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

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 \(\text{OR}=1.737\); 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: 1.01–1.69) 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. 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. HPV infection is associated with more than 90%3 and 60%4 cases of cervical and oropharyngeal cancers, respectively, and in contrast, EBV is associated with nearly 90% of nasopharyngeal cancer,6,7 Burkitt’s,8 and Hodgkin’s lymphoma.5,7,9 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.10 Coinfection of EBV and HPV was observed in 30%–50% of patients with oral cancer11,12 and cervical cancer.13,14 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.\textsuperscript{15} Interindividual differences of innate antiviral immunity that is affected by hereditary factors might be involved in the underlying pathological process of HEACs.\textsuperscript{16}

Among the antiviral-relevant immune factors, interleukin-10 (IL-10) is a key cytokine that determines viral clearance or persistence\textsuperscript{17} and is involved in carcinogenesis.\textsuperscript{18,19} Recent studies have observed a marked high level of circulating IL-10 in patients with HEACs and its association with poor prognosis.\textsuperscript{20,21} 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 \textit{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.\textsuperscript{22,23} 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.\textsuperscript{24,25} 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 \textit{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 \textit{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.

\textbf{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).

\textbf{Search strategy}

A literature search for observational studies investigating the association between \textit{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.

\textbf{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.

\textbf{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 \textit{IL-10} three variants \((-592C>A, -819C>T, \text{and} -1082A>G\). 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.

\textbf{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
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 ≥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. 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.26-49 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
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:

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**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, %) |
|------------------|-----------|------------------------|---------|--------------------|-------------|-------------|---------------|
| Andrie et al⁵⁸   | Caucasian | Lymphoma               | Yes     | Hospital           | 37          | 85          | NA            |
| Barbisan et al⁵⁷ | Latinos   | Cervical cancer        | NA      | Hospital           | 176         | 122         | 40.0          |
| Chagas et al⁵⁶   | Latinos   | Cervical cancer        | NA      | Population         | 171         | 193         | 34.7          |
| Cunningham et al⁵⁹| Caucasian | Lymphoma               | NA      | Hospital           | 49          | 164         | NA            |
| da Silva et al⁶⁰ | Latinos   | Lymphoma               | NA      | Hospital           | 65          | 50          | 31.0          |
| Farhat et al⁶¹   | Caucasian | Nasopharyngeal cancer  | Yes     | Population         | 160         | 156         | 41.9          |
| Fernandes et al⁶²| Caucasian | Cervical cancer        | Yes     | Hospital           | 42          | 87          | 27.0          |
| Govan et al⁶³    | Mixed     | Cervical cancer        | NA      | Hospital           | 197         | 182         | NA            |
| Ivansson et al⁶⁴ | Caucasian | Cervical cancer        | NA      | Population         | 1,282       | 288         | NA            |
| Matsumoto et al⁶⁵| Asian     | Cervical cancer        | NA      | Hospital           | 104         | 173         | 51.7          |
| Minnicelli et al⁶⁶| Latinos   | Lymphoma               | Yes     | Hospital           | 61          | 230         | NA            |
| Munro et al⁶⁷    | Caucasian | Lymphoma               | No      | Hospital           | 146         | 111         | 44.0          |
| Nieters et al⁶⁸  | Caucasian | Lymphoma               | Yes     | Population         | 108         | 660         | NA            |
| Oduor et al⁶⁹    | African   | Lymphoma               | Yes     | Hospital           | 117         | 88          | 5.0           |
| Pratesi et al⁷⁰  | Caucasian | Nasopharyngeal cancer  | Yes     | Population         | 89          | 130         | NA            |
| Roh et al⁷¹      | Asian     | Cervical cancer        | Yes     | Hospital           | 144         | 179         | NA            |
| Shekari et al⁷²  | Asian     | Cervical cancer        | NA      | Hospital           | 200         | 200         | 48.6          |
| Singh et al⁷³    | Asian     | Cervical cancer        | Yes     | Hospital           | 150         | 162         | 48.3          |
| Stanczuk et al⁷⁴ | African   | Cervical cancer        | Yes     | Hospital           | 77          | 69          | 47.5          |
| Tsai et al⁷⁵     | Asian     | Nasopharyngeal cancer  | Yes     | Population         | 176         | 522         | 48.2          |
| Tsai et al⁷⁶     | Asian     | Oral cancer            | Yes     | Population         | 788         | 956         | 55.8          |
| Wang et al⁷⁷     | Asian     | Cervical cancer        | NA      | Hospital           | 186         | 200         | 54.0          |
| Wei et al⁷⁸      | Asian     | Nasopharyngeal cancer  | Yes     | Population         | 198         | 210         | 48.7          |
| Zoodama et al⁷⁹  | Caucasian | Cervical cancer        | NA      | Hospital           | 667         | 563         | NA            |

