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Cigarette Smoking and the Risk of Nasopharyngeal Carcinoma: A Meta-Analysis of Epidemiological Studies

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Abstract

Objective The role of cigarette smoking as an independent risk factor for patients with nasopharyngeal carcinoma (NPC) is controversial. We attempted to provide evidence of reliable association between cigarette smoking and the risk of NPC.

Design Meta-analysis.

Data sources PubMed online and the Cochrane Library of relevant studies published up to February 2016.

Eligibility criteria All studies had to evaluated the relationship between NPC and cigarette smoking with nonsmokers as the reference group.

Outcomes The primary outcome was the adjusted OR, RR or HR of NPC patients comparing smoking with nonsmoking; the second was the crude OR, RR or HR.

Results We identified 17 case-control studies and four cohort studies including 5960 NPC cases and 429464 subjects. Compared with never smokers, current smokers and ever smokers had a 59% and a 56% greater risk of NPC respectively. A dose-response relation was identified in that risk estimate rose by 15% (P<0.001) with every additional 10 pack-years of smoking, and risk increased with intensity of cigarette smoking (>30 cigarettes per day). Significantly increased risk was only found among male smokers (Odds Ratio (OR), 1.36; 95% confidence interval (CI), 1.15-1.60), not among female smokers (OR, 1.58; 95% CI, 0.99-2.53). This finding also existed in the differentiated (OR, 2.34; 95% CI, 1.77-3.09) and the undifferentiated type of NPC (OR, 1.15; 95% CI, 0.90-1.46). Moreover, people started smoking at younger age (<18y) had a greater risk for developing NPC (OR, 1.78; 95% CI, 1.41-2.25).

Conclusions Cigarette smoking was associated with increased risk of NPC, especially for young smokers. However, we did not find statistical significant risks of NPC in females and in undifferentiated type, which might warrant further researches.

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Strengths and limitations of this study

- Major strengths of our meta-analysis comprise new published studies being included, strict selection criteria, careful literature search, data extraction and analyses by two authors separately.
- The main limitations of our meta-analysis are study design, characteristics and size of study population, different outcome and variables used in eligible studies.

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Introduction

There were approximately 86,691 incident cases of NPC and 50,831 NPC-related deaths in 2012 worldwide [1]. Despite NPC is rare in developed countries, the overall incidence rate in Southeastern Asia is 6.5/100,000 person-years among males and 2.6/100,000 person-years among females [2]. Particularly, an age-standardized incidence rate of 20-50 per 100,000 males in south China presented a remarkably high incidence compared to that among white populations [3].

Cigarette smoking has been regarded as a risk factor for occurrence of a wide variety of malignancies, including respiratory tract, gastrointestinal and urogenital systems [4, 5]. Over the decades, some reports have suggested that cigarette smoking is associated with NPC risk [6]. However, the association has not been consistently demonstrated, some studies failed to find such a positive association [7-10]. The discrepancies of inconsistent outcome might be owing to variations in study population, methodology, definitions of cigarette smoking and so on. Furthermore, inevitable recall bias and confounding in case-control studies might further complicate the scenario [11, 12].

One recent meta-analysis of 28 case-control studies and 4 cohort studies reported the adverse effect of cigarette smoking on the incidence of NPC [13]. The pooled analysis showed that ever smokers had a 60% greater risk of developing the disease than never smokers. And there was a significant dose-dependent association. However, between-study heterogeneity was strikingly high across the overall analysis and still remained after stratified analyses. Specifically, some included studies might not be appropriate to be combined for synthetic analysis because of their inadequate reports about association between cigarette smoking and NPC risk [14-17], unclear definition of cigarette smoking and health condition of controls [18, 19], controls with a history of cancer [20], and inappropriate reference group [21, 22]. These might result in overestimating or underestimating the association of cigarette smoking on NPC risk, and thus the conclusions might be hard to interpret. In addition, new studies have been published recently which warrant an up-to-date analysis [23-26].

In this meta-analysis, we sought to provide a summary of available literature of

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high quality to examine the association between cigarette smoking and the risk of NPC, we also assessed the gender and histological type differences in effects of cigarette smoking on the NPC risk.

Methods

Literature search

This meta-analysis was performed on the basis of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) [27]. To identify all relevant publications on NPC and cigarette smoking, firstly, we searched the PubMed and Cochrane Library databases with terms "(((nasopharyngeal carcinoma OR nasopharyngeal cancer OR cancer of nasopharynx)) AND (smoking OR cigarette OR tobacco OR nicotine)) AND (etiology OR epidemiology OR environment OR risk factor) AND (Humans [Mesh])", then we scrutinized the references of articles obtained from the database search for additional studies. Only publications in English were included.

Selection criteria

The following criteria were applied for literature selection: (1) the study was case-control or cohort design; (2) controls were cancer-free; (3) cases were patients who were histopathologically confirmed NPC and had no other malignances; (4) the study evaluated the relationship between NPC and one of various aspects of cigarette smoking, including cigarette smoking status, smoking intensity, cumulative amount of cigarette smoking, age at onset and duration of smoking; (5)studies used nonsmokers as the reference group; (6)studies provided enough information to estimate the odds ratios (ORs) or the relative risk (RR) or hazard ratios (HRs) with 95% confidence interval (CI) for cigarette smoking variable. If multiple articles were on the same study population, the one with adequate information or most related or largest sample size was finally selected; furthermore, when there were separate data for gender or histologic type of NPC in one study, they were considered for additional subgroup analysis.

Data extraction

The following data were extracted from eligible studies: first author, publication

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year, study region, study design, sample size, control source, age of participants (range, mean), gender distribution, categories of smoking (status, intensity, pack-years, age at onset, et al.), method of questionnaire survey, duration of follow-up, end-point (for cohort study), covariates for adjustment, OR, RR or HR with their 95% CIs for each category of smoking exposure. In case the above effect sizes were not available, crude effect estimates and 95% CIs were calculated by provided number of subjects. All data were independently extracted and analyzed by two investigators; any inconsistency was resolved by consensus.

Quality assessment

The qualities of eligible studies were assessed by using the Newcastle-Ottawa Scale (NOS) [28], which comprised three parts assigned with a maximum of 9 points: selection, comparability, exposures and outcome condition. Two investigators evaluated all eligible publications separately and discrepancies were resolved by discussion.

Data integration

Not all studies included in this meta-analysis provided consistent information about cigarette smoking, so we stipulated smoking status as follows: ever smokers, current smokers and former smokers. With regard to smoking quantity, we combined data extracted from all eligible publications into new categories: subjects with cigarettes consumption of <30 pack-years were assigned to light smokers, while those who consumed ≥30 pack-years were designated to heavy smokers. Similarly, for age at smoking onset, early group meant that subjects began smoking at <18 years old while later group defined as smoking at ≥18 years old. We also defined that regions with NPC incidence less than 1 per 100,000 person-years was low incidence rate group, 1-10 per 100,000 person-years was intermediate incidence rate group and greater than 10 per 100,000 was high incidence rate group.

Statistical analysis

Since NPC is considered as a relatively rare outcome, relative risk and odds ratio were not differentiated, the odds ratios were used as effect size for all studies. We conducted fixed and random effects meta-analyses and the synthetic estimates did not

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differ substantially between the two models. Therefore, random-effects (Der Simonian-Laird) model [29], generally regarded as the more conservative method, was applied to calculate point estimates for all analyses. Heterogeneity among articles was estimated by using the I^2 statistic and p value associated with Q statistics [30].

We conducted dose-response meta-analyses using the generalized least-squares method for trend estimation of summary dose-response data, as described by Greenland and Longnecker [31]. For non-linearity relationship, restricted cubic splines with four knots at percentiles 5%, 35%, 65% and 95% of the distribution were created and P value for non-linearity was computed by testing the null hypothesis that the coefficient of the second and the third splines were equal to zero [32].

To assess the robustness of our findings and the source of heterogeneity, meta-regression methods and stratified analyses were performed according to study design, incidence rate of regions, adjustment, score of eligible studies, categories of cigarette smoking, gender and NPC histological type (the latter three were only evaluated in stratified analysis). Sensitivity analysis was also conducted by deleting each study in turn to reflect the influence of every single study to the overall estimate. In addition, we evaluated the publication bias in the pooled analysis by Egger's test and the trim-and-fill method [33]. All statistical analyses were performed with Stata SE 12.0 software, and p value <0.05 (two sides) was considered statistically significant.

Patient involvement

No patients were involved in this study.

Results

Study characteristics

Figure 1 shows the flow chart describing the sequential selection procedures of eligible studies. A total of 342 articles were identified, of which 302 articles were deemed irrelevant after reviewing the titles and abstracts. Subsequently, 40 articles were further scanned by full-text. Meanwhile, by searching all references of relevant articles, three additional articles were considered as potentially eligible. Among them 22 were excluded because of following reasons: five studies with inadequate

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information for data extraction, four studies without report of the association between cigarette smoking and NPC risk, four studies with overlapped data, four studies did not designate never smokers as reference group, two studies included improper controls (for example, controls with malignancies or without description of health conditions), one without clear definition of cigarette smoking, one systematic review and one meta-analysis. Finally, 21 articles were eligible for qualitative synthesis, including seventeen case-control studies (5673 cases and 8653 controls) and four cohort studies (287 cases and 420,811 participants).

All of the studies in the overall analysis were published between 1985 and 2015. Of these included studies, not all studies reported the estimates for all risk estimates. Nineteen studies reported on ever smoking [7-10, 23, 25, 26, 34-45], ten on former smoking [7-9, 23, 26, 38, 39, 41, 46], eleven on current smoking [7-10, 23, 24, 26, 38, 39, 41, 46], ten on pack-years of smoking [7, 23, 24, 26, 35, 37-39, 41, 43] and six on age onset of smoking [7, 9, 10, 23, 26, 46]. Additionally, five studies provided separate data of gender [35, 38, 41-43] and five studies reported the risk of NPC histological type associated with cigarette smoking [9, 38, 39, 42, 44]. As regarding to geographic region, eight studies were conducted in China [7, 8, 24, 26, 37, 41, 43, 44], five in the US [34, 35, 38, 39, 46], five in Southeast Asia region [10, 23, 25, 36, 40], two in Europe [9, 45] and one in Africa [42]. The summarized characteristics of the 21 studies are presented in **Tables I** and **II.**

Association between cigarette smoking status and NPC

The pooled analysis of nineteen studies revealed a modest but significant increased risk of NPC among ever smokers against never smokers (OR, 1.56; 95% CI, 1.32-1.83). Heterogeneity was obviously observed across the studies (I^2=66.8%, P<0.01). The pooled estimate for case-control studies was 1.61 (95% CI, 1.36-1.91; heterogeneity: I^2=65.8%, P<0.01), whereas cohort studies presented a null association (OR, 1.11; 95% CI, 0.84-1.48; heterogeneity: I^2=0.0%, P=0.83) (**Figure 2**).

Similarly, eleven studies identified for the comparison of current smokers with NPC risk demonstrated positive result (OR, 1.59; 95% CI, 1.35-1.89; heterogeneity: I^2=32.5%, P=0.14). When analyzed by study design, the risk estimates were both

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statistically significant for case-control and cohort studies. The pooled ORs were 1.67 (95% CI, 1.06-2.61; heterogeneity: I^2=22.6%, P=0.25) and 2.19 (95% CI: 1.02-4.72; heterogeneity: I^2=65.0%, P=0.06), respectively (**Table III**).

When compared with nonsmokers, former smokers from ten studies exhibited an increased risk of NPC (OR, 1.36; 95% CI, 1.15-1.61; heterogeneity: I^2=2.3%, P=0.42). However, stratified analysis presented a void association in cohort studies (OR, 0.87; 95% CI, 0.54-1.41; heterogeneity: I^2=0.0%, P=0.37) but a significant association in case-control studies (OR, 1.45; 95% CI, 1.21-1.73; heterogeneity: I^2=0.0%, P=0.70) (**Table III**).

As for age at cigarette smoking onset, six studies reported the association with NPC risk. The pooled analysis revealed that early group (smoking at <18 years old) had significantly increased risk of NPC (OR, 1.78; 95% CI, 1.41-2.25; heterogeneity: I^2=0.0%, P=0.94), whereas later group (smoking at ≥18 years old) had slightly increased risk of NPC (OR, 1.28; 95% CI, 1.00-1.64; heterogeneity: I^2=0.0%, P=0.86) (**Table III**).

Dose-response analysis

For the cumulative amount of cigarette smoking, no between-study heterogeneity was found (I^2=0.0%, P>0.05) with a pooled OR of 1.34 (95% CI, 1.13-1.58) for light smokers and 2.03 (95% CI, 1.57-2.61) for heavy smokers, respectively (**Table III**). The dose-response analysis showed statistical linear relationship between the cumulative number of pack-years and NPC risk ($P_{\text{for linearity}}$=0.83) (**Figure 3**). Smokers had a 15% (OR, 1.15; 95% CI, 1.11-1.19, P<0.001) increasing risk of NPC for every additional 10 pack-years smoked in comparison with never smokers (data not shown). When comparing the NPC risk for intensity of cigarettes smoked per day with nonsmokers, the non-linear dose-response relationship indicated that smokers with high exposure (>30 cigarettes/day) other than with low exposure have higher risk estimate, which presented an upward tendency in steeply rising trend ($P_{\text{for non-linearity}}$<0.05) (**Figure 4**).

Stratified analysis

When conducted stratified analysis by regions with different incidence rate, there

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were nineteen studies compared NPC risk for ever smokers with that for never smokers. Among them, five studies carried out in regions with low NPC incidence rate yielded the highest risk (OR, 1.68; 95% CI, 1.36-2.07; heterogeneity: I^2=0.0%, P=0.84). The pooled estimates were 1.59 (95% CI, 1.21-2.09; heterogeneity: I^2=78.8%, P<0.01) for regions (ten studies) with intermediate NPC incidence rate and 1.27 (95% CI, 1.05-1.53; heterogeneity: I^2=0.0%, P=0.52) for regions (4 studies) with high incidence rate, respectively (**Table III**).

We also performed stratified analysis by status of adjustment for confounding variables. Thirteen studies provided adjusted ORs for pooled analysis. But six studies either reported unadjusted ORs or reported the number of cases and controls which could be used to calculate the odds ratios. The estimates for the association of cigarette smoking and NPC risk in adjusted group (OR, 1.55; 95% CI, 1.26-1.91; heterogeneity: I^2=75.3%, P<0.01) and in unadjusted group (OR, 1.57, 95% CI, 1.27-1.93; heterogeneity: I^2=0.0%, P=0.68) were similar (**Table III**).

When the meta-regression analyses were applied to assess the sources of heterogeneity and their impacts on the NPC risk, we found that the publication year, study design, regions of different incidence rate and quality of studies were not significant sources of heterogeneity (P=0.55, data not shown).

Association between cigarette smoking and histological type of NPC

Specifically, the effects of cigarette smoking on NPC histological types were different. We found that significant association was only noted for differentiated squamous-cell NPC (OR, 2.34; 95% CI, 1.77-3.09; heterogeneity: I^2=0.0%, P=0.72). Contrarily, the risk estimate for undifferentiated carcinoma of NPC in smokers was pointless in terms of statistics though the odds ratio was 1.15 (95% CI, 0.90-1.46; heterogeneity: I^2=0.0%, P=0.02) (**Table III**).

Association between cigarette smoking of gender and NPC

Seven studies addressed the association between cigarette smoking and NPC risk by gender, including five in males and two in females. Compared with never smokers, increased risk for male smokers was noted (OR, 1.36; 95% CI, 1.15-1.60). However, an insignificant association (OR, 1.58; 95% CI, 0.99-2.53) was observed for female

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smokers (**Table III**).

