Int J Clin Exp Med 2015;8(4):5317-5322
www.ijcem.com /ISSN:1940-5901/IJCEM0004195

*Original Article*

# Association of interleukin-12 gene polymorphisms with cancer susceptibility: a meta-analysis

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Received November 27, 2014; Accepted March 26, 2015; Epub April 15, 2015; Published April 30, 2015

**Abstract:** Objective: To evaluate the association between interleukin-12 (IL-12) gene polymorphism and cervical cancer susceptibility. Methods: We comprehensively retrieved the relevant English and Chinese database to collect case-control studies on the association between the IL-12 gene polymorphism and cervical cancer susceptibility. Data were extracted independently by two researchers respectively, the summary data were analyzed using Revman 5.2 software, the association was described using odds ratios (OR) and 95% confidence intervals (95% CI). Results: According to inclusion and exclusion criteria, 5 case-control studies involving 2552 cervical cancer patients and 2232 healthy controls were included. The meta-analysis results showed that 3'UTR+1188 (rs3212227) polymorphism in IL-12 gene was not associated with cervical cancer risk (C *vs.* A: OR=1.09, 95% CI: 0.88~1.35, *P*=0.45; AA+AC *vs.* CC: OR=0.88, 95% CI: 0.67~1.15, *P*=0.34; AA *vs.* AC+CC: OR=0.89. 95% CI: 0.56-1.42, P=0.62; CC *vs.* AA: OR=1.30. 95% CI: 0.79-2.12, *P*=0.30). Conclusion: The available evidence suggested that rs3212227 polymorphism in IL-12 gene may not be the risk factor to cervical cancer.

**Keywords:** Interleukin 12, single nucleotide polymorphism, cervical cancer, meta-analysis



## Introduction

Cancer has become one of the most important causes of human death. Due to aging, environmental pollution, smoking, lack of exercise, high fat, high calorie diet and lifestyle which will present seriously hazard to health, annual cancer incidence and mortality continue to rise worldwide [1]. Cervical cancer is one of the most common malignant diseases and is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females with approximately 529,800 new cases and 275,100 deaths among females in 2008 worldwide [2]. Cervical cancer is a multifactorial and multistep disease [3, 4]. Innate immune deficiency, environmental aggravation, and genetic mutation have been considered as important pathopoiesis factors [5, 6]. Interleukin-12 (IL-12) is an inflammatory cytokine, which is the bridge connecting the early nonspecific immune responses and secondary antigen-specific immune response. IL-12 is mainly produced by antigen activated monocytes and macrophages and dendritic cells and other antigen presenting cells [7]. In recent years, a number of studies reported that IL-12 gene had important role in the occurrence and development of cervical cancer [8-15], but the results remain controversial. Although a previous meta-analysis [10] indicated that IL-12 rs3212227 was associated with cervical cancer, the authors only included two studies related to cervical cancer in the meta-analysis. In the present study, we comprehensively collected the relevant case-control study to clarify the association between the IL-12 gene polymorphism and susceptibility to cervical cancer utilized meta-analysis.

## Materials and methods

### *Searching strategy*

We retrieved literatures written in English or Chinese from PubMed, EMBASE, Web of Science, CBM, Wanfang, Weipu and CNKI databases. Publication year was from January 1998 to October 2014, we manually retrieved all the documents from references to obtain additional information. The association between IL-12 gene polymorphisms and cervical cancer sus-

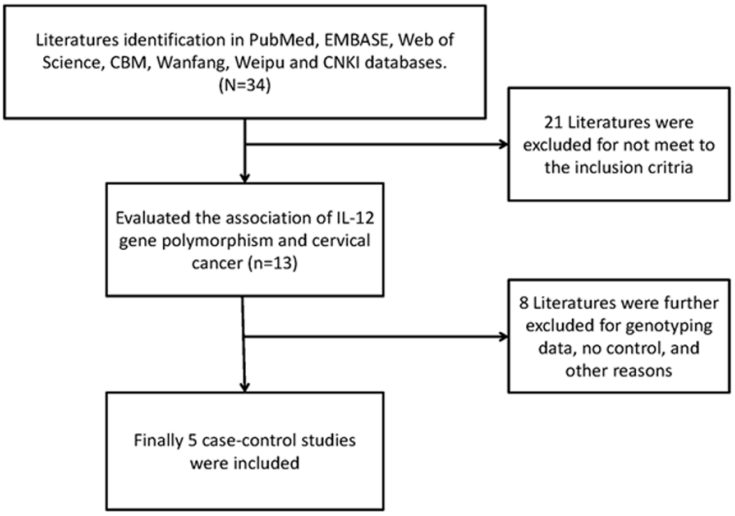


**Figure 1.** Flow chart of literatures identification.

ceptibility were comprehensively collected. Literature search strategy was based on principles of combination of keywords and free words. The search terms included "Interleukin-12", "IL-12", "IL-12A", "IL-12B", "cervical cancer", "tumor", "carcinoma", "neoplasms", "SNPs", "mutation", "variant", "polymorphism".

