Nucleotide variation in *ATG4A* and susceptibility to cervical cancer in Southwestern Chinese women

JIN-JU MAO[1*], LI-XIANG WU[2*], WEI WANG[2], YUAN-YUAN YE[1], JUN YANG[1], HONG CHEN[1],
QIAN-FAN YANG[1], XIU-YU ZHANG[1], BO WANG[1] and WEI-XIAN CHEN[1]

[1]Department of Laboratory Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 410010;
[2]Department of Laboratory Medicine, Chongqing Cancer Institute, Chongqing 400030, P.R. China

Abstract. Early detection of human papillomavirus (HPV) is important for the clinical diagnosis of cervical cancer. However, to date, the pathogenesis of cervical cancer is still unclear. Autophagy is a dynamic process that contributes to the maintenance of cellular homeostasis. Here, we investigate whether variants of autophagy genes affect the occurrence of cervical cancer. In this study, our results indicate that single nucleotide polymorphisms (SNPs) of autophagy-related protein 4 (*ATG4*), including rs4036579, rs5973822, rs807181, rs807182 and rs807183, have a significant relationship with cervical cancer risk. Furthermore, stratified analysis suggests that the homozygous variant genotype could decrease the risk of cervical cancer and should be considered when investigating the role of HPV in cervical cancer. We aim to investigate whether SNPs of *ATG4A* contribute to HPV infection in the population of Southwestern China. The association of both single SNPs and SNP-SNP interactions with HPV was evaluated in a sample of cancer cases and healthy control subjects. The interaction of rs807181 and rs807183 was associated with HPV infection in case and control subjects (combined P=2.00x10^{-3} and 3.22x10^{-2}, respectively). This result showed that *ATG4A* SNP interactions may affect HPV infection in the population of Southwestern China. Notably, the autophagy gene *ATG4A* may contribute to cervical cancer.

Introduction

A woman's health is seriously affected by cervical cancer, which is the second most common malignancy in women.

Correspondence to: Dr Wei-Xian Chen, Department of Laboratory Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 410010, P.R. China
E-mail: chenweixian75@163.com

[*]Contributed equally

Importantly, with more than 520,000 new cervical cancer patients each year, 260,000 cancer-related deaths occur in women aged 15 to 44 years annually. Cervical cancer ranks as the fourth leading cause of female cancer deaths worldwide. In developing regions, the death toll is twice that of developed regions (1). Nevertheless, approximately 131,500 women suffer from cervical cancer every year in China, which accounts for approximately 28.8% of the new incident cases globally. The incidence rate is 6 times higher than that of developed regions. The development of cervical cancer not only exhibits a complex multiple-factor etiology but also involves multiple phases, and many studies suggest that the paramount factor in the development of cervical cancer is a persistent human papillomavirus (HPV) infection (2). However, according to estimates, 30 to 60% of women infected with genital HPV do not develop cancer (3). In most women who have been infected with HPV, the infection is a significant rather than sufficient risk factor for the development of cervical cancer. In recent years, several studies have revealed that changes in individual genetic factors, such as the immune regulatory genes, oncogenes and tumor suppressor genes, play a very important role in the pathogenesis of cervical cancer (4). Increasing evidence has indicated that genetic polymorphisms are associated with the occurrence of cervical cancer, and single nucleotide polymorphisms (SNPs) of the cell surface molecules of the *CD83* gene, including rs750749, rs9296925 and rs9370729, are associated with cervical cancer susceptibility (5,6). In this context, it should be mentioned that the polymorphisms of other genes may also be associated with cervical cancer susceptibility, including *p53* codon 72 and the *IL-8* and *MMP* genes (7,8).

Macroautophagy (hereafter referred to as autophagy) is an extreme degradative process that is necessary to conserve homeostasis in eukaryotic cells (9). Relevant studies show autophagy could modulate the expression level of PA proteins in cervical cancer. Currently, it is widely accepted that the autophagy system might have ambiguous effects in the development of cancer, such as promotion and antagonization (10). Furthermore, autophagy is mainly regulated by autophagy-related genes (*ATGs*). To date, more than 30 *ATGs* have been reported. Previous studies have suggested that initiation of autophagosomes requires the participation of

the two ubiquitin-like conjugated systems, *ATG12-ATG5* and *ATG8*-PE (11). This process is indispensable for *ATG4* splitting, permitting conjugation of *ATG8* to PE on phagophore membranes, deconjugation of the compound *ATG8*-PE, and acceleration of autophagosome maturation (12). Therefore, *ATG4* is a significant factor for the autophagy process and may be a target for cancer prevention and intervention. To date, *ATG4A* has been reported to be related to breast tumorigenesis (13) and the risk of lung cancer (14). The same vital functions of *ATG4A* have been well-documented in ovarian cancer (15). While numerous steps of autophagy have been well described at the molecular level, the role of *ATG4A* in cervical cancer is less well understood. Our group conducted a case-control study to evaluate the association of *ATG4A* variants with the risk of cervical cancer.