**Abbreviations:** IL-10, interleukin 10; HEAC, human papilloma virus and Epstein–Barr virus-associated cancers; NA, not available.
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).\textsuperscript{37,41,50,51} 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
### A

| Study ID                | By ethnicity | OR (95% CI)        | % weight |
|------------------------|--------------|--------------------|----------|
| **African**            |              |                    |          |
| Stanczuk et al<sup>aa</sup> | 3.15 (1.52, 6.51) | 3.20               |
| Odru et al<sup>aa</sup> | 0.94 (0.62, 1.42) | 4.91               |
| Subtotal (P=0.004)     | 1.65 (0.50, 5.43) | 8.11               |
| **Asian**              |              |                    |          |
| Wei et al<sup>aa</sup> | 2.25 (1.53, 3.29) | 5.12               |
| Matsumoto et al<sup>ab</sup> | 3.81 (2.10, 6.92) | 3.86               |
| Wang et al<sup>aa</sup> | 1.33 (0.98, 1.80) | 5.60               |
| Tsai et al<sup>ab</sup> | 1.99 (1.43, 2.76) | 5.45               |
| Subtotal (P=0.020)     | 2.01 (1.57, 2.58) | 26.21              |
| **Caucasian**          |              |                    |          |
| Cunningham et al<sup>ab</sup> | 0.75 (0.48, 1.18) | 4.67               |
| Munro et al<sup>ab</sup> | 1.11 (0.78, 1.57) | 5.31               |
| Zoodmsa et al<sup>aa</sup> | 0.97 (0.63, 1.43) | 6.32               |
| Pratesi et al<sup>aa</sup> | 1.09 (0.74, 1.60) | 5.10               |
| Nieters et al<sup>ab</sup> | 0.80 (0.60, 1.08) | 5.65               |
| Fernandes et al<sup>ab</sup> | 1.17 (0.67, 2.04) | 4.09               |
| Farhat et al<sup>ab</sup> | 1.13 (0.62, 1.96) | 5.48               |
| Andrie et al<sup>ab</sup> | 0.92 (0.52, 1.64) | 3.97               |
| Subtotal (P=0.658)     | 0.97 (0.88, 1.08) | 40.60              |
| **Mixed**              |              |                    |          |
| Govan et al<sup>ab</sup> | 0.80 (0.59, 1.07) | 5.64               |
| Subtotal (P=0.003)     | 0.80 (0.59, 1.07) | 5.64               |
| **Latinos**            |              |                    |          |
| da Silva et al<sup>ab</sup> | 1.58 (0.90, 2.79) | 4.01               |
| Barbisan et al<sup>ab</sup> | 1.12 (0.79, 1.58) | 5.33               |
| Minneci et al<sup>ab</sup> | 1.73 (1.15, 2.61) | 4.94               |
| Chagas et al<sup>ab</sup> | 0.93 (0.64, 1.35) | 5.17               |
| Subtotal (P=0.177)     | 1.26 (0.94, 1.76) | 19.45              |
| **Overall**            |              |                    |          |
| (P=0.177)              | 1.29 (1.07, 1.54) | 100                |

