine cervical carcinogenesis

Introduction

The development of uterine cervical cancer is a multistep process. Cervical intraepithelial neoplasias (CINs) are precancerous lesions that may progress into invasive cancer of the uterine cervix.[1] In Taiwan, uterine cervical cancer is the fifth most common type of cancer in women. The age-standardized incidence rate in 2009 was reported to be 11.86 per 100 000 women, with an age-standardized mortality rate reported to be 3.72 per 100 000 women in 2011, making it the seventh leading cause of cancer death in Taiwanese women.

The *intercellular adhesion molecule 1* (*ICAM-1*) gene is located on chromosome 19 and consists of 7 exons separated by 6 introns.[2-5] Its protein product is a 90-kDa transmembrane glycoprotein belonging to the immunoglobulin superfamily of cell adhesion molecules.[6] The ICAM-1 is comprised of 505 amino acids that are divided into 3 parts: an extracellular portion (453 amino acids) containing 5 immunoglobulin-like domains (D1-5) functioning in adhesive interactions, a transmembrane region consisting of 24 residues, and a short intracellular tail made up of 28 amino acids relating to signal transduction.[7] It is expressed in various cell types such as leukocytes, epithelial, and endothelial cells and is upregulated by inflammatory cytokines.[3,8] It is involved in

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signal transduction after binding to β2 integrin ligands through activation of the mitogen-activated protein kinase and AP-1 pathways and regulates cell proliferation.[6]

Genetic polymorphisms in the *ICAM-1* gene have been identified such as the nonsynonymous single-nucleotide polymorphism (SNP) rs5498 (A1548G in exon 6), which has been reported to be involved in functional activity.[9] It has also been reported that variations in the genetic polymorphisms in exon 6 (rs5498; K469E) and exon 2 (rs5491; K56M) or intron 2 (rs281432; C8823G) as well as in the region between the *ICAM-1* and the *ICAM-4* genes (rs3093030; C-286T) are associated with an increased risk of various types of cancer including prostate, gastric, and breast cancers.[10-12] However, to the best of our knowledge, no published studies have investigated the role of *ICAM-1* SNPs on the risk of uterine cervical neoplasias. We therefore conducted this study to investigate the association of *ICAM-1* genetic polymorphisms with cervical tumorigenesis.

Materials and Methods

Participants

Four hundred forty four women, including 91 with invasive cancer, 63 with precancerous lesions of the uterine cervix, and 290 normal controls, were recruited consecutively into this study. The patients with invasive cervical cancer were clinically staged based on the International Federation of Gynecology and Obstetrics Classification and received routine treatment protocols at the Department of Obstetrics and Gynecology in Chung Shan Medical University Hospital, Taiwan, from August 1999 to August 2013. Cervical intraepithelial neoplasias include CIN1 (mitoses and immature cells that are confined to the basal one-third of the epithelium and constitute low-grade squamous intraepithelial lesions, low-grade dysplasia, or low-grade CIN), CIN2 (mitoses and immature cells that are confined to the basal two-thirds of the epithelium and constitute moderate dysplasia), and CIN3 (mitoses and immature cells in more than basal two-thirds of the epithelium known as CIN3 or severe dysplasia, and mitoses and immature cells in whole epithelial thickness especially called as carcinoma in situ) based on the Bethesda system.[13] High-grade squamous intraepithelial lesions (high-grade dysplasia or high-grade CIN) include both CIN2 and CIN3. The CIN1 (low-grade CIN), CIN2, and CIN3 (high-grade CIN) are all regarded as precancerous lesions. Few samples could be obtained by colposcopy-directed biopsy for CIN1. In order to prevent misdiagnosis by the pathologist, CIN1 cases were not included in the study. The patients with high-grade CIN underwent colposcopy-directed cervical punch biopsy, large loop excision of the transformation zone, total abdominal hysterectomy, or total vaginal hysterectomy, and large samples could be obtained from these patients. Therefore, only the patients with high-grade CIN, which included moderate and severe dysplasia and carcinoma in situ, were recruited for the precancerous lesions. The normal Papanicolaou smears of the 290 controls during general examinations at the outpatient department of our hospital were further verified by the same gynecologic oncologist (P-H. Wang) using colposcopy at the Department of Obstetrics and Gynecology as described in detail previously.[14] The ages of the women with cervical invasive cancer, precancerous lesions, and normal controls were 54.0 ± 11.9 years, 42.8 ± 12.6 years, and 44.5 ± 10.2 years (mean ± standard deviation [SD]), respectively. All of the women were Taiwanese who resided in central Taiwan. The median parity of the patients with cervical cancer was 2, those with precancerous lesions 2, and normal controls also 2. Rare studied participants (<5%) had the smoking. The women in this study almost refused to reply to the question multiple partners due to conservative attitude in Taiwan culture. The Chung Shan Medical University Hospital Institutional Review Board approved this study (CSMUH IRB: CS12219, CS14014), and written informed consent was obtained from each individual.