Materials and methods

Study samples. For this study, a total of 542 peripheral blood samples from 285 incident cervical cancer patients and 257 healthy control subjects were collected and analyzed. We used the guidelines of the International Federation of Gynecology and Obstetrics to assess the tumor type, stage and histological features of the cervical cancer cases. The control subjects in this study were ThinPrep cytology test (TCT)-negative in a physical examination. All subjects participated in specialized tests such as the TCT and HPV subtype tests. A definitive diagnosis was based on histopathology results.

Study population. Between January 2012 and December 2016, subjects from The First and Second Affiliated Hospitals of Chongqing Medical University and Chongqing Cancer Institute (Chongqing, China) were enrolled in this study; all subjects were members of the Han Chinese population. The types of cancers in the cervical cancer group were as follows: squamous cell carcinoma (SCC) (n=236), adenocarcinoma (n=39) and other histologic types of tumors (n=10) (Table I). All patients were females, aged 27.0 to 67.0 years. Information on demographic characteristics, family history of cancer, occupational exposure, tobacco smoking, pregnancy, oral contraceptive use, induced abortion, embryo number, menarche and menopause, and reproductive histories were obtained for all participants using a quasi-questionnaire survey. Informed consent was obtained from all participants. Control group frequency matching of cases was conducted using 257 age- and region-matched married females, aged 27.0 to 67.0 years, consisting of healthy individuals with no history of cancer who were randomly selected during the same time period as the case study.

This study was approved by the ethics committees of the hospitals. Data obtained using structured questionnaires were saved into databases. All procedures performed in studies were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

DNA extraction and genotyping. The genomic DNA of all patients was isolated from the clinical peripheral blood samples using the phenol-chloroform extraction method. The DNA was sent to Bio Miao Biological Science and Technology Co., Ltd. (Beijing, China) to detect the SNP genotype. A large number of literature and site related researches were analyzed and used to assist with the process of genotyping. We selected candidate gene expression studies via bioinformatics and examined *ATG4*, *LC3*, *ATG9*, and *ATG16*; we found a total of 39 donor splice sites, including rs4036579: A>G, rs5973822: A>G, rs807181: G>C, rs807182: A>C, rs807183: G>A, and others.

Analysis of SNP-SNP interactions. Multiple dimension reduction (MDR) is a method used to reduce the dimensionality of multilocus information (16); this method was used to improve the identification of polymorphism combinations associated with the risk of cervical cancer. MDR analysis was also used to help determine the correlation between SNPs and cervical cancer. Similarly, the Bonferroni correction was used, and P<0.05 was considered to indicate a statistically significant difference for the permutation test. All the SNP combinations are evaluated in the training dataset based on 10-fold CV. Two selection criteria are used to choose the best SNP combination, the cross validation consistency (CVC) and the average of test balanced accuracy (TA), consequently, the chosen SNP combination has the highest CVC and/or test TA. This gene predictor summarizes the effects of individual genes on each gene. Meta-analyses were performed to determine significant SNP-SNP interactions associated with HPV infection, and multiple testing corrections were performed using the Bonferroni method.

Statistical analysis. All statistical analyses were performed using PLINK version 1.07 (17). Allele and genotype frequencies of the SNPs were compared using the chi-square test and Fisher's exact test. Odds Ratio (OR) and 95% Confidence Interval (CI) were calculated using unconditional logistic regression and by adjusting the relevant confounding factors, such as age, pregnancy, abortion, BMI, menopausal status, and family history of cancer (15). A P-value less than 0.05 was considered statistically significant. Because all participants in the experiment were Han Chinese, the population was not stratified.

Results

Clinical features. The basic information of these subjects is listed in Table I. Cases were, on average, a greater frequency of eutocia (83.2/70.8%) and fertility births (49.9/32.3%) were observed than control. And the proportion of blood type B (40.7/33.1%) was also higher than that of the control. HPV16 and 18 were associated with the highest rate of infection (55.5%), with HPV16 accounting for 32% of infections and HPV18 accounting for 23.5% of infections. We found that HPV types were related to pathology, and HPV16 was the predominant strain associated with SCC (72%). However, the major strain involved in adenocarcinomas was HPV18 (53%). A descriptive analysis of selected polymorphisms was carried out (Table II), establishing the polymorphisms of the *ATG4A* gene and their association with cervical cancer. In addition, polymorphic loci were mainly located in intron, and most of them were mutated from A to G.

Gene sequencing. Among the 39 loci tested, the statistically significant loci are shown in Fig. 1, and the remaining sites

Table I. Clinical and demographic characteristics of patients and controls.