#### Allelic model

#### B

| Study ID                | By ethnicity | OR (95% CI)        | % weight |
|------------------------|--------------|--------------------|----------|
| **African**            |              |                    |          |
| Stanczuk et al<sup>aa</sup> | 3.76 (1.71, 8.24) | 3.58               |
| Odru et al<sup>aa</sup> | 0.96 (0.55, 1.68) | 4.90               |
| Subtotal (P=0.006)     | 1.84 (0.49, 6.99) | 8.48               |
| **Asian**              |              |                    |          |
| Wei et al<sup>aa</sup> | 2.37 (1.52, 3.68) | 5.66               |
| Matsumoto et al<sup>ab</sup> | 3.90 (2.03, 7.40) | 4.30               |
| Wang et al<sup>aa</sup> | 1.50 (1.01, 2.25) | 5.93               |
| Tsai et al<sup>ab</sup> | 2.05 (1.40, 3.00) | 6.08               |
| Subtotal (P=0.172)     | 2.05 (1.65, 2.55) | 7.10               |
| **Mixed**              |              |                    |          |
| Govan et al<sup>ab</sup> | 0.89 (0.59, 1.33) | 5.90               |
| Subtotal (P=0.003)     | 0.89 (0.59, 1.33) | 5.90               |
| **Caucasian**          |              |                    |          |
| Cunningham et al<sup>ab</sup> | 0.69 (0.34, 1.38) | 4.07               |
| Munro et al<sup>ab</sup> | 1.08 (0.59, 1.97) | 4.59               |
| Zoodmsa et al<sup>aa</sup> | 0.91 (0.70, 1.19) | 6.83               |
| Pratesi et al<sup>aa</sup> | 1.13 (0.64, 2.00) | 4.80               |
| Nieters et al<sup>ab</sup> | 0.85 (0.55, 1.30) | 5.76               |
| Fernandes et al<sup>ab</sup> | 1.19 (0.57, 2.50) | 3.81               |
| Farhat et al<sup>ab</sup> | 1.43 (0.91, 2.25) | 5.60               |
| Subtotal (P=0.652)     | 1.07 (0.35, 3.28) | 2.30               |
| **Latinos**            |              |                    |          |
| da Silva et al<sup>ab</sup> | 1.76 (0.84, 3.71) | 3.79               |
| Barbisan et al<sup>ab</sup> | 1.17 (0.73, 1.87) | 5.49               |
| Minneci et al<sup>ab</sup> | 1.70 (0.94, 3.08) | 4.67               |
| Chagas et al<sup>ab</sup> | 0.96 (0.54, 1.75) | 4.85               |
| Subtotal (P=0.426)     | 1.29 (0.97, 1.71) | 18.79              |
| **Overall**            |              |                    |          |
| (P=0.000)              | 1.38 (1.13, 1.69) | 100                |

#### Dominant model

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**Figure 2 (Continued)**
### C

