

# Circulating interleukin-10 levels and human papilloma virus and Epstein–Barr virus-associated cancers: evidence from a Mendelian randomization meta-analysis based on 11,170 subjects

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**Abstract:** Recent studies have showed interleukin 10 (IL-10) is a critical cytokine that determines antiviral immune response and is related to virus-associated cancers. However, whether genetically elevated circulating IL-10 levels are associated with the risk of human papilloma virus and Epstein–Barr virus-associated cancers (HEACs) is still unclear. Mendelian randomization method was implemented to meta-analyze available observational studies by employing *IL-10* three variants (–592C>A, –819C>T, and –1082A>G) as instruments. A total of 24 articles encompassing 11,170 subjects were ultimately eligible for the meta-analysis. Overall, there was a significant association between *IL-10* promoter variant –1082A>G and HEACs under allelic and dominant models (both  $P<0.01$ ). Subgroup analysis by cancer type indicated that the risk estimate of –1082A>G was significant for nasopharyngeal cancer under allelic, homozygous genotypic and dominant models (all  $P<0.001$ ). Moreover by ethnicity, carriers of –1082G allele had a 74% increased risk for nasopharyngeal cancer in Asians under dominant model (odds ratio [OR]=1.737; 95% confidence interval [CI]: 1.280–2.358;  $P<0.001$ ). In further Mendelian randomization analysis, the predicted OR for 10 pg/mL increment in IL-10 levels was 1.14 (95% CI: 1.01–16.99) in HEACs. Our findings provided strong evidence for a critical role of genetically elevated circulating IL-10 levels in the development of HEACs, especially in Asian population and for nasopharyngeal cancer.

**Keywords:** interleukin-10, human papilloma virus, Epstein–Barr virus, meta-analysis, Mendelian randomization

## Introduction

There is growing recognition that viruses are capable of causing cancer in humans, and approximately 15% of all human malignancies are estimated to be attributable to viruses, creating a major global health burden.<sup>1</sup> Among various cancer viruses, human papilloma virus (HPV) and Epstein–Barr virus (EBV) are exhaustively investigated and are considered to account for 38% of all virus-associated cancers.<sup>2</sup> HPV infection is associated with more than 90%<sup>3</sup> and 60%<sup>4,5</sup> cases of cervical and oropharyngeal cancers, respectively, and in contrast, EBV is associated with nearly 90% of nasopharyngeal cancer,<sup>6,7</sup> Burkitt's,<sup>8</sup> and Hodgkin's lymphoma.<sup>5,7,9</sup> Moreover, the two viruses could also exhibit synergistic or cooperative effects on carcinogenesis. For example, EBV is deemed as a “helper virus” for HPV-induced carcinogenesis.<sup>10</sup> Coinfection of HPV and EBV was observed in 30%–50% of patients with oral cancer<sup>11,12</sup> and cervical cancer.<sup>13,14</sup> Although the obvious association between HPV/EBV and human papilloma virus and



Epstein–Barr virus-associated cancers (HEACs) has been universally accepted, the inherited procancer mechanisms so far remain unclear. It should be pointed out that the infection of HPV and EBV affects more than 40% of general population; however, only a small proportion of infected cases develop cancer.<sup>15</sup> Interindividual differences of innate antiviral immunity that is affected by hereditary factors might be involved in the underlying pathological process of HEACs.<sup>16</sup>

Among the antiviral-relevant immune factors, interleukin-10 (IL-10) is a key cytokine that determines viral clearance or persistence<sup>17</sup> and is involved in carcinogenesis.<sup>18,19</sup> Recent studies have observed a marked high level of circulating IL-10 in patients with HEACs and its association with poor prognosis.<sup>20,21</sup> Emerging evidence suggested that interindividual differences in circulating IL-10 levels might be due to the polymorphic defects of *IL-10*. Recently, three variants located within *IL-10* promoter region, viz,  $-592C>A$  (rs1800872),  $-819C>T$  (rs1800871), and  $-1082A>G$  (rs1800896), have been well defined in association with the changes of IL-10 production.<sup>22,23</sup> It is, therefore, reasonable to hypothesize that if IL-10 is involved in the carcinogenesis process of HEACs, the inherited genetic determinants that alter IL-10 production should affect cancer susceptibility in the direction and magnitude predicted by its circulating levels.

Mendelian randomization approach, which is based on Mendel's second law, uses measured variation in genes of known function to examine the effect of a modifiable exposure on disease in observational studies.<sup>24,25</sup> This method could partially provide evidence for the causal nature of the target phenotype influenced by genetic defects. To test the hypothesis that genetically elevated levels of IL-10 due to *IL-10* genetic defects cause an increased risk of cancer, in this study, we first decided to perform a meta-analysis to evaluate the association of *IL-10* three variants with both circulating IL-10 levels and the risk for HEACs. If the variant under study is found to be predictive of both cancer and circulating IL-10 levels, Mendelian randomization approach will be further implemented to test the possible association of circulating IL-10 levels with HEACs.

## Materials and methods

This meta-analysis was undertaken according to the guidelines set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Table S1).

### Search strategy

A literature search for observational studies investigating the association between *IL-10* three variants ( $-592C>A$

[rs1800872],  $-819C>T$  [rs1800871], and  $-1082A>G$  [rs1800896]) and all types of HEACs was conducted of PubMed and Google Scholar databases covering the period from the earliest possible year to August 1, 2015. Subject terms used for the search were: “interleukin 10”, “interleukin-10”, “IL 10”, “IL-10”, “oral or mouth cancer”, “nasopharyngeal cancer”, “oropharyngeal cancer”, “Hodgkin or Burkitt lymphoma”, “cervical or vaginal or vulvar cancer”, “anus or anal cancer”, combined with “polymorphism”, “genetic”, “variant”, “mutation”, “allele”, or “genotype”. The reference lists of all the retrieved articles as well as those of reviews on the same topic were also searched to supplement the additional missing articles. Searching results was limited to studies with a case–control design and articles published in the English language.

### Trial selection

Two investigators (Kai Qu and Ming Zhang) independently obtained the full texts of potentially eligible articles based on the titles and abstracts. If necessary, we emailed the corresponding authors to avoid double counting of participants recruited in more than one publication. In case of more than one publication from the same study population, we abstracted data from the most recent or most complete publication.

### Inclusion/exclusion criteria

For inclusion, the studies should strictly fulfill the following inclusion criteria (all points must be satisfied for inclusion): 1) clinical endpoint (dependent variable): HEACs including oropharyngeal cancer, nasopharyngeal cancer, cervical cancer, Hodgkin's lymphoma, and Burkitt's lymphoma; 2) study design: either retrospective or prospective case–control design; and 3) independent variables: the genotype and/or allele counts of at least one of *IL-10* three variants ( $-592C>A$ ,  $-819C>T$ , and  $-1082A>G$ ). Studies were excluded (one point was sufficient for exclusion) if they investigated the gene function, disease progression, severity, and the response to treatment or survival. Additionally, conference abstracts, case reports or series, editorials, narrative reviews, meta-analysis, and the non-English articles were also excluded.

### Data extraction

Two investigators (Kai Qu and Ming Zhang) independently extracted data using a standardized Excel template. Disagreements were resolved by consensus or by a third investigator (Wenquan Niu). Data were collected on the

first author, year of publication, ethnicity and country of the study population, cancer type, case–control status, study design, sample size, the genotypes/alleles of *IL-10* three variants (–592C>A, –819C>T, and –1082A>G) between cases and controls, and characteristics of participants, if available, including age, sex, body mass index, smoking, drinking, family history of cancer, and virus infection status.