Characteristic	Patients, n=285 (%)	Controls, n=257 (%)
Age, years (mean ± SD[a])	47.3±9.5	47.1±8.7
Tumor stage		
I	70 (24.8)	
II	141 (49.6)	
III	63 (21.8)	
IV	11 (3.8)	
Labor presentation		
Eutocia	237 (83.2)	182 (70.8)
Caesarean section	48 (16.8)	75 (29.2)
Histological type		
Squamous cell carcinoma	236 (82.9)	
Adenocarcinoma	39 (13.7)	
Other	10 (3.4)	
Parity		
Never	10 (3.5)	15 (5.8)
1	132 (46.6)	159 (61.9)
2	94 (32.9)	56 (21.8)
3	36 (12.6)	19 (7.4)
≥4	13 (4.4)	8 (3.1)
Abortion frequency		
Never	98 (34.4)	112 (43.6)
1	49 (17.2)	51 (19.8)
2	53 (18.6)	57 (22.2)
≥3	85 (29.8)	37 (14.4)
Oral contraceptive pill use		
Never	239 (83.8)	235 (91.4)
Ever	46 (16.2)	22 (8.6)
Liquor and tobacco		
Never	177 (62.1)	168 (65.4)
Ever	108 (37.9)	89 (34.6)
Menopausal status		
Premenopausal	205 (71.9)	187 (72.7)
Postmenopausal	80 (28.1)	70 (27.3)
Blood type		
O	54 (18.9)	49 (19.1)
A	95 (33.3)	78 (30.3)
B	116 (40.7)	85 (33.1)
AB	20 (7.1)	45 (17.5)
HPV genotypes[b]		
Negative	79 (27.7)	198 (77.0)
16 or 18	158 (55.5)	31 (12.1)
Others[c]	48 (16.8)	28 (10.9)
BMI		
<18.5	6 (2.1)	5 (1.9)
18.5-23.9	203 (71.2)	211 (82.1)
≥24	76 (26.7)	41 (17.0)

Table I. Continued.

Characteristic	Patients, n=285 (%)	Controls, n=257 (%)
Family history of cancer		
No	220 (77.1)	223 (86.8)
Yes	65 (22.9)	34 (13.2)

[a]Age at first diagnosis; [b]HPV genotypes were determined by a Cobas HPV Test (Roche Molecular Systems, Inc., Alameda, CA, USA); [c]HPV genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. HPV, human papillomavirus; BMI, body mass index.

were not statistically significant. These statistically significant sites after screening will be followed by subsequent tests to determine their effect.

Characterization of SNPs in the ATG4A gene. As shown in Table III, for rs4036579, the frequency of the dominant model allele A in the case group was higher than that in the control group (94.39 vs. 90.66%, respectively, P=0.0057). The OR was 0.461 (95% CI 0.154 to 0.697). In the recessive model study, the frequency of the variant allele G was higher in control subjects than in the case group (38.91 vs. 26.67%, respectively, P=0.0098). The OR was 0.682 (95% CI 0.386 to 0.991). Compared with the homozygous wildtype genotype (AA), the homozygous variant genotype (GG) was associated with a significant decrease in the incidence of cervical cancer (P=0.0013, OR=0.427, 95% CI 0.184 to 0.848), whereas no significant correlation was found among carriers of the heterozygous variant genotype (AG). Unconditional logical analysis showed that the wildtype A allele of rs4036579 was associated with a reduced risk of cervical cancer (P<0.001, OR=0.504, 95% CI 0.401 to 0.707).

Similarly, for the donor splice site of rs5973822, the frequency of the wildtype A allele in the case group was higher than that of the control group (96.14 vs. 92.61%, respectively, P<0.0001, OR=3.927, 95% CI 1.896 to 7.652). In the recessive model, the frequency of the variant G allele in control subjects was higher than that in the case group (28.40 vs.17.89%, respectively, P=0.096, OR 0.684, 95% CI 0.364 to 0.949). Compared with the homozygous variant genotype (GG), the homozygous wildtype genotype (AA) was associated with a significantly decreased incidence of cervical cancer (P=0.0046, OR=0.384, 95% CI 0.148 to 0.733). Analogously, the unconditional logical analysis demonstrated that the A allele of rs4036579 was associated with a decreased risk of cervical cancer (P<0.001, OR=0.407, 95% CI 0.253 to 0.648). The results for rs807182 were identical to the above-mentioned results in that the wildtype A allele was associated with a decreased risk of cervical cancer (P=0.0074, OR=2.6627, 95% CI 2.4900 to 2.8964).

An analysis of rs807181 indicated that the variant C allele was associated with a lower risk of cervical cancer (P=0.0051, OR=0.596, 95% CI 0.403 to 0.821). A greater frequency was observed for the recessive model of polymorphism among case (96.14%) than among control (83.66%). No significant deviations were observed for dominant model. Furthermore,

Table II. Polymorphisms of the ATG4A gene and their relationship to cervical cancer.

Gene	Chromosomal region	Region	Function	Chromosomal Location	Nucleotide position	Change[a]	dbSNP ID[b]
ATG4A	Xq22.1-22.3	5'near gene[e]	TFBS[c]	intron	108090623	A/G	rs4036579
			miRNA	3'UTR[d]	108153728	A/G	rs5973822
			TFBS	intron	108090354	C/G	rs807181
			TFBS	intron	108092031	A/C	rs807182
			TFBS	intron	108094263	A/G	rs807183

[a]The nucleotide change is compared with matched normal genomic DNA. [b]The chromosome position is based on data from http://www.ncbi.nih.gov/snp; [c]Transcription Factor Binding Site; [d]Untranslated region; [e]Near the 5' end of genes. miRNA, microRNA; UTR, untranslated region; ATG4A, autophagy-related protein 4A.

