

# Association of Three Micro-RNA Gene Polymorphisms with the Risk of Cervical Cancer: A Meta-Analysis and Systematic Review

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## Research Article

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# Abstract

**Objective:** Regulation of single nucleotide polymorphisms (SNP) in micro-RNA ( miRNA) on the host cells may be one of the most important factors influencing the occurrence of cervical cancer based on the prevalence of HPV infection and the development of cervical cancer. In order to explore the contribution of miRNA polymorphism to the occurrence and development of cervical cancer, we conducted an analytical study.

**Methods:** We selected the polymorphisms of three widely studied miRNAs (miRNA-146a rs2910164, miRNA-499 rs3746444 and miRNA-196a2 rs11614913). Then we conducted a meta-analysis (for the first time) to investigate their susceptibility to cervical cancer. Case control studies on the correlation between these three miRNAs and cervical cancer susceptibility were investigated by searching on from Pubmed, The Cochrane Library, Embase, CBM, CNKI, Wanfang database and VIP database. Basic characteristics were recorded and meta-analysis of the case studies was performed using STATA 15.1 software.

**Results:** The miRNA-146a rs2910164 mutation significantly reduced the risk of cervical cancer in both recessive model (OR= 0.804, 95%CI= 0.652-0.992,  $P=0.042$  CC vs. CG+GG) and allelic model (OR= 0.845, 95%CI= 0.721-0.991,  $P=0.038$  C vs. G). There was no significant correlation between miRNA -499 rs3746444 and the risk of cervical cancer. The miRNA -196a2 rs11614913 mutation was significantly associated with a reduced risk of cervical cancer in homozygous model (OR= 0.641, 95%CI= 0.447-0.919,  $P=0.016$  TT vs. CC), dominant model (OR= 0.795, 95%CI= 0.636-0.994,  $P=0.045$  CT+TT vs. CC), recessive model (OR= 0.698, 95%CI= 0.532-0.917,  $P=0.01$  TT vs. CC+CT), and allelic models (OR= 0.783, 95%CI= 0.643-0.954,  $P=0.015$  T vs. C).

**Conclusion:** In summary, this meta-analysis shows that the mutant genotypes of miRNA -146a rs2910164 and miRNA -196a2 rs11614913 are associated with a reduced risk of cervical cancer. Therefore, they may be two gene regulatory points for the prevention of cervical cancer.

## 1. Introduction

Cervical cancer is the second most common malignancy in women; whose incidence is second only to the breast cancer<sup>[1]</sup>. About 500,000 new cases of cervical cancer occur globally each year, accounting for 5% of all new cancer cases. Every year, more than 260,000 women die of cervical cancer<sup>[2]</sup>, accounting for 7.5% of all female cancer deaths. The number is increasing year by year, and the patient population is getting younger. Therefore, cervical cancer is an important disease endangering women's health and life<sup>[3, 4]</sup>. Focusing on women's health is an important step in human reproduction and development. At present, in the face of cervical cancer, we still focus on prevention. Hence, the factors affecting the occurrence and development of cervical cancer are worthy of our research and discussion.

It is well known that human papillomavirus (HPV) is associated with the occurrence of cervical cancer. HPV is the major risk factor for cervical cancer<sup>[5]</sup>. Women infected with HPV can get rid of it by their own immune system, but there are still some women who develop cervical cancer due to persistent infection with high-risk HPV<sup>[6]</sup>. In the process of continuous infection with the high-risk HPV until the development of cervical cancer, these women are also affected by environmental, genetic and other factors<sup>[7]</sup>.

Many diseases have been found to be related to genes. Nowadays, the genetic changes of patients and their influence on the occurrence and development of diseases are the focus of research. MicroRNAs (miRNAs) are a class of non-coding single-stranded RNA molecules with a length of about 22 nucleotides that regulate various intracellular activities such as cell proliferation, apoptosis and carcinogenesis<sup>[8-10]</sup>. Each miRNA can have multiple target genes, and several miRNAs can regulate the same gene. MicroRNAs have been reported to regulate about one-third of human genes<sup>[11]</sup>. The length of miRNA is usually shorter than the length of the encoding gene. Its small genetic changes may alter human miRNA expression and target selection<sup>[12]</sup>. MicroRNAs have been shown to be associated with cervical cancer<sup>[13]</sup>. Therefore, single nucleotide polymorphisms (SNPs) in miRNA genes may lead to abnormal gene expression by changing miRNA maturation and expression<sup>[14, 15]</sup>. This may be related to cervical cancer susceptibility. In the past few years, several studies have investigated the association between some miRNA polymorphisms and cervical cancer risk, but the results were different<sup>[16-23]</sup>. Therefore, three miRNA polymorphisms (miRNA-146a rs2910164, miRNA-499 rs3746444 and miRNA-196a2 rs11614913) associated with cervical cancer risk were selected for meta-analysis. The polymorphism sites and base pair locations of the above three miRNAs are shown in Table 1.

## 2. Materials And Methods

### 2.1 Study Registration

This protocol of systematic review and meta-analysis has been drafted under the guidance of the preferred reporting items for systematic reviews and meta-analyses protocols (PRISMA-P). It has been registered on open science framework (OSF) (Registration number: DOI 10.17605 / OSF.IO / P38H2).