Sensitivity analysis and publication bias

Sensitivity analysis revealed that Ji 2011 study [42] was the source of statistical heterogeneity in the pooled analysis for ever smokers. When this outlier study was removed, between-study heterogeneity dropped strikingly to 27.3% in the remaining studies, whereas the odds ratios (OR, 1.47; 95% CI, 1.31-1.66) changed moderately but remained significant. As for case-control studies, the OR changed from 1.61 (95% CI: 1.36-1.91) to 1.52 (95% CI: 1.35-1.72) with heterogeneity fallen from 65.8% to 23.5% (**Figure 5**). The findings were further verified in the intermediated incidence rate group (OR, 1.49, 95% CI, 1.21-1.82; heterogeneity: I^2=49.6%, P=0.04) and in the adjusted group (OR, 1.45; 95% CI, 1.25-1.69; heterogeneity: I^2=41.8%, P=0.05) (data not shown).

Publication bias was evaluated by Egger's test and Trim-and-Fill method. Except for subgroup analyses with ever smokers and heavy smokers, no prominently significant publication bias (with P>0.05 in Egger's test) was observed in our meta-analysis. After adjusted for publication bias, the risk of NPC remained stable with an OR of 1.56 (95% CI, 1.32-1.84) for ever smokers, but changed slightly (OR, 1.80, 95% CI, 1.37-2.36) for heavy smokers (**Table III**).

Discussion

The results from this meta-analysis, based on seventeen case-control studies and four cohort studies, supported that there was moderate association between cigarette smoking and nasopharyngeal carcinoma risk, which was consistent with the result of previous meta-analysis [13].

Interpretation

The pooled risk estimate for cohort studies comparing ever smokers to never smokers was not statistically significant. When conducted similar stratified analyses for current smokers and former smokers, we found that current smoking was significantly related to the risk of NPC while former smoking had an insignificant association with NPC risk. Considering the findings of stratified analyses, it might be the result from former smoking that contributed to the discrepancy between pooled

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analysis for cohort studies and overall analysis. In addition, this meta-analysis demonstrated relatively high heterogeneity both for the overall analysis and subgroup analyses. When the Ji 2011 study [44] was removed from the synthetic analysis, heterogeneity was strikingly reduced in stratified analysis by study design and regions with different NPC incidence rate. Furthermore, the meta-regression analyses indicated that heterogeneity did not prominently result from publication year, study design, regions of different incident rate and quality of studies. To our knowledge, multiple lines of epidemiological studies had found that the development of NPC could be influenced by varieties of etiologies including Epstein-Barr virus (EBV), genetic components and other environmental factors, like preserved food, socioeconomic status, occupation, so on and so forth [6, 47-50]. Therefore, it might be its inappropriate subjects that contributed to selection bias which resulted in the high heterogeneity in the Ji 2011 study, though it had a large sample size with risk estimates adjusted by age, gender, alcohol intake and family history.

One large cohort study [10], conducted in high-incidence region and comprised the majority of undifferentiated NPC (nearly 90% cases), did not reported statistically increased risk of NPC among current smokers compared with never smokers. The difference in the effect of current smoking on NPC risk may be due to its histological type of NPC because undifferentiated carcinoma in high-risk areas seemed more strongly related to Epstein-Barr virus infection other than cigarette smoking [48]. Meanwhile, some case-control studies with small sample size of current smokers also had null results [7-9, 38, 39], of which two studies pointed out that significantly higher risk only existed for smokers with considerable levels of cigarette smoking (>20 cigarettes/day or >30 pack-years) [38, 39]. Nonetheless, the result of our integrated analysis for current smokers versus never smokers was generally consistent with that of the previous meta-analyses [13].

For former smokers, the less consistent risk estimates might result from small number of studies with adequate sample size. The estimates for former smokers in eight studies [7-10, 26, 39, 41, 46] presented null association on NPC risk which was parallel to the results of stratified analysis by study design, and only two studies [23,

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38] demonstrated statistically positive results. The discrepancies in the effects of former cigarette smoking on NPC risk might arise from the following aspects: the group of former smokers may have included people who had quit for a long time, and thus their risk might diminish or even reach the level of never smokers; the minimum period of time since quitting smoking in former smokers varied by study, which could result in judgement bias on the interviewed subjects in some studies.

This meta-analysis revealed that there was a clear dose-response relationship between cigarette smoking and the risk of NPC. That is, the more cigarette smoking (intensity of cigarettes smoked per day and the cumulative amount of pack-years), the higher risk for the development of NPC. Note that similar results have been widely observed for pancreatic cancer, liver cancer, renal carcinoma and gallbladder disease [51-54]. The exact explanation of this dose-dependent effect remains vague, it could be hypothesized that the more cigarette smoking, the greater impact on the epithelial cells of nasopharynx. Therefore, the risk of NPC would be higher in those who smoked more cigarettes. The actual mechanism about the relationship of the amount of smoking and NPC risk had been searched by molecular studies [55, 56], which pointed out that smoking is a factor for tumor growth and acts as a mutagen and DNA damaging agent that drives tumor initiation in normal epithelial cells of nasopharynx.

In this analysis, a statistically significant effect of smoking on NPC risk was observed in males but not in females. The gender difference in response to smoking might be related to interaction between protective endogenous or exogenous estrogens among women compared with men [57], and could also be explained by maturity of smoking trends among males and but not among females. Men might exposure to smoking for a longer duration as compared to women (34% of the male vs. 11% of the female had started smoking before the age of 15 years) [58]. However, the result of female ever smokers might not be adequately stable because only two studies reported the association between cigarette smoking and the risk of NPC for females [35, 41].

Additionally, we found that the younger age people began to smoke, the higher risk they developed NPC. Our results showed that the pooled ORs were 1.78 (95% CI, 1.41-2.25) for smokers in early group and 1.28 (95% CI, 1.00-1.64) in later group,

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respectively. Interestingly, the findings of previous meta-analysis appeared totally opposite with ORs of 1.17 (95% CI, 0.78-1.75) for early group and 1.58 (95% CI, 1.10-2.26) for later group [13]. Like many other cancers, NPC may take decades to develop from premalignant cells to detectable solid tumor. Thus, the exposure to carcinogenic agents early in life could have substantial impacts on the development of NPC [6, 59]. Moreover, the incidence of NPC peaks at age of 50-59 years in high-risk regions, while in western countries, the incidence of NPC peaks somewhat later (≥65-year-olds) [59]. As a result, the number of NPC patients in terms of age distribution could considerably vary in our eligible studies that were conducted in different countries.

When stratified by histological type of NPC, the pooled analysis presented a higher risk of differentiated NPC than that of undifferentiated NPC, and the later had an insignificant risk estimate. This difference might be owing to fewer studies included in the pooled analysis for undifferentiated NPC because we excluded those ineligible studies either for no report of the association between cigarette smoking and NPC risk [16] or for overlapped data [60]. It might avoid incorrect estimation of smoking effects on NPC risk. Moreover, we found that the risk estimates adversely associated with the NPC incidence rate. For example, the pooled OR for high incidence rate areas to low incidence rate areas ranged from 1.27 to 1.68. This might suggest there are substantial heterogeneity between NPC risk and smoking by histological types and geographic variations. Undifferentiated carcinoma of the nasopharynx is the predominant type in high-risk areas, and it is consistently associated with EBV infection, which may increase the carcinogenic effect of cigarette smoking [48].

Generalizability

The magnitude of association between cigarette smoking and the NPC risk was not as big as those for other smoking-related cancers like lung cancer and gastrointestinal malignancies [4]. However, NPC was quite epidemic in southeastern Asia especially in cities in southern China, and China was one of the largest tobacco producing and consuming countries in the world [61]. Besides, we found current

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smokers are more related to the development of NPC with a higher risk estimate as compared to former smokers. These emphasized the importance and urgency of efforts to initiate the control of cigarette smoking to improve public health. Any efficient tobacco control programs would be helpful to reduce morbidity and mortality of smoking-related cancers worldwide.

Limitations

The results of this meta-analysis should be explicated in the context of several limitations. For example, the design of included studies varied in source of subjects recruited, standardization for categories of cigarette smoking, in adjusted factors. Additionally, our meta-analysis was a mix of retrospective studies and prospective studies, and was lack of individual participant data for adjustment of potential confounders. Generally, Epstein-Barr virus infection was thought to be highly related to NPC risk [62]. However, a 22-year follow-up study carried out by Hsu et al. revealed that Epstein-Barr virus was less likely to modify the estimate for smoking associated with NPC risk [43]. And the links of other risk factors like dietary and social practices were often inconsistent between studies [62]. Moreover, the risk estimates of NPC resembled both in the group with adjusted odds ratio and in the group with unadjusted odds ratio in our meta-analysis.

Conclusions

This meta-analysis demonstrated that cigarette smoking associated with a modest, but statistically significant increased risk of NPC. Yet, further prospective studies are needed to elucidate the NPC risk in terms of gender, histological type, and for former smokers and smoking onset age.

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Contributor ship statement: LMJ (Mengjuan Long) and LP (Ping Li) did the literate research and selected the eligible articles separately; LP (Ping Li) and NZH (Zhihua Nie) extracted the whole data and assessed the quality of our selected articles; LMJ integrated and analyzed data, and wrote the manuscript. FZM (Zhenming Fu) examined and revised the manuscript. All authors read and approved the final manuscript.

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References

1. Ferlay J SI, et al. Cancer Incidence and Mortality Worldwide: IARC Cancer Base. *GLOBOCAN 2012 v1.0*.
2. Curado MP EB, et al. Cancer incidence in five continents. *Geneva: WHO Press 2007 vol. IX.*.
3. Jemal A, Bray F, Center MM et al. Global cancer statistics. *CA Cancer J Clin 20116*; 1: 69-90.
4. Sasco AJ, Secretan MB, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung Cancer 2004*; 45 Suppl 2: S3-9.
5. Sandler RS, Sandler DP, Comstock GW et al. Cigarette smoking and the risk of colorectal cancer in women. *J Natl Cancer Inst 1988*; 80: 1329-1333.
6. Jia WH, Qin HD. Non-viral environmental risk factors for nasopharyngeal carcinoma: a systematic review. *Semin Cancer Biol 2012*; 22: 117-126.
7. Cheng YJ, Hildesheim A, Hsu MM et al. Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. *Cancer Causes Control 1999*; 10: 201-207.
8. Zou J, Sun Q, Akiba S et al. A case-control study of nasopharyngeal carcinoma in the high background radiation areas of Yangjiang, China. *J Radiat Res 2000*; 41 Suppl: 53-62.
9. Polesel J, Franceschi S, Talamini R et al. Tobacco smoking, alcohol drinking, and the risk of different histological types of nasopharyngeal cancer in a low-risk population. *Oral Oncol 2011*; 47: 541-545.
10. Friborg JT, Yuan JM, Wang R et al. A prospective study of tobacco and alcohol use as risk factors for pharyngeal carcinomas in Singapore Chinese. *Cancer 2007*; 109: 1183-1191.
11. Grimes DA, Schulz KF. Bias and causal associations in observational research. *Lancet 2002*; 359: 248-252.
12. Weinberg CR. Toward a clearer definition of confounding. *Am J Epidemiol 1993*; 137: 1-8.
13. Xue WQ, Qin HD, Ruan HL et al. Quantitative association of tobacco smoking with the risk of nasopharyngeal carcinoma: a comprehensive meta-analysis of studies conducted between 1979 and 2011. *Am J Epidemiol 2013*; 178: 325-338.
14. Lin TM, Yang CS, Tu SM et al. Interaction of factors associated with cancer of the nasopharynx. *Cancer 1979*; 44: 1419-1423.
15. Chen CJ, Liang KY, Chang YS et al. Multiple risk factors of nasopharyngeal carcinoma: Epstein-Barr virus, malarial infection, cigarette smoking and familial tendency. *Anticancer Res 1990*; 10: 547-553.
16. Nesic V, Sipetic S, Vlajinac H et al. Risk factors for the occurrence of undifferentiated carcinoma of nasopharyngeal type: a case-control study. *Srp Arh Celok Lek 2010*; 138: 6-10.
17. Ren ZF, Liu WS, Qin HD et al. Effect of family history of cancers and environmental factors on risk of nasopharyngeal carcinoma in Guangdong, China. *Cancer Epidemiol 2010*; 34: 419-424.
18. Armstrong RW, Armstrong MJ, Yu MC, Henderson BE. Salted fish and inhalants as risk factors for nasopharyngeal carcinoma in Malaysian Chinese. *Cancer Res 1983*; 43: 2967-2970.
19. West S, Hildesheim A, Dosemeci M. Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. *Int J Cancer 1993*; 55: 722-727.
20. Ng TP. A case-referent study of cancer of the nasal cavity and sinuses in Hong Kong. *Int J Epidemiol 1986*; 15: 171-175.
21. Guo X, Johnson RC, Deng H et al. Evaluation of nonviral risk factors for nasopharyngeal carcinoma in a high-risk population of Southern China. *Int J Cancer 2009*; 124: 2942-2947.
22. Ma F, Zhang H, Zhai Y et al. Functional polymorphism -31C/G in the promoter of BIRC5 gene

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and risk of nasopharyngeal carcinoma among chinese. *PLoS One 2011*; 6: e16748.

23. Fachiroh J, Sangrajrang S, Johansson M et al. Tobacco consumption and genetic susceptibility to nasopharyngeal carcinoma (NPC) in Thailand. *Cancer Causes Control 2012*; 23: 1995-2002.

24. Lin JH, Jiang CQ, Ho SY et al. Smoking and nasopharyngeal carcinoma mortality: a cohort study of 101,823 adults in Guangzhou, China. *BMC Cancer 2015*; 15: 906.

25. Lye MS, Visuvanathan S, Chong PP et al. Homozygous Wildtype of XPD K751Q Polymorphism Is Associated with Increased Risk of Nasopharyngeal Carcinoma in Malaysian Population. *PLoS One 2015*; 10: e0130530.

26. Xie SH, Yu IT, Tse LA et al. Tobacco smoking, family history, and the risk of nasopharyngeal carcinoma: a case-referent study in Hong Kong Chinese. *Cancer Causes Control 2015*; 26: 913-921.

27. Stroup DF, Berlin JA, Morton SC et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *Jama 2000*; 283: 2008-2012.

28. Wells G Sbea. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Ottawa, Ontario, *Canada: Ottawa Health Research Institute 2000*.

29. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials 1986*; 7: 177-188.

30. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med 2002*; 21: 1539-1558.

31. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol 1992*; 135: 1301-1309.

32. Orsini N GS, et al. A procedure to tabulate and plot results after flexible modeling of a quantitative covariate. *Stata J 2011*; 11: 29.

33. Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. Bmj 315: 629-634, 1997.

34. Mabuchi K, Bross DS, Kessler, II. Cigarette smoking and nasopharyngeal carcinoma. *Cancer 1985*; 55: 2874-2876.

35. Nam JM, McLaughlin JK, Blot WJ. Cigarette smoking, alcohol, and nasopharyngeal carcinoma: a case-control study among U.S. whites. *J Natl Cancer Inst 1992*; 84: 619-622.

36. Sriamporn S, Vatanasapt V, Pisani P et al. Environmental risk factors for nasopharyngeal carcinoma: a case-control study in northeastern Thailand. *Cancer Epidemiol Biomarkers Prev 1992*; 1: 345-348.

37. Yu MC, Garabrant DH, Huang TB, Henderson BE. Occupational and other non-dietary risk factors for nasopharyngeal carcinoma in Guangzhou, China. *Int J Cancer 1990*; 45: 1033-1039.

38. Zhu K, Levine RS, Brann EA et al. A population-based case-control study of the relationship between cigarette smoking and nasopharyngeal cancer (United States). *Cancer Causes Control 1995*; 6: 507-512.

