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Original Article

CD192 gene variant and susceptibility to cervical cancer: a meta analysis

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Abstract: Background: Several molecular epidemiological studies have explored the association between G46295A variant of *CD192* gene and cervical cancer susceptibility in distinct populations. However, the results are contradictory. To provide convincing evidence for the association, we performed the present meta-analysis incorporating all case-control studies. Methods and findings: The Cochrane, Google Scholar and PubMed databases were systematically searched to identify the potentially relevant studies. Statistical analyses were performed using R software. Crude odds ratio (OR) was calculated to estimate the risk of cervical cancer. Five case-control studies were considered in the final analysis. Using the homozygous, heterogeneous, allele, dominant, and recessive model, we found the association between *CD192* G46295A variant and cervical cancer was not statistically significant. Conclusions: This meta-analysis shows that the *CD192* G46295A variant may not confer genetic susceptibility towards cervical cancer.

Keywords: *CD192*, polymorphism, cervical cancer

Introduction

Chemokines first reported nearly 30 year ago were basic heparin-binding polypeptides and have proinflammatory and reparative functions [1, 2]. These small (8-10 kd) and inducible cytokines are reportedly engaged in embryogenic, organogenic and homeostatic processes [3]. Chemokines and their receptors as a complex network mediate a series of cancerous phenotypes, including angiogenesis, invasion, tumorigenesis and metastasis [3, 4]. The significant role of chemokines played in multiple biological activities provides novel insights into an association of host-cell genomic variations and human carcinogenesis.

CD192 controls the recruitment of activated macrophages into various types of cancer and it is these active macrophages that exhibit dual biological functions: promoting tumor cytotoxicity on the one hand, and blocking tumor growth and eliminating neoplastic cells on the other hand [5]. A number of cancer-related agents identified as potent tumor promoters or immunosuppressors have been linked with macrophages [6]. Expression of *CD192*, the 7-transmembrane G protein-coupled receptor of CC family ligand CCL2 (corresponds to MCP-1), is regulated via basophils, dendritic cells, mast cells, monocytes/macrophages, NK cells, and T lymphocytes, all of which are immune cells [7-10]. Several women-specific cancers have shown higher expression of *CD192* [11].

Cervical cancer is an extremely invasive disease for women; about 371,000 newly-diagnosed cases accounting for almost 10% of total female cancers have been reported in the past ten years. Delineating the genetic variations associated with *CD192* may help to identify individuals at a higher risk of cervical cancer. A G-to-A single nucleotide polymorphism (G46295A) results in a valine to isoleucine substitution at position 64 within the *CD192* gene [12, 13]. This amino acid transition enables

overexpression of its gene and an extended half-life of CCR2A, a transcribed isoform of *CD192* [14]. We therefore hypothesized that this *CD192* gene variant may contribute to the individual susceptibility towards cervical cancer. Available epidemiological data have shown an increased risk of cervical cancer related to the 46295A allele [15, 16], a finding that was inconsistent with several other studies [17, 18]. In this work, we incorporated all case-control studies into one pooling-dataset and performed a meta-analysis to test the hypothesis and to clarify the association between the G46295A variant and cervical cancer susceptibility.

Materials and methods

Literature source and search strategy

The Cochrane, Google Scholar and PubMed databases were systematically searched by two investigators using the key words 'Chemokine receptor-2', '*CD192*', 'polymorphism', 'variants', and 'cervical cancer', to identify all possibly relevant studies. Additional original articles were identified by hand searching the reference lists of all studies reporting *CD192* gene variants and cervical cancer.

Study selection

The studies were considered eligible when satisfying both of the following two criteria, without any consideration of the language or a minimum of sample size: 1. The authors designed a case-control study where the association of *CD192* gene variant and cervical cancer susceptibility was investigated; 2. The original article must provide detailed genotype frequency or related information to estimate risk of cervical cancer (odds ratio).

For the studies on the genetic variant of interest and cervical cancer employed the same population, we selected the largest study with extractable genetic data.

Data extraction

Three investigators independently extracted authors, country of origin, ethnicity, publication years, number of cases and controls, genotyping assay, matching and adjustment factors, and genotype frequency for all eligible studies. The extracted data were then cross-checked and disagreements, if any, were resolved by discussion.