### 2.2 Literature search and selection

We searched Pubmed, The Cochrane Library, Embase, CBM, CNKI, Wanfang Database and VIP database; all of which were established until May 2020. The retrieval method adopted the combination of subject words and free words. The search words were miRNA-146a, miRNA-499, miRNA-196a2, cervical cancer and so on. The retrieved articles were screened by inclusion and exclusion criteria. The included literature was then determined. Where necessary, we contacted the original study author by email or phone for the undetermined but important information required in this study.

Inclusion criteria: (1) The association between miRNA-146a rs2910164 and/or miRNA-499 rs3746444 and/or miRNA-196a2 rs11614913 and the cervical cancer risk was assessed; (2) Studies with relevant genotype OR allele frequency, information to calculate OR and 95% CI; (3) The subjects were humans ; (4) The study was designed as a case control.

Exclusion criteria: (1) Specific information on genotype or allele frequency of miRNA-146a rs2910164 and/or miRNA-499 rs3746444 and/or miRNA-196a2 rs11614913 was not provided in the literature; (2) The genotype frequency of the control group was not in Hardy-Weinberg equilibrium; (3) Cervical abscess cell pathology in the control group showed cervical intraepithelial neoplasia (CIN) ; (4) Literatures with less than 6 stars in the Newcastle-Ottawa Scale.

## 2.3 Data extraction and quality evaluation

The meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Table S1). The final included literature recorded the following information: first author name, year of publication, patient nationality, genotyping method, genotype and allele frequency, and tabulated the basic characteristics. The selected literature was evaluated using the Newcastle-Ottawa Scale.

## 2.4 Statistical analysis

Meta-analysis was used to assess the relationship between three miRNA polymorphisms and cervical cancer susceptibility. The genotype frequency of the control group was tested by the Hardy-Weinberg equilibrium (HWE) test using the Chi square test. If  $P > 0.05$ , it was regarded to be in the Hardy-Weinberg equilibrium. The odds ratio (OR) and 95% confidence interval (95%CI) were calculated to evaluate the relationship between the six gene models (homozygous, heterozygous, dominant, recessive, over dominant and allelic genetic models) of the polymorphisms of miRNA-146a rs2910164, miRNA-499 rs3746444, and miRNA-196a2 rs11614913 and the risk of cervical cancer. The strength of association between each polymorphism and the risk of cervical cancer was evaluated by the combined odds ratio (OR) and its 95% confidence interval (95%CI). The statistical significance of combined OR was tested by z test; and  $P < 0.05$  showed a significant statistical difference. We assessed heterogeneity between studies by chi-square test and  $I^2$ : If  $P \geq 0.1$  and  $I^2 < 50\%$ , we used a fixed-effect model to assess OR and 95% CI. If  $P < 0.1$  and  $I^2 > 50\%$ , we evaluated OR and 95% CI using a random effect model.

This meta-analysis quantitatively assessed publication bias using the Begg's test and the Egger's test. Sensitivity analysis assessed the stability of the results by excluding each study in turn. All analyses were performed using the STATA 15.1 software (STATA Corporation, College Station, TX, USA).

## 3. Results

### 3.1 Literature screening results

In total, 8 articles were included in this meta-analysis<sup>[16–23]</sup>. From the studies, 14 data sets, comprising 3142 patients and 3971 controls were retrieved. Among these, five studies related to the correlation between miRNA-146a rs2910164 and cervical cancer; three related to the correlation between miRNA-499 rs3746444 and cervical cancer; and six related to the correlation between miRNA-196a2 rs11614913 and cervical cancer. The flow chart of literature screening is as shown in Fig. 1.

## 3.2 Basic Characteristics of the Included Literature and Results of Quality Assessment

The basic characteristics of the literature are described in Table 2. Eight literatures were evaluated by Newcastle-Ottawa Scale, and the scores were all above 7 (full score was 9). The quality evaluation results of the included literature are as shown in Table 3.

## 3.3 Meta-analysis of the relationship between three miRNAs and cervical cancer

### 3.3.1 Relationship between miRNA-146a rs2910164 and cervical cancer

The combined analysis showed that the mutant C allele significantly reduced the risk of cervical cancer in both the recessive genetic model (OR = 0.804, 95%CI = 0.652–0.992,  $P = 0.042$ ; CC vs. CG + GG) and the allele genetic model (OR = 0.845, 95%CI = 0.721–0.991,  $P = 0.038$ ; C vs. G), and the difference was statistically significant (Table 4 and Fig. 2).

Subgroup analysis was performed according to the analysis methods used for genotyping and countries. In the study involving PCR-RFLP analysis, the polymorphism of miRNA-146a rs2910164 reduced the risk of cervical cancer in the homozygote (OR = 0.626, 95% CI = 0.453–0.865,  $P = 0.004$ ; CC vs. GG), heterozygote (OR = 0.787, 95%CI = 0.637–0.972,  $P = 0.026$ ; CG vs. GG), dominant (OR = 0.726, 95%CI = 0.590–0.893,  $P = 0.002$ ; CC + CG vs. GG), recessive (OR = 0.753, 95%CI = 0.581–0.976,  $P = 0.032$ ; CC vs. CG + GG), allele (OR = 0.795, 95%CI = 0.681–0.928,  $P = 0.004$ ; C vs. G) genetic models, and the difference was statistically significant. The difference was not statistically significant in the analysis of China and India (Table 5).