39. Vaughan TL, Shapiro JA, Burt RD et al. Nasopharyngeal cancer in a low-risk population: defining risk factors by histological type. *Cancer Epidemiol Biomarkers Prev 1996*; 5: 587-593.

40. Chelleng PK, Narain K, Das HK et al. Risk factors for cancer nasopharynx: a case-control study from Nagaland, India. *Natl Med J India 2000*; 13: 6-8.

41. Yuan JM, Wang XL, Xiang YB et al. Non-dietary risk factors for nasopharyngeal carcinoma in Shanghai, China. *Int J Cancer 2000*; 85: 364-369.

42. Feng BJ, Khyatti M, Ben-Ayoub W et al. Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. *Br J Cancer 2009*; 101: 1207-1212.

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43. Hsu WL, Chen JY, Chien YC et al. Independent effect of EBV and cigarette smoking on nasopharyngeal carcinoma: a 20-year follow-up study on 9,622 males without family history in Taiwan. *Cancer Epidemiol Biomarkers Prev 2009*; 18: 1218-1226.

44. Ji X, Zhang W, Xie C et al. Nasopharyngeal carcinoma risk by histologic type in central China: impact of smoking, alcohol and family history. *Int J Cancer 2011*; 129: 724-732.

45. Turkoz FP, Celenkoglu G, Dogu GG et al. Risk factors of nasopharyngeal carcinoma in Turkey-an epidemiological survey of the Anatolian Society of Medical Oncology. *Asian Pac J Cancer Prev 2011*; 12: 3017-3021.

46. Chow WH, McLaughlin JK, Hrubec Z et al. Tobacco use and nasopharyngeal carcinoma in a cohort of US veterans. *Int J Cancer 1993*; 55: 538-540.

47. Hildesheim A, West S, DeVeyra E et al. Herbal medicine use, Epstein-Barr virus, and risk of nasopharyngeal carcinoma. *Cancer Res 1992*; 52: 3048-3051.

48. Tsao SW, Tsang CM, Pang PS et al. The biology of EBV infection in human epithelial cells. *Semin Cancer Biol 2012*; 22: 137-143.

49. Polesel J, Serraino D, Negri E et al. Consumption of fruit, vegetables, and other food groups and the risk of nasopharyngeal carcinoma. *Cancer Causes Control 2013*; 24: 1157-1165.

50. He YQ, Xue WQ, Shen GP et al. Household inhalants exposure and nasopharyngeal carcinoma risk: a large-scale case-control study in Guangdong, China. *BMC Cancer 2015*; 15: 1022.

51. Zou L, Zhong R, Shen N et al. Non-linear dose-response relationship between cigarette smoking and pancreatic cancer risk: evidence from a meta-analysis of 42 observational studies. *Eur J Cancer 2014*; 50: 193-203.

52. Lee YC, Cohet C, Yang YC et al. Meta-analysis of epidemiologic studies on cigarette smoking and liver cancer. *Int J Epidemiol 2009*; 38: 1497-1511.

53. Hunt JD, van der Hel OL, McMillan GP et al. Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. *Int J Cancer 2005*; 114: 101-108.

54. Aune D, Vatten LJ, Boffetta P. Tobacco smoking and the risk of gallbladder disease. *Eur J Epidemiol 2016*; 31: 643-653.

55. Furmanski P. Revealing the mechanism of tissue damage due to tobacco use: finally, a smoking gun? *Am J Pathol 2013*; 182: 1489-1493.

56. Salem AF, Al-Zoubi MS, Whitaker-Menezes D et al. Cigarette smoke metabolically promotes cancer, via autophagy and premature aging in the host stromal microenvironment. *Cell Cycle 2013*; 12: 818-825.

57. Corrao G, Zambon A, Conti V et al. Menopause hormone replacement therapy and cancer risk: an Italian record linkage investigation. *Ann Oncol 2008*; 19: 150-155.

58. Peto R, Darby S, Deo H et al. Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *Bmj 2000*; 321: 323-329.

59. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev 2006*; 15: 1765-1777.

60. Nazar-Stewart V, Vaughan TL, Burt RD et al. Glutathione S-transferase M1 and susceptibility to nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev 1999*; 8: 547-551.

61. Mackay J, Ritthiphakdee B, Reddy KS: Tobacco control in Asia. *Lancet 2013*; 381: 1581-1587.

62. Chua ML, Wee JT, Hui EP, Chan AT. Nasopharyngeal carcinoma. *Lancet 2016*; 387: 1012-1024.

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Figure Legends

Figure 1: Summary of literature search.

Figure 2: Forest plots for comparing the risk for NPC between ever smokers versus never smokers.

Figure 3: A linear relationship between the cumulative number of pack-years and NPC risk ($P_{\text{for linearity}}$=0.83), with a 15% (95% CI: 1.11-1.19, P<0.001) increasing risk of NPC for every additional 10 pack-years smoked in comparison with never smokers (The solid line depicts the pooled risk estimate of NPC associated with each 1-pack-year increment of cigarette smoking, the dashed line depicts the upper confidence interval, the dot line depicts the lower confidence interval).

Figure 4: A non-linear association between intensity of cigarette smoking and NPC risk ($P_{\text{for non-linearity}}$<0.05) (The solid line depicts the pooled risk estimate of NPC associated with each 1 cigarette/day increment, the dashed lines depict the upper and the lower confidence interval, respectively).

Figure 5: Forest plots for comparing the risk for NPC between ever smokers versus never smokers after deleting the Ji 2011 study.

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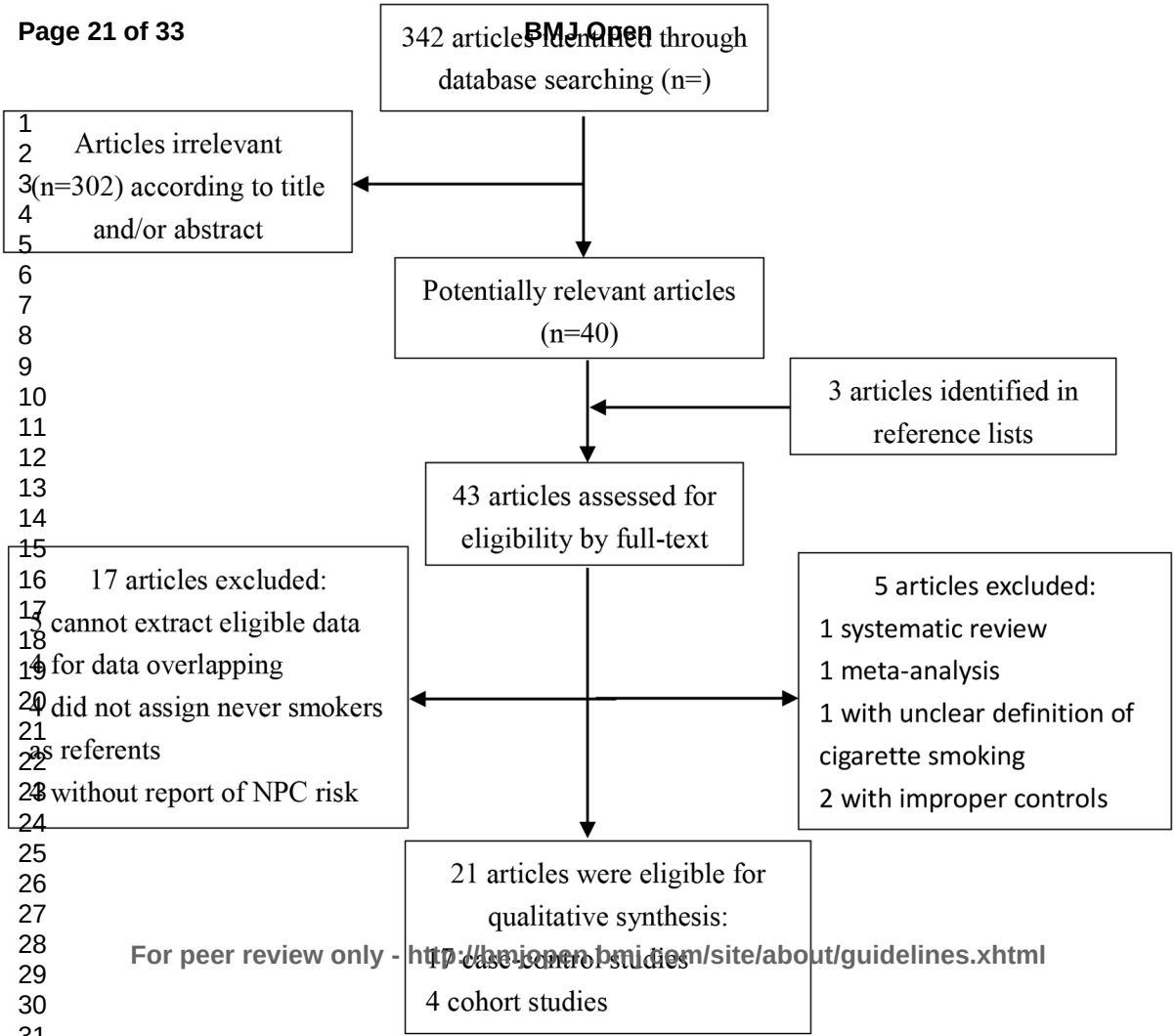

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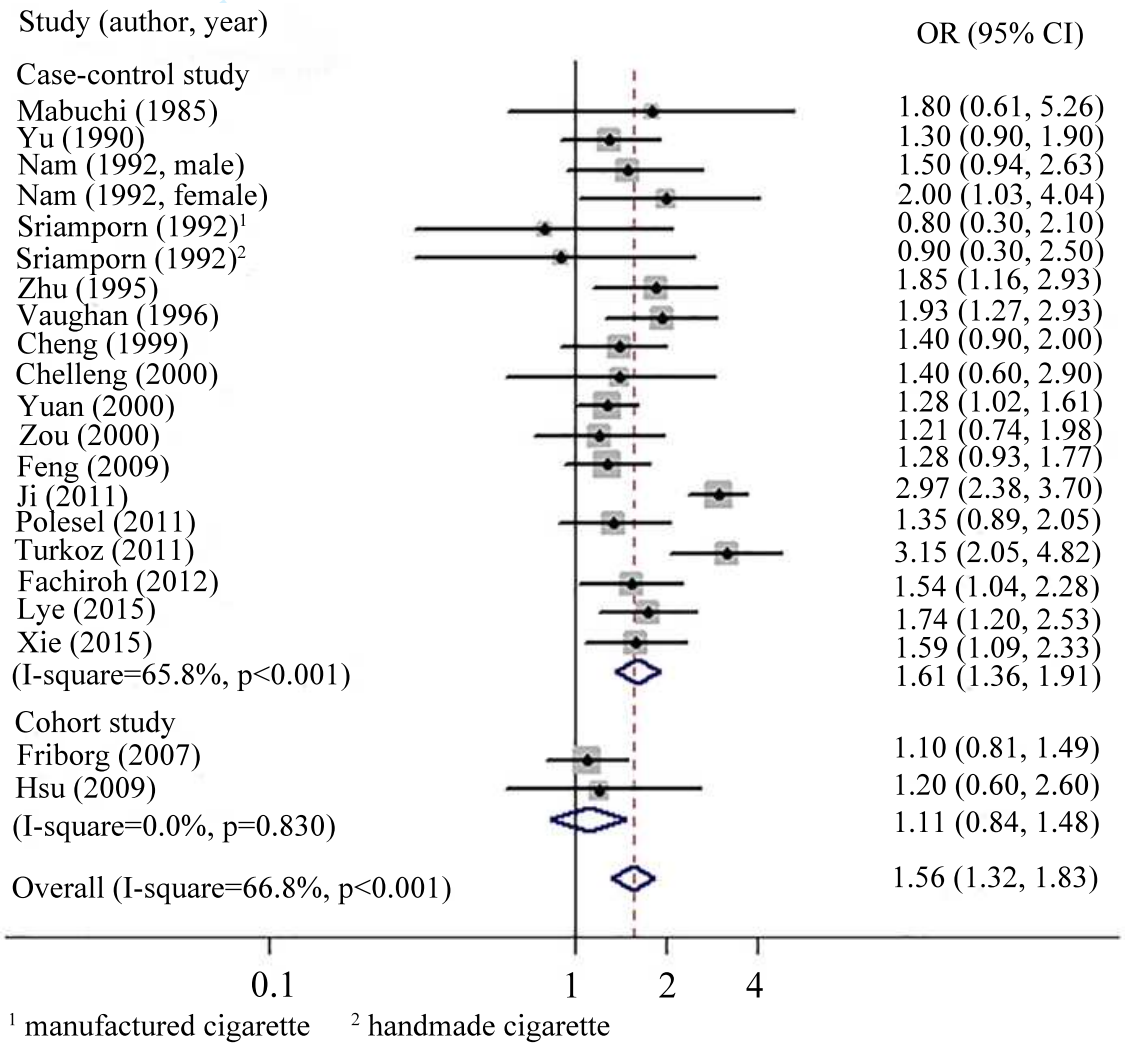
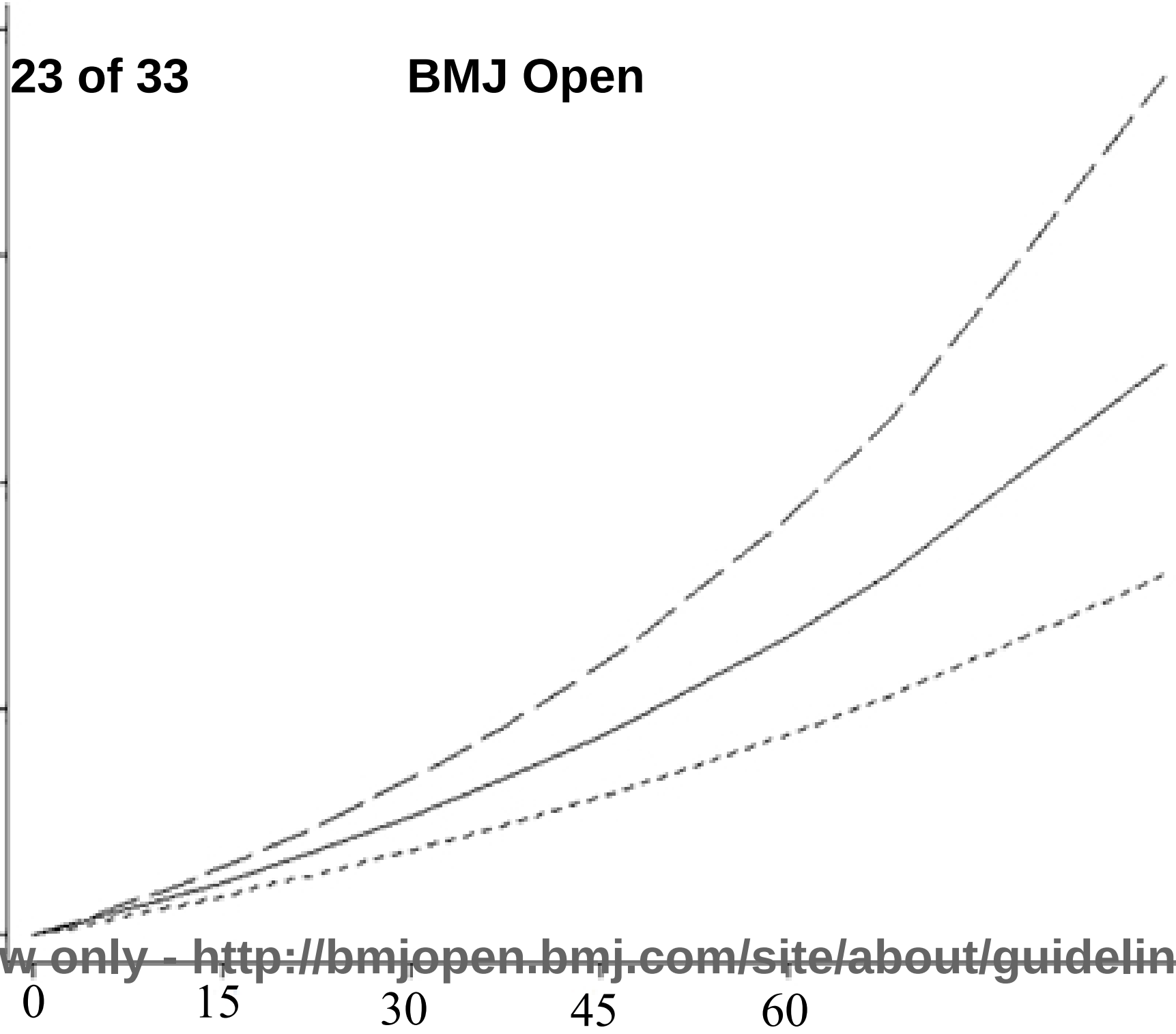