Statistical analysis

Crude odds ratios were calculated to assess the association between the G46295A variant and cervical cancer susceptibility. The genetic models tested in most meta-analyses were also assumed in the present analysis. Significance of combined odds ratios was determined using the Z test. The extent of inconsistency between studies, namely heterogeneity, was measured using the χ^2-based Q test and I^2 metric, with I^2 <50% indicating absence of between-study heterogeneity [19]. The pooled odds ratios were summarized using the Mantel-Haenszel method when there was no obvious heterogeneity [20]; alternatively, the DerSimonian and Laird method was used [21]. Sensitivity analysis was performed to examine the stability of the combined estimates. Begg's test and Egger's test were utilized to assess the publication bias in this analysis [22]. Statistical analyses were performed using R software (R version 2.15.0, the R Foundation for Statistical Computing). All two-sided *P* values at 5% were considered significant.

Results

Study characteristics

Systematic search in electronic databases and reference lists yielded 91 records. Evaluating the titles and abstracts excluded 70 records apparently irrelevant to *CD192* gene variants and cervical cancer, and we were left 21 articles. We subsequently reviewed the texts throughout and discarded another 17 articles that had no genetic data or was designed as a case-case study. Our final pooling dataset therefore included four articles containing five independent populations, providing 1,929 cancerous cases and 1,827 healthy controls [15-18].

Table 1 summarizes the main characteristics of studies that analyzed association between the *CD192* G46295A variant and cervical cancer. The five studies finally considered in this analysis consisted of two Caucasian studies, one Asian study, one African study and one study of mixed populations. The sample size varied substantially, ranging from 100 to 1,580.

Table 1. A summary of studies that analyzed association between the *CD192* G46295A variant and cervical cancer

Study	Year	Study country	Ethnicity	No. of cases-controls	Genotyping assay	Matching	Adjustment
Zheng et al	2006	Sweden	Caucasian	149-148	PCR	Age, date of sampling of smear	/
Ivansson et al	2007	Sweden	Caucasian	1294-286	TaqMan, RT-PCR	/	Family structure
Ding et al	2013	China	Asian	40-60	PCR-SSP	Age, ethnicity	/
Chatterjee et al	2010	Africa	African	106-305	PCR-SSP	Age, ethnicity, domicile status	Smoking status, ethnicity
Chatterjee et al	2010	Africa	Mixed	340-1073	PCR-SSP	Age, ethnicity, domicile status	Smoking status, ethnicity

RT-PCR: real time-PCR; PCR-SSP: PCR-sequence-specific primers.

Table 2. Overall and stratified analyses results with the random-effects model

Groups	OR (95% CI)	*P* heterogeneity (I^2 statistic)	No. of subjects (cases-controls)	No. of studies
Homozygous model (AA versus GG)	1.71 (0.52, 5.57)	0.0007 (0.793)	1328-1290	5
Heterozygous model (AG versus GG)	1.79 (0.59, 5.41)	<0.0001 (0.968)	1881-1827	5
Allele model (A versus G)	1.68 (0.86, 3.29)	<0.0001 (0.953)	3858-3744	5
Dominant model (AA + AG versus GG)	2.12 (0.72, 6.23)	<0.0001 (0.969)	1929-1872	5
Recessive model (AA versus AG + GG)	1.27 (0.46, 3.50)	0.0041 (0.738)	1929-1872	5

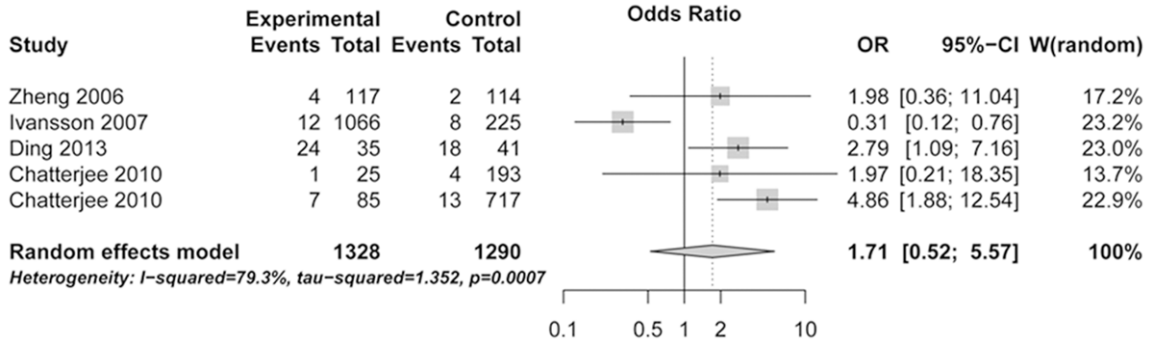

Figure 1. ORs of overall cervical cancer risk associated with the *CD192* G46295A variant under the homozygote model by random effects for each of the published studies. For each study, the estimates of OR and its 95% CI were plotted with a box and a horizontal line. The symbol filled diamond indicates pooled OR and its 95% CI.