## Statistical analysis

In this meta-analysis, three genetic models of inheritance were performed for *IL-10* variants, including allelic model (the A allele versus the C allele for –592C>A SNP [single nucleotide polymorphism]; the T allele versus the C allele for –819C>T SNP; and the G allele versus the A allele for –1082A>G SNP), homozygous (the AA genotype versus the CC genotype for –592C>A SNP; the TT genotype versus the CC genotype for –819C>T SNP; and the GG genotype versus the AA genotype for –1082A>G) and dominant model (the AA genotype plus the AC genotype versus the AA genotype for –592C>A SNP; the TT genotype plus the TC genotype versus the CC genotype for –819C>T SNP; and the GG genotype plus the GA genotype versus the AA genotype for –1082A>G).

Weighted odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were evaluated by a random-effects model using the DerSimonian and Laird method. Heterogeneity between studies was computed by the  $\chi^2$  test, and was quantified by the inconsistency index ( $I^2$ ) statistic, which ranges from 0% to 100% and is defined as the percentage of the observed between-study variability that is due to heterogeneity rather than chance.

Predefined subgroup analyses were performed a priori according to the cancer types (oral cancer, nasopharyngeal cancer, cervical cancer or lymphoma [including Hodgkin and Burkitt lymphoma]), ethnicity of the study populations (Caucasian, Asian, Latinos, or African), study design (population-based or hospital-based), and the total sample size (<300 subjects or  $\geq$ 300 subjects). The data were presented and summarized if there were three or more independent studies that provided the genotype or allele counts of the *IL-10* three variants between cases and controls.

Genetic association studies have been considered more closely relevant to randomized trials than other types of epidemiological study due to independent assortment of alleles that theoretically should not be confounded by environmental or behavioral factors.<sup>25</sup> Therefore, we employed Mendelian randomization model to test the hypothesis that genetically

elevated level of IL-10 because of variants in *IL-10* cause an increased risk of HEACs. In Mendelian randomization analysis, risk estimate was computed from the ratio of the coefficient of the association between a variant and a disease to that of the association between the variant and biomarker as a reflection of the potential effect of circulating IL-10 levels on cancer risk.

Publication bias was assessed by visual inspection of Begg's and Egger's funnel plots, accompanied by the corresponding Begg's and Egger's tests. The trim and fill method was implemented to estimate the number and outcomes of potentially missing trials resulting from publication bias. Data management and statistical analyses were conducted using STATA software (StataCorp, College Station, TX, USA, version 11.2 for Windows).  $P<0.05$  was considered statistically significant. For Begg's and Egger's statistics, a significance level was defined as  $P<0.10$ .

## Results

### Eligible articles

A flow diagram schematizing the process of article selection with specific reasons is presented in Figure 1. In total, 103 potentially relevant articles were identified after the initial search, and 24 of them that satisfied inclusion/exclusion criteria were deemed as eligible.<sup>26–49</sup> All 24 qualified articles written in English were published between 2001 and 2014.

### Study characteristics

The basic characteristics of all 24 qualified articles are listed in Table 1, and the genotype distributions and allele frequencies of *IL-10* three variants (–592C>A, –819C>T, and –1082A>G) between cases and controls are listed in Table S2. In this meta-analysis, 12 articles were conducted for cervical cancer, 7 for lymphoma (including Hodgkin's lymphoma and Burkitt's lymphoma), 4 for nasopharyngeal cancer, and 1 for oral cancer. Additionally, there were 8 articles involving Asians, 9 involving Caucasians, 3 involving Latinos, 2 involving Africans, and 1 involving the mixed populations. There were 11 articles conducted on a population-based design and 13 on a hospital-based design. Of 24 qualified articles, 14 (58.33%) had the total sample size (the sum of patients and controls) of at least 300 subjects.

### Overall and subgroup analysis of IL-10 variants and HEACs

Pooling all 24 qualified articles together indicated a significant association between *IL-10*–1082A>G variant and HEACs

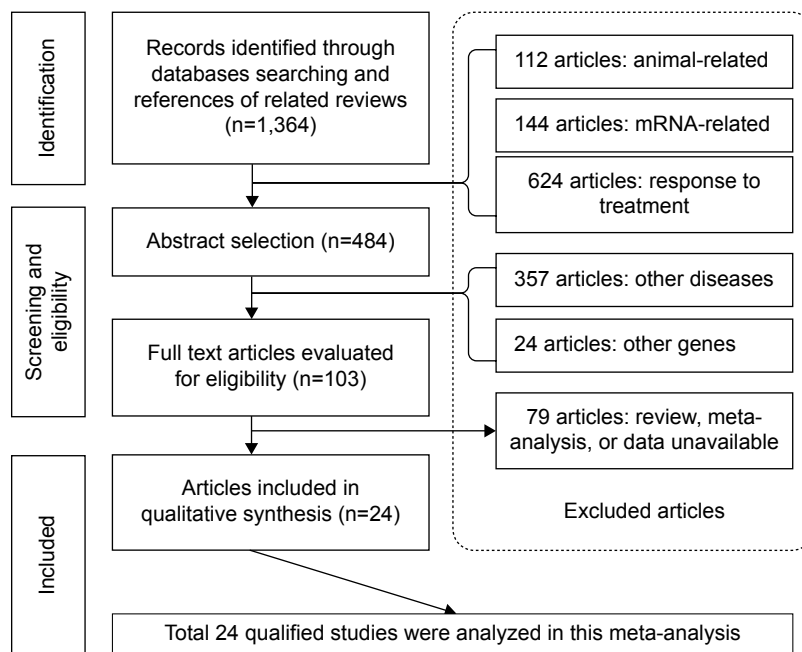


Figure 1 Flow diagram of search strategy and study selection.

under allelic (OR=1.283; 95% CI: 1.071–1.537;  $P=0.007$ ) and dominant models (OR=1.382; 95% CI: 1.128–1.694;  $P=0.002$ ; Table 2). Conversely, we failed to find any significance for the other two variants (–592C>A and –819C>T) in association with HEACs (Tables S3 and S4).

To account for the potential sources of between-study heterogeneity, a set of predefined subgroup analyses were conducted (Table 2). By ethnicity, an extremely significant association between *IL-10*–1082G allele and HEACs in Asians was observed under allelic (OR=2.009; 95% CI:

Table 1 Baseline characteristics of eligible studies for association of *IL-10* three variants with HEACs