Selection of ICAM-1 Gene Polymorphisms

The dbSNP database (SNP database) has documented over 20 SNPs in the 7-exon region of the *ICAM-1* gene. We selected the nonsynonymous SNPs rs5491 (K56M in exon 2) and rs5498 (A1548G in exon 6) in the coding sequences as well as rs281432 (C8823G in intron 2) of the gene based on Chinese HapMap (Han Chinese in Beijing, China) data. Moreover, another SNP between the *ICAM*-1 and *ICAM-4* genes (rs3093030, C-286T) was selected in this study, since this SNP has been found to affect the production of soluble ICAM-1 (sICAM-1) in Chinese patients.[15] Because rs3093030 is in linkage disequilibrium with rs5498 ($R^2 = 0.84$) based on Chinese HapMap data, the haplotypes of rs3093030 and rs5498 were established and included into the analysis.

Blood Sample Collection and Genomic DNA Extraction

Blood specimens were collected from all of the patients and controls. Genomic DNA was extracted from EDTA anticoagulated venous blood using a QIAamp DNA blood mini kit (Qiagen, Valencia, California) based on the manufacturer's protocol as described in detail previously.[14] The DNA was dissolved in Tris ethylene buffer (10 mmol/L Tris and 1 mmol/L EDTA; pH 7.8) and then quantified by measurement at OD260. The final preparation was stored at −20°C and applied as the template in polymerase chain reactions (PCRs).

Analysis of SNPs by Real-Time PCR and Genotyping

Allelic discrimination of rs3093030 (C-286T), rs5491(K56M), rs281432 (C8823G in intro 2), and rs5498 (A1548G) was performed using an ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, California) and analyzed by SDS version 3.0 software (Applied Biosystems) using the TaqMan assay as described in detail previously.[14] The final volume (10 μL) for each reaction contained 5 μL TaqMan

Genotyping Master Mix, 0.25 μL TaqMan probe mix, and 10 ng genomic DNA. The real-time PCR included an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and then 60°C for 1 minute.

Statistical Analysis

Analysis of variance was used to analyze the age distribution of the study population and control participants as described in detail previously.[14] Scheffe test was used for post hoc analysis, and Hardy–Weinberg equilibrium was used to analyze the genotype distributions of rs3093030, rs5491, rs281432, and rs5498 in the normal controls (degrees of freedom [*df*] = 2). Chi-square or Fisher exact tests were used to examine the relationships among the frequencies of *ICAM-1* gene SNPs, alleles, and haplotypes and the incidence of cervical neoplasias (including invasive cancer and precancerous lesions). Logistic regression and multiple logistic regression models were used to analyze multiple comparisons of genotypes of the *ICAM-1* gene polymorphisms before and after controlling for age between the patients with cervical neoplasias and the controls and among the patients with invasive cancer or precancerous lesions and the controls. The sample size was estimated with a power (1-β) of 0.8 and an α value of .05, with the power being calculated when comparisons among the subgroups reached a statistical significance ($P < .05$) or a different tendency with this study sample size using WinPepi software, version 10.0. A P value of less than .05 was considered to indicate statistical significance. Statistical analyses including odds ratios (ORs) and adjusted odds ratios (aORs; controlling for age) and their 95% confidence intervals (CIs) were calculated using SPSS software version 12.0 and WinPepi Software version 10.0.