Study (author, year)
OR (95% CI)
Case-control study
Mabuchi (1985) 1.80 (0.61, 5.26)
Yu (1990) 1.30 (0.90, 1.90)
Nam (1992, male) 1.50 (0.94, 2.63)
Nam (1992, female) 2.00 (1.03, 4.04)
Sriamporn (1992)[1] 0.80 (0.30, 2.10)
Sriamporn (1992)[2] 0.90 (0.30, 2.50)
Zhu (1995) 1.85 (1.16, 2.93)
Vaughan (1996) 1.93 (1.27, 2.93)
Cheng (1999) 1.40 (0.90, 2.00)
Chelleng (2000) 1.40 (0.60, 2.90)
Yuan (2000) 1.28 (1.02, 1.61)
Zou (2000) 1.21 (0.74, 1.98)
Feng (2009) 1.28 (0.93, 1.77)
Ji (2011) 2.97 (2.38, 3.70)
Polesel (2011) 1.35 (0.89, 2.05)
Turkoz (2011) 3.15 (2.05, 4.82)
Fachiroh (2012) 1.54 (1.04, 2.28)
Lye (2015) 1.74 (1.20, 2.53)
Xie (2015) 1.59 (1.09, 2.33)
(I-square=65.8%, p<0.001) 1.61 (1.36, 1.91)
Cohort study
Friborg (2007) 1.10 (0.81, 1.49)
Hsu (2009) 1.20 (0.60, 2.60)
(I-square=0.0%, p=0.830) 1.11 (0.84, 1.48)
Overall (I-square=66.8%, p<0.001) 1.56 (1.32, 1.83)
0.1
1
2
4
[1] manufactured cigarette
[2] handmade cigarette



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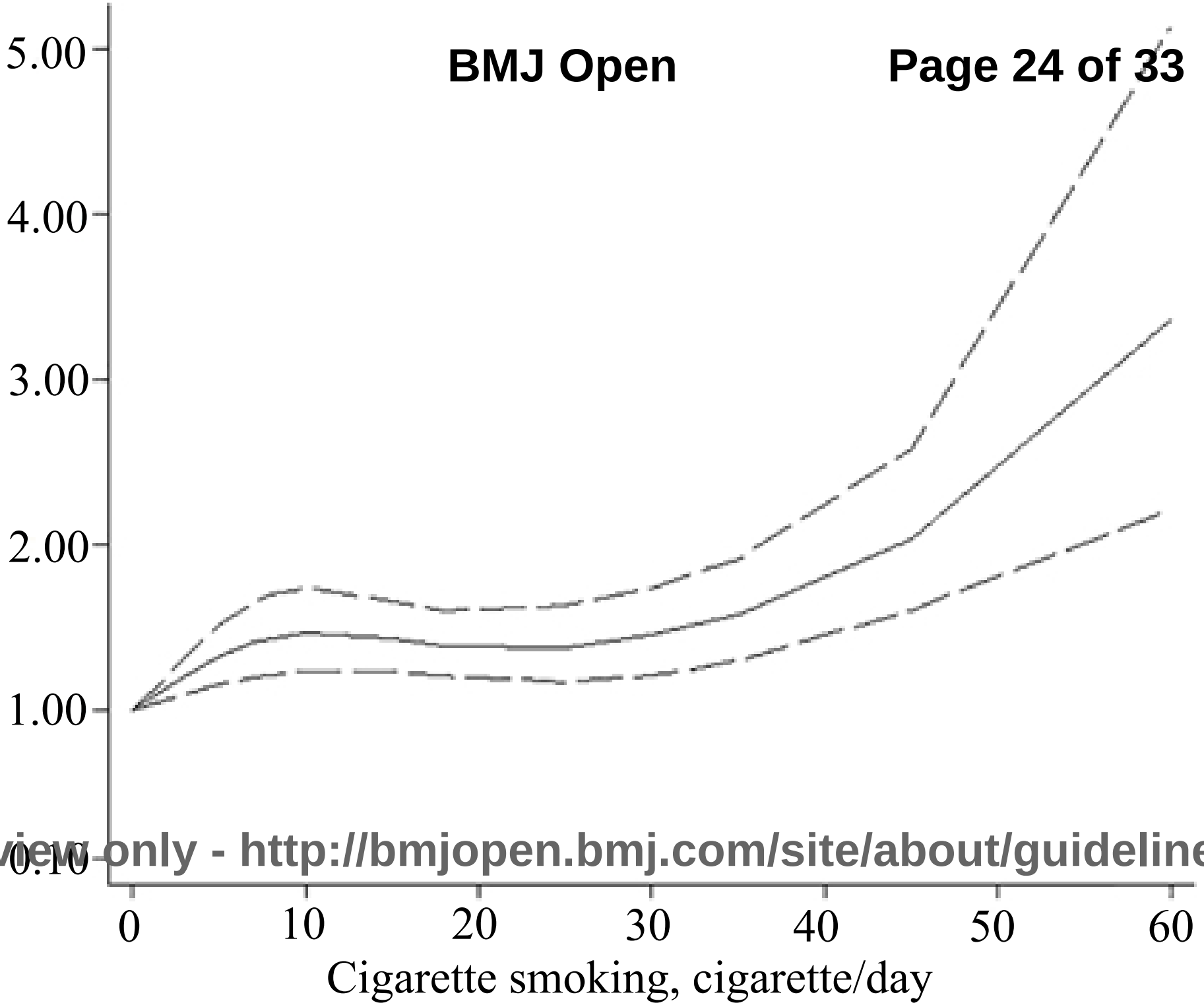

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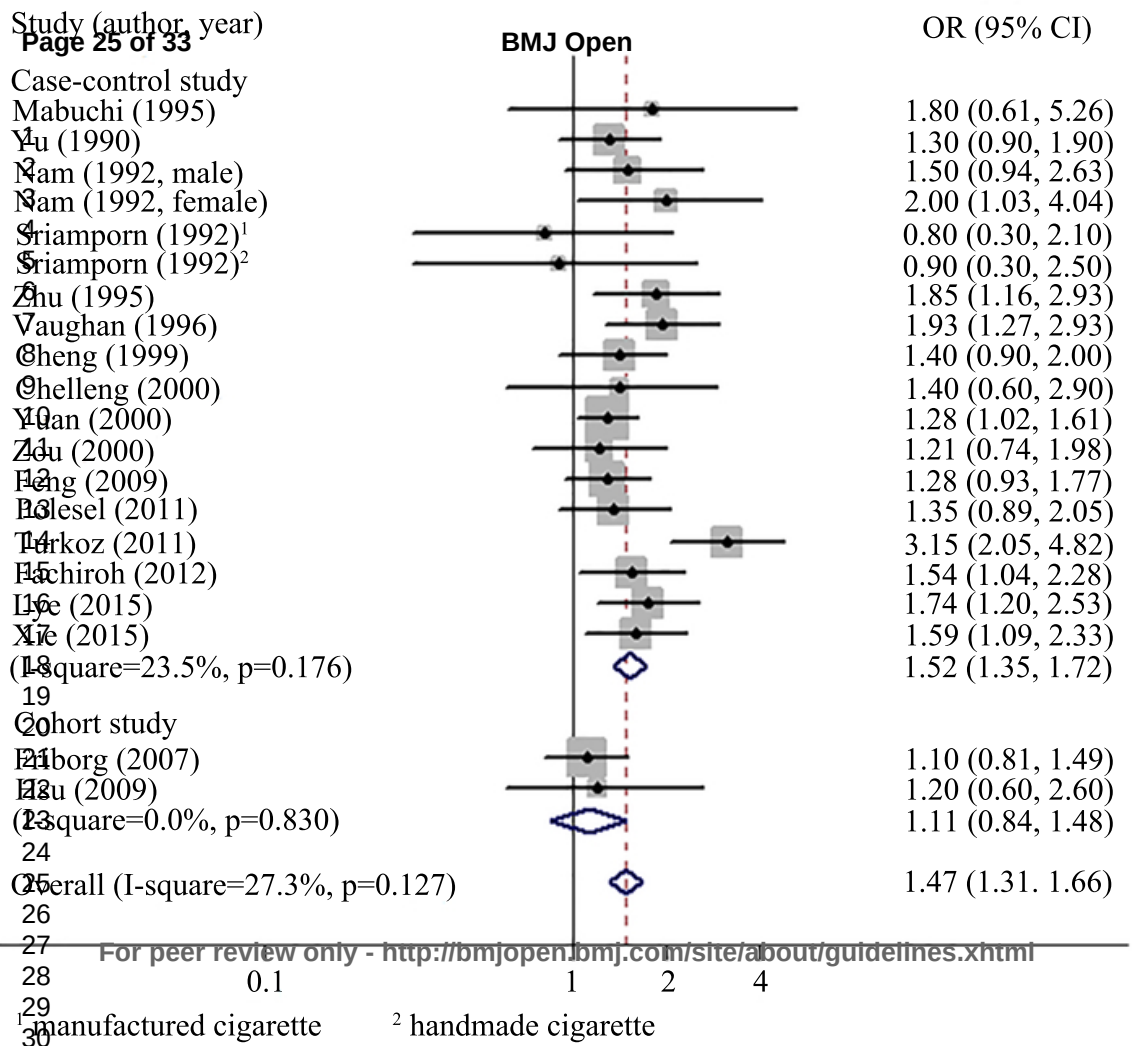

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[1] manufactured cigarette [2] handmade cigarette

Table 1. General characteristics of case-control studies used for meta-analysis

Study	Region	Period	Incidence rate	Cases/ Controls	Male/ Female	Age range (years old)	Quality score	Source of controls	Matching factors	Adjusting factors
Mabuchi et al (34)	the US	—	Low	39/39	—	—	7	Hospital-based	Age, sex, race, education, occupation, marital status	—
Yu et al (37)	Guangzhou	1983-1985	High	306/306	209/97	Under 45	7	Population-based	Age, sex, residence, education	Birth place, marital status, dietary risk
Nam et al (35)	the US	1983-1986	Low	204/408	141/63	Under 65	5	Hospital-based	Age, sex	By multiple logistic regression analysis
Sriamporn et al (36)	Thailand	1987-1990	Moderate	120/120	81/39	mean 47.2	6	Hospital-based	Age, sex	Age, sex, education, residence, occupation ,consumption of salted fish and alcohol
Zhu et al (38)	the US	1984-1988	Low	113/1910	male	—	8	Population-based	—	Birth, education, background, medical history, occupation, alcohol intake
Vaughan et al (39)	the US	1987-1993	Low	231/244	154/77	mean 55.2	9	Population-based	Age, sex, region	Age, sex, alcohol use, education
Cheng et al (7)	Taiwan	1991-1994	Moderate	375/327	260/115	mean 46 (15-74)	7	Population-based	Age, sex, residence, education, marital status	Age, sex, race, education, family history of NPC, drinking status
Chelleng et al (40)	India	1996-1997	Moderate	47/94	34/13	mean 43.7	6	Population-based	Age, sex, ethnicity	—
Yuan et al (41)	Shanghai	1987-1991	Moderate	935/1032	668/267	mean 50	8	Population-based	Age, sex, residence	Age, gender, education, intake frequencies of preserved foods, occupational exposure history of chronic ear and nose condition, family history of NPC

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Table 1. Continued

Study	Region	Period	Incidence rate	Cases/ Controls	Male/ Female	Age range (years old)	Quality score	Source of controls	Matching factors	Adjusting factors
Zou et al (51)	Yangjiang	1987-1995	High	97/192	83/14	mean 52.6 (30-82)	7	Population-based	Age, sex, occupation	—
Feng et al (42)	North Africa	2002-2005	Moderate	440/409	male	—	7	Hospital-based	Age, sex, ethnicity, center, childhood household type	Age, socioeconomic status, dietary risk factors
Ji et al (44)	Wuhan	1991-2009	Moderate	1044/1095	755/289	—	5	—	Age, sex, ethnicity	Age, gender, cigarette, alcohol intake, family history
Polesel et al (9)	Italy	1992-2008	Low	150/450	119/31	median 52 (18-76)	6	Hospital-based	Age, sex, residence	Age, sex, place of residence, education, alcohol intake
Turkoz et al (45)	Turkey	—	Moderate	183/183	122/61	mean 44.9 (18-75)	6	Hospital-based	Age, sex	Age, sex
Fachiroh et al (23)	Thailand	2005-2010	Moderate	681/1078	504/177	mean 49.8	6	Hospital-based	Age, sex, residence	Age group, sex, center , education, alcohol drinking
Lye et al (25)	Malaysia	2007	Moderate	356/356	276/80	mean 53.2	6	Hospital-based	Age, sex, ethnicity	Age, sex, ethnicity, salted fish and alcohol intake
Xie et al (26)	Hong Kong	2010-2012	High	352/410	253/99	mean 51.6	8	Population-based	Age, sex, ethnicity, residence district	Age, sex, education, house type, family history of NPC, environmental tobacco smoke exposure, dietary risk, occupational exposure and cooking experience

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Table 2. General characteristics of cohort studies used for meta-analysis

Study	Region	Period	Incidence rate	Cohort size	No. of cases	Years of follow-up	End-point	Quality score	Source of cohort	Adjusting factors
Chow et al (46)	the US	1954-1980	Low	248046	48	26	Mortality	6	Veterans	Age, calendar year
Friborg et al (10)	Singapore	1993-2005	High	61320	173	12	Morbidity	9	Population-based	Age, sex, dialect group, year of interview, education
Hsu et al (43)	Taiwan	1984-2006	Moderate	9622	32	mean 18.1	Incidence	9	Population-based	Age, two anti-EBV viral serum-markers
Lin et al (24)	Guangzhou	1988-1999	High	101823	34	mean 7.3	Incidence	8	Factory workers and drivers	Age, sex, education, drinking status, occupation

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Table 3. Subgroup analysis on pooled ORs for the association between cigarette smoking and nasopharyngeal carcinoma.