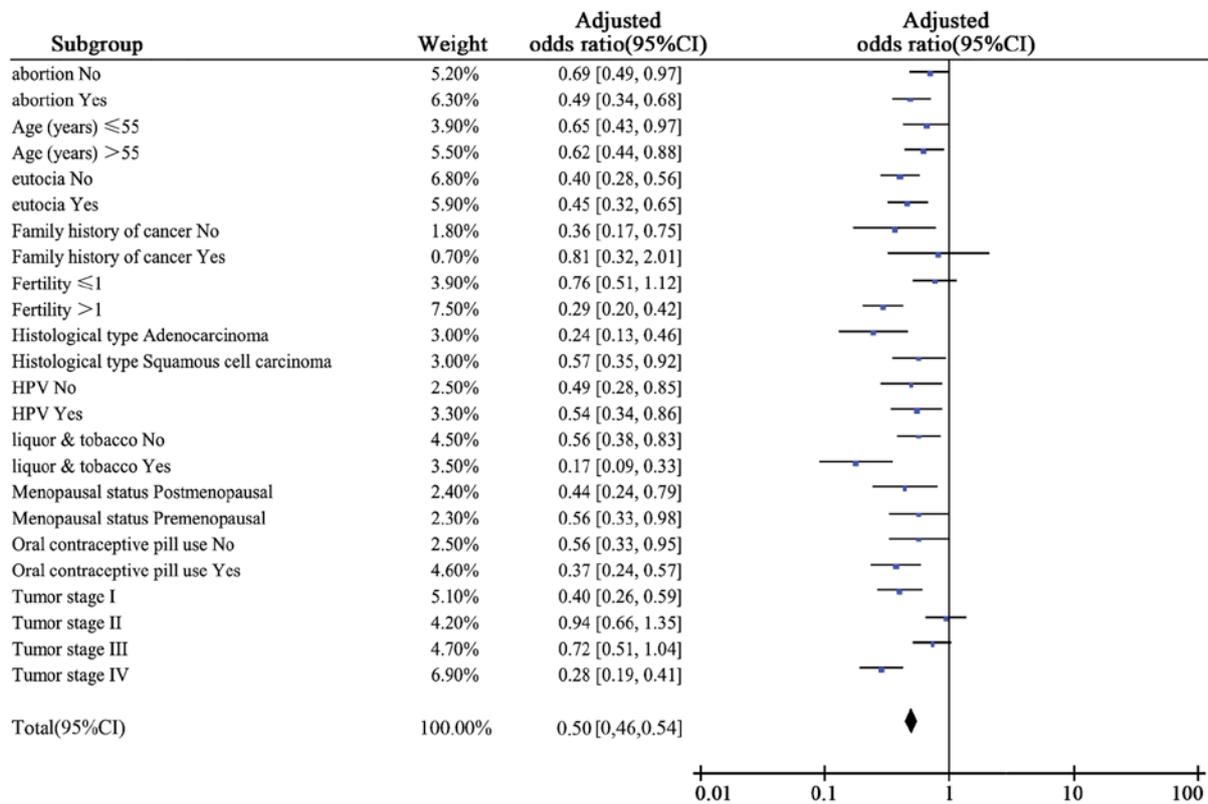| Subgroup	Weight	Adjusted odds ratio(95%CI)
abortion No	5.20%	0.69 [0.49, 0.97]
abortion Yes	6.30%	0.49 [0.34, 0.68]
Age (years) ≤55	3.90%	0.65 [0.43, 0.97]
Age (years) >55	5.50%	0.62 [0.44, 0.88]
eutocia No	6.80%	0.40 [0.28, 0.56]
eutocia Yes	5.90%	0.45 [0.32, 0.65]
Family history of cancer No	1.80%	0.36 [0.17, 0.75]
Family history of cancer Yes	0.70%	0.81 [0.32, 2.01]
Fertility ≤1	3.90%	0.76 [0.51, 1.12]
Fertility >1	7.50%	0.29 [0.20, 0.42]
Histological type Adenocarcinoma	3.00%	0.24 [0.13, 0.46]
Histological type Squamous cell carcinoma	3.00%	0.57 [0.35, 0.92]
HPV No	2.50%	0.49 [0.28, 0.85]
HPV Yes	3.30%	0.54 [0.34, 0.86]
liquor & tobacco No	4.50%	0.56 [0.38, 0.83]
liquor & tobacco Yes	3.50%	0.17 [0.09, 0.33]
Menopausal status Postmenopausal	2.40%	0.44 [0.24, 0.79]
Menopausal status Premenopausal	2.30%	0.56 [0.33, 0.98]
Oral contraceptive pill use No	2.50%	0.56 [0.33, 0.95]
Oral contraceptive pill use Yes	4.60%	0.37 [0.24, 0.57]
Tumor stage I	5.10%	0.40 [0.26, 0.59]
Tumor stage II	4.20%	0.94 [0.66, 1.35]
Tumor stage III	4.70%	0.72 [0.51, 1.04]
Tumor stage IV	6.90%	0.28 [0.19, 0.41]
Total(95%CI)	100.00%	0.50 [0,46,0.54]