Results

No significant difference in parity was found among the patients with cervical cancer (median: 2, range: 0-7), those with precancerous lesions (median: 2, range: 0-6), and the control women (median: 2, range: 0-5) using Kruskal–Wallis H test ($P = .186$). There was a significant difference in age between the patients with cervical neoplasias and the controls (49.4 ± 13.3 years vs 44.5 ± 10.2 years, $P < .001$). After classifying the cervical neoplasias into invasive cancer and precancerous lesions, there were significant differences in age between the patients with cervical cancer and those with precancerous lesions (54.0 years ± 11.9 vs 42.8 years ± 12.6 years, $P < .001$) and between those with cervical cancer and the controls (54.0 ± 11.9 years vs 44.5 ± 10.2 years, $P < .001$) but not between those with precancerous lesions and the controls (42.8 ± 12.6 years vs 44.5 ± 10.2 years, $P = .56$). The minor allele frequencies of *ICAM-1* SNPs rs3093030 (C-286T), rs5491 (K56M), rs281432 (C8823G), and rs5498 (A1548G) of the controls were all >5% (16.4%, 5.7%, 21.9%, and 21.4%, respectively). In these participants, the genotypic frequency of *ICAM-1* SNP rs3093030 met the Hardy–Weinberg equilibrium ($P > .05$, χ^2 value: 1.47, *df*: 2). The frequencies of *ICAM-1* SNPs rs5491, rs281432, and rs5498 were also in the Hardy–Weinberg equilibrium ($P > .05$, χ^2 value: 1.48; $P > .05$, χ^2 value: 0.16; and $P > .05$, χ^2 value: 1.40, respectively).

Association of ICAM-1 Polymorphisms With Uterine Cervical Neoplasias

The genotypic distribution of *ICAM-1* SNPs in the patients with cervical neoplasias and the controls is summarized in Table 1. There was a significant difference in the genotypic distribution of SNPs in rs281432 ($P = .001$) and rs5498 ($P = .032$). Using CC as a comparison reference in rs281432, the individuals with the heterozygous genotype CG or homozygous mutant genotype GG had an increased risk of developing cervical neoplasias (OR: 1.95, 95% CI: 1.28-2.95 for CG; OR: 2.83, 95% CI: 1.33-6.05 for GG; Table 1). Even after adjusting for age, the ORs were still 1.86 (95% CI: 1.22-2.86) and 3.00 (95% CI: 1.38-6.54), respectively. The participants who had a genotype with at least 1 mutant allele G (CG/GG) also had an elevated risk of developing cervical neoplasias (OR: 2.06, 95% CI: 1.36-3.13) even after adjusting for age (aOR: 2.01, 95% CI: 1.34-3.02), using wild CC as a reference. Similarly, the individuals with homozygous mutant GG in rs5498 had a higher risk of developing cervical neoplasias, using wild AA as a comparison reference (OR: 2.74, 95% CI: 1.14-6.62) even adjusting for age (aOR: 2.70, 95% CI: 1.08-6.73; Table 1). The participants who had genotypes with at least 1 mutant allele G (AG/GG) still had this risk (OR, 1.54 and aOR, 1.50 after adjusting for age). However, the power was 0.59 in rs5498, which was not as strong as in rs281432 (0.97).