#### By cancer type

| Study ID | OR (95% CI) | % weight |
|----------|-------------|----------|
| **Cervical cancer** | | |
| Stanczuk et al. | 3.15 (1.52, 6.51) | 3.20 |
| Govan et al. | 0.80 (0.59, 1.07) | 6.64 |
| Zoodsma et al. | 0.97 (0.83, 1.13) | 6.32 |
| Fernandes et al. | 1.17 (0.67, 2.04) | 4.09 |
| Matsumoto et al. | 3.81 (2.10, 6.92) | 3.86 |
| Wang et al. | 1.33 (0.98, 1.80) | 5.60 |
| Barbisan et al. | 1.12 (0.79, 1.56) | 5.33 |
| Chagas et al. | 0.93 (0.64, 1.35) | 5.17 |
| **Subtotal (I^2=79.5%, P=0.000)** | 1.27 (0.97, 1.68) | 39.21 |
| **Lymphoma** | | |
| Cunningham et al. | 0.75 (0.48, 1.18) | 4.67 |
| Munro et al. | 1.11 (0.78, 1.57) | 5.31 |
| Nieters et al. | 0.80 (0.60, 1.08) | 5.65 |
| da Silva et al. | 1.58 (0.90, 2.79) | 4.01 |
| Andrei et al. | 0.92 (0.52, 1.64) | 3.97 |
| Minnicelli et al. | 1.73 (1.52, 2.14) | 4.94 |
| Odor et al. | 0.84 (0.62, 1.12) | 4.91 |
| **Subtotal (I^2=55.3%, P=0.037)** | 1.05 (0.83, 1.33) | 33.46 |
| **Nasopharyngeal cancer** | | |
| Pratesi et al. | 1.09 (0.74, 1.60) | 5.10 |
| Wei et al. | 2.25 (1.53, 3.29) | 5.12 |
| Farhat et al. | 1.13 (0.82, 1.56) | 5.48 |
| Tsai et al. | 1.96 (1.43, 2.67) | 5.45 |
| **Subtotal (I^2=76.5%, P=0.005)** | 1.53 (1.06, 2.20) | 21.14 |
| **Oral cancer** | | |
| Tsai et al. | 2.00 (1.66, 2.42) | 6.19 |
| **Subtotal (I^2=%, P=.)** | 2.00 (1.66, 2.42) | 6.19 |
| **Overall (I^2=81.6%, P=0.000)** | 1.28 (1.07, 1.54) | 100 |

#### Allelic model

### D

#### By cancer type

| Study ID | OR (95% CI) | % weight |
|----------|-------------|----------|
| **Cervical cancer** | | |
| Stanczuk et al. | 3.75 (1.71, 8.24) | 5.09 |
| Govan et al. | 0.89 (0.59, 1.33) | 5.90 |
| Zoodsma et al. | 0.91 (0.70, 1.19) | 6.83 |
| Fernandes et al. | 1.19 (0.57, 2.50) | 3.81 |
| Matsumoto et al. | 3.90 (2.03, 7.49) | 4.30 |
| Wang et al. | 1.50 (1.01, 2.25) | 5.93 |
| Barbisan et al. | 1.17 (0.73, 1.87) | 5.49 |
| Chagas et al. | 0.95 (0.54, 1.68) | 4.85 |
| **Subtotal (I^2=75.6%, P=0.000)** | 1.39 (0.98, 1.98) | 40.67 |
| **Lymphoma** | | |
| Cunningham et al. | 0.69 (0.34, 1.38) | 4.07 |
| Munro et al. | 1.08 (0.59, 1.97) | 4.59 |
| Nieters et al. | 0.85 (0.55, 1.30) | 5.76 |
| da Silva et al. | 1.76 (0.84, 3.71) | 3.79 |
| Andrei et al. | 1.07 (0.35, 3.28) | 2.30 |
| Minnicelli et al. | 1.70 (0.94, 3.08) | 4.67 |
| Odor et al. | 0.96 (0.55, 1.68) | 4.90 |
| **Subtotal (I^2=13.3%, P=0.328)** | 1.00 (0.83, 1.37) | 30.08 |
| **Nasopharyngeal cancer** | | |
| Pratesi et al. | 1.13 (0.64, 2.00) | 4.80 |
| Wei et al. | 2.37 (1.52, 3.68) | 5.66 |
| Farhat et al. | 1.43 (0.91, 2.25) | 5.60 |
| Tsai et al. | 2.05 (1.40, 3.00) | 6.08 |
| **Subtotal (I^2=44.9%, P=0.142)** | 1.74 (1.28, 2.36) | 22.15 |
| **Oral cancer** | | |
| Tsai et al. | 2.05 (1.65, 2.55) | 7.10 |
| **Subtotal (I^2=%, P=.)** | 2.05 (1.65, 2.55) | 7.10 |
| **Overall (I^2=72.0%, P=0.000)** | 1.38 (1.13, 1.69) | 100 |

#### Dominant model

**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.
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, nasopharyngeal cancer, cervical cancer, and some subtypes of lymphoma such as Hodgkin’s and Burkitt’s lymphoma. 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 and in vivo, 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.