Figure 1. The LD block of the *ATG4A* gene. A total of 29 loci were analyzed using a software program. The SNPs tagged with Haploview are shown at the top of the graph (arrows), and the following inserts indicate different sites. The color depth in the graph reflects the LD intensity between the sites. Moreover, the locations of the 'Block' are marked in the figure, where Block 1 is <1 kb, Block 2 is >1 kb, and Block 3 is 56 kb. SNP, single nucleotide polymorphism; CI, confidence interval;

to exclude the influence of other factors, we used a stratified analysis to separately evaluate the influence of the homozygous variant genotype (CC) of rs807181 on the risk of cervical cancer in different subgroups in a dominant model (Fig. 2).

For rs807183, the variant A allele was associated with an increased risk of cervical cancer (P<0.0001, OR=1.573, 95% CI 1.305 to 2.085). In assessing the correlation in the recessive model shown that the frequency of the wildtype G allele was higher in the case group than in the control group (95.44 vs. 83.66%, P<0.0001, OR=4.025, 95% CI 2.039 to 7.402). Others did not show significant differences. When we performed the association analysis of SNPs with the risk of cervical cancer,

the stratified analysis did not lead to considerable changes in any of the results, no statistically significant association was found for any of the models analyzed (data no show).

The association between ATG4A SNPs and HPV infection. In order to explore whether the SNPs could act together to increase HPV infection risk, a total of 29 SNPs were identified with an average density of 1 SNP per 0.55 kb. An r^2 threshold of 0.8 was used to tag pairs with Haploview (18), with the ultimate goal of selecting 3 tagged SNPs. The results of haplotype analysis are shown in Fig. 1. When we evaluated the interaction between each of the SNPs expression at

Table III. Association of the SNPs of ATG4A with the risk of cervical cancer in the case and control groups.

SNP	Genotype	Cases [n (%)]	Controls [n (%)]	P-value	Crude OR	(95% CI)	Adjusted P	Adjusted OR[a]	(95% CI)
		285	257						
ATG4A rs4036579									
	GG	16 (5.61)	34 (13.23)						
	AA	209 (73.33)	175 (68.09)	0.0028	0.394	0.2104 to 0.7379	0.0013	0.427	0.184 to 0.848
	AG	60 (21.05)	48 (18.68)	0.8351	0.9554	0.6218 to 1.468	0.796	0.997	0.603 to 1.521
Dominant model	Any A vs. GG	269 (94.39)	233 (90.66)	0.0002	0.33	0.1807 to 0.6025	0.0057	0.461	0.154 to 0.697
Recessive model	Any G vs. AA	76 (26.67)	100 (38.91)	0.0135	0.6364	0.4441 to 0.9118	0.0098	0.682	0.386 to0.991
Additive model	AA vs. AG vs. GG	209/60/16	58/42/175	<0.0001	0.64	0.526 to 0.807	0.001	0.603	0.463 to 0.801
Allele OR	A vs. G	478/92	408/142	<0.0001	0.553	0.4122 to 0.7418	<0.0001	0.504	0.401 to 0.707
ATG4A rs5973822									
	GG	11 (3.86)	29 (11.28)						
	AA	234 (82.11)	202 (78.60)	0.0015	0.3274	0.1595 to 0.6722	0.0046	0.384	0.148 to 0.733
	AG	40 (14.04)	26 (10.12)	0.2915	0.753	0.4438 to 1.277	0.254	0.802	0.407 to 1.288
Dominant model	Any A vs. GG	274 (96.14)	238 (92.61)	<0.0001	3.872	1.932 to 7.762	<0.0001	3.927	1.896 to 7.652
Recessive model	Any G vs. AA	51 (17.89)	73 (28.4)	0.0137	0.6031	0.4025 to 0.9036	0.096	0.684	0.364 to 0.949
Additive model	AA vs. AG vs. GG	234/40/11	202/26/29	0.003	1.803	1.204 to 2.622	0.006	1.987	1.224 to 3.132
Allele OR	A vs. G	478/92	398/116	<0.0001	0.4882	0.3487 to 0.6835	<0.0001	0.407	0.253 to 0.648
ATG4A rs807181									
	CC	11 (3.86)	42 (16.34)						
	GG	204 (71.58)	170 (66.15)	<0.0001	4.582	2.288 to 9.176	<0.0001	4.245	2.023 to 9.003
	CG	70 (24.56)	45 (17.51)	0.2321	1.296	0.8463 to 1.985	0.216	1.313	0.805 to 1.998
Dominant model	Any C vs. GG	81 (28.42)	87 (33.85)	0.1722	0.7759	0.5387 to 1.117	0.138	0.814	0.485 to 1.377
Recessive model	Any G vs. CC	274 (96.14)	215 (83.66)	<0.0001	4.866	2.446 to 9.678	<0.0001	4.657	2.326 to 9.453
Additive model	CC vs. CG vs. GG	11/70/204	42/45/170	0.297	1.203	1.056 to 2.799	0.225	1.323	1.003 to 2.826
Allele OR	C vs. G	92/478	129/385	0.0003	0.5744	0.4258 to 0.7749	0.0051	0.596	0.403 to 0.821
ATG4A rs807182									
	AA	202 (70.88)	175 (68.09)						
	CC	11 (3.86)	36 (14.01)	<0.0001	0.2647	0.1308 to 0.5358	<0.0001	0.225	0.104 to 0.497
	AC	72 (25.26)	46 (17.9)	<0.0001	5.123	2.372 to 11.06	<0.0001	5.021	2.113 to 10.866
Dominant model	Any A vs. CC	274 (96.14)	221 (85.99)	<0.0001	4.058	2.018 to 8.157	<0.0001	4.002	1.996 to 8.022
Recessive model	Any C vs. AA	83 (29.12)	82 (31.91)	0.4819	1.14	0.7906 to 1.645	0.448	1.185	0.732 to 1.694
Additive model	AA vs. AC vs. CC	202/72/11	175/46/36	0.007	2.335	1.857 to 4.536	0.024	2.383	1.824 to 4.776
Allele OR	A vs. C	476/94	396/118	0.0074	0.6627	0.4900 to 0.8964	0.012	0.715	0.446 to 0.913