When the cervical neoplasias were further subdivided into invasive cancer and precancerous lesions, the homozygous mutant TT in rs3093030 exhibited a different distribution between the individuals with cervical invasive cancer and the controls, using homozygous CC as a reference ($P = .044$; OR: 3.70, 95% CI: 1.03-13.33). The presence of TT increased the risk of invasive cancer to 4.83 (aOR: 4.83, 95% CI: 1.21-19.20; Table 2) after adjusting for age. There were no differences in the distribution of TT or CT between the participants with precancerous lesions and the normal controls, using CC as a reference. Only individuals with both mutant alleles TT had an increased risk of developing invasive cancer after adjusting for age (aOR: 4.61, 95% CI: 1.17-18.18; Table 2). Moreover, compared to homozygous wild CC, the presence of heterozygous CG, homozygous mutant GG, and genotypes with 1 or 2 mutant alleles (CG/GG) led to an elevated risk or a tendency of precancerous lesions (aOR: 2.35, 95% CI: 1.30-4.24; aOR: 3.23, 95% CI: 1.19-8.70; and aOR: 2.48, 95% CI: 1.41-4.35, respectively) or invasive cancer (aOR: 1.58, 95% CI: 0.93-2.70; aOR: 2.72, 95% CI: 1.04-7.14; and aOR: 1.72, 95% CI: 1.04-2.87, respectively) after adjusting for age in rs281432 (Table 2). With regard to rs5498, the women with the homozygous mutant GG had a higher risk of developing cervical precancerous lesions compared to those with the wild genotype AA (aOR: 4.02, 95% CI: 1.42-11.4) or AA/AG (aOR: 3.48,

Table 1. Genotypic Distribution of the Single-Nucleotide Polymorphisms of the *Intercellular Adhesion Molecule-1* Gene in Patients With Neoplasias of the Uterine Cervix and Normal Controls.[a]

Variables	Normal Controls (n = 290)	Cervical Neoplasias[b](n = 154)	*P* value (Power)[c]	OR (95% CI)	aOR (95% CI)[d]
rs3093030					
CC[e]	200	94	.140	1.00	1.00
CT	85	54		1.35 (0.89-2.06)	1.20 (0.78-1.85)
TT	5	6		2.55 (0.76-8.58)	2.64 (0.76-9.16)
CC[e]	200	94	.093	1.00	1.00
CT/TT	90	60		1.42 (0.92-2.18)	1.28 (0.84-1.94)
CC/CT[e]	285	148	.201	1.00	1.00
TT	5	6		2.31 (0.58-9.72)	2.49 (0.73-8.58)
rs5491					
AA[e]	259	135	.449	1.00	1.00
AT	29	19		1.26 (0.68-2.32)	1.27 (0.68-2.38)
TT	2	0		UA	UA
AA[e]	259	135	.601	1.00	1.00
AT/TT	31	19		1.18 (0.60-2.24)	1.21 (0.65-2.24)
AA/AT[e]	288	154	.546	1.00	1.00
TT	2	0		UA	UA
rs281432					
CC[e]	178	67	.001[c]	1.00	1.00
CG	97	71	(.94)	1.95 (1.28-2.95)	1.86 (1.22-2.86)
GG	15	16		2.83 (1.33-6.05)	3.00 (1.38-6.54)
CC[e]	178	67	<.001[c]	1.00	1.00
CG/GG	112	87	(.97)	2.06 (1.36-3.13)	2.01 (1.34-3.02)
CC/CG[e]	275	138	.04[c]	1.00	1.00
GG	15	16	(.54)	2.13 (0.95-4.76)	2.31 (1.08-4.91)
rs5498					
AA[e]	176	77	.032[c]	1.00	1.00
AG	104	65	(.65)	1.43 (0.95-2.15)	1.38 (0.91-2.11)
GG	10	12		2.74 (1.14-6.62)	2.70 (1.08-6.73)
AA[e]	176	77	.030[c]	1.00	1.00
AG/GG	114	77	(.59)	1.54 (1.02-2.33)	1.50 (1.00-2.24)
AA/AG[e]	280	142	.045	1.00	1.00
GG	10	12	(.52)	2.37 (0.91-6.26)	2.36 (0.96-5.79)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio; UA, unavailable.
[a]Statistical analysis: logistic regression model, χ^2, or Fisher exact tests.
[b]Cervical neoplasias included precancerous lesions and invasive cancer of the uterine cervix.
[c]$P < .05$; the sample size was estimated with a power (1-β) of 0.8 and an α value of 0.05, and the power was calculated when the comparisons among the subgroups reached a statistical significance ($P < .05$) or a different tendency with this study sample size using WinPepi software, version 10.0. *P* values are presented and important powers are given in parenthesis.
[d]The aOR and their 95% CIs were estimated by logistic regression model after controlling for age.
[e]Used as a reference for comparison to evaluate the odds ratio of other genotypes.

