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Original Article

Genetic association between *CD95* rs2234767 polymorphism and cervical cancer risk: a meta analysis

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Abstract: Background: *CD95* rs2234767 polymorphism in the promotor region of *CD95* gene has been implicated in several studies of cervical cancer. However, the results have not been conclusively established. Objective: The main aim of this study was to deal with the controversy with respect to the correlation between *CD95* rs2234767 polymorphism and risk of cervical cancer through a meta-analysis. Methods: Association studies that pertain to *CD95* rs2234767 polymorphism and risk of cervical cancer were identified up to May 26, 2014. ORs and 95% CIs were calculated assuming AA versus GG, AA + AG versus GG, AA versus AG + GG, A versus G and AG versus GG genetic models. Results: A total of 5 case-control studies were included in this meta-analysis. Overall, no significant effect modification of cervical cancer risk was revealed either at the genotypic or the allelic level for *CD95* rs2234767 polymorphism. This null association persisted in the stratified analysis of Asian population. Conclusions: These findings revealed that *CD95* rs2234767 polymorphism may not act as a causative agent of cervical cancer. Further evidence is needed to confirm our findings.

Keywords: *CD95*, polymorphism, cervical cancer

Introduction

Following breast cancer and colorectal cancer, cervical cancer has been the third most commonly diagnosed cancer and the fourth major cause of cancer-related deaths among women worldwide, with 9% (529,800) of the total new cancer cases and 8% (275,100) of the total cancer deaths estimated in 2008 [1]. Human papillomavirus (HPV) infection and cigarette consumption have been recognized as independent risk factors for cervical cancer [2, 3]. However, cervical cancer is considered as a genetic component disease attracting widespread attention since the genetic epidemiological studies based on biological relatives showed that cervical cancer is nearly two-fold more likely to favor the individuals with biological full-sisters of cervical cancer than the controls without [4, 5]. Apoptosis is a regulated form of cell death involved in the development of organisms and pathophysiology of certain disorders [6, 7]. The *CD95* signaling system is essential for apoptotic signaling in cells of the immune response [8] and for the activation of a major extrinsic cell death pathway which plays a key role in the maintenance of immuno-privileged sites and fulfillment of other regulatory functions [9, 10]. *CD95* induces apoptosis in susceptible cells by interacting with the well-characterized death-inducing ligand *CD95*. Apoptosis caused by FASL leads to immune homeostasis and cell-mediated cytotoxicity [11]. *CD95* has been shown to be commonly expressed in liver, spleen, cardiac, thymus and ovarian tissues [11, 12].

A growing body of evidence has suggested the importance of the genetic polymorphisms of *CD95* rs2234767 polymorphism death pathway genes in determining predisposition to human cancers, such as lung cancer, pancreatic cancer and esophageal squamous-cell carcinoma [13-15]. In the promoter region of the *CD95* gene, there is a G to A substitution at nucleotide position-1377 (rs2234767) located in the stimulatory protein-1 (Sp1). This polymorphism deregulates cell death signaling, result-