Subgroup	No. of Studies	Effect estimate (95% CI)	Heterogeneity I^2, *P*	Egger's Test *P value*	Adjusted for Publication Bias
Smoking status					
Ever smokers	19	1.56(1.32-1.83)	66.8%, <.01	0.29	1.56(1.32-1.84)
Current smokers	11	1.59 (1.35-1.89)	32.5%, .14	0.10	
Former smokers	10	1.36 (1.15-1.61)	2.3%, .42	0.97	
Design					
Case-control					
Current smokers	8	1.67(1.06-2.61)	22.6%, .25	0.58	
Former smokers	8	1.45(1.21-1.73)	0.0%, .70	0.98	
Cohort					
Current smokers	3	2.19(1.02-4.72)	65%, .06	0.16	
Former smokers	2	0.87(0.54-1.41)	0.0%, .37	—	
Pack-years					
<30	7	1.34 (1.13-1.58)	0.0%, .73	0.54	
⩾30	6	2.03 (1.57-2.61)	0.0%, .45	<0.01	1.80(1.37-2.36)
Age at onset					
<18y	5	1.78 (1.41-2.25)	0.0%, .94	0.46	
⩾18y	5	1.28 (1.00-1.64)	0.0%, .86	0.93	
Incidence rate					
Low	5	1.68 (1.36-2.07)	0.0%, .84	0.64	
Intermediate	10	1.59 (1.21-2.09)	78.8%, <.01	0.29	
High	4	1.27 (1.05-1.53)	0.0%, .52	0.63	
Gender					
Male	5	1.36 (1.15-1.60)	0.0%, .68	0.48	
Female	2	1.58 (0.99-2.53)	0.0%, .64	—	
Histological type					
Differentiated	5	2.34 (1.77-3.09)	0.0%, .72	0.64	
Undifferentiated	4	1.15 (0.90-1.46)	0.0%, .02	0.28	
Adjustment					
Adjusted	13	1.55 (1.26-1.91)	75.4%, <.01	0.33	
Unadjusted	6	1.57 (1.27-1.93)	0.0%, .68	0.93	

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MOOSE Checklist for Meta-analyses of Observational Studies

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	4
2	Hypothesis statement	5
3	Description of study outcome(s)	5
4	Type of exposure or intervention used	4
5	Type of study designs used	4
6	Study population	8
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	None
8	Search strategy, including time period included in the synthesis and key words	5
9	Effort to include all available studies, including contact with authors	5
10	Databases and registries searched	5
11	Search software used, name and version, including special features used (eg, explosion)	5
12	Use of hand searching (eg, reference lists of obtained articles)	5
13	List of citations located and those excluded, including justification	7
14	Method of addressing articles published in languages other than English	5
15	Method of handling abstracts and unpublished studies	7
16	Description of any contact with authors	None
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	5
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	5
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	6
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	6
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	6
22	Assessment of heterogeneity	7
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	6
24	Provision of appropriate tables and graphics	7
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	8
26	Table giving descriptive information for each study included	20
27	Results of sensitivity testing (eg, subgroup analysis)	9
28	Indication of statistical uncertainty of findings	11

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Item No	Recommendation	Reported on Page No
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	12, 14
30	Justification for exclusion (eg, exclusion of non-English language citations)	None
31	Assessment of quality of included studies	12
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	15
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	14
34	Guidelines for future research	15
35	Disclosure of funding source	16

From: Stroup DF, Berlin JA, Morton SC, et al, for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. Meta-analysis of Observational Studies in Epidemiology. A Proposal for Reporting. *JAMA*. 2000;283(15):2008-2012. doi: 10.1001/jama.283.15.2008.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstrac	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **Page 1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Page 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported: Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses **Page 4-5**
Methods		
Study design	4	Present key elements of study design early in the paper **Page 1-2**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page 5-7**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case **Page 6 and Table 1&2**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page 5**
Bias	9	Describe any efforts to address potential sources of bias **Page 3**
Study size	10	Explain how the study size was arrived at **Page 2 or 7**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Page 5-6**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses **Page 6-7**

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Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram **Page 7-8**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **Page 8**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures **Page 7-8**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **Page 8-11**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **Page 10-11**
Discussion		
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Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Page 15**
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Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Page 16**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Cigarette Smoking and the Risk of Nasopharyngeal Carcinoma: A Meta-Analysis of Epidemiological Studies

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Abstract

Objective The role of cigarette smoking as an independent risk factor for patients with nasopharyngeal carcinoma (NPC) is controversial. We attempted to provide evidence of a reliable association between cigarette smoking and the risk of NPC.

Design Meta-analysis.

Data sources PubMed online and the Cochrane Library of relevant studies published up to February 2016.

Eligibility criteria All studies had to evaluate the relationship between NPC and cigarette smoking with never smokers as the reference group.

Outcomes The primary outcome was the adjusted OR, RR or HR of NPC patients comparing smoking with never-smoking; the second was the crude OR, RR or HR.

Results We identified 17 case-control studies and four cohort studies including 5960 NPC cases and 429464 subjects. Compared with never smokers, current smokers and ever smokers had a 59% and a 56% greater risk of NPC respectively. A dose-response relation was identified in that the risk estimate rose by 15% (P<0.001) with every additional 10 pack-years of smoking, and risk increased with intensity of cigarette smoking (>30 cigarettes per day). Significantly increased risk was only found among male smokers (Odds Ratio (OR), 1.36; 95% confidence interval (CI), 1.15-1.60), not among female smokers (OR, 1.58; 95% CI, 0.99-2.53). Significantly increased risk also existed in the differentiated (OR, 2.34; 95% CI, 1.77-3.09) and the undifferentiated type of NPC (OR, 1.15; 95% CI, 0.90-1.46). Moreover, people started smoking at younger age (<18y) had a greater risk than those starting later for developing NPC (OR, 1.78; 95% CI, 1.41-2.25).

Conclusions Cigarette smoking was associated with increased risk of NPC, especially for young smokers. However, we did not find statistical significant risks of NPC in females and in undifferentiated type, which might warrant further researches.

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Strengths and limitations of this study

- Major strengths of our meta-analysis comprise new published studies being included, strict selection criteria, careful literature search, data extraction and analyses by two authors separately.
- The main limitations of our meta-analysis are study design, characteristics and size of study population, different outcome and variables used in eligible studies.

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Introduction

There were approximately 86,691 incident cases of NPC and 50,831 NPC-related deaths in 2012 worldwide [1]. Despite NPC being rare in developed countries, the overall incidence rate in Southeastern Asia is 6.5/100,000 person-years among males and 2.6/100,000 person-years among females [2]. Particularly, an age-standardized incidence rate of 20-50 per 100,000 males in south China presented a remarkably high incidence compared to that among white populations [3].

Cigarette smoking has been regarded as a risk factor for the occurrence of a wide variety of malignancies, including respiratory tract, gastrointestinal and urogenital systems [4, 5]. Over the decades, some reports have suggested that cigarette smoking is associated with NPC risk [6]. However, the association has not been consistently demonstrated, some studies failing to find such a positive association [7-10]. The discrepancies of inconsistent outcome might be owing to variations in study population, methodology, definitions of cigarette smoking and so on. Furthermore, inevitable recall bias and confounding in case-control studies might further complicate the scenario [11, 12].

One recent meta-analysis of 28 case-control studies and 4 cohort studies reported the adverse effect of cigarette smoking on the incidence of NPC [13]. The pooled analysis showed that ever smokers had a 60% greater risk of developing the disease than never smokers. And there was a significant dose-dependent association. However, between-study heterogeneity was strikingly high across the overall analysis and still remained after stratified analyses. Specifically, some included studies might not be appropriate to be combined for synthetic analysis because of their inadequate reports about association between cigarette smoking and NPC risk [14-17], unclear definition of cigarette smoking and health condition of controls [18, 19], controls with a history of cancer [20], and inappropriate reference group [21, 22]. These might result in overestimating or underestimating the association of cigarette smoking on NPC risk, and thus the conclusions might be hard to interpret. In addition, new studies have been published recently which warrant an up-to-date analysis [23-26].

In this meta-analysis, we sought to provide a summary of available literature to

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examine the association between cigarette smoking and the risk of NPC, we also assessed the gender and histological type differences in effects of cigarette smoking on the NPC risk.

Methods

Literature search

This meta-analysis was performed on the basis of the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) [27]. To identify all relevant publications on NPC and cigarette smoking, firstly, we used the engine "Windows Internet Explorer 10.0" to search the PubMed and Cochrane Library databases with terms "(((nasopharyngeal carcinoma OR nasopharyngeal cancer OR cancer of nasopharynx)) AND (smoking OR cigarette OR tobacco OR nicotine)) AND (etiology OR epidemiology OR environment OR risk factor) AND (Humans [Mesh])", then we scrutinized the references of articles obtained from the database search for additional studies. Only publications in English were included.

Selection criteria

The following criteria were applied for literature selection: (1) the study was case-control or cohort design; (2) controls were cancer-free; (3) cases were patients who were histopathologically confirmed NPC and had no other malignances; (4) the study evaluated the relationship between NPC and one of various aspects of cigarette smoking, including cigarette smoking status, smoking intensity, cumulative amount of cigarette smoking, age at onset and duration of smoking; (5)studies used never smokers as the reference group; (6)studies provided enough information to estimate the odds ratios (ORs) or the relative risk (RR) or hazard ratios (HRs) with 95% confidence interval (CI) for cigarette smoking variable. If multiple articles were on the same study population, the one with adequate information or most related or largest sample size was finally selected; furthermore, when there were separate data for gender or histologic type of NPC in one study, they were considered for additional subgroup analysis.

Data extraction

The following data were extracted from eligible studies: first author, publication

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year, study region, study design, sample size, control source, age of participants (range, mean), gender distribution, categories of smoking (status, intensity, pack-years, age at onset of smoking, et al.), method of questionnaire survey, duration of follow-up, end-point (for cohort study), covariates for adjustment, OR, RR or HR with their 95% CIs for each category of smoking exposure. In case the above effect sizes were not available, crude effect estimates and 95% CIs were calculated by provided number of subjects. All data were independently extracted and analyzed by two investigators; any inconsistency was resolved by consensus.

Quality assessment

The qualities of eligible studies were assessed by using the Newcastle-Ottawa Scale (NOS) [28], which comprised three parts assigned with a maximum of 9 points: selection, comparability, exposures and outcome condition. Two investigators evaluated all eligible publications separately and discrepancies were resolved by discussion.

Data integration

Not all studies included in this meta-analysis provided consistent information about cigarette smoking, so we stipulated smoking status as follows: never smokers (people that did not smoke any tobacco product), ever smokers, current smokers and former smokers. With regard to smoking quantity, we combined data extracted from all eligible publications into new categories: subjects with cigarettes consumption of <30 pack-years were assigned to light smokers, while those who consumed ≥30 pack-years were designated to heavy smokers. Similarly, for age at smoking onset, early group meant that subjects began smoking at <18 years old while later group defined as smoking at ≥18 years old. We also defined that regions with NPC incidence less than 1 per 100,000 person-years was low incidence rate group, 1-10 per 100,000 person-years was intermediate incidence rate group and greater than 10 per 100,000 was high incidence rate group.

Statistical analysis

Since NPC is considered as a relatively rare outcome, relative risk and odds ratio were not differentiated, the odds ratios were used as effect size for all studies. We

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conducted fixed and random effects meta-analyses and the synthetic estimates did not differ substantially between the two models. Therefore, random-effects (Der Simonian-Laird) model [29], generally regarded as the more conservative method, was applied to calculate point estimates for all analyses. Heterogeneity among articles was estimated by using the I^2 statistic and p value associated with Q statistics [30].

We conducted dose-response meta-analyses using the generalized least-squares method for trend estimation of summary dose-response data, as described by Greenland and Longnecker [31]. For non-linearity relationship, restricted cubic splines with four knots at percentiles 5%, 35%, 65% and 95% of the distribution were created and P value for non-linearity was computed by testing the null hypothesis that the coefficient of the second and the third splines were equal to zero [32].

To assess the robustness of our findings and the source of heterogeneity, meta-regression methods and stratified analyses were performed according to study design, incidence rate of regions, adjustment, score of eligible studies, categories of cigarette smoking, gender and NPC histological type (the latter three were only evaluated in stratified analysis). Sensitivity analysis was also conducted by deleting each study in turn to reflect the influence of every single study to the overall estimate. In addition, we evaluated the publication bias in the pooled analysis by Egger's test and the trim-and-fill method [33]. All statistical analyses were performed with Stata SE 12.0 software, and p value <0.05 (two sides) was considered statistically significant.

Patient involvement

No patients were involved in this study.

Results

Study characteristics

Figure 1 shows the flow chart describing the sequential selection procedures of eligible studies. A total of 342 articles were identified, of which 302 articles were deemed irrelevant after reviewing the titles and abstracts. Subsequently, 40 articles were further scanned by full-text. Meanwhile, by searching all references of relevant articles, three additional articles were considered as potentially eligible. Among them

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22 were excluded because of following reasons: five studies with inadequate information for data extraction, four studies without report of the association between cigarette smoking and NPC risk, four studies with overlapped data, four studies did not designate never smokers as reference group, two studies included improper controls (for example, controls with malignancies or without description of health conditions), one without clear definition of cigarette smoking, one systematic review and one meta-analysis. Finally, 21 articles were eligible for qualitative synthesis, including seventeen case-control studies (5673 cases and 8653 controls) and four cohort studies (287 cases and 420,811 participants).

All of the studies in the overall analysis were published between 1985 and 2015. Of these included studies, not all studies reported the estimates for all risk estimates. Nineteen studies reported on ever smoking [7-10, 23, 25, 26, 34-45], ten on former smoking [7-9, 23, 26, 38, 39, 41, 46], eleven on current smoking [7-10, 23, 24, 26, 38, 39, 41, 46], ten on pack-years of smoking [7, 23, 24, 26, 35, 37-39, 41, 43] and six on age at onset of smoking [7, 9, 10, 23, 26, 46]. Additionally, five studies provided separate data of gender [35, 38, 41-43] and five studies reported the risk of NPC histological type associated with cigarette smoking [9, 38, 39, 42, 44]. As regarding to geographic region, eight studies were conducted in China [7, 8, 24, 26, 37, 41, 43, 44], five in the US [34, 35, 38, 39, 46], five in Southeast Asia region [10, 23, 25, 36, 40], two in Europe [9, 45] and one in Africa [42]. The summarized characteristics of the 21 studies are presented in **Tables I** and **II.**

Association between cigarette smoking status and NPC

The pooled analysis of nineteen studies revealed a modest but significant increased risk of NPC among ever smokers against never smokers (OR, 1.56; 95% CI, 1.32-1.83). Heterogeneity was obviously observed across the studies (I^2=66.8%, P<0.01). The pooled estimate for case-control studies was 1.61 (95% CI, 1.36-1.91; heterogeneity: I^2=65.8%, P<0.01), whereas cohort studies presented a null association (OR, 1.11; 95% CI, 0.84-1.48; heterogeneity: I^2=0.0%, P=0.83) (**Figure 2**).

Similarly, eleven studies identified for the comparison of current smokers with NPC risk demonstrated positive result (OR, 1.59; 95% CI, 1.35-1.89; heterogeneity:

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I^2=32.5%, P=0.14). When analyzed by study design, the risk estimates were both statistically significant for case-control and cohort studies. The pooled ORs were 1.67 (95% CI, 1.06-2.61; heterogeneity: I^2=22.6%, P=0.25) and 2.19 (95% CI: 1.02-4.72; heterogeneity: I^2=65.0%, P=0.06), respectively (**Table III**).

When compared with never smokers, former smokers from ten studies exhibited an increased risk of NPC (OR, 1.36; 95% CI, 1.15-1.61; heterogeneity: I^2=2.3%, P=0.42). However, stratified analysis presented a void association in cohort studies (OR, 0.87; 95% CI, 0.54-1.41; heterogeneity: I^2=0.0%, P=0.37) but a significant association in case-control studies (OR, 1.45; 95% CI, 1.21-1.73; heterogeneity: I^2=0.0%, P=0.70) (**Table III**).

As for age at cigarette smoking onset, six studies reported the association with NPC risk. The pooled analysis revealed that early group (smoking at <18 years old) had significantly increased risk of NPC (OR, 1.78; 95% CI, 1.41-2.25; heterogeneity: I^2=0.0%, P=0.94), whereas later group (smoking at ≥18 years old) had slightly increased risk of NPC (OR, 1.28; 95% CI, 1.00-1.64; heterogeneity: I^2=0.0%, P=0.86) (**Table III**).