Table III. Continued.

SNP	Genotype	Cases [n (%)]	Controls [n (%)]	P-value	Crude OR	(95% CI)	Adjusted P	Adjusted OR[a]	(95% CI)
ATG4A rs807183									
	GG	206 (72.28)	166 (64.59)						
	AA	13 (4.56)	42 (16.34)	<0.0001	0.2494	0.1296 to 0.4801	<0.0001	0.215	0.069 to 0.427
	AG	66 (23.16)	49 (19.07)	<0.0001	4.352	2.110 to 8.974	<0.0001	4.068	1.995 to 8.484
Dominant model	Any A vs. GG	79 (27.72)	91 (35.41)	0.054	0.6996	0.4860 to 1.007	0.035	0.722	0.443 to 1.356
Recessive model	Any G vs. AA	272 (95.44)	215 (83.66)	<0.0001	4.087	2.139 to 7.809	<0.0001	4.025	2.039 to 7.402
Additive model	AA vs. AG vs. GG	13/66/206	42/49/166	0.066	0.746	0.577 to 0.896	0.101	0.769	0.514 to 0.931
Allele OR	A vs. G	92/478	133/381	<0.0001	1.814	1.346 to 2.443	<0.0001	1.573	1.305 to 2.085

SNP, single-nucleotide polymorphism; CI, confidence interval; ATG, autophagy-related gene; [a]Adjusted in an unconditional logistic regression model that includes age, labor presentation, family history of cancer, fertility, parity, abortion frequency, and BMI.

Table IV. The interactions of ATG4A SNPs and their relationship to HPV infection.

Factor model	TA	CVC	Permutation P-value
CCC vs. HC			
rs1130905, rs1130906	0.4613	9/10	0.992
rs26532, rs26534, rs26537	0.4832	10/10	0.166
rs807181, rs807182, rs807183, rs807185[a]	0.4771	10/10	0.002

ATG4A, autophagy-related protein 4A; SNP, single nucleotide polymorphisms; HPV, human papillomavirus; TA, testing accuracy; CVC, cross-validation consistency; CCC, cervical cancer case; HC, healthy control. [a]The best factor model.

the systemic level, as well as the SNP-SNP interaction, we conducted 9 single SNP analyses, and 27 (3x9) SNP-SNP analyses shown different results. No significant associations of SNP-SNP interactions were observed (P>0.05) after multiple testing corrections. As shown in Table IV, the MDR program (v3.0.2) was used to analyze the interactions between *ATG4A* SNPs and HPV infection. Four SNPs (rs807181, rs807182, rs807183, and rs807185) showed significant interactions with HPV infection (permutation P=0.002). In addition, the results of the meta-analyses that revealed a correlation between SNP rs807181 and rs807183 were verified by multiple testing corrections (combined P=2.00 x10^{-4}, Table V). Both SNPs are located in the intron of *ATG4A*. The results showed that the interaction of rs807181 and rs807183 was significantly associated with HPV infection (P=0.0120).

Discussion

Autophagy is a well-conserved and functionally complex pathway that is essential for cellular homeostasis. Many studies have suggested that autophagy has a tumor-suppressive function in cancer. In contrast, other studies have argued that autophagy might have beneficial effects for the survival of cancer cells (19). The process of autophagy in the maintenance of coordination of cell functions depends on complex reaction systems, including *ATG4*, which is a cysteine protease that plays diverse functional roles. The *ATG4* family includes four members, *ATG4A*, *ATG4B*, *ATG4C* and *ATG4D*, which are orthologs of yeast *ATG4* (20). Recent reports have shown that *ATG4B* efficiently binds to and cleaves *LC3* proteins, and the C-terminal LC3-interacting region of *ATG4B* plays an important role in this process (21). Furthermore, dysregulation proteins of *ATG4* have been observed in other diseases. One study found that *ATG4B* is a biomarker, has a latent function for predicting the response of therapeutic treatment using CML stem/progenitor cells and may serve as a drug target for these cells (22). SNPs have become the third generation of genetic markers. The human body exhibits many phenotypic differences, different susceptibilities to a drug or a disease and other differences. All of these differences may be mainly associated with SNPs.

