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Original Article

Genetic association between the *HIF-1α* P582S polymorphism and cervical cancer risk: a meta analysis

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Abstract: Background: Hypoxia-inducible factor-1 alpha (*HIF-1α*) P582S polymorphism has been reported to increase transactivation capacity of *HIF-1α*, which is prone to tumorigenesis. Several published case-control studies on the association between P582S polymorphism and cervical cancer have shown mixed results. In this study, we chose to perform a meta-analysis to assess the association. Methodology/Principal findings: We conducted a meta-analysis consisting of four studies with a total of 846 cases and 991 controls. All data were collected and overall comparison was performed among all subjects. Using the fixed effects model, the homozygous and the recessive models showed a significant increase in the risk of cervical cancer (the pooled OR=6.32, 95% CI=2.28-17.55, Phet=0.348; the pooled OR=5.86, 95% CI=2.13-16.11, Phet=0.394 respectively). Publication bias was not significantly indicated in this analysis. Conclusions: This meta-analysis demonstrates that *HIF-1α* P582S polymorphism may be associated with the risk of cervical cancer.

Keywords: *HIF-1α*, cervical cancer, polymorphism, risk

Introduction

Hypoxia-inducible factor-1 (HIF-1) plays a pivotal role in cellular responses to hypoxia, including the regulation of genes with involvement in angiogenesis, cell survival, energy metabolism, apoptotic process and proliferation [1, 2]. HIF-1 consists of α and β subunits [3]; the subunit is prone to proteasomal degradation (von Hippel-Lindau tumor suppressor protein) under normal circumstances, but hypoxia facilitates stabilization [4]. Lack of oxygen causes the enzymatic inactivation of the prolyl hydroxylase domain-containing proteins, leading to accumulated *HIF-1α* and translocation to the nucleus in which it combines with HIF-1β to form HIF-1. Overexpression of *HIF-1α* has been reported in a variety of cancers [5, 6], and investigations into the pathogenesis of human cancer at different sites demonstrate there is an evident association between mortality and overexpressed *HIF-1α* [1]. The aforementioned evidence implies that *HIF-1α* is very likely to have biological involvement in cancer, including cervical cancer.

Many single nucleotide polymorphisms (SNPs) have been identified in the oxygen-dependent degradation domain of the *HIF-1α* gene. P582S polymorphism (also known as C1772T, rs11549465) causes an amino acid substitution from proline to serine [7]. Such a substitution serves as a transcription factor associated with enhanced transactivation activity of *HIF-1α* under both normoxic and hypoxic conditions, as compared with the CC genotype; cancer patients with the CT genotype have significantly increased numbers of microvessels compared to those with CC genotype [8]. We hypothesized that the P582S genotypes may modulate the risk of cancer. A number of researchers had significant concerns with the genetic effects of P582S polymorphism on the initiation of cancer [9-12]. The relationship of this polymorphism and cervical cancer was also evaluated in several previous studies [13-15]. But these studies showed mixed results, with the most recent findings suggesting that cervical cancer risk should not be assigned to the presence of P582S genotypes [16]. Notably, the role of P582S polymorphism in the *HIF-1α* pathway remains to be elucidated. To our knowledge, no