*Inclusion and exclusion criteria*

*Literature inclusion criteria:* 1) Study type: case-control studies on the association between IL-12 gene polymorphism and susceptibility to cervical cancer 2) All case patients were diagnosed via clinical and pathological methods as the tumor of different parts; 3) Literature included provided the complete clinical data, allele or genotype frequencies and other data; 4) The genotype frequencies of control group in studies included were in line with the genetic Hardy-Weinberg equilibrium (HWE); 5) All included studies were in Chinese or English language.

*Exclusion criteria:* 1) Case reports, abstracts, reviews, lectures, commentary and other non-case-control studies; 2) Studies unrelated with research theme or purpose; 3) Genotype frequencies in control group of studies did not meet the HWE or familial genetic research; 4) Data was incorrect or can't be extracted; 5) Repeat published literature.

*Data extraction*

A unified data collection forms were developed, literature data were extracted by two investigators (Chang Su-Wen and Ju-Qin Xu) independently, the main data included first author, published time, country, language, race, study design, number of patient in case group and control group, the source of the control population, genotyping methods, allele or genotype frequencies. If the included study was involved in multiple races such as Asians, Europeans, Africans, data were extracted according to the different races. If there were disputes, differences shall be resolved via discussion with a third party.

*Quality assessment included literatures*

According to STROBE rating scale, the two researchers (Chang Su-Wen and Ju-Qin Xu) evaluated the quality of the included studies [16]. There were 40 evaluation items for the meta-analyzed quality evaluation of included literatures. Rating intervals were from 0 to 40, 0 to 19 was considered as low quality, 20 to 29 was considered as medium quality, 30 to 40 was considered as high quality. If there is a dispute, we should resolve their differences through discussion.

*Data analysis*

Association between IL-12A and IL-12B gene polymorphisms and cervical cancer susceptibility were evaluated with odds ratio (OR) and 95% confidence intervals (95% CI). The statistical significance of OR values were aggregated using Z test evaluation. HWE of genotypes in control population was evaluated using the chi-square test. Heterogeneity among studies was evaluated using the Cochran Q test, If $P<0.05$, heterogeneity was suggested. In addition, the quantitative evaluation test $I^2$ was used to evaluate the size of heterogeneity, $I^2$ values ranged from 0% to 100%, the greater $I^2$ value, the more obvious heterogeneity. If heterogeneity test $P<0.05$ or $I^2>50\%$, it suggested that heterogeneity among studies existed. Therefore, the random effects model such as DerSimonian Laird method analysis was performed. Otherwise the fixed effects model such as Mantel-Haenszel method analysis was performed. Included studies were deleted one by

**Table 1.** The characteristics of included studies

| Author | Year | Country | Genotyping | Groups | No. | 3'UTR+1188 (rs3212227) Genotypes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | AA | AC | CC | A | C |
| Han et al. | 2008 | Korea | SNaPShot | case | 154 | 32 | 87 | 31 | 151 | 494 |
| | | | | control | 191 | 52 | 88 | 39 | 192 | 837 |
| Chen et al. | 2009 | China | PCR-RFLP | case | 404 | 127 | 199 | 78 | 453 | 1391 |
| | | | | control | 404 | 150 | 185 | 69 | 485 | 1219 |
| de Carvalho | 2012 | Brazil | PCR-RFLP | case | 162 | 100 | 49 | 13 | 249 | 597 |
| | | | | control | 76 | 31 | 37 | 8 | 99 | 796 |
| Roszak et al. | 2012 | Poland | PCR-RFLP | case | 405 | 212 | 174 | 19 | 598 | 1925 |
| | | | | control | 450 | 289 | 151 | 10 | 729 | 3713 |
| Hussain et al. | 2013 | USA | TaqMan | case | 1427 | - | - | - | 2255 | 599 |
| | | | | control | 1111 | - | - | - | 1778 | 444 |