Table V. Significant SNP-SNP interactions in ATG4A associated with HPV infection.

SNP[a]-SNP[b]	Subjects	Combined P	Allele A	Allele B	MAF A	MAF B	Beta	SE	P-value
rs807181-rs807183	CCC	2.00×10^{-3}	C/G	G/A	0.242	0.275	0.6245	0.0286	0.012
	HC	3.22×10^{-2}	C/G	G/A	0.136	0.154	2.153	0.0058	0.037

Only SNP pairs that were significantly associated after multiple testing corrections are shown. SNP, single nucleotide polymorphism; ATG4A, autophagy-related protein 4A; HPV, human papillomavirus; CCC, cervical cancer case; HC, healthy control; [a]rs807181; [b]rs807183; SE, standard error; Allele A, alleles of SNP[a]; Allele B, alleles of SNP[b]; MAF, minor allele frequency of SNPs.
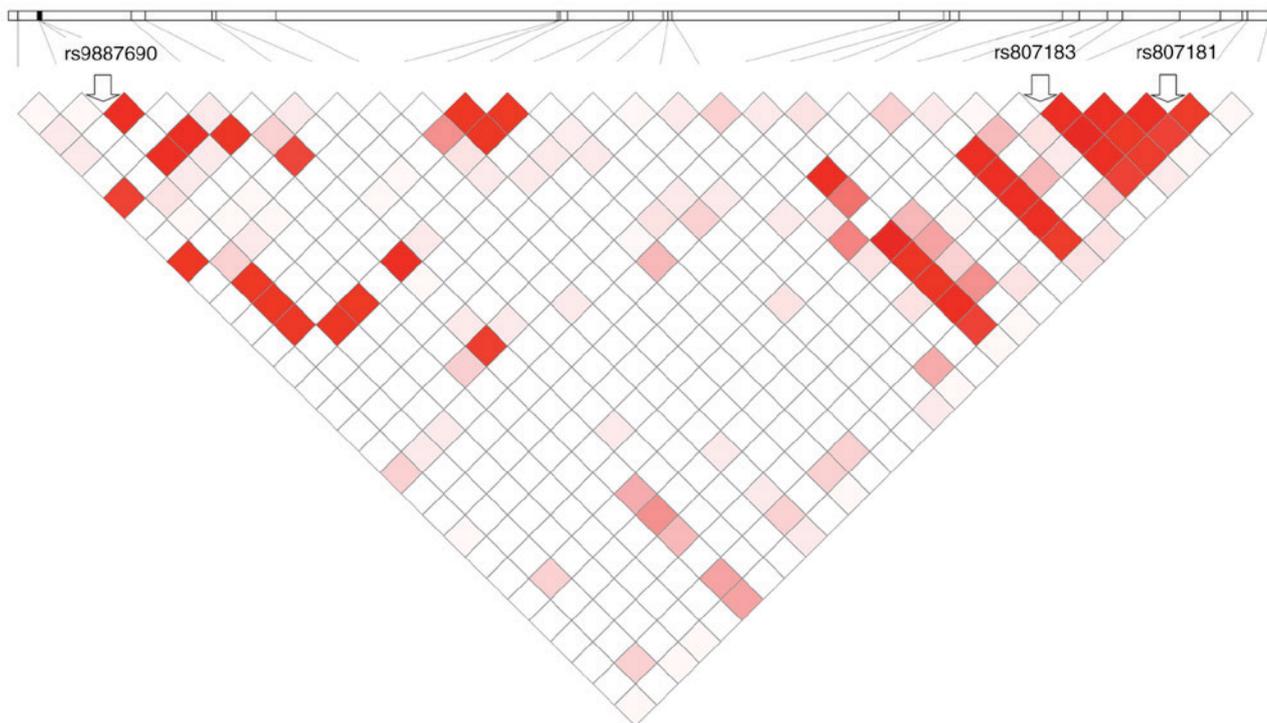
Figure 2. Forest plot of an *ATG4A* SNP in stratified groups. A stratified analysis of rs807181 using a dominant model to examine the relationship between the homozygous variant genotype (CC) and the risk of cervical cancer in different subgroups. ATG, autophagy-related gene; SNP, single-nucleotide polymorphism; CI, confidence interval; HPV, human papillomavirus.

Dysregulation of cysteine protease activity has great relevance as a therapeutic target in some diseases, such as cancer and inflammatory bowel disease (23). Some researchers have examined the dysfunctional expression of these proteases in cervical cancer and recommended potential therapies based on their modulation (24). The rs5973822 SNP of *ATG4A* has been associated with granulomas in Crohn's disease (25). Furthermore, little is known about the relationship between the locus mutations of *ATG4* and cervical cancer. In this study, we highlighted the key role of human *ATG4* in autophagy and its relationship with cervical cancer. In addition, *ATG4A* has the potential to become a new target for cancer treatment.