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.
oropharyngeal cancer,\textsuperscript{54} and Hodgkin’s lymphoma,\textsuperscript{55} 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),\textsuperscript{56} 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,\textsuperscript{57} with its incidence rates peaking at 30 cases/100,000 in males and at 10 cases/100,000 in females.\textsuperscript{58} 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.\textsuperscript{58} 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 \textsuperscript{IL-10}–1082A\textsuperscript{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 \textsuperscript{IL-10}–1082A\textsuperscript{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\textsuperscript{59} 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.\textsuperscript{60} 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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| Table S1 PRISMA 2009 Checklist |
|-------------------------------|
| **Section/topic**             | **#** | **Checklist item**                                                                 |
| Title                         |      | Identify the report as a systematic review, meta-analysis, or both.                      |
| Abstract                      |      | 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. |
| Introduction                  |      | Describe the rationale for the review in the context of what is already known.            |
| Methods                       |      | 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. |
| Eligibility criteria          |      | 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. |
| Information sources           |      | 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. |
| Search                        |      | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. |
| Study selection               |      | State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). |
| Data collection process       |      | Describe method of data extraction from reports (eg, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. |
| Data items                    |      | List and define all variables for which data were sought (eg, PICOS, funding sources) and any assumptions and simplifications made. |
| Risk of bias in individual studies | | 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. |
| Summary measures              |      | State the principal summary measures (eg, risk ratio, difference in means). |
| Synthesis of results          |      | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (eg, I²) for each meta-analysis. |
| Risk of bias across studies   |      | Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies). |
| Additional analyses           |      | Describe methods of additional analyses (eg, sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. |
| Results                       |      | 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. |

Reported on page #
### Study characteristics
For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.

### Risk of bias within studies
Present data on risk of bias of each study and, if available, any outcome level assessment (see Item 12).

### Results of individual studies
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.

### Synthesis of results
Present results of each meta-analysis done, including confidence intervals and measures of consistency.

### Risk of bias across studies
Present results of any assessment of risk of bias across studies (see Item 15).

### Additional analysis
Give results of additional analyses, if done (eg, sensitivity or subgroup analyses, meta-regression [see Item 16]).

### Discussion
**Summary of evidence**
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).

**Limitations**
Discuss limitations at study and outcome level (eg, risk of bias), and at review-level (eg, incomplete retrieval of identified research, reporting bias).

**Conclusions**
Provide a general interpretation of the results in the context of other evidence, and implications for future research.