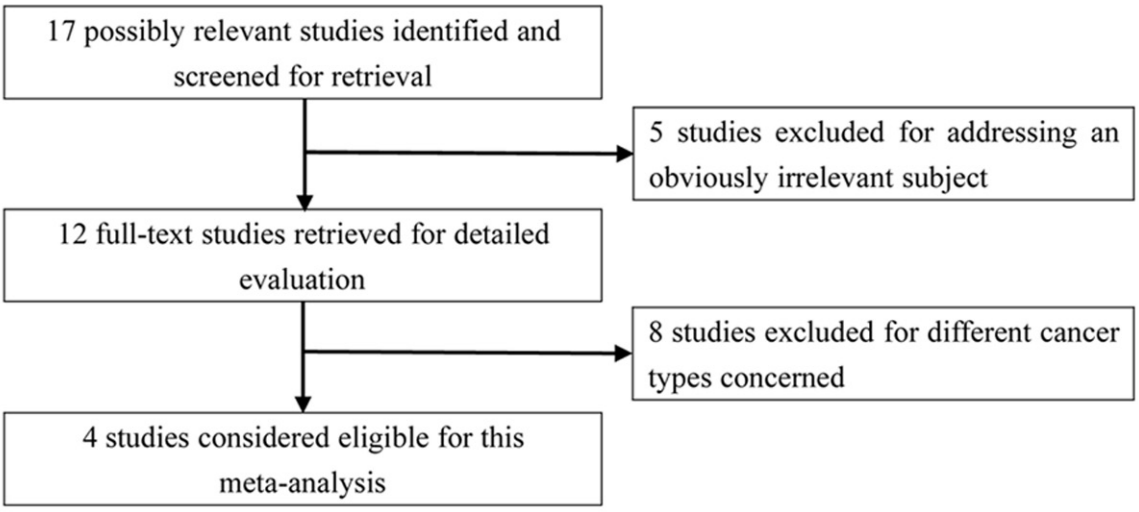

Figure 1. Flow chart for the literature search in the meta-analysis.

Table 1. Studies on the association of P582S polymorphism and cervical cancer risk

Study	Year	Ethnicity of subjects	Source of controls	Cancer cases	No. of cases/ controls	Cervical cancer cases			Controls		
						CC	CT	TT	CC	CT	TT
Konac [13]	2007	Caucasian	Hospital	Cervical cancer	32/107	10	14	8	68	37	2
Chai [14]	2010	Asian	Hospital	Cervical cancer	97/117	65	25	7	94	21	2
Kim [15]	2011	Asian	Hospital	Cervical cancer	199/214	177	22	0	187	27	0
Fu [16]	2013	Asian	Hospital	Cervical squamous cell carcinoma	518/553	467	49	2	492	60	1

meta-analysis has reported the association of interest. Therefore, we chose to perform a meta-analysis to assess the association between P582S polymorphism and cervical cancer risk.

Methods

Identification of eligible studies

We carried out a literature search in the PubMed and EMBASE up to January 10, 2014, to identify the possibly relevant studies using the combinations of "*HIF-1α*", "polymorphism", "C1772T", "P582S", "rs11549465" and "cervical cancer". Although there was no restrictions, we only obtained English and Chinese language articles regarding the association of P582S polymorphism and cervical cancer risk. The reference lists of all studies were checked for additional relevant articles. Studies that fulfilled the following conditions were considered eligible: 1) investigated the role of P582S polymorphism in cervical cancer risk; 2) had genotype frequency of TT, CT, and CC that facilitated successful calculation of odds ratio (OR) with its 95% confidence interval (95% CI); 3) used a case-control design; However, we excluded the studies if they were published as abstracts, reviews articles, and case-case studies.

Data extraction

After all eligible articles were identified by two investigators according to the pre-described inclusion criteria, the following data were extracted from each of them: first author's name, publication year, ethnicity of subjects (Asian or Caucasian), source of controls (population- or hospital-based controls), cancer cases (cervical cancer or cervical squamous cell carcinoma), total number of cases and controls, and genotyping data. Discussion among investigators was conducted once there were discrepancies.

Statistical analysis

OR with 95% CI was calculated to evaluate the association between P582S polymorphism and cervical cancer risk. The calculation of pooled