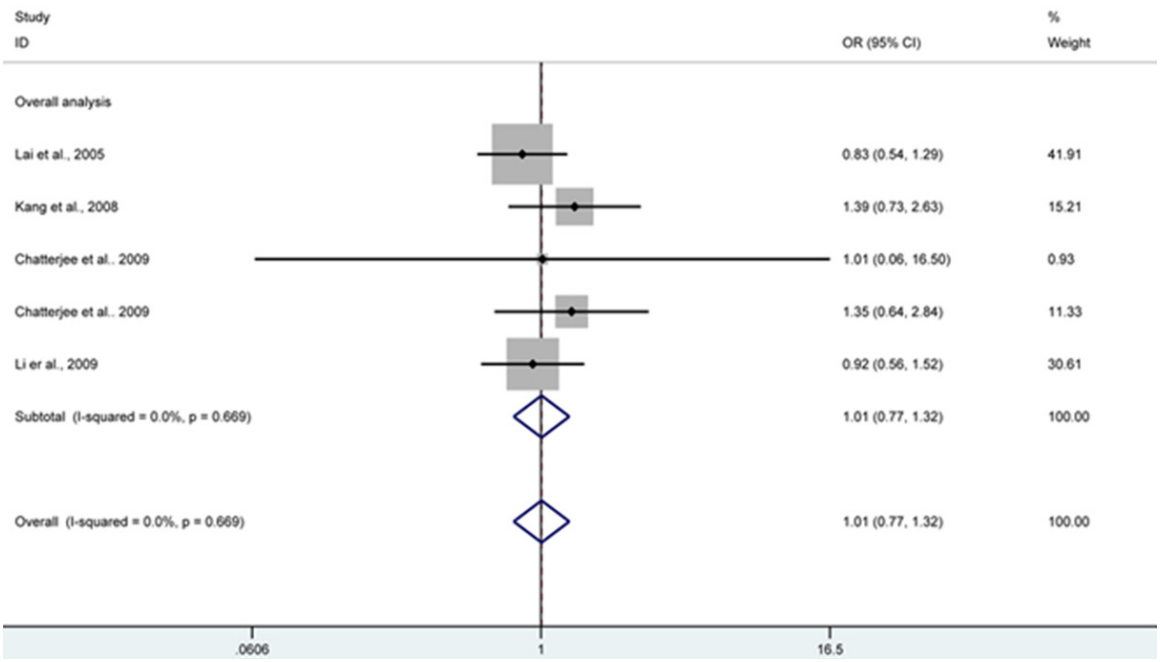

Figure 1. *CD95* rs2234767 polymorphism and risk of cervical cancer by AA versus GG genetic model, forest plot shows no significant association between *CD95* rs2234767 polymorphism and risk of cervical cancer under AA genotype compared with GG. CI confidence interval; OR odds risk.

ing in a consequent reduce in *CD95* rs2234767 polymorphism expression [16, 17], which is frequently reported in cancer occurrence, including cervical cancer. The role of *CD95* rs2234767 polymorphism in cervical cancer development has once been frequently studied with uncertain results [18-21]. These results could be better convinced if a sufficiently large study is performed. In this investigation, therefore, we conducted a meta-analysis to identify the association between *CD95* rs2234767 polymorphism and cervical cancer risk and to promote more studies to continually concern this association.

Materials and methods

Data sources

We carried out a search in electronic databases including Embase, PubMed and CNKI (China National Knowledge Infrastructure) up to May 26, 2014 to identify all case-control studies on the association between *CD95* rs2234767 polymorphism and cervical cancer risk without using any restriction. The search strategy used was: (*CD95*) OR (TNFRSF6) OR (APO-1) AND (polymorphism) OR (genotype) OR (polymorphisms) AND (cervical cancer), in combination with (-1377 A/G) OR (rs2234767). We also conducted a manual review of the bibliographic references cited in the selected articles for additional data available for the present meta-analysis.

Study selection

For this meta-analysis, we selected the studies that: a) included both cases and well-matched controls; b) assessed the association of *CD95* rs2234767 polymorphism and cervical cancer risk; c) contained clear genotype frequencies for odds ratios (ORs) and 95% confidence intervals (CIs) calculation; and d) no significant departure from Hardy-Weinberg equilibrium (HWE) for the genotype distribution of controls. When several studies selected the same cases series, we considered the most updated one.

Data extraction

Two independent investigators (Ping Liu, Xiaofeng He) extracted the following information from the selected studies: the last name of first authors, year of publication, study country, matching criteria, ethnicity, total counts of genotyped cases and controls, genotype frequency distributing between cases and controls, and