Our observations offer meaningful insight into five SNPs of *ATG4A*, including rs4036579, rs5973822, rs807181, rs807182 and rs807183, and the role that they play in the susceptibility to cervical cancer. Carriers with a homozygous wildtype genotype of AA or an A allele have a lower the risk of morbidity from cervical cancer. Similar phenomena have been observed with the rs4036579, rs5973822 and rs807182

SNPs. However, the homozygous variant genotype CC and the C allele are associated with a decreased risk of cervical cancer in rs807181 carriers. In contrast, for rs807183, the homozygous variant genotype AA and the A allele are associated with an increased risk of cervical cancer. According to the results of recent studies, intron and transcription factor binding site polymorphisms are associated with the risk of cancer or other diseases due to a coupling imbalance with functional or genetic transcription. Currently, the sequences in *TP53* intron 1 encode transcripts that may play a significant role in modulating the *R249S* mutation rate in HCC, and the *TCF21* rs12190287 polymorphism may increase the genetic susceptibility to breast cancer by regulating the expression of *TCF21* (26,27). Moreover, some studies report a focus on gene targeting therapy for tumors; for example, a target therapy drug, bevacizumab, targets the VEGF signaling pathway and is currently used to treat advanced cervical cancer. Scholars agree that ROS (reactive oxygen species)-modulated core autophagic pathways involved in ATG4-ATG8/LC3, Beclin-1, p53, and

MAPK signaling in cancer. It is worth noting that Beclin1 could enhance the expression levels of ATG4, overexpression of Beclin1 inhibited proliferation, migration and invasion. Furthermore, VEGF was involved in Beclin1-mediated inhibition of migration and invasion. The current studies have shown that, the MAPK and P53 pathways should be targeted by drugs (28,29). We believe that ATG4, which has an intricate relevance, may become a target gene for cancer drug therapy. Additional treatments for cervical cancer related to the gene targets have not been researched and discussed; gene target therapies cannot be replaced by other treatments.

Notably, our study is the first exploration that reveals the correlation among the intron SNPs rs4036579, rs807182, rs807181 and rs807183 and the untranslated region SNP rs5973822 in *ATG4A* and the risk of cervical cancer in the Han Chinese population. The variants of intron SNPs rs4036579 and rs807182 and the untranslated region SNP rs5973822 might be non-conservation factors, and the variant of the intron SNP rs807181 might be a protective factor against cervical cancer. Notably, the variant of the intron SNP rs807183 might be a risk factor for cervical cancer. Intriguingly, the SNPs rs4036579, rs807182, rs807181 and rs807183 of *ATG4A* are located in the intron, and all are transcription factor binding sites. An miRNA binding site is located in the untranslated region SNP rs5973822. Some studies revealed that protein expression can be controlled via AUG sequences located upstream to the initiation codon in the 5' UTR of autophagy related genes. Thus, the ATG4A protein level may be correlated with the SNPs. Possibly, these SNPs exert their effects by regulating gene transcription or function. These variants SNPs might regulate *ATG4A* and indirectly increase or reduce the expression of proteins that directly influence autophagy.

Although *ATG4A* is involved in autophagy, we aimed to determine whether SNPs in *ATG4A* contribute to HPV infection in the Han Chinese population. Based on the results, we speculated that the interaction of rs807181 and 807183 affected the *ATG4A* gene and down-regulated or inhibited its role in autophagy, which led to an increased susceptibility and ability of HPV to bind to the host. Some studies have shown that SNP-SNP interactions were notably associated with BMI (30,31), as well as colon cancer. The association between *ATG4A* genetic variations and HPV infection is still unknown. Analyses of SNP-SNP interactions and the association with HPV infection were performed. According to our study, rs807181 and rs807183 interaction, which is a haplotype of *ATG4A* SNPs, is significantly associated with HPV infection. Many types for HPV, the experiment mainly involves 16/18 type. At present, no research reports correlation between *ATG4A* and HPV, based on our results, genetic variants of *ATG4A* is likely to be tightly related to 16 or 18, and this view needs further exploration. HPV infection is the most important prerequisite for the occurrence and development of most cervical cancers in clinical practice, therefore, *ATG4A* SNPs are also a great correlation with cervical cancer susceptibility. Our results reveal the association between genes and HPV in terms of disease mechanisms, therefore, we suggest that could aid in disease-related genetic diagnosis and providing clinical control, prevention and treatment strategies for HPV-related cervical cancer. In the future, we will examine the genetic variants of the *ATGs* and their relationship to cervical cancer.

Furthermore, these studies will confirm the fundamental mechanism by which these SNPs regulate gene expression. The sample size of this study was limited, and further analysis to address the prognosis of cervical cancer in relation to different mutations was not performed.

In summary, our study determined the main role of several SNPs, which may serve as direct inducers and intermediates in different modalities of autophagy associated with *ATG4A*. The results of our study may serve to provide a new approach for cervical cancer prevention and treatment strategies.

Acknowledgements

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