Study	Ethnicity	Cancer type	Matched	Source of controls	Sample size		Age (years)		Sex (male, %)	
					Cases	Controls	Cases	Controls	Cases	Controls
Andrie et al <sup>26</sup>	Caucasian	Lymphoma	Yes	Hospital	37	85	NA	NA	61.9	71.8
Barbisan et al <sup>27</sup>	Latinos	Cervical cancer	NA	Hospital	176	122	44.0	37.0	0.00	0.00
Chagas et al <sup>28</sup>	Latinos	Cervical cancer	NA	Hospital	171	193	34.7	34.7	0.00	0.00
Cunningham et al <sup>29</sup>	Caucasian	Lymphoma	NA	Population	49	164	NA	NA	NA	NA
da Silva et al <sup>30</sup>	Latinos	Lymphoma	NA	Hospital	65	50	31.0	7.6	64.28	50.00
Farhat et al <sup>31</sup>	Caucasian	Nasopharyngeal cancer	Yes	Population	160	156	41.9	40.4	72.50	74.68
Fernandes et al <sup>32</sup>	Caucasian	Cervical cancer	Yes	Hospital	42	87	27.0	29.0	0.00	0.00
Govan et al <sup>33</sup>	Mixed	Cervical cancer	NA	Hospital	197	182	NA	NA	0.00	0.00
Ivansson et al <sup>34</sup>	Caucasian	Cervical cancer	NA	Population	1,282	288	NA	NA	0.00	0.00
Matsumoto et al <sup>35</sup>	Asian	Cervical cancer	NA	Hospital	104	173	51.7	35.6	0.00	0.00
Minnicelli et al <sup>36</sup>	Latinos	Lymphoma	Yes	Population	61	230	NA	NA	NA	NA
Munro et al <sup>37</sup>	Caucasian	Lymphoma	No	Hospital	146	111	44.0	58.3	47.26	45.94
Nieters et al <sup>38</sup>	Caucasian	Lymphoma	Yes	Population	108	660	NA	NA	NA	NA
Oduor et al <sup>39</sup>	African	Lymphoma	Yes	Hospital	117	88	5.0	7.0	65.80	55.70
Pratesi et al <sup>40</sup>	Caucasian	Nasopharyngeal cancer	Yes	Population	89	130	NA	NA	78.70	76.90
Roh et al <sup>41</sup>	Asian	Cervical cancer	Yes	Hospital	144	179	NA	NA	0.00	0.00
Shekari et al <sup>42</sup>	Asian	Cervical cancer	NA	Population	200	200	48.6	48.8	0.00	0.00
Singh et al <sup>43</sup>	Asian	Cervical cancer	Yes	Hospital	150	162	48.3	47.2	0.00	0.00
Stanczuk et al <sup>44</sup>	African	Cervical cancer	Yes	Hospital	77	69	47.5	48.0	0.00	0.00
Tsai et al <sup>46</sup>	Asian	Nasopharyngeal cancer	Yes	Population	176	522	48.2	48.9	72.20	72.60
Tsai et al <sup>45</sup>	Asian	Oral cancer	Yes	Population	788	956	55.8	56.6	76.00	76.00
Wang et al <sup>47</sup>	Asian	Cervical cancer	NA	Population	186	200	54.0	42.0	0.00	0.00
Wei et al <sup>48</sup>	Asian	Nasopharyngeal cancer	Yes	Population	198	210	48.7	47.9	72.22	66.19
Zoodsma et al <sup>49</sup>	Caucasian	Cervical cancer	NA	Hospital	667	563	NA	NA	0.00	0.00

Abbreviations: IL-10, interleukin 10; HEAC, human papilloma virus and Epstein–Barr virus-associated cancers; NA, not available.

**Table 2** Overall and subgroup analyses of *IL-10*-1082A>G with HEAC risk

Groups	Studies	Allelic model		Homozygous genotypic model		Dominant model	
		OR; 95% CI; P-value	I <sup>2</sup> (%)	OR; 95% CI; P-value	I <sup>2</sup> (%)	OR; 95% CI; P-value	I <sup>2</sup> (%)
Overall	20	<b>1.283; 1.071–1.537; 0.007</b>	81.6	1.329; 0.993–1.779; 0.056	63.6	<b>1.382; 1.128–1.694; 0.002</b>	72.0
Ethnicity							
Caucasian	8	0.974; 0.876–1.083; 0.630	0.0	0.921; 0.742–1.142; 0.453	0.0	0.991; 0.837–1.173; 0.916	0.0
Asian	5	<b>2.009; 1.566–2.578; 0.000</b>	65.7	<b>2.832; 1.831–4.379; 0.000</b>	27.7	<b>2.101; 1.694–2.607; 0.000</b>	37.4
Latinos	4	1.259; 0.942–1.682; 0.119	49.0	1.575; 0.841–2.949; 0.156	49.7	1.287; 0.968–1.710; 0.082	0.0
African	2	1.652; 0.503–5.428; 0.408	87.7	0.917; 0.368–2.283; 0.853	0.0	1.843; 0.486–6.994; 0.369	87.0
Mixed	1	0.796; 0.593–1.068; 0.128	–	0.611; 0.347–1.076; 0.088	–	0.888; 0.591–1.334; 0.568	–
Sample size							
<300 subjects	11	<b>1.315; 1.023–1.689; 0.032</b>	68.7	1.336; 0.927–1.924; 0.120	27.0	<b>1.417; 1.042–1.928; 0.026</b>	58.7
≥300 subjects	9	1.252; 0.957–1.638; 0.102	88.8	1.315; 0.848–2.040; 0.222	79.0	<b>1.353; 1.027–1.800; 0.038</b>	81.6
Cancer type							
Cervical	8	1.275; 0.969–1.677; 0.083	79.5	1.046; 0.749–1.462; 0.792	34.5	1.389; 0.983–1.965; 0.063	75.6
Oral	1	2.003; 1.659–2.419; 0.000	–	3.268; 1.953–5.471; 0.000	–	2.054; 1.654–2.552; 0.000	–
Nasopharyngeal	4	<b>1.530; 1.063–2.200; 0.022</b>	76.5	1.814; 0.945–3.481; 0.074	60.3	<b>1.737; 1.280–2.358; 0.000</b>	44.9
Lymphoma	7	1.050; 0.828–1.311; 0.688	55.3	1.087; 0.663–1.782; 0.741	54.6	1.062; 0.827–1.365; 0.637	13.3
Case-control matched							
NA	8	1.145; 0.895–1.464; 0.282	76.2	1.014; 0.715–1.438; 0.937	44.9	1.218; 0.893–1.661; 0.213	70.0
Yes	11	<b>1.407; 1.091–1.814; 0.009</b>	80.8	<b>1.612; 1.024–2.536; 0.039</b>	66.4	<b>1.557; 1.214–1.999; 0.000</b>	64.5
No	1	1.107; 0.780–1.571; 0.569	–	1.200; 0.597–2.413; 0.609	–	1.076; 0.588–1.969; 0.813	–
Study design							
Population	9	<b>1.365; 1.047–1.780; 0.022</b>	83.7	1.598; 0.983–2.597; 0.059	74.7	<b>1.492; 1.157–1.924; 0.002</b>	68.6
Hospital	11	1.191; 0.958–1.481; 0.116	71.0	0.984; 0.770–1.259; 0.899	10.0	1.293; 0.972–1.722; 0.078	65.2

**Note:** Data in bold indicates statistical significance.

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein-Barr virus-associated cancers; OR, odds ratio; CI, confidence interval; NA, not available; I<sup>2</sup>, inconsistency index.

1.566–2.578;  $P < 0.001$ ), homozygous genotypic (OR=2.832; 95% CI: 1.831–4.379;  $P < 0.001$ ), and dominant (OR=2.101; 95% CI: 1.694–2.607;  $P < 0.001$ ) models (Figure 2A and B). In Caucasians, Latinos, Africans, or mixed populations, there was no significant association observed in this meta-analysis. By cancer type, the magnitude of risk estimates was significant for nasopharyngeal cancer under allelic (OR=1.530; 95% CI: 1.063–2.200;  $P = 0.022$ ) and dominant models (OR=1.737; 95% CI: 1.280–2.358;  $P < 0.001$ ; Figure 2C and D), whereas no significance was reached for the other cancer types under investigation. By study design, there was a significant association between *IL-10*-1082G allele and HEACs in population-based studies under allelic (OR=1.365; 95% CI: 1.047–1.780;  $P = 0.022$ ) and dominant (OR=1.492; 95% CI: 1.157–1.924;  $P = 0.002$ ) models. Additionally, *IL-10*-1082G allele was more significantly associated with HEACs in those case-control-matched studies under allelic (OR=1.407; 95% CI: 1.091–1.814;  $P = 0.009$ ), homozygous genotypic (OR=1.612; 95% CI: 1.024–2.536;  $P = 0.039$ ), and dominant (OR=1.557; 95% CI: 1.214–1.999;  $P < 0.001$ ) models (Table 2).