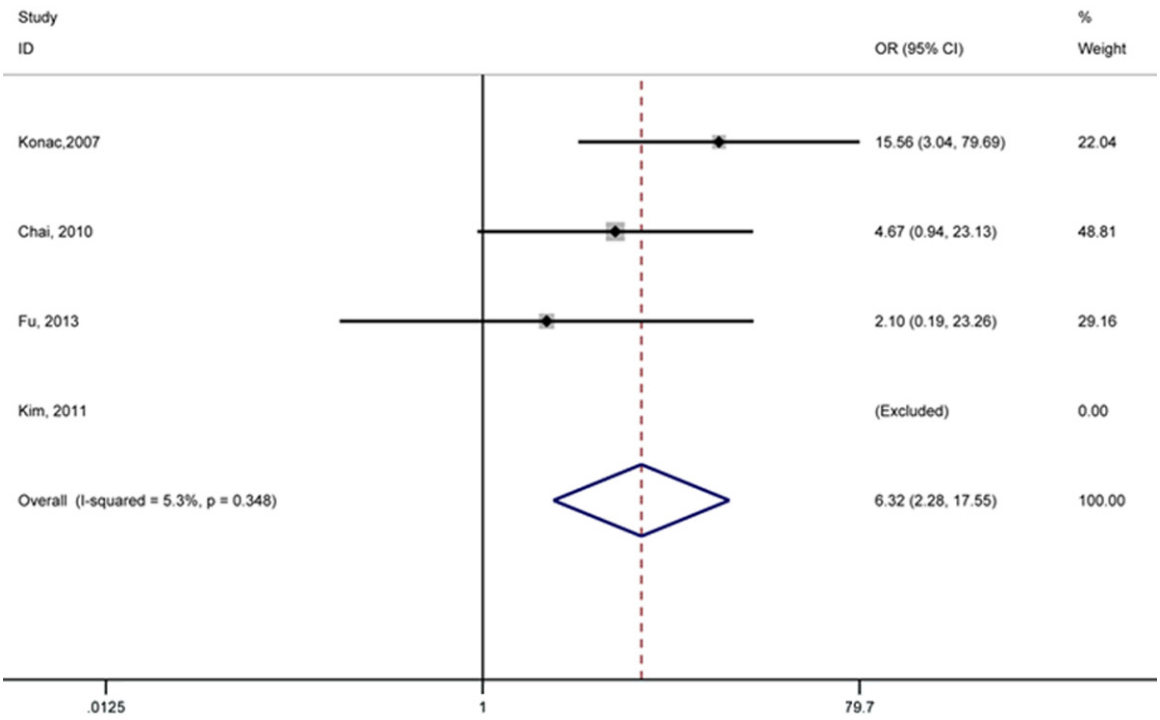

Figure 2. Forest plot for the overall association between *HIF-1α* P582S polymorphism and risk of cervical cancer using the TT vs. CC model.

ORs was first performed for the homozygous model (TT vs. CC), followed by the heterogeeous model (CT vs. CC), dominant model (TT+CT vs. CC) and recessive model (TT vs. CT+CC). Heterogeneity assumptions were checked using the Chi square-test based on Q-statistic [17] and I^2 index [I^2=100 % × (Q - df)/Q] [18], with a *P* value above 0.05 and I^2 bellow 50% being deemed significant. In case of absence of heterogeneity, the fixed effects model was used [19], while if there was obvious heterogeneity between studies, the random effects model was selected [20]. One-way sensitivity analysis was carried out to evaluate the stability of the combined results. Publication bias was tested using the funnel plot described by Begg and the linear regression test by Egger [21]. To determine the deviation from Hardy-Weinberg equilibrium (HWE), we applied the goodness-of-fit X^2-test. All statistical analyses were performed using STATA software (version 12.0; Stata Corporation, College Station, TX). A *P* value < 0.05 was taken to be significant.

Results

Study characteristics

The electronic and manual literature search helped to identify 17 studies. Of these, four studies were ultimately included after excluding those on an obviously irrelevant subject or on different cancer types. The procedures of study selection are represented graphically in a flow diagram shown in **Figure 1**. The eligible studies had 846 cases and 991 controls [13-16]. Among these, only Konac et al. [13] examined the association between P582S polymorphism and cervical cancer risk among Caucasians, with the remainder having Asians (**Table 1**). The *P* values yielded using goodness-of-fit X^2-test showed that the distribution of genotypes in controls was all consistent with HWE (greater than 0.05, data not shown).