### 3.3.2 Relationship between miRNA-499 rs3746444 and cervical cancer

The pooled analysis showed that among the six genetic models, there was no correlation between miRNA-499 rs3746444 and the risk of cervical cancer (Table 4). Subgroup analysis indicated that this correlation remained absent in different genotyping assays and countries (Table 5).

### 3.3.3 Relationship between miRNA-196a2 rs11614913 and cervical cancer

The combined analysis showed that mutant TT was significantly associated with a lower risk of cervical cancer compared to the wild homozygous CC (OR = 0.641, 95%CI = 0.447–0.919,  $P = 0.016$ ; TT vs. CC). The Mutant gene T significantly reduced the risk of cervical cancer in both dominant (OR = 0.795, 95%CI =

0.636–0.994,  $P = 0.045$  CT + TT vs. CC) and recessive (OR = 0.698, 95%CI = 0.532–0.917,  $P = 0.01$  TT vs. CC + CT) genetic models. In the allele model, T significantly reduced the risk of cervical cancer as compared to C (OR = 0.783, 95%CI = 0.643–0.954,  $P = 0.015$  T vs. C) (Table 4 and Fig. 3).

Subgroup analysis by genotyping method and country in the study of genotype analysis using TaqMan showed that, the polymorphism of miRNA-196a2 rs11614913 significantly reduced the risk of cervical cancer in homozygous (OR = 0.762, 95%CI = 0.625–0.929,  $P = 0.007$ ; TT vs. CC), recessive (OR = 0.807, 95%CI = 0.707–0.921,  $P = 0.001$  TT vs. CT + CC) and allele (OR = 0.871, 95%CI = 0.795–0.955,  $P = 0.003$  T vs. C) genetic models (Table 5). Studies in China showed that, the polymorphism of miRNA-196a2 rs11614913 significantly reduces the risk of cervical cancer in homozygous (OR = 0.787, 95%CI = 0.672–0.921,  $P = 0.003$  TT vs. CC), recessive (OR = 0.817, 95%CI = 0.724–0.923,  $P = 0.001$  TT vs. CT + CC) and allele (OR = 0.883, 95%CI = 0.817–0.954,  $P = 0.002$  T vs. C) genetic models. Furthermore, studies in India showed that have shown that the risk of cervical cancer is significantly reduced in homozygous (OR = 0.167, 95%CI = 0.086–0.323,  $P < 0.001$  TT vs. CC), dominant (OR = 0.389, 95%CI = 0.241–0.628,  $P < 0.001$  CT + TT vs. CC), recessive (OR = 0.209, 95%CI = 0.114–0.381,  $P < 0.001$  TT vs. CT + CC) and allele (OR = 0.362, 95%CI = 0.259–0.506,  $P < 0.001$  T vs. C) genetic models.

### 3.4 Heterogeneity

MiRNA-146a rs2910164 showed no heterogeneity in the super-dominant genetic model, and heterogeneity in the homozygote and the dominant genetic models. The results of subgroup analysis showed that the heterogeneity decreased in the studies using the PCR-RFLP analysis. No heterogeneity was observed in the homozygous and recessive genetic models for miRNA-499 rs3746444, but considerable heterogeneity was observed in the remaining models. The heterogeneity persisted after subgroup analysis. MiRNA-196a2 rs11614913 showed no heterogeneity in heterozygote and hyper-dominant genetic models, but it showed heterogeneity in the homozygote, recessive and allele models. Subgroup analysis showed that heterogeneity decreased in studies using TaqMan analysis (Table 4).

### 3.5 Sensitivity analysis

In order to explore whether the meta-analysis results were stable, sensitivity analysis was conducted. The meta-analysis results appeared to be stable.

### 3.6 Publication bias

Potential publication bias of this meta-analysis was tested by Begg's test and Egger's test (Table 4). The results shown in the table indicate that no publication bias existed among the polymorphisms of miRNA-146a rs2910164, miRNA-499 rs3746444 and miRNA-196a2 rs11614913 in all genetic models.

## 4. Discussion

Research on the impact of single nucleotide polymorphisms (SNPs) on the occurrence and development of diseases has increased over the years. With the development of molecular biology, discussions on genetic problems have become necessary. In the face of multiple pathogenic factors such as: HPV infection, risky living environment, and host factors; which together lead to the occurrence of cervical cancer<sup>[2]</sup>, while studying this process in depth, we found that the microenvironment formed by the regulation of certain genes in the human body seems to guide the occurrence of cervical cancer<sup>[24, 25]</sup>.