95% CI: 1.27-9.62) rather than invasive cancer after adjusting for age (Table 2).

Association of Allele Frequencies of ICAM-1 Polymorphisms With Uterine Cervical Neoplasias

With regard to the allele frequencies of the 4 *ICAM-1* gene polymorphisms in the 444 samples collected, the mutant allele G in SNP rs281432 increased the risk of developing cervical precancerous lesions, using C as a comparison reference (aOR: 2.01, 95% CI: 1.32-3.05; Table 3) and also increased the risk of developing cervical invasive cancer (aOR: 1.62, 95% CI: 1.09-2.40). Moreover, the mutant allele G in rs5498 increased the risk of precancerous lesions (aOR: 1.68, 95% CI: 1.09-2.58) rather than invasive cancer.

Association of the Haplotypes of ICAM-1 SNPs With Uterine Cervical Carcinogenesis

Based on the established haplotypes of rs3093030 and rs5498, we investigated the association of the haplotypes with cervical tumorigenesis. Four haplotypes (CA, CG, TA, and TG) were found, and the haplotype without a mutant allele was used as a reference. There was a significant difference in the frequency of haplotype distribution among the women with cervical invasive cancer, precancerous lesions, and the controls ($P = .034$). There was also a significant difference in the distribution of haplotypes CG/TA/TG between the women with invasive cancer and the controls ($P = .034$; OR: 1.49, 95% CI: 1.03-2.15). However, these haplotypes tended to increase the risk of precancerous lesions (OR: 1.52, 95% CI: 1.00-2.31), although the difference did not reach a

Table 2. Genotypic Distribution of the Single-Nucleotide Polymorphisms of the *Intercellular Adhesion Molecule* 1 Gene in the Patients With Invasive Cancer and Precancerous Lesions of the Uterine Cervix and Normal Controls.[a]

Variables	Normal Controls (n = 290)	Precancerous Lesions (n = 63)	Invasive Cancer (n = 91)	*P* value (Power)[b]	aOR (95% CI)[c]	aOR (95% CI)[d]
rs3093030						
CC[e]	200	40	54	.176	1.00	1.00
CT	85	22	32	(.48)	1.23 (0.68-2.22)	1.15 (0.67-1.99)
TT	5	1	5		0.94 (0.11-8.26)	4.83 (1.21-19.20)
CC[e]	200	40	54	.211	1.00	1.00
CT/TT	90	23	37		1.21 (0.68-2.16)	1.31 (0.77-2.20)
CC/CT[e]	285	62	86	.116	1.00	1.00
TT	5	1	5	(.43)	0.88 (0.10-7.69)	4.61 (1.17-18.18)
rs5491						
AA[e]	259	52	83	.342	1.00	1.00
AT	29	11	8		1.93 (0.90-4.12)	0.87 (0.37-2.05)
TT	2	0	0		UA	UA
AA[e]	259	52	83	.215	1.00	1.00
AT/TT	31	11	8		1.79 (0.84-3.80)	0.83 (0.35-1.95)
AA/AT[e]	288	63	91	.587	1.00	1.00
TT	2	0	0		UA	UA
rs281432						
CC[e]	178	25	42	.006[b]	1.00	1.00
CG	97	31	40	(.89)	2.35 (1.30-4.24)	1.58 (0.93-2.70)
GG	15	7	9		3.23 (1.19-8.70)	2.72 (1.04-7.14)
CC[e]	178	25	42	.001[b]	1.00	1.00
CG/GG	112	38	49	(.93)	2.48 (1.41-4.35)	1.72 (1.04-2.87)
CC/CG[e]	275	56	82	.116	1.00	1.00
GG	15	7	9		2.23 (0.86-5.75)	2.26 (0.88-5.78)
rs5498						
AA[e]	176	31	46	.050	1.00	1.00
AG	104	25	40	(.69)	1.41 (0.79-2.54)	1.37 (0.81-2.30)
GG	10	7	5		4.02 (1.42-11.4)	1.39 (0.39-4.90)
AA[e]	176	31	46	.095	1.00	1.00
AG/GG	114	32	45	(.47)	1.64 (0.95-2.86)	1.37 (0.83-2.27)
AA/AG[e]	280	56	86	.038[b]	1.00	1.00
GG	10	7	5	(.62)	3.48 (1.27-9.62)	1.22 (0.35-4.22)

Abbreviations: aOR, adjusted odds ratio; 95% CI, 95% confidence interval; UA: unavailable.