There was no publication bias of three *IL-10* promoter variants either in overall or subgroup analysis as reflected by the funnel plots, Egger's tests, and Begg-Mazendam tests (Figure 3).

## Association of IL-10 variants with circulating IL-10 levels

Genotype-phenotype association was based on four articles with circulating IL-10 levels measured in HEAC cancer patients (Table S5).<sup>37,41,50,51</sup> We compared averaged circulating IL-10 levels under homozygous genotypic and dominant models. Circulating IL-10 level was significantly elevated in -1082G allele carriers under homozygous genotypic model (standard mean difference [SMD] =25.692; 95% CI: 1.303–50.081;  $P = 0.039$ ) and dominant model (SMD =13.313; 95% CI: 0.901–25.725;  $P = 0.036$ ; Figure 4). There were low probabilities of publication bias for both models as reflected by the Begg's funnel plots (both  $P = 0.296$ ) and the Egger's tests ( $P = 0.308$  and  $P = 0.442$ , respectively). As expected, there were no significant differences in the changes of circulating IL-10 level for -592C>A and -819C>T under both models.

## Predicted association of circulating IL-10 levels with HEACs from Mendelian randomization

We assumed a linear-logistic relationship between difference of circulating IL-10 level and odds of HEACs when implementing Mendelian randomization method. The predicted OR for 5 and 10 pg/mL IL-10 increment were 1.13

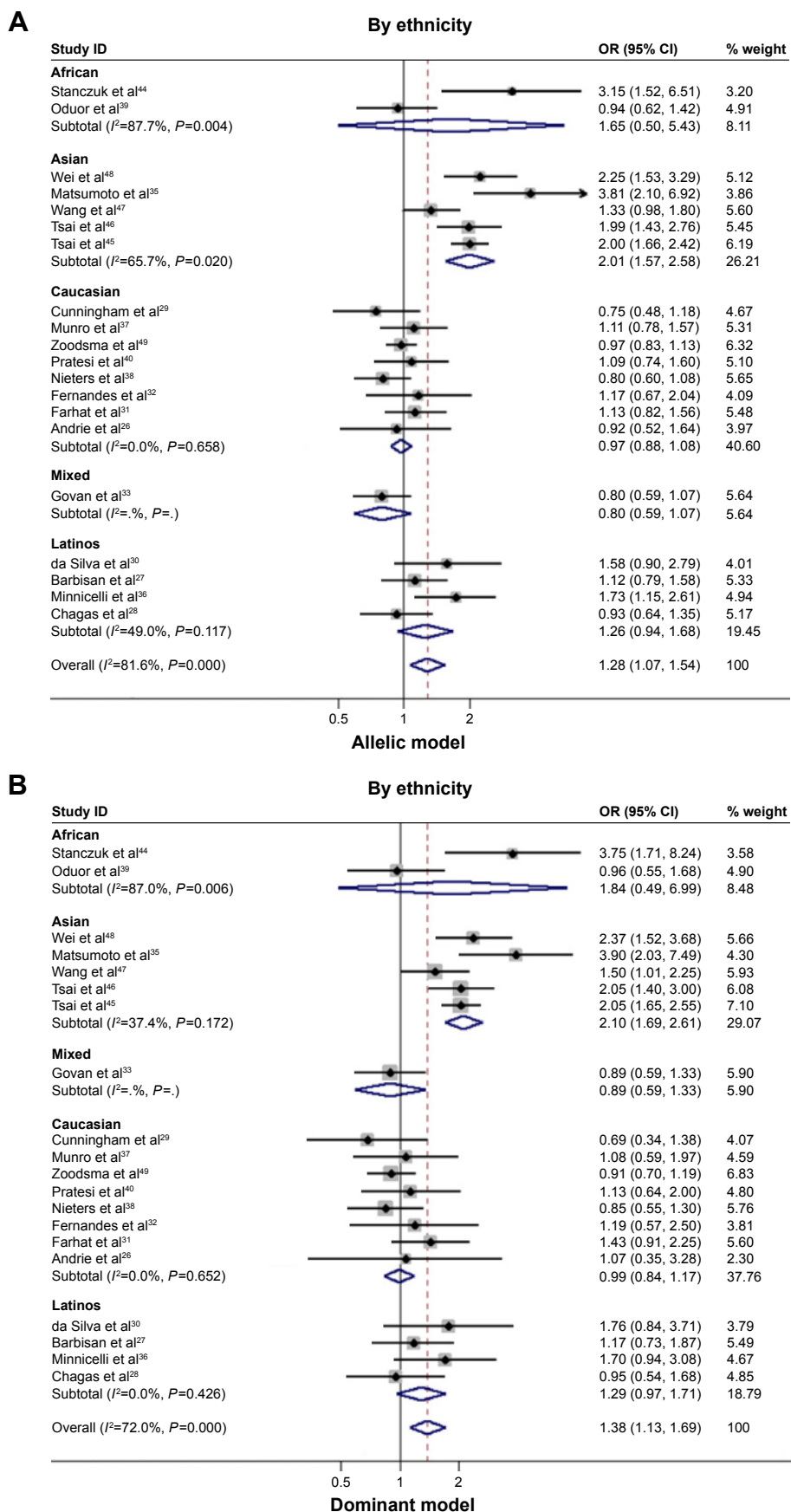
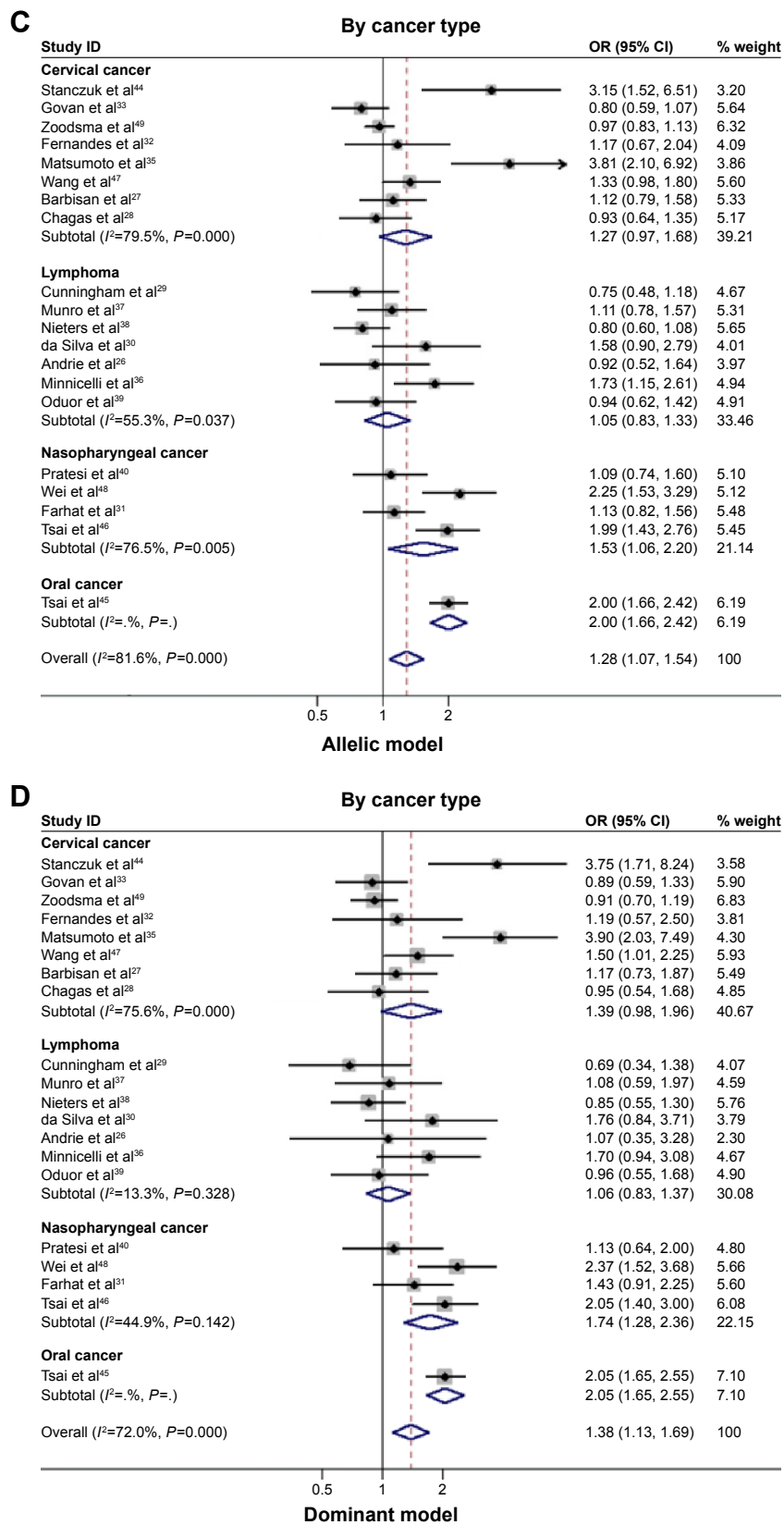