Dose-response analysis

For the cumulative amount of cigarette smoking, no between-study heterogeneity was found (I^2=0.0%, P>0.05) with a pooled OR of 1.34 (95% CI, 1.13-1.58) for light smokers and 2.03 (95% CI, 1.57-2.61) for heavy smokers, respectively (**Table III**). The dose-response analysis showed statistical linear relationship between the number of pack-years and NPC risk ($P_{\text{for linearity}}$=0.83) (**Figure 3**). Smokers had a 15% (OR, 1.15; 95% CI, 1.11-1.19, P<0.001) increasing risk of NPC for every additional 10 pack-years smoked in comparison with never smokers (data not shown). When comparing the NPC risk for intensity of cigarettes smoked per day with never smokers, the non-linear dose-response relationship indicated that smokers with high exposure (>30 cigarettes/day) other than with low exposure have higher risk estimate, which presented an upward tendency in steeply rising trend ($P_{\text{for non-linearity}}$<0.05) (**Figure 4**).

Stratified analysis

When conducted stratified analysis by regions with different incidence rate, there

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were nineteen studies compared NPC risk for ever smokers with that for never smokers. Among them, five studies carried out in regions with low NPC incidence rate yielded the highest risk (OR, 1.68; 95% CI, 1.36-2.07; heterogeneity: I^2=0.0%, P=0.84). The pooled estimates were 1.59 (95% CI, 1.21-2.09; heterogeneity: I^2=78.8%, P<0.01) for regions (ten studies) with intermediate NPC incidence rate and 1.27 (95% CI, 1.05-1.53; heterogeneity: I^2=0.0%, P=0.52) for regions (4 studies) with high incidence rate, respectively (**Table III**).

We also performed stratified analysis by status of adjustment for confounding variables. Thirteen studies provided adjusted ORs for pooled analysis. But six studies either reported unadjusted ORs or reported the number of cases and controls which could be used to calculate the odds ratios. The estimates for the association of cigarette smoking and NPC risk in adjusted group (OR, 1.55; 95% CI, 1.26-1.91; heterogeneity: I^2=75.3%, P<0.01) and in unadjusted group (OR, 1.57, 95% CI, 1.27-1.93; heterogeneity: I^2=0.0%, P=0.68) were similar (**Table III**).

When the meta-regression analyses were applied to assess the sources of heterogeneity and their impacts on the NPC risk, we found that the publication year, study design, regions of different incidence rate and quality of studies were not significant sources of heterogeneity (P=0.55, data not shown).

Association between cigarette smoking and histological type of NPC

Specifically, the effects of cigarette smoking on NPC histological types were different. We found that significant association was only noted for differentiated squamous-cell NPC (OR, 2.34; 95% CI, 1.77-3.09; heterogeneity: I^2=0.0%, P=0.72). Contrarily, the risk estimate for undifferentiated carcinoma of NPC in smokers was statistically insignificant though the odds ratio was 1.15 (95% CI, 0.90-1.46; heterogeneity: I^2=0.0%, P=0.02) (**Table III**).

Association between cigarette smoking of gender and NPC

Seven studies addressed the association between cigarette smoking and NPC risk by gender, including five in males and two in females. Compared with never smokers, increased risk for male smokers was noted (OR, 1.36; 95% CI, 1.15-1.60). However, an insignificant association (OR, 1.58; 95% CI, 0.99-2.53) was observed for female

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smokers (**Table III**).

Sensitivity analysis and publication bias

Sensitivity analysis revealed that Ji 2011 study [42] was the source of statistical heterogeneity in the pooled analysis for ever smokers. When this outlier study was removed, between-study heterogeneity dropped strikingly to 27.3% in the remaining studies, whereas the odds ratios (OR, 1.47; 95% CI, 1.31-1.66) changed moderately but remained significant. As for case-control studies, the OR changed from 1.61 (95% CI: 1.36-1.91) to 1.52 (95% CI: 1.35-1.72) with heterogeneity fallen from 65.8% to 23.5% (**Figure 5**). The findings were further verified in the intermediated incidence rate group (OR, 1.49, 95% CI, 1.21-1.82; heterogeneity: I^2=49.6%, P=0.04) and in the adjusted group (OR, 1.45; 95% CI, 1.25-1.69; heterogeneity: I^2=41.8%, P=0.05) (data not shown). However, the heterogeneity reduced partly when the study of Turkoz 2001 was removed (OR, 1.50; 95% CI, 1.28-1.76; heterogeneity: I2=62.4%, P<0.01) (data not shown).

Publication bias was evaluated by Egger's test and Trim-and-Fill method. Except for subgroup analyses with ever smokers and heavy smokers, no prominently significant publication bias (with P>0.05 in Egger's test) was observed in our meta-analysis. After adjusted for publication bias, the risk of NPC remained stable with an OR of 1.56 (95% CI, 1.32-1.84) for ever smokers, but changed slightly (OR, 1.80, 95% CI, 1.37-2.36) for heavy smokers (**Table III**).

Discussion

The results from this meta-analysis, based on seventeen case-control studies and four cohort studies, supported that there was moderate association between cigarette smoking and nasopharyngeal carcinoma risk, which was consistent with the result of previous meta-analysis [13].

Interpretation

The pooled risk estimate for cohort studies comparing ever smokers to never smokers was not statistically significant. When conducted similar stratified analyses for current smokers and former smokers, we found that current smoking was significantly related to the risk of NPC while former smoking had an insignificant

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association with NPC risk. Considering the findings of stratified analyses, it might be the result from former smoking that contributed to the discrepancy between pooled analysis for cohort studies and overall analysis. In addition, this meta-analysis demonstrated relatively high heterogeneity both for the overall analysis and subgroup analyses. When the Ji 2011 study [44] was removed from the synthetic analysis, heterogeneity was strikingly reduced in stratified analysis by study design and regions with different NPC incidence rate. Furthermore, the meta-regression analyses indicated that heterogeneity did not prominently result from publication year, study design, regions of different incident rate and quality of studies. To our knowledge, multiple lines of epidemiological studies had found that the development of NPC could be influenced by varieties of etiologies including Epstein-Barr virus (EBV), genetic components and other environmental factors, like preserved food, socioeconomic status, occupation, so on and so forth [6, 47-50]. Therefore, it might be its inappropriate subjects that contributed to selection bias which resulted in the high heterogeneity in the Ji 2011 study, though it had a large sample size with risk estimates adjusted by age, gender, alcohol intake and family history.

One large cohort study [10], conducted in high-incidence region and comprised the majority of undifferentiated NPC (nearly 90% cases), did not reported statistically increased risk of NPC among current smokers compared with never smokers. The difference in the effect of current smoking on NPC risk may be due to its histological type of NPC because undifferentiated carcinoma in high-risk areas seemed more strongly related to Epstein-Barr virus infection other than cigarette smoking [48]. Meanwhile, some case-control studies with small sample size of current smokers also had null results [7-9, 38, 39], of which two studies pointed out that significantly higher risk only existed for smokers with considerable levels of cigarette smoking (>20 cigarettes/day or >30 pack-years) [38, 39]. Nonetheless, the result of our integrated analysis for current smokers versus never smokers was generally consistent with that of the previous meta-analyses [13].

For former smokers, the less consistent risk estimates might result from small number of studies with adequate sample size. The estimates for former smokers in

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eight studies [7-10, 26, 39, 41, 46] presented null association on NPC risk which was parallel to the results of stratified analysis by study design, and only two studies [23, 38] demonstrated statistically positive results. The discrepancies in the effects of former cigarette smoking on NPC risk might arise from the following aspects: the group of former smokers may have included people who had quit for a long time, and thus their risk might diminish or even reach the level of never smokers; the minimum period of time since quitting smoking in former smokers varied by study, which could result in judgement bias on the interviewed subjects in some studies.

This meta-analysis revealed that there was a clear dose-response relationship between cigarette smoking and the risk of NPC. That is, the more cigarette smoking (intensity of cigarettes smoked per day and the amount of pack-years), the higher risk for the development of NPC. Note that similar results have been widely observed for pancreatic cancer, liver cancer, renal carcinoma and gallbladder disease [51-54]. The exact explanation of this dose-dependent effect remains vague, it could be hypothesized that the more cigarette smoking, the greater impact on the epithelial cells of nasopharynx. Therefore, the risk of NPC would be higher in those who smoked more cigarettes. The actual mechanism about the relationship of the amount of smoking and NPC risk had been searched by molecular studies [55, 56], which pointed out that smoking is a factor for tumor growth and acts as a mutagen and DNA damaging agent that drives tumor initiation in normal epithelial cells of nasopharynx.

In this analysis, a statistically significant effect of smoking on NPC risk was observed in males but not in females. The gender difference in response to smoking might be related to interaction between protective endogenous or exogenous estrogens among women compared with men [57], and could also be explained by maturity of smoking trends among males and but not among females. Men might exposure to smoking for a longer duration as compared to women (34% of the male vs. 11% of the female had started smoking before the age of 15 years) [58]. However, the result of female ever smokers might not be adequately stable because only two studies reported the association between cigarette smoking and the risk of NPC for females [35, 41].

Additionally, we found that the younger age people began to smoke, the higher

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risk they developed NPC. Our results showed that the pooled ORs were 1.78 (95% CI, 1.41-2.25) for smokers in early group and 1.28 (95% CI, 1.00-1.64) in later group, respectively. Interestingly, the findings of previous meta-analysis appeared totally opposite with ORs of 1.17 (95% CI, 0.78-1.75) for early group and 1.58 (95% CI, 1.10-2.26) for later group [13]. Like many other cancers, NPC may take decades to develop from premalignant cells to detectable solid tumor. Thus, the exposure to carcinogenic agents early in life could have substantial impacts on the development of NPC [6, 59]. Moreover, the incidence of NPC peaks at age of 50-59 years in high-risk regions, while in western countries, the incidence of NPC peaks somewhat later (≥65-year-olds) [59]. As a result, the number of NPC patients in terms of age distribution could considerably vary in our eligible studies that were conducted in different countries.

When stratified by histological type of NPC, the pooled analysis presented a higher risk of differentiated NPC than that of undifferentiated NPC, and the later had an insignificant risk estimate. This difference might be owing to fewer studies included in the pooled analysis for undifferentiated NPC because we excluded those ineligible studies either for no report of the association between cigarette smoking and NPC risk [16] or for overlapped data [60]. It might avoid incorrect estimation of smoking effects on NPC risk. Moreover, we found that the risk estimates adversely associated with the NPC incidence rate. For example, the pooled OR for high incidence rate areas to low incidence rate areas ranged from 1.27 to 1.68. This might suggest there are substantial heterogeneity between NPC risk and smoking by histological types and geographic variations. Undifferentiated carcinoma of the nasopharynx is the predominant type in high-risk areas, and it is consistently associated with EBV infection, which may increase the carcinogenic effect of cigarette smoking [48].

Generalizability

The magnitude of association between cigarette smoking and the NPC risk was not as big as those for other smoking-related cancers like lung cancer and gastrointestinal malignancies [4]. However, NPC was quite epidemic in southeastern

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Asia especially in cities in southern China, and China was one of the largest tobacco producing and consuming countries in the world [61]. Besides, we found current smokers are more related to the development of NPC with a higher risk estimate as compared to former smokers. These emphasized the importance and urgency of efforts to initiate the control of cigarette smoking to improve public health. Any efficient tobacco control programs would be helpful to reduce morbidity and mortality of smoking-related cancers worldwide.

Limitations

The results of this meta-analysis should be explicated in the context of several limitations. For example, the design of included studies varied in source of subjects recruited, standardization for categories of cigarette smoking, ambiguous definition of tobacco products and adjusted factors. Additionally, our meta-analysis was a mix of retrospective studies and prospective studies, and was lack of individual participant data for adjustment of potential confounders. Generally, Epstein-Barr virus infection was thought to be highly related to NPC risk [62]. However, a 22-year follow-up study carried out by Hsu et al. revealed that Epstein-Barr virus was less likely to modify the estimate for smoking associated with NPC risk [43]. And the links of other risk factors like dietary and social practices were often inconsistent between studies [62]. Moreover, the risk estimates of NPC resembled both in the group with adjusted odds ratio and in the group with unadjusted odds ratio in our meta-analysis.

Conclusions

This meta-analysis demonstrated that cigarette smoking associated with a modest, but statistically significant increased risk of NPC. Yet, further prospective studies are needed to elucidate the NPC risk in terms of gender, histological type, and for former smokers and smoking onset age.

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Contributor ship statement: LMJ (Mengjuan Long) and LP (Ping Li) did the literate research and selected the eligible articles separately; LP (Ping Li) and NZH (Zhihua Nie) extracted the whole data and assessed the quality of our selected articles; LMJ integrated and analyzed data, and wrote the manuscript. FZM (Zhenming Fu) examined and revised the manuscript. All authors read and approved the final manuscript.

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References

1. Ferlay J SI, et al. Cancer Incidence and Mortality Worldwide: IARC Cancer Base. *GLOBOCAN 2012 v1.0*.
2. Curado MP EB, et al. Cancer incidence in five continents. *Geneva: WHO Press 2007 vol. IX.*.
3. Jemal A, Bray F, Center MM et al. Global cancer statistics. *CA Cancer J Clin 20116*; 1: 69-90.
4. Sasco AJ, Secretan MB, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung Cancer 2004*; 45 Suppl 2: S3-9.
5. Sandler RS, Sandler DP, Comstock GW et al. Cigarette smoking and the risk of colorectal cancer in women. *J Natl Cancer Inst 1988*; 80: 1329-1333.
6. Jia WH, Qin HD. Non-viral environmental risk factors for nasopharyngeal carcinoma: a systematic review. *Semin Cancer Biol 2012*; 22: 117-126.
7. Cheng YJ, Hildesheim A, Hsu MM et al. Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. *Cancer Causes Control 1999*; 10: 201-207.
8. Zou J, Sun Q, Akiba S et al. A case-control study of nasopharyngeal carcinoma in the high background radiation areas of Yangjiang, China. *J Radiat Res 2000*; 41 Suppl: 53-62.
9. Polesel J, Franceschi S, Talamini R et al. Tobacco smoking, alcohol drinking, and the risk of different histological types of nasopharyngeal cancer in a low-risk population. *Oral Oncol 2011*; 47: 541-545.
10. Friborg JT, Yuan JM, Wang R et al. A prospective study of tobacco and alcohol use as risk factors for pharyngeal carcinomas in Singapore Chinese. *Cancer 2007*; 109: 1183-1191.
11. Grimes DA, Schulz KF. Bias and causal associations in observational research. *Lancet 2002*; 359: 248-252.
12. Weinberg CR. Toward a clearer definition of confounding. *Am J Epidemiol 1993*; 137: 1-8.
13. Xue WQ, Qin HD, Ruan HL et al. Quantitative association of tobacco smoking with the risk of nasopharyngeal carcinoma: a comprehensive meta-analysis of studies conducted between 1979 and 2011. *Am J Epidemiol 2013*; 178: 325-338.
14. Lin TM, Yang CS, Tu SM et al. Interaction of factors associated with cancer of the nasopharynx. *Cancer 1979*; 44: 1419-1423.
15. Chen CJ, Liang KY, Chang YS et al. Multiple risk factors of nasopharyngeal carcinoma: Epstein-Barr virus, malarial infection, cigarette smoking and familial tendency. *Anticancer Res 1990*; 10: 547-553.
16. Nesic V, Sipetic S, Vlajinac H et al. Risk factors for the occurrence of undifferentiated carcinoma of nasopharyngeal type: a case-control study. *Srp Arh Celok Lek 2010*; 138: 6-10.
17. Ren ZF, Liu WS, Qin HD et al. Effect of family history of cancers and environmental factors on risk of nasopharyngeal carcinoma in Guangdong, China. *Cancer Epidemiol 2010*; 34: 419-424.
18. Armstrong RW, Armstrong MJ, Yu MC, Henderson BE. Salted fish and inhalants as risk factors for nasopharyngeal carcinoma in Malaysian Chinese. *Cancer Res 1983*; 43: 2967-2970.
19. West S, Hildesheim A, Dosemeci M. Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. *Int J Cancer 1993*; 55: 722-727.
20. Ng TP. A case-referent study of cancer of the nasal cavity and sinuses in Hong Kong. *Int J Epidemiol 1986*; 15: 171-175.
21. Guo X, Johnson RC, Deng H et al. Evaluation of nonviral risk factors for nasopharyngeal carcinoma in a high-risk population of Southern China. *Int J Cancer 2009*; 124: 2942-2947.
22. Ma F, Zhang H, Zhai Y et al. Functional polymorphism -31C/G in the promoter of BIRC5 gene

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and risk of nasopharyngeal carcinoma among chinese. *PLoS One 2011*; 6: e16748.