Genotyping assay, matching and adjustment factors were also differently used across the studies.

Meta-analysis results

Main meta-analysis results are presented in **Table 2**. We first used the homozygous model to test the association of *CD192* G46295A variant and cervical cancer susceptibility, without any statistical evidence of a significant association observed (odds ratios 1.71, 95% confidence interval 0.52-5.57). We then analyzed the heterogeneous model following the initially null finding, and found similar results (odds ratios 1.79, 95% confidence interval 0.59-5.41). Subsequent analyses using the allele, dominant and recessive model all revealed that the *CD192* G46295A variant was not associated with risk of cervical cancer. The forest plots for the homozygous and recessive model are shown in **Figures 1**, **2**, respectively.

Heterogeneity test and sensitivity analysis

Heterogeneity was apparently indicated in this analysis when all studies were combined. Sensitivity analysis revealed that the two Caucasian studies in Sweden contributed a large part to the apparent heterogeneity, suggesting ethnicity is the main cause of heterogeneous results.

Bias diagnostics

Funnel plots of all genetic models were visually symmetrical, and the Egger's test provided no evidence of obvious publication bias in this study. Funnel plot of the homozygous model is shown in **Figure 3** (t = 1.48, P = 0.192).

Discussion

In the present meta-analysis, we systematically evaluated the association of *CD192* G46295A variant and cervical cancer susceptibility for 1,929 cancerous cases and 1,827 healthy controls from all case-control studies published in the past decade. Odds ratios of genetic models tested were all insignificant, suggesting the presence of G46295A alleles or genotypes did not represent a risk factor for cervical cancer. Although this is the largest assessment of the literature regarding *CD192* G46295A variant and cervical cancer conducted to date, the findings should be taken as preliminary and require validating in further investigations.

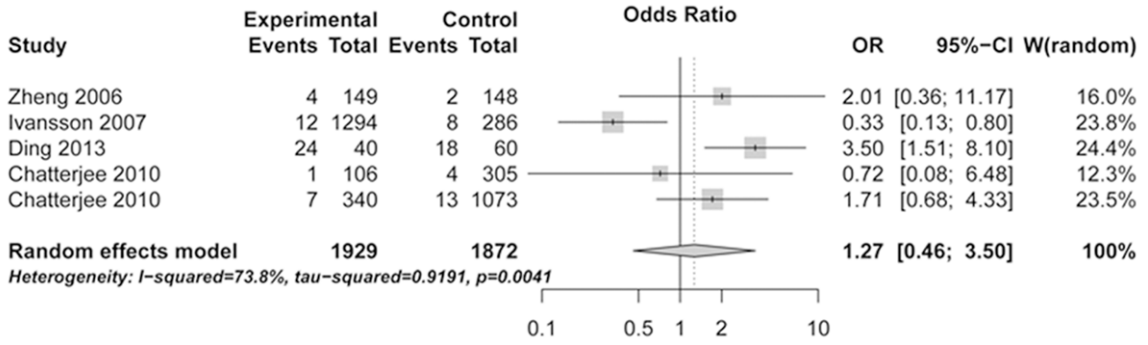

Figure 2. ORs of overall cervical cancer risk associated with the *CD192* G46295A variant under the recessive model by random effects for each of the published studies. For each study, the estimates of OR and its 95% CI were plotted with a box and a horizontal line. The symbol filled diamond indicates pooled OR and its 95% CI.