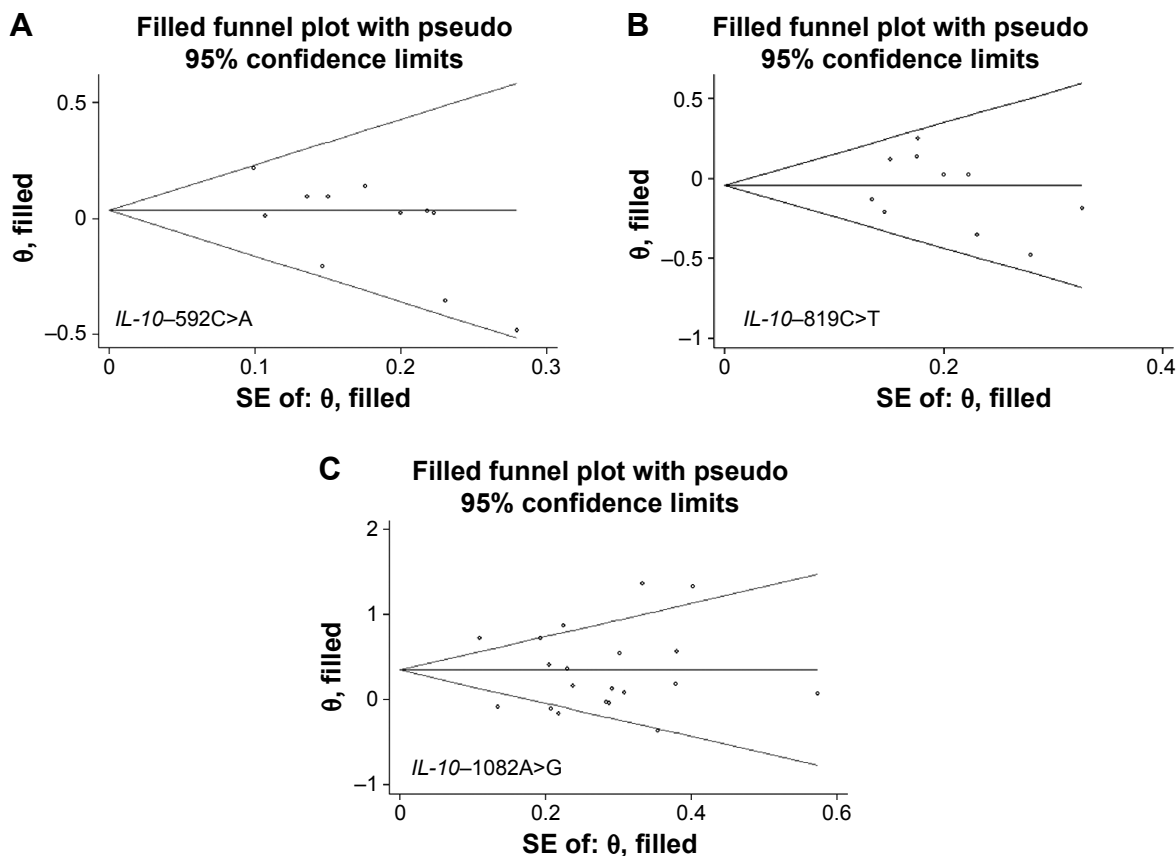
Figure 2 (Continued)



**Figure 2** Risk estimates of *IL-10*-1082A>G for cancer risk by subgroup analysis.

**Notes:** (A) By ethnicity under allelic model; (B) by ethnicity under dominant model; (C) by cancer type under allelic model; and (D) by cancer type under dominant model. The summary OR is shown by the middle of a solid diamond whose left and right extremes represent the corresponding 95% CI. Horizontal axis represents OR values, which were calculated against healthy controls. Weights are from random effects analysis.

**Abbreviations:** IL-10, interleukin 10; OR, odds ratio; CI, confidence interval;  $I^2$ , inconsistency index.



**Figure 3** Funnel plots for studies investigating the effect of *IL-10* three variants on HEAC risk.

**Notes:** (A) *IL-10-592C>A*; (B) *IL-10-819C>T*; and (C) *IL-10-1082A>G*. Vertical axis represents the log of OR; horizontal axis represents the SE of log(OR). Funnel plots are drawn with 95% confidence limits. The graphic symbols represents the data in the plot which is sized proportional to the inverse variance.

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein-Barr virus-associated cancers; OR, odds ratio; SE, standard error.

(95% CI: 1.02–18.64) and 1.28 (95% CI: 1.05–347.26), respectively. Because the 95% CIs of both estimated OR did not include the null hypothesis value of 1.00, it was safe to reject the null hypothesis at a 5% significance level, and reveal a potential role of elevated circulating IL-10 level in development of HEACs.