[a]Statistical analysis: multiple logistic regression model, χ^2, or Fisher exact tests. The aORs with their 95% CIs were estimated by the multiple logistic regression model after controlling for age.

[b]$P < .05$; the sample size was estimated with a power (1-β) of 0.8 and an α value of 0.05, and the power was calculated when the comparisons among the subgroups reached a statistical significance ($P < .05$) or a different tendency with this study sample size using WinPepi software, version 10.0. *P* values are presented and important powers are given in parenthesis.

[c]Comparison between patients with precancerous cervical lesions and normal controls after adjusting for age.

[d]Comparison between patients with cervical cancer and normal controls after adjusting for age.

[e]Used as a reference for comparisons to evaluate the odds ratio of other genotypes.

statistical significance ($P = .052$). After controlling for age, the women with haplotypes CG/TA/TG had a tendency to be more susceptible to precancerous lesions (aOR: 1.49, 95% CI: 0.97-2.29) and invasive cancer (aOR: 1.38, 95% CI: 0.93-2.05; Table 4).

Discussion

To date, few studies have investigated associations of the SNPs and haplotypes of *ICAM-1* with uterine cervical carcinogenesis. In this study, we found that there are no differences in the distribution of homozygous mutant TT in rs3093030 (C-286T) between the patients with cervical neoplasias and normal controls, using CC or CC/CT as comparison references. However, the presence of TT exerted a significant difference between the women with invasive cancer and the controls. The effect of both mutant alleles TT in rs3093030 on invasive cancer was probably masked by the precancerous lesions. Kim et al reported that systemic lupus erythematosus was strongly associated with rs3093030 located in the 0.4-kb intergenic region between *ICAM-1* and *ICAM-4* in their study, which included patients from multiple institutions worldwide as part of the Lupus Association Study-2.[16] Although *ICAM-1* SNP rs5491 was not significantly associated with cervical carcinogenesis in our study, Lin et al found that participants with at least 1 allele T in rs3093030 or 1 allele T in rs5491 as well as 1 allele G in rs281432 or 1 allele G in rs5498 in those who chewed betel nut had a higher risk of developing oral cancer, using those

Table 3. Allele Distribution of *Intercellular Adhesion Molecule-1* Genetic Polymorphisms in Patients With Invasive Cancer and Precancerous Lesions of the Uterine Cervix and Normal Controls.[a]

Variables	Normal Controls (n = 290)	Precancerous Lesions (n = 63)	Invasive Cancer (n = 91)	*P* value (Power)[b]	aOR (95% CI)[c]	aOR (95% CI)[d]
rs3093030						
C[e]	485	102	140	.118	1.00	1.00
T	95	24	42	(.43)	1.15 (0.69-1.90)	1.40 (0.90-2.18)
rs5491						
A[e]	547	115	174	.270	1.00	1.00
T	33	11	8	(.27)	1.59 (0.78-3.26)	0.80 (0.35-1.84)
rs281432						
C[e]	453	81	124	.001[b]	1.00	1.00
G	127	45	58	(.95)	2.01 (1.32-3.05)	1.62 (1.09-2.40)
rs5498						
A[e]	456	87	132	.035[b]	1.00	1.00
G	124	39	50	(.63)	1.68 (1.09-2.58)	1.26 (0.84-1.91)

Abbreviations: aOR, adjusted odds ratio; 95% CI, 95% confidence interval.
[a]Statistical analysis: multiple logistic regression model or the χ^2test. The aORs with their 95% CIs were estimated by the multiple logistic regression model after controlling for age.
[b]*P*< 0.05; the sample size was estimated with a power (1-β) of 0.80 and an α value of 0.05, and the power was calculated when the comparison among the subgroups was performed with this study sample size using WinPepi software, version 10.0. *P* values are presented and important powers are given in parenthesis.
[c]Comparison between patients with precancerous lesions and normal controls after adjusting for age.
[d]Comparison between patients with cervical cancer and normal controls after adjusting for age.
[e]Used as a reference to evaluate the odds ratio.