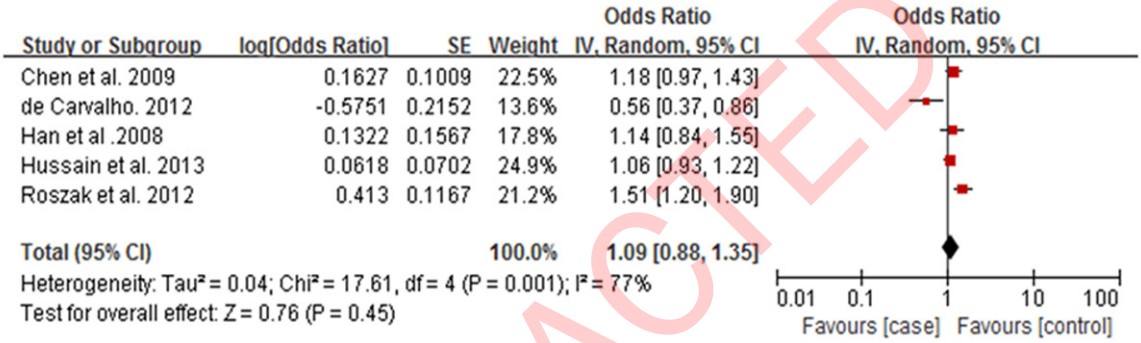


**Figure 2.** Forest plot of cervical cancer susceptibility and IL-12 rs3212227 polymorphism (C allele *vs.* A allele), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

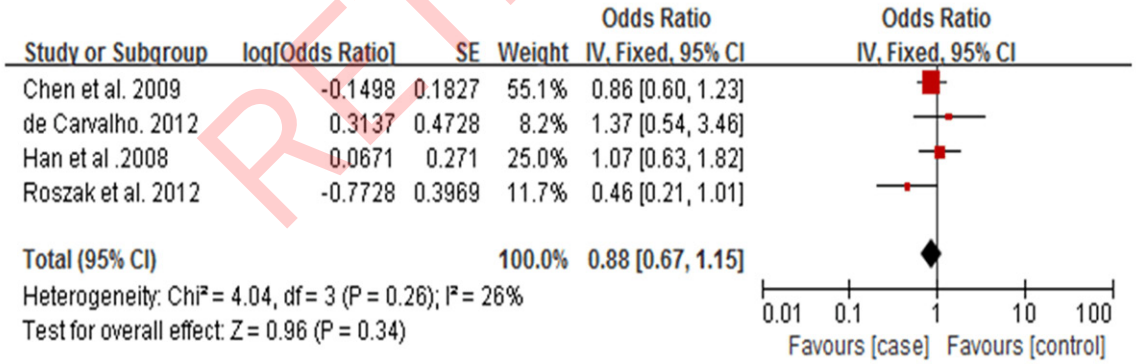


**Figure 3.** Forest plot of cervical cancer susceptibility and IL-12 rs3212227 polymorphism (AA+AC *vs.* CC), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

one to perform the sensitivity analysis. Begger funnel plot and Egger analysis were used to assess whether the literature had publishing bias and whether the literature results were reliable. Meta-analysis was performed using Revman 5.2 software.

## Results

### *Characteristics of the included study*

Initially we retrieved 34 literatures, based on strict inclusion and exclusion criteria for litera-

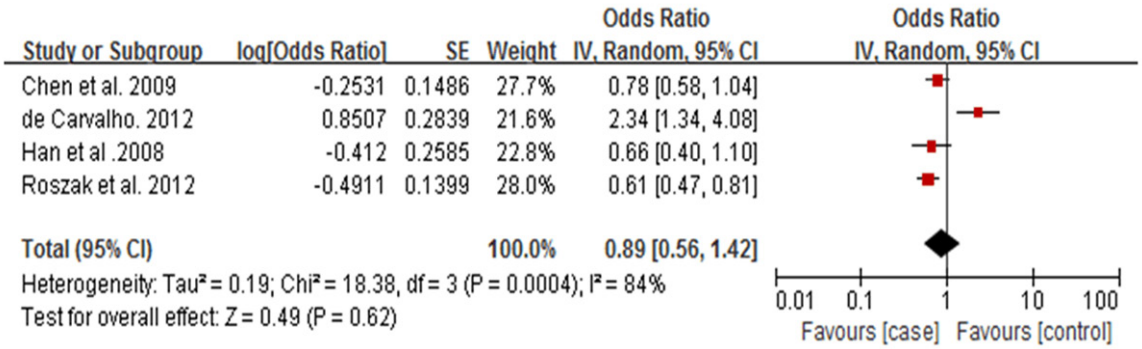


**Figure 4.** Forest plot of cervical cancer susceptibility and IL-12 rs3212227 polymorphism (AA *vs.* CC+AC), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