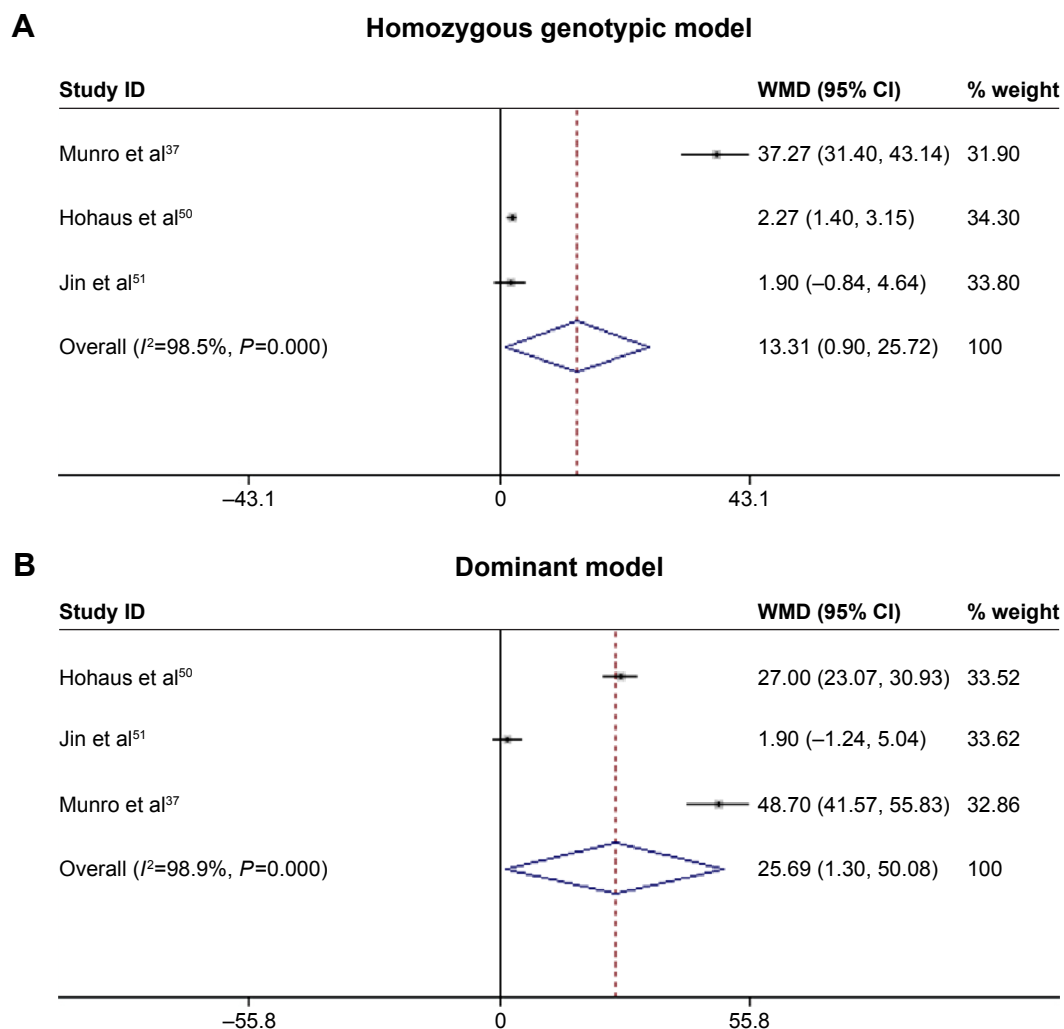
## Discussion

On the basis of a meta-analysis of the data from 24 studies involving 5,390 cases and 5,780 controls, we investigated *IL-10* three promoter variants ( $-592C>A$ ,  $-819C>T$ , and  $-1082A>G$ ) and circulating IL-10 levels in relation to the risk for HEACs. One principal finding of this study was the significant association of *IL-10-1082A>G* variant with HEACs, especially in Asians and for nasopharyngeal cancer. On the basis of aforementioned results, we further employed  $-1082A>G$  variant as an instrument to surrogate circulating IL-10 levels, and revealed the association of IL-10 levels with risk for HEACs. To our knowledge, this is the first meta-analysis demonstrating the association between

circulating IL-10 levels and HEACs by implementing Mendelian randomization approach.

Previous studies have revealed that HPV and EBV infection is the main etiologic risk factors for many epithelial malignancies such as oropharyngeal cancer,<sup>4</sup> nasopharyngeal cancer,<sup>6</sup> cervical cancer,<sup>3</sup> and some subtypes of lymphoma such as Hodgkin's<sup>9</sup> and Burkitt's lymphoma.<sup>8</sup> HPV and EBV are ubiquitous, double-stranded DNA viruses, which can be found in the upper aerodigestive tract. Epidemiologic data showed HPV and EBV infection affected over 10% and 90% of the general population, respectively, but only a small percentage of those infected developed cancer, probably because of lowered immune response and virus clearance due to interindividual genetic variations that result in persistent virus infection. IL-10, a cytokine with multiple effects in immunoregulation and inflammation, has a central role in infection by limiting the immune response to pathogens. Given the essential role of IL-10 in the antiviral response in vitro<sup>52</sup> and in vivo,<sup>17</sup> it is reasonable to expect that IL-10 is implicated in the tumorigenesis of HEACs. Indeed, elevation of circulating IL-10 levels was detected in patients with cervical cancer,<sup>53</sup>





**Figure 4** Comparison of circulating IL-10 levels across *IL-10*-1082A>G genotypes under homozygous (A) and dominant (B) models.

**Notes:** The summary treatment effect (SMD) is shown by the middle of a solid diamond with the left and right extremes representing the corresponding 95% CI. Weights are from random effects analysis.

**Abbreviations:** IL-10, interleukin 10; SMD, standard mean difference; CI, confidence interval; WMD, weighted mean differences.

oropharyngeal cancer,<sup>54</sup> and Hodgkin's lymphoma,<sup>55</sup> and was associated with poor prognoses of these patients. Consistent with above evidence, in this study, our pooled results using Mendelian randomization approach indicated that 5 and 10 pg/mL increments in circulating IL-10 levels were 1.13 and 1.28 times more likely to develop HEACs in a significant manner, respectively. However, considering the unstable status of circulating IL-10 levels in time as previously described (plasma half-life ranged from 2.7 to 4.5 hours),<sup>56</sup> which may cause a weak association between IL-10 level and HEAC risk, well-designed studies with precise IL-10 measurement are required to quantify this effect size reliably.

Nasopharyngeal cancer is a quite common malignancy in Eastern Asians, especially in Chinese, as well as in migrants from those areas,<sup>57</sup> with its incidence rates peaking at 30 cases/100,000 in males and at 10 cases/100,000

in females.<sup>58</sup> Conversely, it is rare in Europe and North America, accounting for less than 1% of all cancer cases, with incidence rates generally below 2 cases/100,000 in males and 1 case/100,000 in females.<sup>58</sup> The obvious various incidence of nasopharyngeal cancer among different ethnic groups suggests this cancer is influenced by heredity factors. In this study, our results robustly confirmed the association between -1082A>G allele and nasopharyngeal cancer, especially in Asian population with relative low between-study heterogeneity. Our findings added a potential explanation for varying incidence of nasopharyngeal cancer worldwide. Further studies are necessary to confirm our findings, and *IL-10*-1082A>G allele, once validated, might be a specific biomarker for patients with nasopharyngeal cancer.