MiRNAs regulate gene expression at the post-transcriptional level, participate in the regulation of various cellular functions. They affect cell proliferation, apoptosis and carcinogenesis<sup>[26]</sup>. Variation of a certain miRNA, such as the conversion, insertion, deletion or mismatch of a single base, can cause the polymorphism of the whole DNA sequence. This may in turn affect the function of cells and tissues and the whole host microenvironment<sup>[27]</sup>. Therefore, miRNAs may be used to predict the occurrence and development of cervical cancer<sup>[25]</sup>. We selected three kinds of miRNA polymorphisms (miRNA-146a rs2910164, miRNA-499 rs3746444 and miRNA-196a2 rs11614913), that have been studied more frequently in order to analyze their relationship with susceptibility to cervical cancer<sup>[16–23]</sup>. The results showed that, the polymorphisms of miRNA-146a rs2910164 (mutant C allele) and miRNA-196a2 rs11614913 (mutant T allele) significantly reduced the risk of cervical cancer. There was no significant correlation between the polymorphisms of miRNA-499 rs3746444 and cervical cancer.

In the study using PCR-RFLP analysis, the polymorphism of miRNA-146a rs2910164 significantly reduced the risk of cervical cancer in homozygote, heterozygote, dominant, recessive, allele models. With regard to the TaqMan analysis of the genotypes, the polymorphism of miRNA-196a2 rs11614913 significantly reduced the risk of cervical cancer in homozygous, recessive, and allelic models. According to the Chinese studies, miRNA-196a2 rs11614913 reduced cervical cancer risk in homozygous, recessive and allelic models, whereas in Indian studies, it significantly reduced cervical cancer risk in homozygous, dominant, recessive and allelic models. Thus, using different genotype analysis methods and in different countries, the correlation between the polymorphisms of miRNA-146a rs2910164 and miRNA-196a2 rs11614913 and cervical cancer is somehow different. However, it cannot be denied that they can both reduce the risk of cervical cancer.

Meanwhile, a study by Yan et al showed that the mutant T allele in the miRNA-126 rs4636297 polymorphism was associated with cervical cancer susceptibility, and the mutant T gene increased cervical cancer susceptibility compared to the wild C gene<sup>[21]</sup>. Wang et al reported that the mutant C allele in the polymorphism of miRNA-149 rs2292832 was associated with cervical cancer susceptibility, and the mutant C gene increased cervical cancer susceptibility relative to the original T gene<sup>[22]</sup>. Furthermore, Chuntao noted that mutation of A allele to G allele in the polymorphism of miRNA-30c rs928508 could reduce the susceptibility to cervical cancer<sup>[20]</sup>. However, due to few studies on the related miRNA polymorphisms, they were not included in the meta-analysis. These loci may present more in-depth studies in the future.

Persistent infection with high-risk HPV is a major factor in the development of cervical cancer<sup>[29, 30]</sup>. However, the occurrence of cervical cancer is a multigene, multistage abnormal regulation process<sup>[31]</sup>. Studies have also shown that the abnormal expression of miRNAs affects the cervical epithelial cell carcinogenesis<sup>[32]</sup>. Different miRNAs regulate different biological activities; some target to regulate the activity of tumor suppressor genes to inhibit

development of the cancer cells. Others accelerate the growth and metastasis of cancer cells by stimulating proto-oncogenes and metastasis genes<sup>[32]</sup>. Studies have shown that the polymorphism of miRNA-146a rs2910164 G to C leads to increased expression of miR-146a, and the mutant CC genotype is associated with reduced risk of cervical cancer<sup>[33]</sup>. As a mediator of the pro-apoptotic transcription nuclear factor kappaB, miRNA-146a affects apoptosis and hence influences the development of cancer<sup>[34]</sup>. Each cancer tissue has its specific miRNA target<sup>[35]</sup>, so the miRNA-146a polymorphism may affect the occurrence and development of cervical cancer through certain pathways. MiRNA-196a2 rs11614913 is a polymorphic site in the mature miR-196a2 sequence, and its mutation expression in situ gene C, the T allele, was found to be associated with reduced risk of cervical cancer<sup>[36]</sup>. Studies have shown that C gene increases the expression of mature miR-196a2 in cervical cancer patients, and this overexpression changes the state of binding of miR-196a2 to its target genes, thereby affecting the development of cervical cancer<sup>[19]</sup>. Tracking the changes of the two genetic polymorphism loci in human may help to understand how to reduce the incidence of cervical cancer, by changing the internal human environment. Therefore, these two sites may become the targets for the prevention and treatment of cervical cancer in the future.

However, this meta-analysis had several limitations: (1) Due to the fewer factors controlled by relevant studies, the relationship between age, number of abortions and the expression of the three miRNAs in cervical cancer patients was not analyzed. (2) Heterogeneity in some polymorphisms could have arose from different types of cervical cancer and other factors associated with the participants themselves. (3) This meta-analysis did not establish whether the mixed effects of genetic polymorphisms were associated with susceptibility to cervical cancer.

## 5. Conclusion

In conclusion, this meta-analysis showed that the polymorphisms of miRNA-146a rs2910164 (mutant C allele) and miRNA-196a2 rs11614913 (mutant T allele) could reduce the susceptibility to cervical cancer.

## Abbreviations

SNP, single nucleotide polymorphisms; miRNA, micro-RNA; HPV, human papillomavirus; NCTV, non\_coding\_transcript\_variant; CIN, cervical intraepithelial neoplasia; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; HB, Hospital-based; PCR-RFLP, Restriction fragment length polymorphism polymerase chain reaction; PCR-LDR, Polymerase chain reaction-Ligase detection reaction.