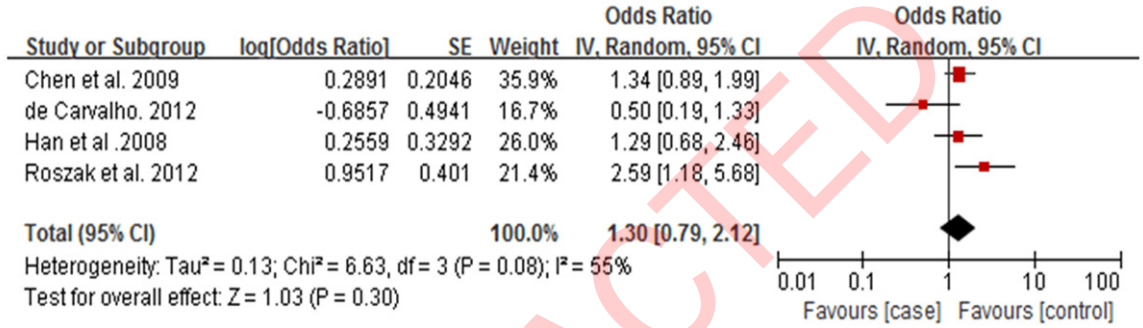


**Figure 5.** Forest plot of cervical cancer susceptibility and IL-12 rs3212227 polymorphism (CC *vs.* AA), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.



ture screening, 21 unrelated literatures were excluded according to title and abstract. Further abstract and full text reading removed 8 documents. Finally, 5 studies involving 2552 cervical cancer patients and 2232 control subjects [8, 11, 12, 14, 16] were included (**Figure 1**). The publication year was from 2008 to 2013. All patients were clinically and histologically confirmed to have cervical cancer. The baseline characteristics of the included studies were shown in **Table 1**.

*Association between IL-12 geners3212227 and cervical cancer susceptibility*

In all 5 included studies, a total of 4 studies provided the genotype and allele frequency of rs3212227. One study only provided allele frequency. Q test indicated there were significant heterogeneity among the included studies (all $P<0.05$, and $I^2>50\%$), the random effects model analysis was performed. Meta-analysis showed that IL-12 gene rs3212227 was not associated with cervical cancer risk (C *vs.* A: OR =1.09, 95% CI: 0.88~1.35, P=0.45; AA+AC *vs.* CC: OR=0.88, 95% CI: 0.67~1.15, P=0.34; AA *vs.* AC+CC: OR=0.89. 95% CI: 0.56-1.42, P=0.62; CC *vs.* AA: OR=1.30. 95% CI: 0.79-2.12, P=0.30) (**Figures 2**-**5**).

*Sensitivity analysis and publication bias evaluation*

We performed the sensitivity analysis by deletion of single study one by one. The results showed that no single study can significantly affect statistical significance of the original analysis, which indicated the results were stable. We also evaluated the publication bias by Begger symmetrical funnel plot. The result showed no significant publication bias (data not shown).

## Discussion

Currently, new tumor markers in genetics and molecular biology have gradually become important monitoring indicators in cancer pre-

vention and early diagnosis and treatment. A number of genetic studies showed that cytokine gene mutation was closely associated with cervical cancer [17-20]. IL-12 gene polymorphism may significantly alter the expression or function of the protein level to result in immune system disorders and autoimmune diseases and may ultimately lead to malignant tumors [21]. In recent years, IL-12 gene polymorphism was considered to be the risk factors contributing to the occurrence of many cancers, including cervical cancer. But the relevant findings were controversial. It was necessary to adopt meta-analysis to clarify the specific association between IL-12 genetic polymorphism and cervical cancer. The present meta- analyses were focus on the relation between 3'UTRA>C (rs3212227) polymorphism in IL-12 gene and cervical cancer. However, we did not find any difference between case and control group in allele and genotype distribution. Previously, Zhou et al. [10] performed a meta-analysis to investigate the relation between rs3212227 and cancer risk, the authors found that the 3'UTR A>C (rs3212227) polymorphism of IL-12 gene might be a potential biomarker for cancer risk among Asians, especially for cervical and nasopharyngeal cancers. In their study, the authors only included two studies related to cervical cancer. In our study, we included 5 case-control studies and did not verify Zhou's [10] findings.

The present study was performed through scientific and rational design, however, there were still several limitations. Firstly, although Begger funnel plot analysis did not show the presence of publication bias, there may still be selective language bias, since this study only included documents published in English and Chinese. Secondly, due to the sample size limitations of the included studies, the subgroup analyses were not carried out to clarify its association with IL-12 gene, which may lead to the limited clinical value of this research. Finally, the potential confounding factors may exist such as race, age, gender, geographical distribution.

In conclusion, by a comprehensive analysis of the 5 included studies, we did not find association between IL-12 genetic polymorphism and cervical cancer. Due to the number of included literatures, the quality and sample size limitations, the conclusions still need large sample, high-quality, rational designed clinical studies or epidemiological studies for confirmation.

## Disclosure of conflict of interest

None

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