### Funding
**Funding**
Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.

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**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 al19   | NA      | NA      | NA                | NA         | NA         | NA         | 23      | 11      |
| Barbisan et al10 | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Chagas et al11   | NA      | NA      | NA                | NA         | NA         | NA         | 56      | 90      |
| Cunningham et al5 | NA    | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| da Silva et al4  | 30      | 31      | 4                 | 18         | 23         | 9          | 30      | 31      |
| Farhat et al18   | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Fernandes et al6 | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Govan et al17    | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Ivensson et al10 | 736     | 464     | 82                | 162        | 112        | 14         | NA      | NA      |
| Matsumoto et al11| NA     | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Minnicelli et al12| 33   | 24      | 4                 | 90         | 92         | 23         | 33      | 24      |
| Munro et al13    | 88      | 55      | 4                 | 66         | 42         | 2          | NA      | NA      |
| Nieters et al14  | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Oduor et al15    | 32      | 61      | 24                | 28         | 39         | 21         | 32      | 61      |
| Pratesi et al16  | 48      | 36      | 5                 | 70         | 54         | 6          | 48      | 36      |
| Roh et al17      | 11      | 56      | 77                | 15         | 77         | 87         | 11      | 56      |
| Shekari et al18  | 16      | 96      | 88                | 17         | 102        | 81         | NA      | NA      |
| Singh et al19    | NA      | NA      | NA                | NA         | NA         | NA         | 56      | 94      |
| Stanczuk et al20 | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Tsai et al21     | 17      | 66      | 93                | 56         | 205        | 261        | 19      | 69      |
| Tsai et al21     | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Wang et al22     | NA      | NA      | NA                | NA         | NA         | NA         | NA      | NA      |
| Wei et al14      | 35      | 81      | 82                | 24         | 92         | 94         | 35      | 81      |
| Zoodsma et al23  | 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 | OR; 95% CI; P-value | OR; 95% CI; P-value |
| Overall           | 11      | 1.018; 0.913–1.135; 0.751 | 0.986; 0.780–1.247; 0.907 | 1.004; 0.853–1.183; 0.227 |
| Ethnicity         |         |               |                           |               |
| Caucasian         | 4       | 1.110; 0.976–1.264; 0.113 | 1.243; 0.857–1.804; 0.251 | 1.106; 0.909–1.346; 0.315 |
| Asian             | 4       | 1.022; 0.873–1.196; 0.785 | 0.954; 0.668–1.365; 0.798 | 0.905; 0.655–1.250; 0.544 |
| Latinos           | 2       | 0.665; 0.469–0.943; 0.022 | 0.371; 0.157–0.876; 0.024 | 0.661; 0.419–1.044; 0.076 |
| 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 | 0.750; 0.429–1.312; 0.314 | 0.911; 0.703–1.181; 0.482 |
| ≥ 300             | 6       | 1.070; 0.952–1.204; 0.256 | 1.058; 0.818–1.369; 0.665 | 1.042; 0.828–1.312; 0.724 |
| Cancer type       |         |               |                           |               |
| Cervical          | 4       | 1.126; 0.998–1.270; 0.054 | 1.214; 0.877–1.681; 0.242 | 1.136; 0.929–1.388; 0.215 |
| Nasopharyngeal    | 3       | 0.965; 0.792–1.177; 0.728 | 0.884; 0.540–1.445; 0.622 | 0.876; 0.603–1.272; 0.487 |
| Lymphoma          | 4       | 0.854; 0.667–1.093; 0.210 | 0.667; 0.341–1.305; 0.237 | 0.887; 0.660–1.192; 0.427 |
| Case–control matched |     |               |                           |               |
| NA                | 4       | 1.049; 0.859–1.279; 0.640 | 1.040; 0.659–1.640; 0.866 | 1.066; 0.816–1.394; 0.638 |
| Yes               | 6       | 0.970; 0.839–1.122; 0.681 | 0.892; 0.652–1.220; 0.474 | 0.901; 0.704–1.152; 0.404 |
| No                | 1       | 1.032; 0.672–1.583; 0.887 | 1.500; 0.267–8.437; 0.645 | 1.006; 0.853–1.665; 0.982 |
| Study design      |         |               |                           |               |
| Population        | 6       | 0.977; 0.866–1.104; 0.712 | 0.959; 0.700–1.247; 0.797 | 0.901; 0.750–1.082; 0.265 |
| Hospital          | 5       | 1.070; 0.884–1.296; 0.486 | 1.010; 0.667–1.529; 0.964 | 1.211; 1.005–1.459; 0.044 |