Quantitative synthesis

With the aid of the fixed effects model, the homozygous and the recessive models showed a significant increase in the risk of cervical cancer (the pooled OR=6.32, 95% CI=2.28-17.55, Phet=0.348, **Figure 2**, **Table 2**; the pooled OR=5.86, 95% CI=2.13-16.11, Phet=0.394, **Figure 3**, **Table 2**, respectively). Using the heterozygous model and the dominant model, we did not see any association between cervical cancer and the polymorphism tested (the pooled OR=1.05, 95% CI=0.80-1.38, Phet=0.278, **Table 2**; the pooled OR=1.13, 95%

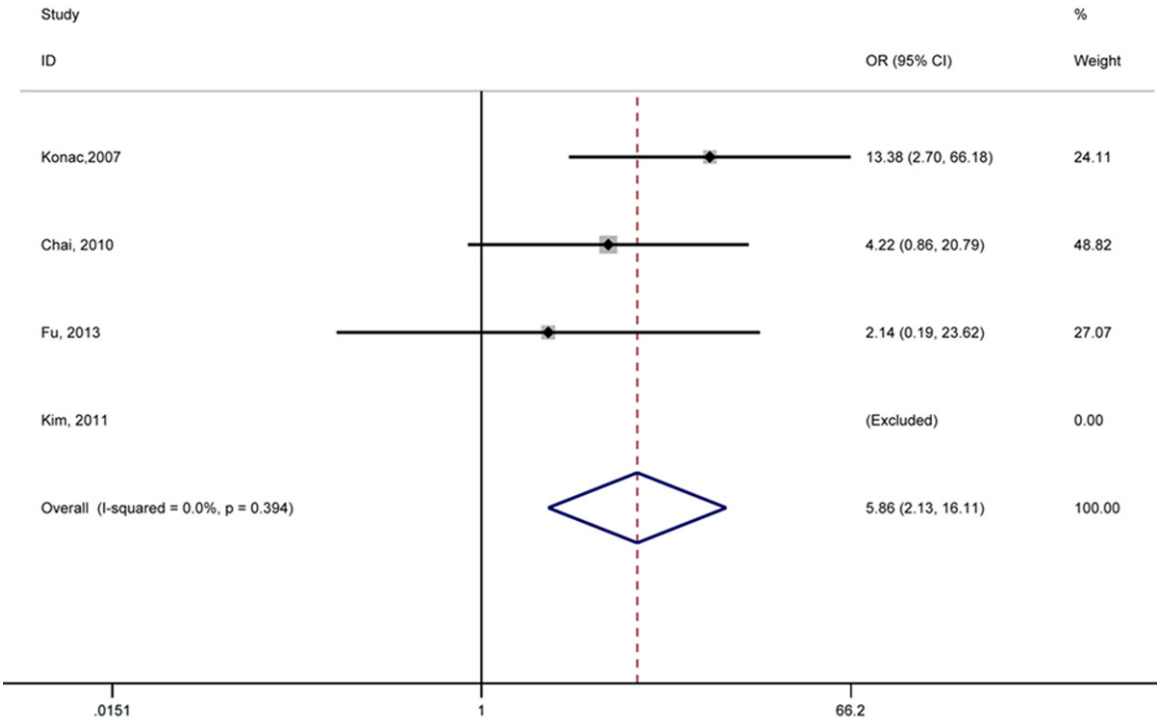

Figure 3. Forest plot for the overall association between *HIF-1α* P582S polymorphism and risk of cervical cancer using the TT vs. CT+CC model.

Table 2. Summary of meta-analysis for P582S polymorphism

Comparisons	Cases	Controls	Heterogeneity test		Model	Summary OR (95% CI)
			I^2	*P*		
Total	846	991				
TT vs. CC	753	851	5.3%	0.348	Fixed	6.32 (2.28, 17.55)
CT vs. CC	939	1131	22.0%	0.278	Fixed	1.05 (0.80, 1.38)
TT + CT vs. CC	973	1141	51.0%	0.106	Fixed	1.13 (0.87, 1.47)
TT vs. CT + CC	863	996	0.0%	0.394	Fixed	5.86 (2.13, 16.11)

CI=0.87-1.47, Phet=0.106, **Table 2**, respectively). For all genetic models adopted, there was no significant heterogeneity across studies (**Table 2**).