Despite the clear strengths including the large sample sizes and implementation of Mendelian randomization

approach, several possible limitations in the present meta-analysis should also be noted. First, to avoid the impact of low-quality studies, we only included articles written in English, which might cause publication bias, even though our funnel plots and statistical tests did not tell. Second, we only examined three promoter variants in IL-10 gene, and investigation on other variants in or flanking IL-10 gene, especially some low-penetrance genes will be encouraged. Third, the single-locus-based nature of meta-analysis precluded the possibility of gene–gene<sup>59</sup> and gene–environment interactions, but whether this variant integrated with other genetic or environmental risk factors will enhance prediction requires additional research. For instance, it is found that different types of HPV proteins exhibited varying abilities in inducing promoter activity of IL-10 gene.<sup>60</sup> Therefore, it is also necessary to perform a HPV type-stratified analysis in further study. Fourth, nearly all involved studies in this meta-analysis had circulating IL-10 measured only once and did not reflect its long-term level in the development of HEACs. Therefore, because of the above limitations, the jury must refrain from drawing a firm conclusion until a large-scale and well-designed study confirms or refutes our findings.

In summary, our findings provided evidence for a critical role of genetically elevated circulating IL-10 in the development of HEACs by employing IL-10 gene –1082A>G as an instrument, and the risk association of this variant with HEACs was more evident in Asian patients with nasopharyngeal cancer. Additional studies examining biological function of elevated circulating IL-10 level in HEACs, as well as studies seeking to provide clinical validations of our findings, are warranted.

## Acknowledgments

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## Disclosure

The authors report no conflicts of interest in this work.

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# Supplementary materials

**Table S1** PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>Title</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title (Page 1)
<b>Abstract</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria; participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract (Page 1)
<b>Introduction</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction (Page 1)
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction (Page 2)
<b>Methods</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (eg, Web address), and, if available, provide registration information including registration number.	Methods (Page 4)
Eligibility criteria	6	Specify study characteristics (eg, PICOS, length of follow-up) and report characteristics (eg, years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods: inclusion/exclusion criteria (Page 2)
Information sources	7	Describe all information sources (eg, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods: search strategy (Page 2)
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Methods: search strategy (Page 2)
Study selection	9	State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods: search strategy (Page 2)
Data collection process	10	Describe method of data extraction from reports (eg, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods: data extraction (Page 2)
Data items	11	List and define all variables for which data were sought (eg, PICOS, funding sources) and any assumptions and simplifications made.	Methods: data extraction (Page 2, 3)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Methods: statistical analysis (Page 3)
Summary measures	13	State the principal summary measures (eg, risk ratio, difference in means).	Methods: statistical analysis (Page 3)
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (eg, $I^2$ ) for each meta-analysis.	Methods: statistical analysis (Page 3)
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies).	Methods: statistical analysis (Page 3)
Additional analyses	16	Describe methods of additional analyses (eg, sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Methods: statistical analysis (Page 3)
<b>Results</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1

Study characteristics	18	For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.	Results: eligible articles Table 1, Tables S1 and S4
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see Item 12).	Results: IL-10 gene three variants with HEACs; IL-10 variants with circulating IL-10 level; Mendelian randomization analysis (Page 3–8) Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 2 and Tables S2, S3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figures 2 and 4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	None
Additional analysis	23	Give results of additional analyses, if done (eg, sensitivity or subgroup analyses, meta-regression [see Item 16]).	
<b>Discussion</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (eg, health care providers, users, and policy makers).	Discussion (Page 8)
Limitations	25	Discuss limitations at study and outcome level (eg, risk of bias), and at review-level (eg, incomplete retrieval of identified research, reporting bias).	Discussion (Page 9, 10)
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion (Page 10)
<b>Funding</b>			
Funding	27	Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.	Funding (Page 10)

**Notes:** Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6(6):e1000097. For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein-Barr virus-associated cancers.

**Table S2** The genotype distributions of three examined variants in *IL-10* between HEAC patients and controls in all qualified studies

Study	IL-10 gene -592 C>A (rs1800872)						IL-10 gene -819C>T (rs1800871)	
	Case_CC	Case_CA	Case_AA	Control_CC	Control_CA	Control_AA	Case_CC	Case_CT
Andrie et al <sup>2</sup>	NA	NA	NA	NA	NA	NA	23	11
Barbisan et al <sup>3</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Chagas et al <sup>4</sup>	NA	NA	NA	NA	NA	NA	56	90
Cunningham et al <sup>5</sup>	NA	NA	NA	NA	NA	NA	NA	NA
da Silva et al <sup>6</sup>	30	31	4	18	23	9	30	31
Farhat et al <sup>7</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Fernandes et al <sup>8</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Govan et al <sup>9</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Ivansson et al <sup>10</sup>	736	464	82	162	112	14	NA	NA
Matsumoto et al <sup>11</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Minnicelli et al <sup>12</sup>	33	24	4	90	92	23	33	24
Munro et al <sup>13</sup>	88	55	4	66	42	2	NA	NA
Nieters et al <sup>14</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Oduor et al <sup>15</sup>	32	61	24	28	39	21	32	61
Pratesi et al <sup>16</sup>	48	36	5	70	54	6	48	36
Roh et al <sup>17</sup>	11	56	77	15	77	87	11	56
Shekari et al <sup>18</sup>	16	96	88	17	102	81	NA	NA
Singh et al <sup>19</sup>	NA	NA	NA	NA	NA	NA	56	94
Stanczuk et al <sup>20</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Tsai et al <sup>22</sup>	17	66	93	56	205	261	19	69
Tsai et al <sup>21</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Wang et al <sup>23</sup>	NA	NA	NA	NA	NA	NA	NA	NA
Wei et al <sup>24</sup>	35	81	82	24	92	94	35	81
Zoodsma et al <sup>25</sup>	393	231	30	405	175	26	NA	NA

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein-Barr virus-associated cancers; NA, not available.

**Table S3** The overall and subgroup analyses of -592C>A in *IL-10* with HEAC risk

Groups	Studies	Allelic model		Homozygous genotypic model		Dominant model	
		OR; 95% CI; P-value	I <sup>2</sup> (%)	OR; 95% CI; P-value	I <sup>2</sup> (%)	OR; 95% CI; P-value	I <sup>2</sup> (%)
Overall	11	1.018; 0.913-1.135; 0.751	24.0	0.986; 0.780-1.247; 0.907	3.8	1.004; 0.853-1.183; 0.227	22.7
Ethnicity							
Caucasian	4	1.110; 0.976-1.264; 0.113	0.0	1.243; 0.857-1.804; 0.251	0.0	1.106; 0.909-1.346; 0.315	27.5
Asian	4	1.022; 0.873-1.196; 0.785	12.2	0.954; 0.668-1.365; 0.798	10.9	0.905; 0.655-1.250; 0.544	1.5
Latinos	2	<b>0.665; 0.469-0.943; 0.022</b>	0.0	<b>0.371; 0.157-0.876; 0.024</b>	0.0	0.661; 0.419-1.044; 0.076	0.0
African	1	1.023; 0.691-1.514; 0.911	-	1.000; 0.461-2.170; 1.000	-	1.240; 0.677-2.271; 0.487	-
Sample size							
<300	5	0.891; 0.728-1.090; 0.262	3.3	0.750; 0.429-1.312; 0.314	15.5	0.911; 0.703-1.181; 0.482	0.0
≥300	6	1.070; 0.952-1.204; 0.256	20.9	1.058; 0.818-1.369; 0.665	0.0	1.042; 0.828-1.312; 0.724	40.7
Cancer type							
Cervical	4	1.126; 0.998-1.270; 0.054	0.0	1.214; 0.877-1.681; 0.242	0.0	1.136; 0.929-1.388; 0.215	19.6
Nasopharyngeal	3	0.965; 0.792-1.177; 0.728	16.3	0.884; 0.540-1.445; 0.622	27.8	0.876; 0.603-1.272; 0.487	25.5
Lymphoma	4	0.854; 0.667-1.093; 0.210	17.8	0.667; 0.341-1.305; 0.237	26.6	0.887; 0.660-1.192; 0.427	0.0
Case-control matched							
NA	4	1.049; 0.859-1.279; 0.640	53.0	1.040; 0.659-1.640; 0.866	38.0	1.066; 0.816-1.394; 0.638	47.6
Yes	6	0.970; 0.839-1.122; 0.681	6.7	0.892; 0.652-1.220; 0.474	0.0	0.901; 0.704-1.152; 0.404	2.2
No	1	1.032; 0.672-1.583; 0.887	-	1.500; 0.267-8.437; 0.645	-	1.006; 0.853-1.166; 0.982	-
Study design							
Population	6	0.977; 0.866-1.104; 0.712	2.8	0.959; 0.700-1.247; 0.797	11.6	0.901; 0.750-1.082; 0.265	0.0
Hospital	5	1.070; 0.884-1.296; 0.486	33.5	1.010; 0.667-1.529; 0.964	14.1	<b>1.211; 1.005-1.459; 0.044</b>	0.0