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, inconsistency index.
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  | OR; 95% CI; P-value       | OR; 95% CI; P-value |
|                 | Overall | 0.955; 0.837–1.088; 0.487 | 0.834; 0.637–1.091; 0.185 | 0.970; 0.778–1.208; 0.783 |
|                 | Ethnicity |                          |                           |               |
| Caucasian       | 2       | 0.958; 0.668–1.373; 0.814 | 1.198; 0.458–3.314; 0.712 | 0.884; 0.566–1.381; 0.588 |
| Asian           | 4       | 0.990; 0.804–1.218; 0.921 | 0.793; 0.547–1.151; 0.222 | 0.991; 0.646–1.519; 0.966 |
| Latinos         | 3       | 0.825; 0.556–1.225; 0.341 | 0.531; 0.251–1.159; 0.264 | 0.800; 0.527–1.172; 0.628 |
| 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 | 0.746; 0.439–1.628; 0.279 | 0.852; 0.642–1.301; 0.265 |
| ≥300            | 5       | 1.013; 0.855–1.201; 0.879 | 0.869; 0.630–1.197; 0.390 | 1.071; 0.767–1.494; 0.687 |
| Cancer type     |         |                          |                           |               |
| Cervical        | 3       | 1.177; 0.975–1.422; 0.090 | 1.158; 0.699–1.918; 0.569 | 1.375; 1.028–1.839; 0.032 |
| Nasopharyngeal  | 3       | 0.873; 0.731–1.043; 0.134 | 0.754; 0.509–1.119; 0.161 | 0.822; 0.598–1.131; 0.229 |
| Lymphoma        | 4       | 0.808; 0.610–1.203; 0.083 | 0.665; 0.355–1.240; 0.202 | 0.803; 0.577–1.118; 0.194 |
| Case–control matched | |                          |                           |               |
| NA              | 2       | 0.872; 0.487–1.564; 0.624 | 0.619; 0.153–2.503; 0.501 | 1.004; 0.508–1.985; 0.990 |
| Yes             | 8       | 0.953; 0.833–1.090; 0.478 | 0.826; 0.609–1.120; 0.212 | 0.945; 0.738–1.209; 0.651 |
| No              |         |                          |                           |               |
| Study design    | 4       | 0.848; 0.719–1.000; 0.500 | 0.718; 0.495–1.401; 0.081 | 0.782; 0.592–1.033; 0.084 |
| Population      | 6       | 1.058; 0.889–1.259; 0.524 | 0.975; 0.649–1.464; 0.903 | 1.164; 0.899–1.506; 0.249 |

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^2, 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) |
|-------|-----------|--------|-------------|--------|---------------------|--------|---------------------|--------|---------------------|
|       |           |        |             | CC     | CA                  | AA     |                     |        |                     |
|       | IL-10 gene -592 C>A |        |             | genotype |                      | genotype |                      | genotype |                      |
| Hohaus et al16 | Caucasian | Cases | 95 | NA | NA | 85 | 29.20 | 10 | 53.10 |
| Jìn et al17 | East Asian | Cases | 180 | 96 | 10.1 | 84 | 13.20 | NA | NA |
| Munro et al18 | Caucasian | Cases | 25 | 15 | 106 | 10 | 35.90 | NA | NA |
| Roh et al19 | East Asian | Cases | 144 | 11 | 2.55 | 56 | 4.22 | 77 | 3.17 |
|       | IL-10 gene -819 C>T |        |             | genotype |                      | genotype |                      | genotype |                      |
| Jìn et al17 | East Asian | Cases | 180 | 99 | 9.60 | 81 | 12.80 | NA | NA |
| Roh et al19 | East Asian | Cases | 144 | 11 | 2.55 | 56 | 4.22 | 77 | 3.17 |
|       | IL-10 gene -1082A>G |        |             | genotype |                      | genotype |                      | genotype |                      |
| Hohaus et al16 | Caucasian | Cases | 95 | NA | NA | 87 | 29.20 | 8 | 56.20 |
| Jìn et al17 | East Asian | Cases | 180 | 68 | 10.70 | 112 | 12.60 | NA | NA |
| Munro et al18 | Caucasian | Cases | 26 | 3 | 43.00 | 11 | 67.80 | 12 | 91.70 |

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

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