## Declarations

### Disclosure

Jingyu Xu and Yihua Fan are co-first authors.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### Author Contribution

Conception and design: Qiang Zhang; Acquisition of data: Yihua Fan; Data extraction: Jingyu Xu, Junze Geng; Drafting of the manuscript: Jingyu Xu; Critical revision of the manuscript for important intellectual content: Yihua Fan.

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## Tables

Table.1 Basic information of three miRNA polymorphisms

Gene name	dpSNP rs#ID	Location	Chromosome	Alleles	Ancestra	Functional
					Allele	Consequence
miRNA-146a	rs2910164	pre-miRNA	5:160485411	G/C	G	NCTV*
miRNA-499	rs3746444	pre-miRNA	20:34990448	T/C	T	NCTV*
miRNA-196a2	rs11614913	pre-miRNA	12:53991815	C/T	C	NCTV*

Note: NCTV,non\_coding\_transcript\_variant;

Table.2 Characteristics of the studies eligible for Meta-analysis

Frist Author	Year	Country	Source of	Genotyping	Case/Control	Case					Control					
			Control	Method												
MiRNA-146a rs2910164						CC	CG	GG	C	G		CC	CG	GG	C	G
Bin Zhou	2011	China	HB*	PCR-RFLP*	226/309	70	113	43	253	199		116	159	34	391	227
Cong Yue	2011	China	HB*	PCR-RFLP*	447/443	105	224	118	434	460		150	206	87	506	380
Li Ma	2015	China	HB*	PCR-RFLP*	205/415	53	102	50	208	202		103	219	93	425	405
ShiZhi Wang	2019	China	HB*	TaqMan	954/1339	318	475	141	1111	757		471	631	212	1573	1055
Nisha Thakur	2019	India	HB*	PCR-RFLP*	150/150	21	49	80	91	209		28	49	73	105	195
MiRNA-499 rs3746444						TT	CT	CC	T	C		TT	CT	CC	T	C
Bin Zhou	2011	China	HB*	PCR-RFLP*	226/309	134	84	8	352	100		223	71	15	517	101
ShiZhi Wang	2019	China	HB*	TaqMan	954/1339	675	228	27	1578	282		946	339	35	2231	409
Nisha Thakur	2019	India	HB*	PCR-RFLP*	150/150	25	47	78	97	203		21	49	80	91	209
MiRNA-196a2 rs11614913						TT	CT	CC	T	C		TT	CT	CC	T	C
Bin Zhou	2011	China	HB*	PCR-RFLP*	226/309	57	123	46	237	215		82	169	58	333	285
Bo Ding	2016	China	HB*	TaqMan*	509/562	133	265	111	531	487		181	278	103	640	484
ChunTao Wang	2016	China	HB*	PCR-LDR*	104/186	31	52	21	114	94		65	82	39	212	160
ZhiLing Yan	2019	China	HB*	TaqMan	547/567	117	277	153	511	583		153	282	132	588	546
ShiZhi Wang	2019	China	HB*	TaqMan	954/1339	271	464	194	1006	852		424	629	269	1477	1167
Nisha Thakur	2019	India	HB*	PCR-RFLP*	150/150	17	58	75	92	208		57	51	42	165	135

Note:

HWE: Hardy-Weinberg equilibrium;

HB: Hospital-based;

PCR-RFLP: Restriction fragment length polymorphism polymerase chain reaction;

Table 3. Newcastle-Ottawa Scale for quality assessment

Author(year)	Selection				Comparability	Exposure			Total
	Case	Case	Selection	Definition	Control	Determination	Same	No	score
	definition	representation	of	of	for factor	of exposure	exposure	response	
			controls	contrast			method	rates	
Zhou(2011)	☐	☐		☐	☐☐	☐	☐	☐	8
Yue(2011)	☐	☐		☐	☐☐	☐	☐	☐	8
ma(2015)	☐	☐		☐	☐	☐	☐	☐	7
Ding(2016)	☐	☐	☐	☐	☐☐	☐	☐	☐	9
Wang(2016)	☐	☐		☐	☐☐	☐	☐	☐	8
Yan(2019)	☐	☐		☐	☐	☐	☐	☐	7
Wang(2019)	☐	☐		☐	☐	☐	☐	☐	7
Thakur(2019)	☐	☐	☐	☐	☐☐	☐	☐	☐	9
☐The article scored one point in the project									
☐☐The article scored two points in the project									

Table.4 Total OR and 95%CI of three MiRNA polymorphisms in relation to cervical cancer susceptibility