**Note:** Data in bold indicates statistical significance.

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein-Barr virus-associated cancers; OR, odds ratio; CI, confidence interval; NA, not available; I<sup>2</sup>, inconsistency index.

				IL-10 gene -1082A>G (rs1800896)					
Case_TT	Control_CC	Control_CT	Control_TT	Case_AA	Case_AG	Case_GG	Control_AA	Control_AG	Control_GG
3	45	35	5	5	16	16	12	32	40
NA	NA	NA	NA	50	61	11	79	83	14
25	76	87	30	56	78	37	26	36	20
NA	NA	NA	NA	16	24	9	41	82	41
4	18	23	9	26	30	9	27	19	4
NA	NA	NA	NA	58	80	22	70	60	26
NA	NA	NA	NA	18	19	5	41	38	8
NA	NA	NA	NA	88	80	29	76	65	41
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	73	26	5	156	16	1
4	90	92	23	21	26	14	102	92	22
NA	NA	NA	NA	30	69	48	24	55	32
NA	NA	NA	NA	38	53	17	208	302	150
24	28	39	21	53	53	11	39	39	10
5	70	54	6	29	41	19	46	58	26
77	15	77	87	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
0	77	85	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	45	31	1	58	11	0
88	52	185	285	117	49	10	419	92	11
NA	NA	NA	NA	522	217	49	766	168	22
NA	NA	NA	NA	77	85	24	103	76	21
82	24	92	94	123	61	14	167	38	5
NA	NA	NA	NA	154	326	187	130	307	169

**Table S4** The overall and subgroup analyses of -819C>T in *IL-10* with HEAC risk

Groups	Studies	Allelic model		Homozygous genotypic model		Dominant model	
		OR; 95% CI; P-value	I <sup>2</sup> (%)	OR; 95% CI; P-value	I <sup>2</sup> (%)	OR; 95% CI; P-value	I <sup>2</sup> (%)
Overall	10	0.955; 0.837–1.088; 0.487	20.7	0.834; 0.637–1.091; 0.185	0.0	0.970; 0.778–1.208; 0.783	30.0
Ethnicity							
Caucasian	2	0.958; 0.668–1.373; 0.814	0.0	1.198; 0.458–3.314; 0.712	0.0	0.884; 0.566–1.381; 0.588	0.0
Asian	4	0.990; 0.804–1.218; 0.921	45.0	0.793; 0.547–1.151; 0.222	0.0	0.991; 0.646–1.519; 0.966	54.6
Latinos	3	0.825; 0.556–1.225; 0.341	61.5	0.606; 0.251–1.459; 0.264	56.8	0.881; 0.527–1.472; 0.628	58.3
African	1	1.023; 0.691–1.514; 0.911	–	1.000; 0.461–2.170; 1.000	–	1.240; 0.677–2.271; 0.487	–
Sample size							
<300	5	0.854; 0.691–1.055; 0.143	0.0	0.746; 0.439–1.268; 0.279	10.3	0.852; 0.642–1.130; 0.265	0.0
≥300	5	1.013; 0.855–1.201; 0.879	35.5	0.869; 0.630–1.197; 0.390	0.0	1.071; 0.767–1.494; 0.687	47.6
Cancer type							
Cervical	3	1.177; 0.975–1.422; 0.090	0.0	1.158; 0.699–1.918; 0.569	0.0	<b>1.375; 1.028–1.839; 0.032</b>	0.0
Nasopharyngeal	3	0.873; 0.731–1.043; 0.134	0.0	0.754; 0.509–1.119; 0.161	0.0	0.822; 0.598–1.131; 0.229	0.0
Lymphoma	4	0.808; 0.635–1.028; 0.083	0.0	0.665; 0.355–1.245; 0.202	20.8	0.803; 0.577–1.118; 0.194	0.0
Case-control matched							
NA	2	0.872; 0.487–1.564; 0.647	72.2	0.619; 0.153–2.503; 0.501	73.5	1.004; 0.508–1.985; 0.990	60.9
Yes	8	0.953; 0.833–1.090; 0.478	9.2	0.826; 0.609–1.120; 0.219	0.0	0.945; 0.738–1.209; 0.651	28.5
No							
Study design	4	0.848; 0.719–1.000; 0.050	0.0	0.718; 0.495–1.041; 0.081	0.0	0.782; 0.592–1.033; 0.084	0.0
Population	6	1.058; 0.889–1.259; 0.524	15.0	0.975; 0.649–1.464; 0.903	6.1	1.164; 0.899–1.506; 0.249	12.4

**Note:** Data in bold indicates statistical significance.

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein-Barr virus-associated cancers; OR, odds ratio; CI, confidence interval; NA, not available; I<sup>2</sup>, inconsistency index.

**Table S5** Changes of circulating IL-10 level across genotypes of three examined variants in *IL-10*

Study	Ethnicity	Status	Sample size	Number	IL-10 level (pg/mL)	Number	IL-10 level (pg/mL)	Number	IL-10 level (pg/mL)
<b>IL-10 gene -592C&gt;A</b>									
Hohaus et al <sup>26</sup>	Caucasian	Cases	95	NA	NA	85	29.20	10	53.10
Jin et al <sup>27</sup>	East Asian	Cases	180	96	10.1	84	13.20	NA	NA
Munro et al <sup>13</sup>	Caucasian	Cases	25	15	106	10	35.90	NA	NA
Roh et al <sup>17</sup>	East Asian	Cases	144	11	2.55	56	4.22	77	3.17
<b>IL-10 gene -819C&gt;T</b>									
Jin et al <sup>27</sup>	East Asian	Cases	180	99	9.60	81	12.80	NA	NA
Roh et al <sup>17</sup>	East Asian	Cases	144	11	2.55	56	4.22	77	3.17
<b>IL-10 gene -1082A&gt;G</b>									
Hohaus et al <sup>26</sup>	Caucasian	Cases	95	NA	NA	87	29.20	8	56.20
Jin et al <sup>27</sup>	East Asian	Cases	180	68	10.70	112	12.60	NA	NA
Munro et al <sup>13</sup>	Caucasian	Cases	26	3	43.00	11	67.80	12	91.70

**Abbreviations:** IL-10, interleukin 10; NA, not available.

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