23. Fachiroh J, Sangrajrang S, Johansson M et al. Tobacco consumption and genetic susceptibility to nasopharyngeal carcinoma (NPC) in Thailand. *Cancer Causes Control 2012*; 23: 1995-2002.

24. Lin JH, Jiang CQ, Ho SY et al. Smoking and nasopharyngeal carcinoma mortality: a cohort study of 101,823 adults in Guangzhou, China. *BMC Cancer 2015*; 15: 906.

25. Lye MS, Visuvanathan S, Chong PP et al. Homozygous Wildtype of XPD K751Q Polymorphism Is Associated with Increased Risk of Nasopharyngeal Carcinoma in Malaysian Population. *PLoS One 2015*; 10: e0130530.

26. Xie SH, Yu IT, Tse LA et al. Tobacco smoking, family history, and the risk of nasopharyngeal carcinoma: a case-referent study in Hong Kong Chinese. *Cancer Causes Control 2015*; 26: 913-921.

27. Stroup DF, Berlin JA, Morton SC et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *Jama 2000*; 283: 2008-2012.

28. Wells G Sbea. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Ottawa, Ontario, *Canada: Ottawa Health Research Institute 2000*.

29. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials 1986*; 7: 177-188.

30. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med 2002*; 21: 1539-1558.

31. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol 1992*; 135: 1301-1309.

32. Orsini N GS, et al. A procedure to tabulate and plot results after flexible modeling of a quantitative covariate. *Stata J 2011*; 11: 29.

33. Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. Bmj 315: 629-634, 1997.

34. Mabuchi K, Bross DS, Kessler, II. Cigarette smoking and nasopharyngeal carcinoma. *Cancer 1985*; 55: 2874-2876.

35. Nam JM, McLaughlin JK, Blot WJ. Cigarette smoking, alcohol, and nasopharyngeal carcinoma: a case-control study among U.S. whites. *J Natl Cancer Inst 1992*; 84: 619-622.

36. Sriamporn S, Vatanasapt V, Pisani P et al. Environmental risk factors for nasopharyngeal carcinoma: a case-control study in northeastern Thailand. *Cancer Epidemiol Biomarkers Prev 1992*; 1: 345-348.

37. Yu MC, Garabrant DH, Huang TB, Henderson BE. Occupational and other non-dietary risk factors for nasopharyngeal carcinoma in Guangzhou, China. *Int J Cancer 1990*; 45: 1033-1039.

38. Zhu K, Levine RS, Brann EA et al. A population-based case-control study of the relationship between cigarette smoking and nasopharyngeal cancer (United States). *Cancer Causes Control 1995*; 6: 507-512.

39. Vaughan TL, Shapiro JA, Burt RD et al. Nasopharyngeal cancer in a low-risk population: defining risk factors by histological type. *Cancer Epidemiol Biomarkers Prev 1996*; 5: 587-593.

40. Chelleng PK, Narain K, Das HK et al. Risk factors for cancer nasopharynx: a case-control study from Nagaland, India. *Natl Med J India 2000*; 13: 6-8.

41. Yuan JM, Wang XL, Xiang YB et al. Non-dietary risk factors for nasopharyngeal carcinoma in Shanghai, China. *Int J Cancer 2000*; 85: 364-369.

42. Feng BJ, Khyatti M, Ben-Ayoub W et al. Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. *Br J Cancer 2009*; 101: 1207-1212.

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43. Hsu WL, Chen JY, Chien YC et al. Independent effect of EBV and cigarette smoking on nasopharyngeal carcinoma: a 20-year follow-up study on 9,622 males without family history in Taiwan. *Cancer Epidemiol Biomarkers Prev 2009*; 18: 1218-1226.

44. Ji X, Zhang W, Xie C et al. Nasopharyngeal carcinoma risk by histologic type in central China: impact of smoking, alcohol and family history. *Int J Cancer 2011*; 129: 724-732.

45. Turkoz FP, Celenkoglu G, Dogu GG et al. Risk factors of nasopharyngeal carcinoma in Turkey-an epidemiological survey of the Anatolian Society of Medical Oncology. *Asian Pac J Cancer Prev 2011*; 12: 3017-3021.

46. Chow WH, McLaughlin JK, Hrubec Z et al. Tobacco use and nasopharyngeal carcinoma in a cohort of US veterans. *Int J Cancer 1993*; 55: 538-540.

47. Hildesheim A, West S, DeVeyra E et al. Herbal medicine use, Epstein-Barr virus, and risk of nasopharyngeal carcinoma. *Cancer Res 1992*; 52: 3048-3051.

48. Tsao SW, Tsang CM, Pang PS et al. The biology of EBV infection in human epithelial cells. *Semin Cancer Biol 2012*; 22: 137-143.

49. Polesel J, Serraino D, Negri E et al. Consumption of fruit, vegetables, and other food groups and the risk of nasopharyngeal carcinoma. *Cancer Causes Control 2013*; 24: 1157-1165.

50. He YQ, Xue WQ, Shen GP et al. Household inhalants exposure and nasopharyngeal carcinoma risk: a large-scale case-control study in Guangdong, China. *BMC Cancer 2015*; 15: 1022.

51. Zou L, Zhong R, Shen N et al. Non-linear dose-response relationship between cigarette smoking and pancreatic cancer risk: evidence from a meta-analysis of 42 observational studies. *Eur J Cancer 2014*; 50: 193-203.

52. Lee YC, Cohet C, Yang YC et al. Meta-analysis of epidemiologic studies on cigarette smoking and liver cancer. *Int J Epidemiol 2009*; 38: 1497-1511.

53. Hunt JD, van der Hel OL, McMillan GP et al. Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. *Int J Cancer 2005*; 114: 101-108.

54. Aune D, Vatten LJ, Boffetta P. Tobacco smoking and the risk of gallbladder disease. *Eur J Epidemiol 2016*; 31: 643-653.

55. Furmanski P. Revealing the mechanism of tissue damage due to tobacco use: finally, a smoking gun? *Am J Pathol 2013*; 182: 1489-1493.

56. Salem AF, Al-Zoubi MS, Whitaker-Menezes D et al. Cigarette smoke metabolically promotes cancer, via autophagy and premature aging in the host stromal microenvironment. *Cell Cycle 2013*; 12: 818-825.

57. Corrao G, Zambon A, Conti V et al. Menopause hormone replacement therapy and cancer risk: an Italian record linkage investigation. *Ann Oncol 2008*; 19: 150-155.

58. Peto R, Darby S, Deo H et al. Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. *Bmj 2000*; 321: 323-329.

59. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev 2006*; 15: 1765-1777.

60. Nazar-Stewart V, Vaughan TL, Burt RD et al. Glutathione S-transferase M1 and susceptibility to nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev 1999*; 8: 547-551.

61. Mackay J, Ritthiphakdee B, Reddy KS: Tobacco control in Asia. *Lancet 2013*; 381: 1581-1587.

62. Chua ML, Wee JT, Hui EP, Chan AT. Nasopharyngeal carcinoma. *Lancet 2016*; 387: 1012-1024.

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Table I. General characteristics of case-control studies used for meta-analysis

Study	Region	Period	Incidence rate	Cases/ Controls	Male/ Female	Age range (years old)	Quality score	Source of controls	Matching factors	Adjusting factors
Mabuchi et al (34)	the US	—	Low	39/39	—	—	7	Hospital-based	Age, sex, race, Education, occupation, marital status	—
Yu et al (37)	Guangzhou	1983-1985	High	306/306	209/97	Under 45	7	Population-based	Age, sex, residence, education	Birth place, marital status, dietary risk
Nam et al (35)	the US	1983-1986	Low	204/408	141/63	Under 65	5	Hospital-based	Age, sex	By multiple logistic regression analysis
Sriamporn et al (36)	Thailand	1987-1990	Moderate	120/120	81/39	mean 47.2	6	Hospital-based	Age, sex	Age, sex, education, residence, occupation ,consumption of salted fish and alcohol
Zhu et al (38)	the US	1984-1988	Low	113/1910	male	—	8	Population-based	—	Birth, education, background, medical history, occupation, alcohol intake
Vaughan et al (39)	the US	1987-1993	Low	231/244	154/77	mean 55.2	9	Population-based	Age, sex, region	Age, sex, alcohol use, education
Cheng et al (7)	Taiwan	1991-1994	Moderate	375/327	260/115	mean 46 (15-74)	7	Population-based	Age, sex, residence, education, marital status	Age, sex, race, education, family history of NPC, drinking status
Chelleng et al (40)	India	1996-1997	Moderate	47/94	34/13	mean 43.7	6	Population-based	Age, sex, ethnicity	—
Yuan et al (41)	Shanghai	1987-1991	Moderate	935/1032	668/267	mean 50	8	Population-based	Age, sex, residence	Age, gender, education, intake frequencies of preserved foods, occupational exposure history of chronic ear and nose condition, family history of NPC

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Table I. Continued

Study	Region	Period	Incidence rate	Cases/ Controls	Male/ Female	Age range (years old)	Quality score	Source of controls	Matching factors	Adjusting factors
Zou et al (51)	Yangjiang	1987-1995	High	97/192	83/14	mean 52.6 (30-82)	7	Population-based	Age, sex, occupation	—
Feng et al (42)	North Africa	2002-2005	Moderate	440/409	male	—	7	Hospital-based	Age, sex, ethnicity, center, childhood household type	Age, socioeconomic status, dietary risk factors
Ji et al (44)	Wuhan	1991-2009	Moderate	1044/1095	755/289	—	5	—	Age, sex, ethnicity	Age, gender, cigarette, alcohol intake, family history
Polesel et al (9)	Italy	1992-2008	Low	150/450	119/31	median 52 (18-76)	6	Hospital-based	Age, sex, residence	Age, sex, place of residence, education, alcohol intake
Turkoz et al (45)	Turkey	—	Moderate	183/183	122/61	mean 44.9 (18-75)	6	Hospital-based	Age, sex	Age, sex
Fachiroh et al (23)	Thailand	2005-2010	Moderate	681/1078	504/177	mean 49.8	6	Hospital-based	Age, sex, residence	Age group, sex, center , education, alcohol drinking
Lye et al (25)	Malaysia	2007	Moderate	356/356	276/80	mean 53.2	6	Hospital-based	Age, sex, ethnicity	Age, sex, ethnicity, salted fish and alcohol intake
Xie et al (26)	Hong Kong	2010-2012	High	352/410	253/99	mean 51.6	8	Population-based	Age, sex, ethnicity, residence district	Age, sex, education, house type, family history of NPC, environmental tobacco smoke exposure, dietary risk, occupational exposure and cooking experience

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Table II. General characteristics of cohort studies used for meta-analysis

Study	Region	Period	Incidence rate	Cohort size	No. of cases	Years of follow-up	End-point	Quality score	Source of cohort	Adjusting factors
Chow et al (46)	the US	1954-1980	Low	248046	48	26	Mortality	6	Veterans	Age, calendar year
Friborg et al (10)	Singapore	1993-2005	High	61320	173	12	Morbidity	9	Population-based	Age, sex, dialect group, year of interview, education
Hsu et al (43)	Taiwan	1984-2006	Moderate	9622	32	mean 18.1	Incidence	9	Population-based	Age, two anti-EBV viral serum-markers
Lin et al (24)	Guangzhou	1988-1999	High	101823	34	mean 7.3	Incidence	8	Factory workers and drivers	Age, sex, education, drinking status, occupation

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Table III. Subgroup analysis on pooled ORs for the association between cigarette smoking and nasopharyngeal carcinoma.

Subgroup	No. of Studies	Effect estimate (95% CI)	Heterogeneity I^2, *P*	Egger's Test *P value*	Adjusted for Publication Bias
Smoking status					
Ever smokers	19	1.56(1.32-1.83)	66.8%, <.01	0.29	1.56(1.32-1.84)
Current smokers	11	1.59 (1.35-1.89)	32.5%, .14	0.10	
Former smokers	10	1.36 (1.15-1.61)	2.3%, .42	0.97	
Design					
Case-control					
Current smokers	8	1.67(1.06-2.61)	22.6%, .25	0.58	
Former smokers	8	1.45(1.21-1.73)	0.0%, .70	0.98	
Cohort					
Current smokers	3	2.19(1.02-4.72)	65%, .06	0.16	
Former smokers	2	0.87(0.54-1.41)	0.0%, .37	—	
Pack-years					
<30	7	1.34 (1.13-1.58)	0.0%, .73	0.54	
⩾30	6	2.03 (1.57-2.61)	0.0%, .45	<0.01	1.80(1.37-2.36)
Age at onset of smoking					
<18y	5	1.78 (1.41-2.25)	0.0%, .94	0.46	
⩾18y	5	1.28 (1.00-1.64)	0.0%, .86	0.93	
Incidence rate					
Low	5	1.68 (1.36-2.07)	0.0%, .84	0.64	
Intermediate	10	1.59 (1.21-2.09)	78.8%, <.01	0.29	
High	4	1.27 (1.05-1.53)	0.0%, .52	0.63	
Gender					
Male	5	1.36 (1.15-1.60)	0.0%, .68	0.48	
Female	2	1.58 (0.99-2.53)	0.0%, .64	—	
Histological type					
Differentiated	5	2.34 (1.77-3.09)	0.0%, .72	0.64	
Undifferentiated	4	1.15 (0.90-1.46)	0.0%, .02	0.28	
Adjustment					
Adjusted	13	1.55 (1.26-1.91)	75.4%, <.01	0.33	
Unadjusted	6	1.57 (1.27-1.93)	0.0%, .68	0.93	

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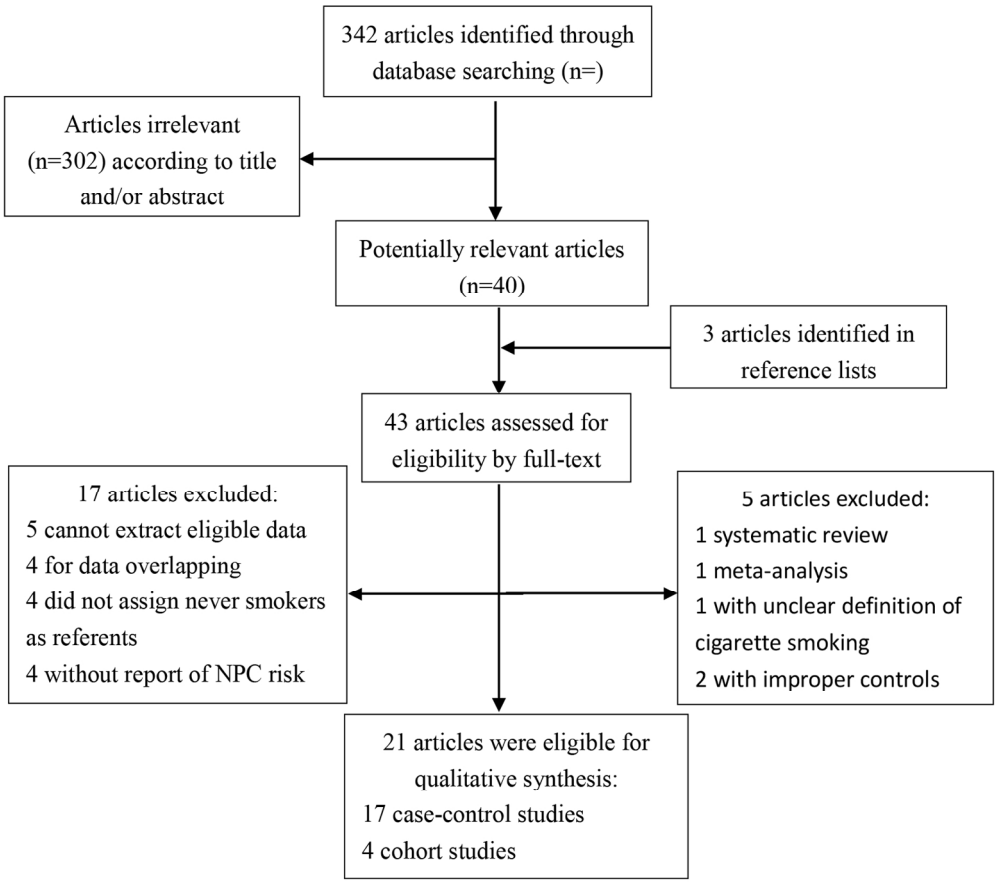

Summary of literature search.