Polymorphism	N	Genetic Model	Association Text			Heterogeneity Text		Publication Bias Test	
			OR [95% CI]	z	P	I <sup>2</sup> (%)	P	Begg's Test P	Egger's Test P
MiRNA-146a rs2910164	5	CC vs.GG	0.713[0.505-1.006]	1.93	0.054	68.8	0.012	0.806	0.330
		CG vs.GG	0.920[0.790-1.080]	1.03	0.304	43.5	0.132	0.462	0.115
		CG+CC vs.GG	0.807[0.627-1.038]	1.67	0.095	59.4	0.043	0.221	0.169
		CC vs.GG+CG	<b>0.804[0.652-0.992]</b>	2.04	0.042	51.2	0.085	1.000	0.563
		CC+GG vs.CG	0.939[0.834-1.056]	1.05	0.293	0.00	0.659	0.462	0.196
		C vs.G	<b>0.845[0.721-0.991]</b>	2.07	0.038	65.1	0.022	0.806	0.336
MiRNA-499 rs3746444	3	CC vs.TT	0.958[0.663-1.383]	0.23	0.818	0.00	0.794	1.000	0.507
		CT vs.TT	1.173[0.678-2.032]	0.57	0.568	83.4	0.002	1.000	0.778
		CT+CC vs.TT	1.14[0.719-1.809]	0.56	0.577	79.3	0.008	1.000	0.796
		CC vs.CT+TT	0.966[0.705-1.324]	0.22	0.829	0.00	0.710	1.000	0.462
		TT+CC vs.CT	0.833[0.511-1.358]	0.73	0.464	83.6	0.002	1.000	0.647
		C vs.T	1.079[0.832-1.401]	0.57	0.566	65.0	0.058	1.000	0.712
MiRNA-196a2 rs11614913	6	TT vs.CC	<b>0.641[0.447-0.919]</b>	2.42	0.016	78.2	☐0.001	0.707	0.270
		CT vs.CC	0.922[0.804-1.056]	1.17	0.24	0.00	0.597	1.000	0.531
		CT+TT vs.CC	<b>0.795[0.636-0.994]</b>	2.01	0.045	60.7	0.026	1.000	0.401
		TT vs.CT+CC	<b>0.698[0.532-0.917]</b>	2.58	0.01	76.1	0.001	0.260	0.187
		TT+CC vs.CT	0.917[0.824-1.020]	1.60	0.11	0.00	0.952	0.260	0.470
		T vs.C	<b>0.783[0.643-0.954]</b>	2.43	0.015	82.5	☐0.001	0.707	0.246



Table.5 A subgroup analysis of the relationship between three MiRNA polymorphisms and susceptibility to cervical cancer

Variable	No.	CC vs.GG		CG vs.GG		CG+CC vs.GG		CC vs.GG+CG		CC+GG vs.CG		C vs.G
MiRNA-146a rs2910164		OR [95% CI]	<i>P</i>	OR [95% CI]	<i>P</i>	OR [95% CI]	<i>P</i>	OR [95% CI]	<i>P</i>	OR [95% CI]	<i>P</i>	OR [95% CI]
PCR-RFLP*	4	<b>0.626</b> [0.453-0.865]	<b>0.004</b>	<b>0.787</b> [0.637-0.972]	<b>0.026</b>	<b>0.726</b> [0.590-0.893]	<b>0.002</b>	<b>0.753</b> [0.581-0.976]	<b>0.032</b>	0.986[0.835-1.164]	0.866	<b>0.795</b> [0.681-0.928]
TaqMan	1	1.015[0.786-1.311]	0.908	1.132[0.887-1.445]	0.320	1.082[0.858-1.364]	0.505	0.924[0.775-1.102]	0.379	0.893[0.755-1.056]	0.185	0.984[0.872-1.111]
China	4	0.715[0.477-1.072]	0.105	0.921[0.779-1.089]	0.334	0.796[0.584-1.048]	0.148	0.813[0.641-1.031]	0.088	0.935[0.828-1.056]	0.278	0.849[0.706-1.021]
India	1	0.684[0.358-1.309]	0.252	0.913[0.549-1.516]	0.724	0.830[0.527-1.305]	0.419	0.709[0.382-1.315]	0.276	1.000[0.617-1.620]	1.000	0.809[0.574-1.138]
MiRNA-499 rs3746444		CC vs.TT		CT vs.TT		CT+CC vs.TT		CC vs.CT+TT		TT+CC vs.CT		C vs.T
PCR-RFLP*	2	0.843[0.497-1.430]	0.527	1.326[0.555-3.164]	0.525	1.258[0.587-2.696]	0.555	0.893[0.598-1.333]	0.579	0.721[0.347-1.496]	0.379	1.159[0.733-1.832]
TaqMan	1	1.081[0.648-1.803]	0.765	0.943[0.776-1.145]	0.552	0.956[0.792-1.152]	0.634	1.098[0.660-1.827]	0.720	1.064[0.877-1.292]	0.531	0.975[0.827-1.149]
China	2	1.028[0.661-1.600]	0.902	1.337[0.650-2.748]	0.430	1.278[0.695-2.349]	0.429	0.983[0.634-1.524]	0.939	0.746[0.359-1.549]	0.432	1.165[0.789-1.720]
India	1	0.819[0.424-1.582]	0.552	0.806[0.398-1.630]	0.548	0.814[0.433-1.529]	0.522	0.948[0.602-1.492]	0.817	1.063[0.654-1.727]	0.805	0.911[0.645-1.287]
MiRNA-196a2 rs11614913		TT vs.CC		CT vs.CC		CT+TT vs.CC		TT vs.CT+CC		TT+CC vs.CT		T vs.C
PCR-RFLP*	2	0.388[0.076-1.970]	0.253	0.788[0.559-1.110]	0.173	0.597[0.261-1.365]	0.221	0.449[0.103-1.956]	0.286	0.939[0.711-1.239]	0.655	0.589[0.230-1.505]
TaqMan	3	<b>0.762</b> [0.625-0.929]	<b>0.007</b>	0.937[0.804-1.092]	0.404	0.872[0.755-1.008]	0.064	<b>0.807</b> [0.707-0.921]	<b>0.001</b>	0.921[0.818-1.037]	0.176	<b>0.871</b> [0.795-0.955]
PCR-LDR*	1	0.886[0.448-1.751]	0.727	1.178[0.625-2.220]	0.613	1.049[0.578-1.901]	0.876	0.791[0.471-1.326]	0.373	0.788[0.487-1.276]	0.333	0.915[0.650-1.288]
China	5	<b>0.787</b> [0.672-0.921]	0.003	0.946[0.822-1.089]	0.439	0.884[0.773-1.010]	0.069	<b>0.817</b> [0.724-0.923]	0.001	0.922[0.827-1.029]	0.148	<b>0.883</b> [0.817-0.954]
India	1	<b>0.167</b> [0.086-0.323]	0.001	0.637[0.374-1.085]	0.097	<b>0.389</b> [0.241-0.628]	0.001	<b>0.209</b> [0.114-0.381]	0.001	0.817[0.510-1.309]	0.401	<b>0.362</b> [0.259-0.506]