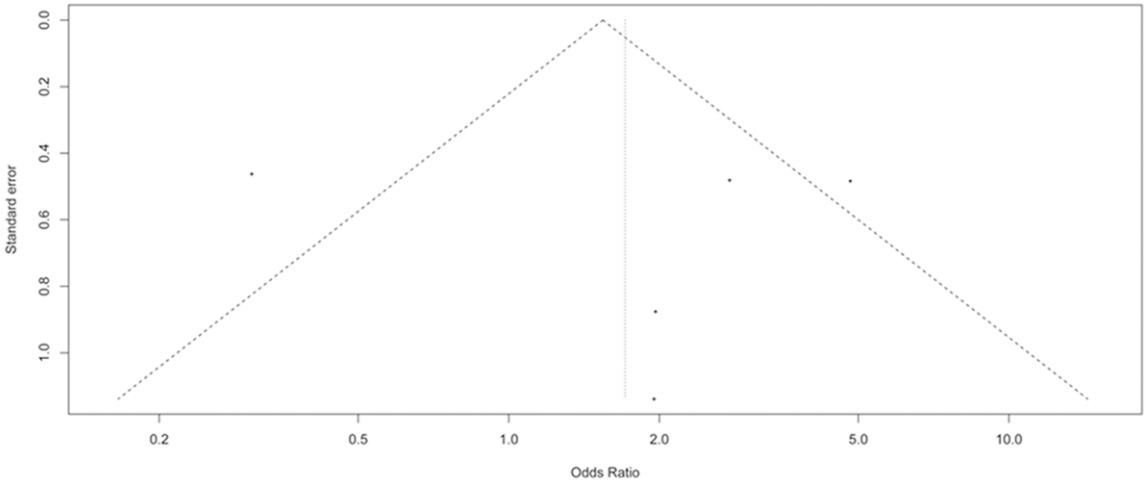

Figure 3. Funnel plot analysis to detect publication bias. Each point represents a separate study for the indicated association.

The *CD192* interacts with many ligands of human CC family and is expressed on a variety of immune cells. Aberrant expression of *CD192* leads to malignant progression into human cancer. Lack of *CD192* expression has been described in ovarian cancer [23]. Earlier research about 20 years ago has linked genetic variability in the chemokine receptors, including the *CD192* gene, to inflammation-related and autoimmune disorders [24]. The G46295A variant within the first transmembrane domain of *CD192* has been reported to increase risk of many malignancies, such as bladder cancer, breast cancer, prostate cancer and hepatocellular carcinoma [25-28]. Smith and colleagues also provide evidence for the effective functioning of this variant in AIDS by delaying the progression for 2-4 years [29], which is in agreement with another larger study published almost at the same time [30]. The experimental evidence along with the epidemiological data suggests that the G46295A variant may have a significant role in the development of cervical cancer, as reported in other human cancers.

The involvement of the G46295A variant in cervical cancer first was described in a case-case study of a Portuguese population by Coelho and colleagues, who found that the A allele was protective against the invasive progression of cervical cancer [31]. This finding was subsequently replicated by several genetic association studies, in which the same allele was found to be associated with a significantly higher risk of cervical cancer in Chinese and African descendants [15, 16]. The 46295A is not an ethnic-specific allele, but present in Caucasians, Asians, and Africans, with a frequency ranging from 0.10 to 0.25 [24]. It is thus possible that the G46295A variant is a potentially risk factor

for any ethnicity and the individuals harboring its genotypes or alleles are at an increased risk of cervical cancer. This hypothesis seems to contradict the negative findings implicated in this meta-analysis. However, the relatively sample size and different design of each study reporting a strong association between the G46295A variant and cervical cancer may lead to a biased or false-positive result. Therefore, further analyses are necessary to provide convincing evidence.

Heterogeneity is an evitable problem when performing a meta-analysis. It may be the result of dissimilarities in methodology, experiment and design. In this analysis, we observed significant between-study heterogeneity that to a large extent should be attributed to ethnicity. As many factors may lead to nonhomogeneous results, the exact cause remains to be identified.

There are several limitations in this study. First, the overall analysis did not reveal any statistical significance for the G46295A variant, but we can't rule out the possibility that the variant has effect modification of cervical cancer in some specific ethnicity. Second, accumulating data have shown increased risk of cervical cancer associated with the 46295A allele, a finding that was not observed in our meta-analysis, which may be underpowered to detect the actual association as a result of the inadequate sample. Third, our results are on the basis of unadjusted estimates, as the published studies provided crude estimates without any adjustment, or presented results adjusted by different confounders. Therefore, whether the estimates become significant after adjustment of the same covariants merit future investigations.

In summary, the present meta-analysis shows that G46295A variant of the *CD192* gene is unlikely to have relevance to the progression of cervical cancer. Although our finding contradicts previous experimental and epidemiological data, this study may provide novel evidence against an association between the G46295A variant and cervical cancer. Future larger study is warranted to validate our findings.

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Disclosure of conflict of interest

None.

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