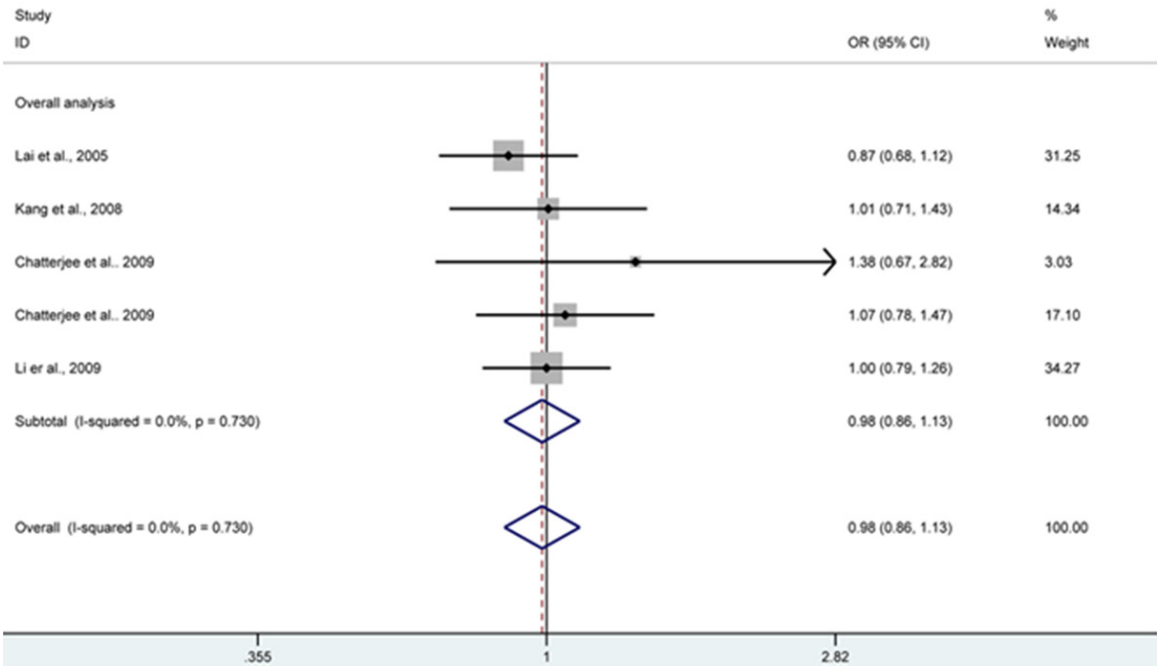

Figure 2. *CD95* rs2234767 polymorphism and risk of cervical cancer by AA + AG versus GG genetic model, forest plot shows no significant association between *CD95* rs2234767 polymorphism and risk of cervical cancer under AA + AG genotype compared with GG. CI confidence interval; OR odds risk.

Table 1. Characteristics of studies included in the meta-analysis

Study (author, year)	Population (country)	Genotyping method	Genotype frequency (case)			Genotype frequency (control)			Matching criteria
			GG	GA	AA	GG	GA	AA	
Lai et al., 2005	Asian (China)	RT-PCR	127	138	53	99	165	54	Age
Kang et al., 2008	Asian (South Korea)	PCR-RFLP	54	69	31	56	82	20	Age
Chatterjee et al., 2009	African (South Africa)	TaqMan	74	21	1	75	14	1	Age, ethnicity, Domicile status
Chatterjee et al., 2009	Mixed (South Africa)	TaqMan	186	87	17	196	86	13	Age, ethnicity, Domicile status
Li et al., 2009	Asian (China)	PCR-RFLP	144	144	26	282	277	56	Age, smoking status

PCR: polymerase chain reaction; PCR-RFLP: PCR-restriction fragment length polymorphism; RT-PCR: real time-PCR.

genotyping method. Each of the extracted items was compared, and a senior investigator (Zibai Wei) was referred to when there was any disagreement.

Statistical analysis

Stata software (version 12.0; StataCorp LP, College Station, TX, USA) was chosen and utilized to perform all statistical analyses involved in this article. The risk of cervical cancer associated with *CD95* rs2234767 polymorphism was indicated as ORs and 95% CIs. We calculated the ORs assuming AA versus GG and AG versus GG comparisons, as well as AA + AG versus GG, AA versus AG + GG, and A versus G genetic models. (**Figures 1**, **2**).

Inter-study heterogeneity was tested using the Q-statistic and $P < 0.10$ was considered as representation of statistically significant heterogeneity [22]. In addition, I2 index was applied to quantify the inter-study variability, with larger values suggesting an increasing degree of heterogeneity (I^2 < 25% low heterogeneity; I^2 = 25-50% moderate heterogeneity; I^2 > 50% large heterogeneity) [23]. Overall ORs with 95% CIs that indicated the association strength were pooled using the fixed effects model, when no heterogeneity presented ($P > 0.10$ and $I^2 < 50\%$) [24]; otherwise, the random effects model was more appropriate to pool the effect sizes [25]. HWE was tested by Pearson's x^2 test for controls in each study. Sensitivity analysis