Note:

PCR-RFLP: Restriction fragment length polymorphism polymerase chain reaction

PCR-LDR: Polymerase chain reaction-Ligase detection reaction.

## Figures

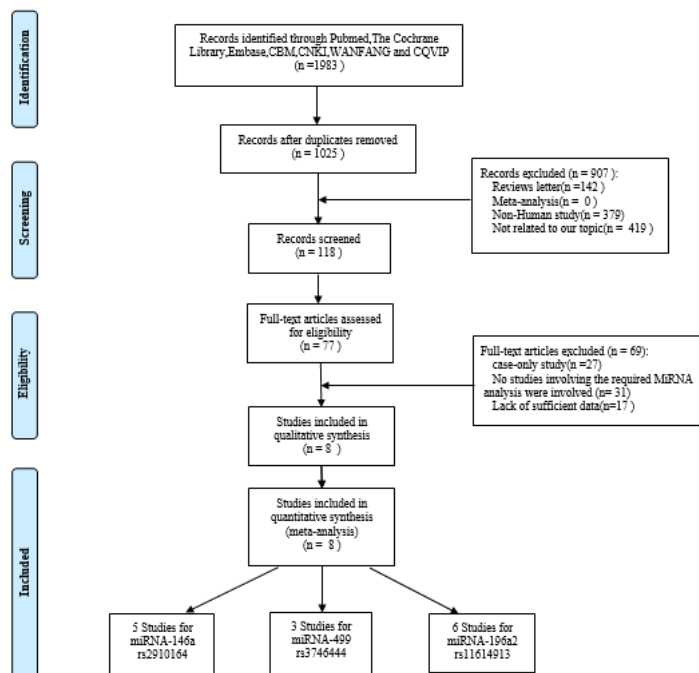


Figure 1

Flow diagram of study selection for this meta-analysis.