Publication bias and sensitivity analyses

Potential publication bias of the included studies in this meta-analysis was determined using Begg's funnel plots and Egger's test. The dots (each dot represents an individual study) of the funnel plots were symmetrically distributed. The statistical evidence provided by the Egger's test indicated that there was no publication bias in this study ($P > 0.05$). The omission of the eligible studies one by one did not result in qualitative change in the pooled ORs, suggesting the results of this meta-analysis were stable.

Discussion

In our meta-analysis of four published articles, the *HIF-1α* P582S polymorphism located in exon 12 was shown to cause a significantly elevated cervical cancer risk. The population appeared to be more susceptible to such cancer when carrying the TT genotype. Considering the limited number of studies included and the complex role of SNPs in the oxygen-dependent degradation domain of the *HIF-1α* gene in cervical carcinogenesis, further studies evaluating the impact of P582S polymorphism on cervical cancer will be required. On the other hand, this study provides the evidence of an increased risk for cervical cancer in women with the *HIF-1α* P582S polymorphism, which may have important implications for the understanding of the SNP's role in the progression of cervical cancer.

Several individual studies have concentrated on the relationship between P582S polymorphism and cervical cancer. One group led by Konac presented the first evidence of an obviously increased risk of cervical cancer attributable to the carriage of T allele (OR=3.84, 95% CI, 1.65-8.93) [13]. But this valuable discovery can only be suitable for the populations with Caucasian ethnicity. The significant association was replicated in a later assessment covering a Chinese population, which is slightly larger than the previous study [14]. Conversely, another two studies, including the one with the most subjects among the four independent studies published recently, indicated no association attributed to the P582S polymorphism either in the Korean or the Chinese patients [15, 16]. Common reasons for this discrepancy include the relatively small sample size, ethnically different populations, histologically different cancer cases, methodological methods and non-standardized threshold for control populations.

HIF-1 is a transcription factor playing a pivotal role in the regulation of angiogenesis and tumorigenesis through activating transcription of the vascular endothelial growth factor (VEGF) gene, which is a biomarker with its expression regulated by *HIF-1α* [8]. Increased expression levels of *HIF-1α*, in comparison to the adjacent histologically benign tissue, have recently been described in human malignancies such as breast and ovarian (two types of gynecologic cancer) [22, 23]. More direct evidence for a role of *HIF-1α* overexpression in tumor was also reported in early-stage invasive cervical cancer [24]. In addition, molecular genetic studies have provided evidence that SNPs in genes involved in the formation of new blood vessels and tumor growth modulate their genetic susceptibility to cervical cancer [25, 26]. Taken together, these data indicate that the *HIF-1α* polymorphisms may have a role in risk of cervical cancer.

Being a first meta-analysis addressing the association between *HIF-1α* P582S polymorphism and cervical cancer risk, our study presents relatively convincing data on the role of P582S polymorphism in the common gynecologic malignancy. However, several limitations should be considered in interpreting the results. Firstly, we found a significantly increased risk of cervical cancer using the homozygous and the recessive models, the estimate of effect size was actually based on three studies (**Figures 2**, **3**), while the evaluation of the heterozygous model and the dominant model was conducted in all four studies. It should be noted that the significant results showed in the former two models were lost in the latter models. Therefore, it is worthwhile to confirm the association in a large-sampled study. Secondly, the current data only allow overall comparison among all subjects. Analyses stratified by ethnicity or other confounding factor are necessary to identify whether they act as covariates in cervical cancer risk.

In conclusion, we have shown that P582S polymorphism of the *HIF-1α* gene is likely to confer increased susceptibility to cervical cancer. Although this finding may expand current understanding of the pathogenesis of this cancer, the association requires further investigation.

Disclosure of conflict of interest

None.

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