Table 2. Meta-analysis of the association between *CD95* rs2234767 polymorphism and cervical cancer risk

	Studies (subjects)	OR (95% CI)	*P* value	Model	P-het
Total studies	5 (2648)				
AA versus GG		1.01 (0.77, 1.32)	0.971	Fixed	0.669
AA + AG versus GG		0.98 (0.86, 1.13)	0.814	Fixed	0.730
AA versus AG + GG		1.09 (0.84, 1.41)	0.512	Fixed	0.631
A versus G		1.00 (0.89, 1.12)	0.996	Fixed	0.594
AG versus GG		0.97 (0.84, 1.12)	0.670	Fixed	0.666
Asians	3 (1871)				
AA versus GG		0.96 (0.72, 1.29)	0.788	Fixed	0.432
AA + AG versus GG		0.95 (0.82, 1.11)	0.518	Fixed	0.689
AA versus AG + GG		1.06 (0.81, 1.40)	0.672	Fixed	0.325
A versus G		0.97 (0.85, 1.10)	0.636	Fixed	0.504
AG versus GG		0.93 (0.79, 1.10)	0.411	Fixed	0.605
Others	2 (777)				
AA versus GG		1.32 (0.64, 2.72)	0.449	Fixed	0.847
AA + AG versus GG		1.11 (0.83, 1.49)	0.465	Fixed	0.529
AA versus AG + GG		1.30 (0.64, 2.66)	0.472	Fixed	0.812
A versus G		1.13 (0.87, 1.47)	0.343	Fixed	0.582
AG versus GG		1.10 (0.81, 1.50)	0.538	Fixed	0.474

by removing a single study at a time was performed to assess if the pooled results were stable and robust. Publication bias was determined by Egger's test which is a liner regression methodology to check the funnel plot asymmetry [26]. All studies were two-sided and $P < 0.10$ was considered statistically significant.

Results

Literature search and study selection

A total of 627 articles appeared to be potentially relevant to our study. By checking the titles, abstracts, and the full texts to examine whether available data were contained, we excluded 623 full-text articles and included 4 eligible articles [18-21], with 5 case-control studies (one record reported two populations) (**Table 1**), resulting in 1,172 cases and 1,476 well-matched controls. Among them, three studies conducted on Asian population [18, 19, 21], and one on both African and mixed populations [20]. Genotyping was completed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), real time-PCR (RT-PCR) and TaqMan. Control subjects of all studies were in agreement with HWE ($P > 0.10$) (**Table 1**).

Meta-analysis

Five studies with 4,271 genotyped subjects were included in the meta-analysis for the assessment of association between *CD95* rs2234767 polymorphism and cervical cancer risk. Overall, no significant effect modification of cervical cancer risk was revealed either at the genotypic or allelic level of *CD95* rs2234767 polymorphism. The null association persisted in the stratified analysis of Asian population. No additional analysis was conducted in the populations of African or mixed descents (**Table 2**).

The heterogeneity for comparisons between cervical cancer subjects and controls was not significant ($P > 0.10$ and $I^2 < 50\%$). Similarly, no obvious indication of between-study heterogeneity was suggested in the analysis of Asian population. To further confirm the stability and credibility of our results, we also conducted sensitivity analysis and all studies turned out to be homogeneous. In addition, no significant publication bias was graphically or statistically suggested by performing the funnel plot and Egger's test (AA versus GG: $t = 0.25$, $P = 0.822$) (**Figure 3**).

Discussion

CD95 rs2234767 polymorphism is a well-characterized genetic variant and has been widely studied in a number of cancers ranging from gastric cancer to lung cancer in various countries all over the world [13, 27, 28]. In contrast, only a small proportion of genetic association studies associate this extensively studied polymorphism with cervical cancer, especially in recent few years. Moreover, a relatively small number of investigations have concerned this point with the association inconclusively established. Considering these problems, we addressed two aims in our meta-analysis. The first aspect dealt with the controversy with respect to the correlation between *CD95* rs2234767 polymorphism and risk of cervical cancer. The second goal related to encouraging