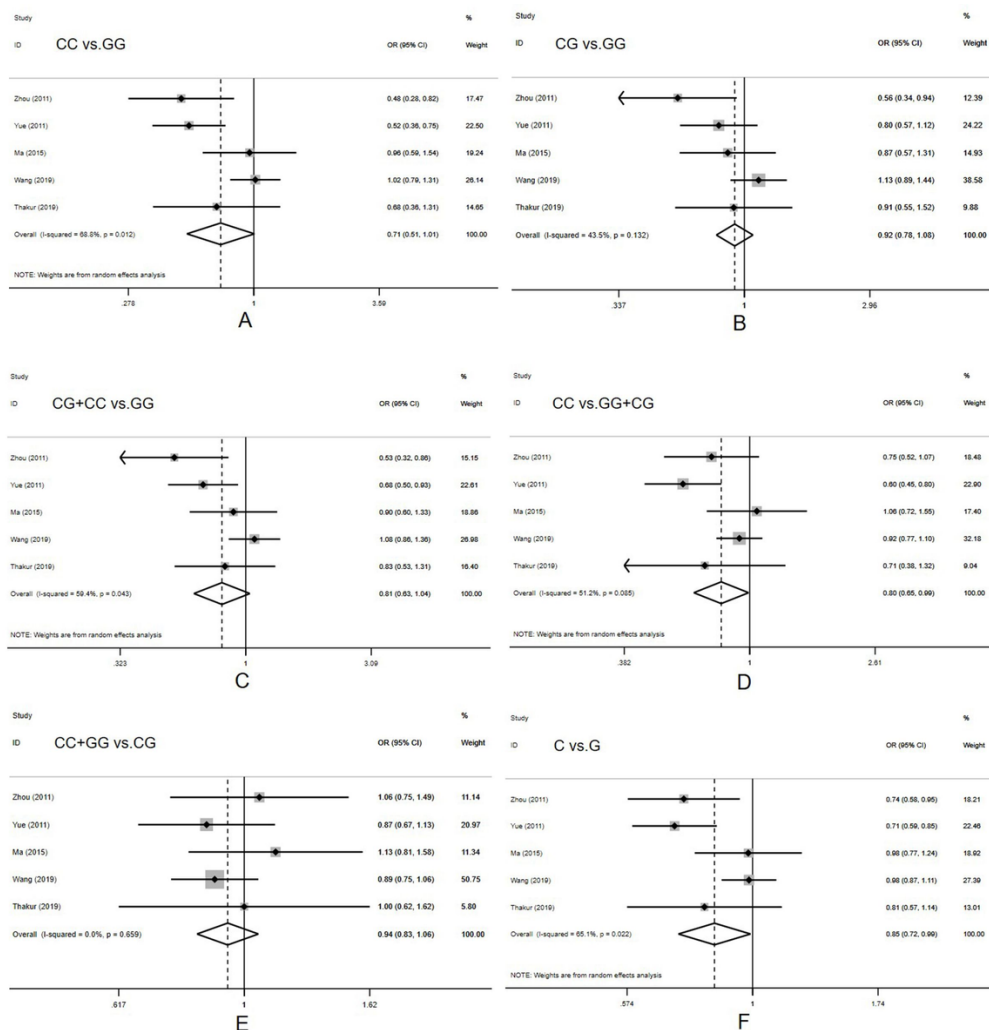
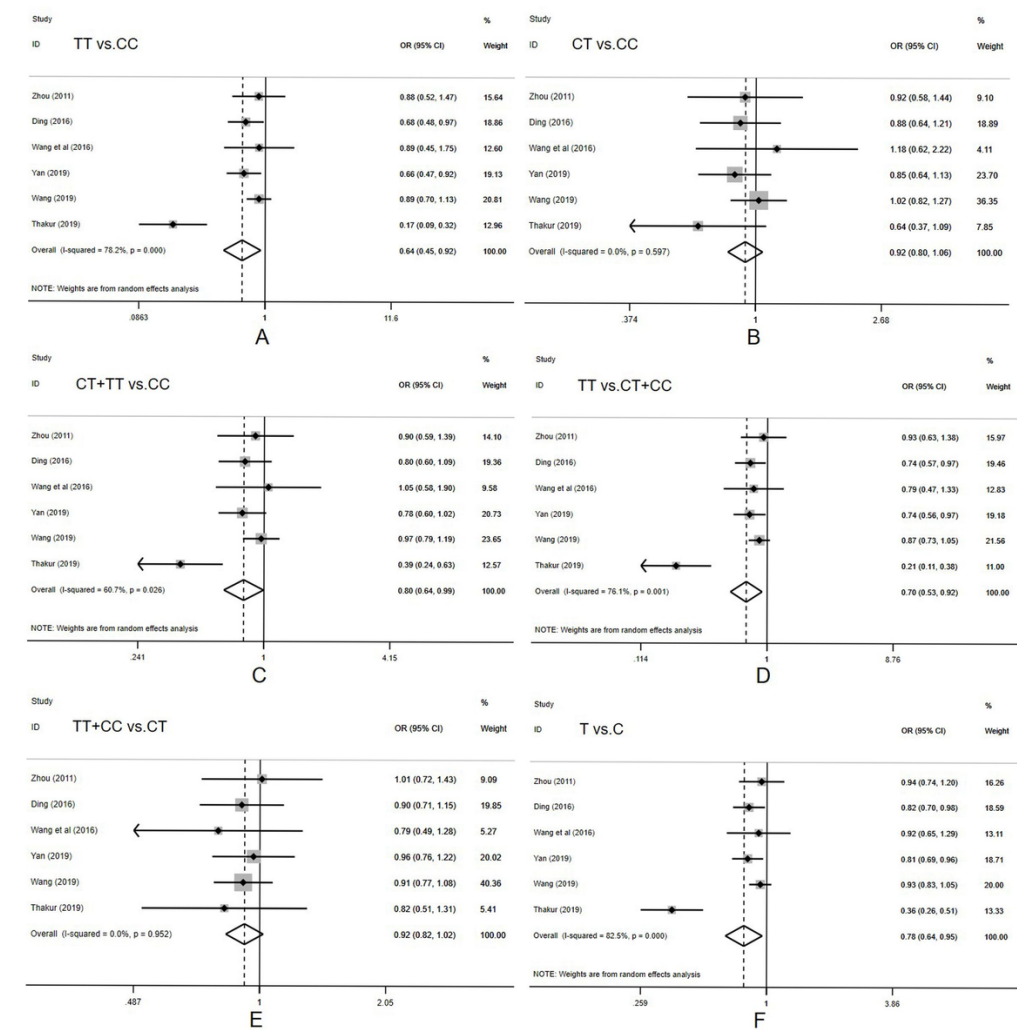


Figure 2

Forest plot for the association between miRNA-146a rs2910164 polymorphism and cervical cancer susceptibility for CC vs. GG (A), CG vs. GG (B), CG+CC vs. GG (C), CC vs. CG+GG (D), CC+GG vs. CG (E) and C vs. G (F) .



**Figure 3**

Forest plot for the association between miRNA-196a2 rs11614913 polymorphism and cervical cancer susceptibility for TT vs. CC (A), CT vs. CC (B), CT+TT vs. CC (C), TT vs. CT+CC (D), CC+TT vs. CT (E) and T vs. C (F) .

Supplementary Files

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- [Table.S1ThePRISMAchecklist.doc](#)