Table 4. Haplotype Distribution of the *ICAM-1* Genetic Polymorphism in the Patients With Invasive Cancer and Precancerous Lesions of the Uterine Cervix and Normal Controls.[a]

Haplotypes for rs3093030 and rs5498	Normal Controls (n = 290)	Precancerous Lesions (n = 63)	Invasive Cancer (n = 91)	*P* value (Power)[b]	aOR (95% CI)[c]	aOR (95% CI)[d]
CA[e]	444	86	125	.034[b]	1.00	1.00
CG/TA/TG	136	40	57	(.63)	1.49 (0.97-2.29)	1.38 (0.93-2.05)

Abbreviations: aOR, adjusted odds ratio; 95% CI, 95% confidence interval; *ICAM-1*, *intercellular adhesion molecule-1*; SNPs, single-nucleotide polymorphisms.
[a]Statistical analysis: multiple logistic regression model or the χ^2test. The aORs with their 95% CIs were estimated by the multiple logistic regression model after controlling for age.
[b]*P*< .05; the sample size was estimated with a power (1-β) of 0.80 and an α value of 0.05, and the power was calculated when the comparison among the subgroups was performed with this study sample size using WinPepi software, version 10.0. *P* values are presented and important powers are given in parenthesis.
[c]Comparison between patients with precancerous lesions and normal controls after adjusting for age.
[d]Comparison between patients with cervical cancer and normal controls after adjusting for age.
[e]A haplotype with one wild allele for each type of *ICAM-1* SNPs rs3093030 and rs5498 was used as a reference for comparison to evaluate the odds ratio of other haplotypes.

with wild genotypes who did not chew betel nut as comparison references among 727 Taiwanese smokers.[17] This emphasizes the importance of the synergistic effects of environmental factors (betel nut and smoking) and *ICAM-1* SNPs on cancer development.

In rs5498, after controlling for age, both mutant allele GG were needed to display the significant increase or the tendency to develop cervical neoplasias, using wild AA or AA/AG as comparison references. However, the women with the homozygous mutant GG carried a higher risk of developing cervical precancerous lesions rather than invasive cancer, using genotype AA or AA/AG as references. The effect of GG on precancerous lesions seemed to be masked by invasive cancer. Lu et al reported that this SNP is located within the 5′ splice donor site and significantly affects splicing, thereby contributing to transcriptome differences.[18] Iwao et al reported that rs5498 (A1548G) may affect *ICAM-1* messenger RNA (mRNA) splicing patterns and modify inflammatory immune responses by changing cell–cell interactions and then regulating apoptosis.[9] In addition, *ICAM-1* has been reported to possibly contribute to tumorigenesis.[19,20] Moreover, Chen et al found that *ICAM-1* SNP rs5498 is associated with the risk of prostate cancer in African Americans with a family history of prostate cancer. The increased risk was pronounced in the individuals who possessed at least 1 G allele of the nonsynonymous K469E variant.[10] In contrast, Kammerer et al found that the *ICAM*-1 SNP rs5498 allele A variant is a potential candidate to influence tumor progression of breast and prostate cancers in German population.[12] However, Thanopoulou et al reported that both the genotype and the allele frequencies of *ICAM-1* did not significantly differ between patients with non–small-cell lung cancer and controls.[21]

Only 1 mutant allele G was needed to significantly increase the risk of developing cervical precancerous lesions and invasive cancer in those with *ICAM-1* SNP rs281432 with strong statistical power. The association of *ICAM-1* SNP rs281432 with disease has also been demonstrated by Ma et al who found that *ICAM-1* gene SNPs rs281432 (C8823G) and rs5498 (A1548G) confer susceptibility to type 1 diabetes and are probably associated with diabetic nephropathy in Swedish caucasians.[22] In addition, rs281432 has been reported to be associated with sICAM-1.[23] Altomonte et al reported that in the presence of a suspicious breast neoplasm, elevated levels of serum sICAM-1 can orient clinical diagnosis toward malignancy.[24]