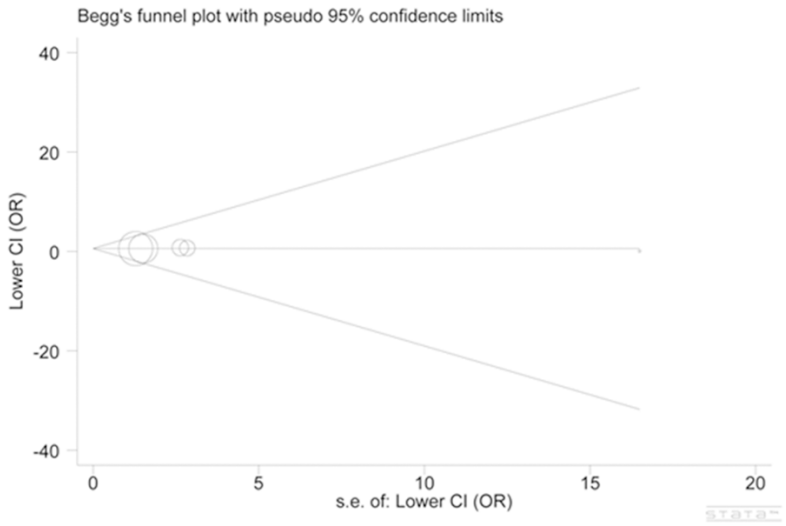

Figure 3. Begg funnel plot for publication bias test (AA versus GG: t = 0.25, P = 0.822). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of odd ratio (OR). Horizontal line, mean effect size. s.e. = Standard error of the mean.

more investigations to supply strong evidence for the association under dispute.

The principle findings of this meta-analysis suggested that the correlation between *CD95* rs2234767 polymorphism and risk of cervical cancer was not statistically significant. In order to identify if the null results could be altered in Asian population, we performed a subsequent stratified analysis implicating similar nonsignificant association. African and mixed populations were not further analyzed, because the insufficient data (each dataset was provided by one single study) made it unnecessary to conduct such analysis.

It is well established that apoptosis plays a vital role in tumorigenesis. Biologically impaired apoptotic function is a prerequisite for cancer development, as more and more evidence implicating that neoplastic mutations appear to act by interfering with apoptosis [29]. The *CD95* system is a mediator of apoptotic cell death. Somatic and germ line mutations within *CD95* contribute to the development of various cancers and hematological malignancies [30-33]. *CD95* rs2234767 polymorphism was revealed to regulate expression level of the *CD95* gene. Patients positively expressed *CD95* have a better prognosis compared with those negatively expressed [34].

Several investigators have correlated risk of cervical cancer with *CD95* rs2234767 polymorphism. But the results were indecisive, even in the studies based on the subjects of the same descent. In an analysis of 318 cervical cancer cases, Lai et al. suggested that no association could be identified [18]. On the contrary, Li et al. (314 patients) believed *CD95* rs2234767 polymorphism is significantly associated with the development of this cancer [21]. Relatively small sample size may be a possible explanation, but the major reason may attribute to the nonstandardized selection of subjects and misclassification of genotypes by using different method in genotyping *CD95* rs2234767 polymorphism. In the meta-analysis performed in this study, the results showed cervical cancer risk was not associated with *CD95* rs2234767 polymorphism. This could be explained by molecular role of the polymorphism in up regulation of the *CD95* gene, precluding malignant transformation and progression.

The presence of significant heterogeneity and/or publication bias may influence the interpretation of the meta-analysis and lead to a potentially misleading conclusion. Corresponding analyses helped to quantitively confirm few effect from these factors in this study. However, several issues should be concerned. First, lack of sufficient data may have mistaken the susceptibility role of *CD95* rs2234767 polymorphism, which can be accurately identified through a larger study. Second, we did not consider gene-gene and gene-environment interactions in this meta-analysis. Although *CD95* rs2234767 polymorphism alone may not change cervical cancer risk, we cannot rule out the possibility that the risk will be influenced by the combination with other genes or environmental factors.

In conclusion, this meta-analysis suggested that no significant association existed between *CD95* rs2234767 polymorphism and cervical cancer risk. The same was true for Asian population. Further large and well-designed studies with gene-gene and gene-environment interac-

tions considered are needed to confirm our findings.

Disclosure of conflict of interest

None.

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