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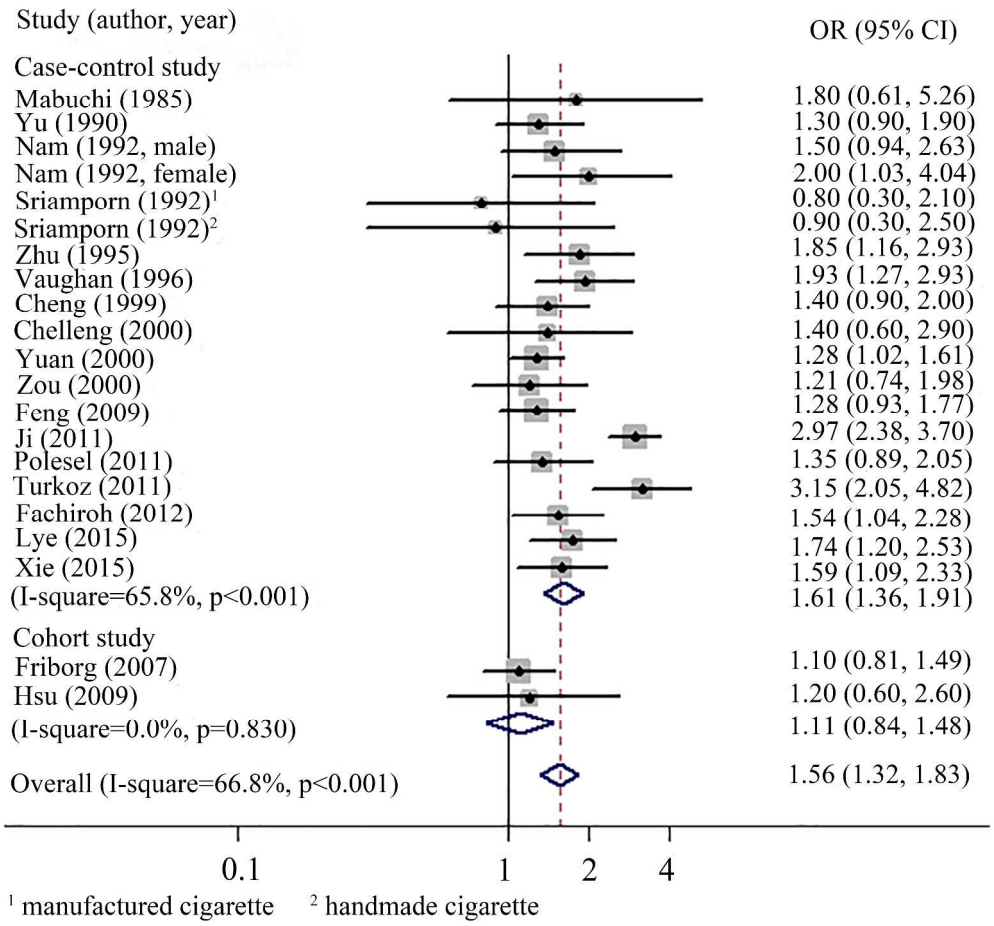
only



Forest plots for comparing the risk for NPC between ever smokers versus never smokers.

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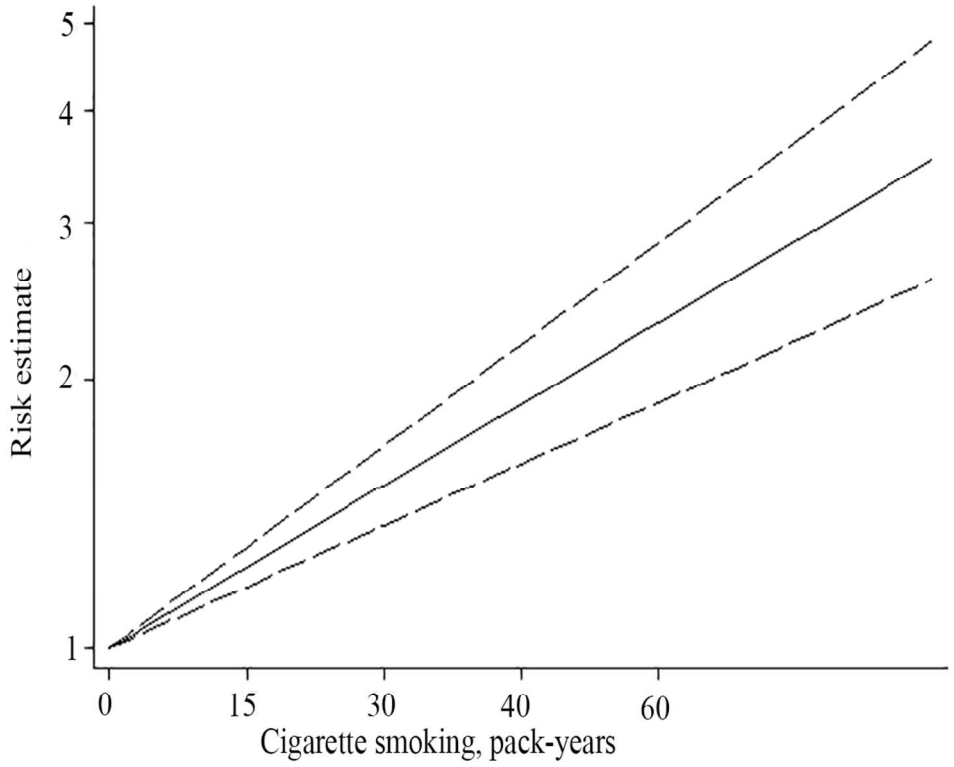

A linear relationship between the cumulative number of pack-years and NPC risk (P for linearity=0.83), with a 15% (95% CI: 1.11-1.19, P<0.001) increasing risk of NPC for every additional 10 pack-years smoked in comparison with never smokers (The solid line depicts the pooled risk estimate of NPC associated with each 1-pack-year increment of cigarette smoking, the dashed line depicts the upper confidence interval, the dot line depicts the lower confidence interval).

99x82mm (300 x 300 DPI)

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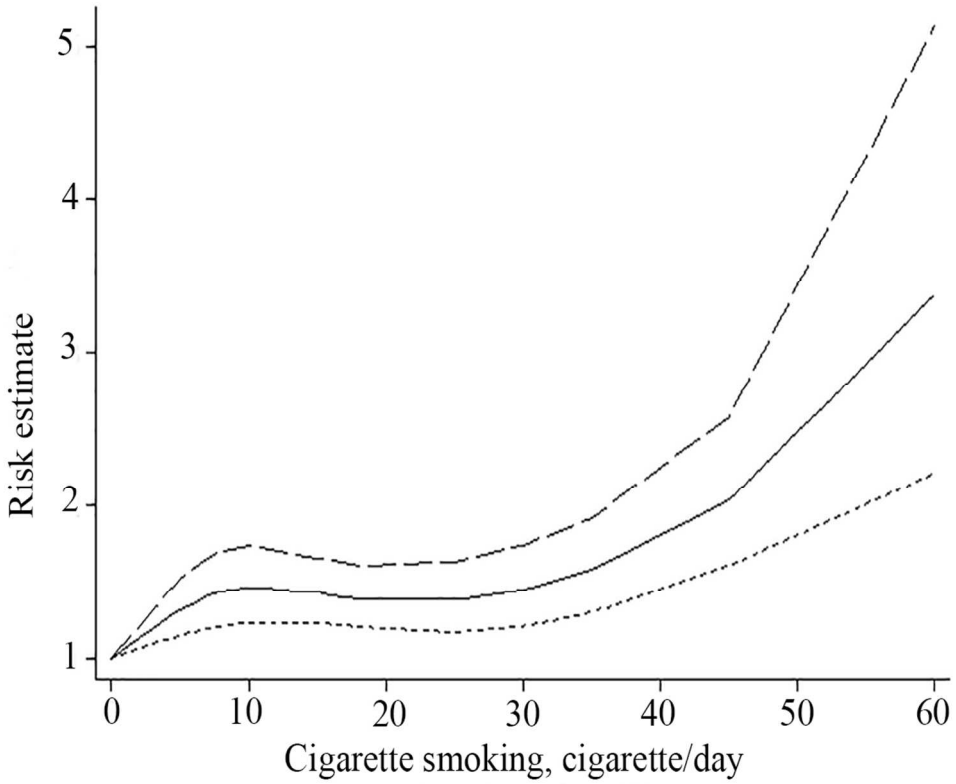

A non-linear association between intensity of cigarette smoking and NPC risk (P for non-linearity<0.05) (The solid line depicts the pooled risk estimate of NPC associated with each 1 cigarette/day increment, the dashed lines depict the upper and the lower confidence interval, respectively).

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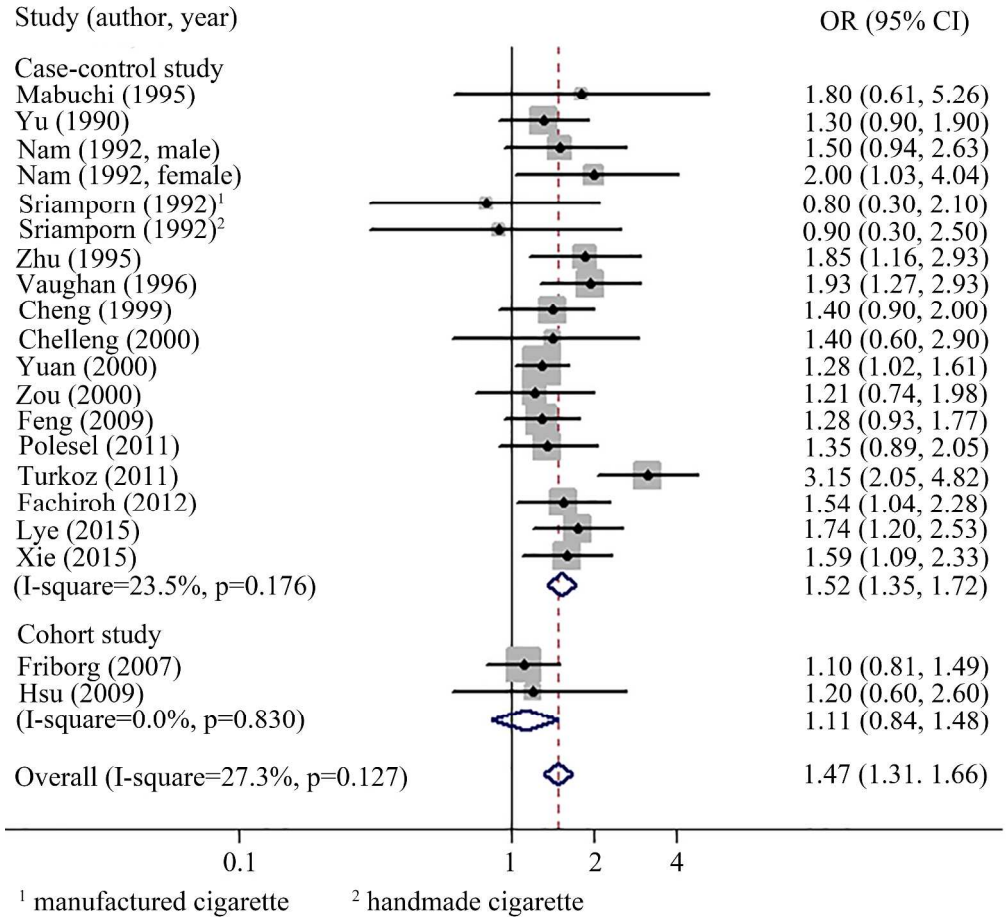

Forest plots for comparing the risk for NPC between ever smokers versus never smokers after deleting the Ji 2011 study.

only

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstrac	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **Page 1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Page 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported: Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses **Page 4-5**
Methods		
Study design	4	Present key elements of study design early in the paper **Page 1-2**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page 5-7**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case **Page 6 and Table 1&2**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page 5**
Bias	9	Describe any efforts to address potential sources of bias **Page 3**
Study size	10	Explain how the study size was arrived at **Page 2 or 7**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Page 5-6**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses **Page 6-7**

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Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram **Page 7-8**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **Page 8**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures **Page 7-8**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **Page 8-11**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **Page 10-11**
Discussion		
Key results	18	Summarise key results with reference to study objectives **Page 11-14**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Page 15**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Page 11-14**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Page 14-15**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Page 16**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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MOOSE Checklist for Meta-analyses of Observational Studies

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	4
2	Hypothesis statement	5
3	Description of study outcome(s)	5
4	Type of exposure or intervention used	4
5	Type of study designs used	4
6	Study population	8
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	None
8	Search strategy, including time period included in the synthesis and key words	5
9	Effort to include all available studies, including contact with authors	5
10	Databases and registries searched	5
11	Search software used, name and version, including special features used (eg, explosion)	5
12	Use of hand searching (eg, reference lists of obtained articles)	5
13	List of citations located and those excluded, including justification	7
14	Method of addressing articles published in languages other than English	5
15	Method of handling abstracts and unpublished studies	7
16	Description of any contact with authors	None
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	5
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	5
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	6
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	6
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	6
22	Assessment of heterogeneity	7
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	6
24	Provision of appropriate tables and graphics	7
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	8
26	Table giving descriptive information for each study included	20
27	Results of sensitivity testing (eg, subgroup analysis)	9
28	Indication of statistical uncertainty of findings	11

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Item No	Recommendation	Reported on Page No
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	12, 14
30	Justification for exclusion (eg, exclusion of non-English language citations)	None
31	Assessment of quality of included studies	12
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	15
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	14
34	Guidelines for future research	15
35	Disclosure of funding source	16

From: Stroup DF, Berlin JA, Morton SC, et al, for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. Meta-analysis of Observational Studies in Epidemiology. A Proposal for Reporting. *JAMA*. 2000;283(15):2008-2012. doi: 10.1001/jama.283.15.2008.

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