The Taiwanese women with CG, GG, or CG/GG in rs281432 were more susceptible to cervical precancerous lesions or invasive cancer. This was reflected by the analysis of allele frequencies of *ICAM-1*, which revealed that 1 mutant allele G increased the risk of developing cervical precancerous lesions and invasive cancer. Both mutant alleles GG in rs5498 were needed to develop cervical precancerous lesions but not invasive cancer, using homozygous wild AA or AA/AG as references. This finding was in agreement with our allele frequency analysis, which showed that mutant G could increase the risk of precancerous lesions rather than invasive cancer.

Because the lower powers of the effect of TT on invasive cancer in rs3093030 and the effect of GG on precancerous lesions in rs5498, as well as rs3093030 being in linkage disequilibrium with rs5498, these haplotypes were established to associate them with cervical carcinogenesis. Bielinski et al also showed that these 2 SNPs were in strong linkage disequilibrium in caucasians ($R^2 = 0.93$) in their cross-sectional study.[23] The Taiwanese women with haplotypes CG/TA/TG of *ICAM-1* SNPs rs3093030 and rs5498 had a tendency of increased susceptibility to precancerous lesions and invasive cancer, although this did not reach a statistical significance in this study. However, Shifman et al demonstrated that when haplotypes consisting of each SNP contribute to the susceptibility of disease, although not obviously, haplotype analysis has a greater statistical power and can be advantageous over individual SNP analysis for the detection of an association between the alleles and a disease phenotype.[25]

The biopsied or excised area always contained transformation zone for the evaluation of cervical carcinogenesis in the study. The squamocolumnar junction is critical to the pathogenesis of cervical neoplasia. It displays a dynamic of epithelial cell growth and differentiation and includes the conversion of columnar epithelium to squamous epithelium via proliferation of reserve cells (squamous metaplasia).[26] The emergence of multiple phenotypes—both invasive and noninvasive phenotypes—associated with human papillomavirus (HPV) infection in this zone indicates not only the virus but also the existence of multipotential stem cells.[27] In this region, there is loss of normal tissue characteristics and basement membrane as the cancer becomes invasive. Nair et al found that the basement membrane is disrupted in high-grade CIN and is completely lost as the lesions progress to invasive cancer during tumor progression in the uterine cervix.[28] Coleman et al have investigated the expression of ICAM-1 in squamous neoplasia of the cervix and have noted a significant induction of the molecule in HSILs (high-grade squamous intraepithelial lesions; high-grade CIN; CIN 2 and CIN3).[29] Nasu et al also noted that the expression of ICAM-1 mRNA was observed in most cervical cancer tissues.[30] Because ICAM-1 SNPs have been reported to be associated with ICAM-1 expression[23] and an increased risk of various types of cancer including prostate, gastric, and breast cancers,[10-12] it is reasonable that ICAM-1 SNPs are involved in cervical carcinogenesis.

There were some limitations in this study. The data of HPV infection were absent. Human papillomavirus may be detected in more than 99% of cases with cervical cancer.[31,32] Taiwan Cooperative Oncologic Group reported that the prevalence of HPV in atypical squamous cells of undetermined significance/atypical glandular cells of undetermined significance–negative histology, HSIL, and invasive cancer were 33.8%, 84.3%, and 100%, respectively.[33] However, the HPV test was not done in this study. Therefore, the occurrence of HPV could not be compared between patients and controls. Another limitation was that the women in this study almost refused to reply to the question multiple partners, an important cofactor that might affect the results, due to conservative attitude in Taiwan culture.

In conclusion, in this study on Taiwanese women, *ICAM-1* SNP rs281432 was significantly associated with uterine cervical carcinogenesis with strong statistical power. In addition, haplotypes CG/TA/TG of *ICAM-1* SNP rs3093030 and rs5498 were also genetic risk factors for cervical tumorigenesis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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