Different Association of Manganese Superoxide Dismutase Gene Polymorphisms with Risk of Prostate, Esophageal, and Lung Cancers: Evidence from a Meta-analysis of 20,025 Subjects

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Abstract

Altered expression or function of manganese superoxide dismutase (MnSOD) has been shown to be associated with cancer risk but assessment of gene polymorphisms has resulted in inconclusive data. Here a search of published data was made and 22 studies were recruited, covering 20,025 case and control subjects, for meta-analyses of the association of MnSOD polymorphisms with the risk of prostate, esophageal, and lung cancers. The data on 12 studies of prostate cancer (including 4,182 cases and 6,885 controls) showed a statistically significant association with the risk of development in co-dominant models and dominant models, but not in the recessive model. Subgroup analysis showed there was no statistically significant association of MnSOD polymorphisms with aggressive or nonaggressive prostate cancer in different genetic models. In addition, the data on four studies of esophageal cancer containing 620 cases and 909 controls showed a statistically significant association between MnSOD polymorphisms and risk in all comparison models. In contrast, the data on six studies of lung cancer with 3,375 cases and 4,050 controls showed that MnSOD polymorphisms were significantly associated with the decreased risk of lung cancer in the homozygote and dominant models, but not the heterozygote model. A subgroup analysis of the combination of MnSOD polymorphisms with tobacco smokers did not show any significant association with lung cancer risk, histological type, or clinical stage of lung cancer. The data from the current study indicated that the Ala allele MnSOD polymorphism is associated with increased risk of prostate and esophageal cancers, but with decreased risk of lung cancer. The underlying molecular mechanisms warrant further investigation.

Keywords: Manganese superoxide dismutase - polymorphism - prostate cancer - esophageal cancer - lung cancer

Introduction

Manganese superoxide dismutase (MnSOD), a nucleus-encoded antioxidant enzyme localized exclusively in the mitochondria, catalyzes the dismutation of two molecules of a superoxide anion into water and hydrogen peroxide, primarily to protect mitochondrial components from superoxide damage (the latter is a normal byproduct of respiration). Thus, MnSOD is one of the most important enzymes to defend against reactive oxygen species (ROS) in the mitochondria. The MnSOD gene is a single-copy gene with five exons and four introns and located on chromosome 6q25.3, the region of which is frequently deleted in various cancer tissues (Zhong et al., 1997; Li et al., 1998; Oberley et al., 2004).

Moreover, a molecular epidemiology study showed that there are at least two functional validated single nucleotide polymorphisms (SNPs) in the MnSOD gene, one of which involves a C to T substitution at nucleotide residue 339 leading to a substitution of isoleucine by threonine at amino acid residue 58 (Ile58Thr), and affects stability of the tetrameric interface of MnSOD and reduces protein amount and thus enzyme activity (Cai et al., 2004). A previous study demonstrated that breast cancer cells overexpressing the Ile58 allele had much higher MnSOD activity than that of the cells overexpressing the Thr58 allele (Zhang et al., 1999). This polymorphism is so rare in the population (< 0.05%) that it does not play any significant role in sporadic breast cancer development (Egan et al., 2003). However, another common polymorphism of MnSOD is Val16Ala (rs4880), a substitution of T to C (thymine to cytosine) at nucleotide 47 resulting in change of the amino acid from valine (Val, GTT) to alanine (Ala, GCT) on the 16th residue of a 24-amino acid signal sequence (Cai et al., 2003; Wang et al., 2009). This residue is 9 amino acids upstream of the
cleavage site and was described as the MnSOD Val9Ala (rs4880) polymorphism. Molecularly, the Val variant is predicted to form a β-sheet structure on the protein, while the Ala variant results in an α-helical conformation. It was demonstrated that MnSOD is synthesized in the cytoplasm and transported into the mitochondria via a N-terminal mitochondrial targeting sequence (MTS); the Ala variant of the MnSOD protein has a decreased rate of protein transportation into the mitochondria and reduced enzymatic activity compared to the Val variant in the mitochondria. The inefficient MnSOD protein in the mitochondria may leave the cell vulnerable to oxidative damage without its full defense against superoxide radicals, which leads to protein oxidation and DNA mutations (Ambrosone et al., 1999). The Ala variant of the MnSOD protein has been reported to increase risk of several types of malignancies, such as breast cancer (Mitrunen et al., 2001), prostate cancer (Choi et al., 2008), esophageal cancer (Murphy et al., 2007), and cervical cancer (Tong et al, 2009), and additional studies showed that it is likely to be associated with alteration of ROS and thus predisposed to a lower risk of liver (Sutton et al., 2003), lung (Wang et al., 2001), and bladder (Hung et al., 2004) cancers. The discrepancy may be partially due to minor effect of this gene polymorphism on cancer risk, or the small sample size reported in each study. Thus, it warrants further investigation to clarify these inconclusive data. In this study, we conducted a meta-analysis of published data to derive a more precise estimation of this gene polymorphism and the risk of prostate, lung and esophageal cancers.

Materials and Methods

Identification and eligibility of relevant studies

We attempted to include all the case-control studies published to date that examined the association of MnSOD gene polymorphism with these three cancers. Eligible studies were included through searching electronic databases, i.e., Pubmed using search terms “manganese superoxide dismutase”, “MnSOD”, “polymorphism(s)”, “prostate cancer”, “esophageal cancer”, and “lung cancer”. Additional publications were included from references of original studies or review papers on the topic by hands-on searches. All full text research papers on this topic, which did not define any minimum number of patients, were included in this meta-analysis. However, we only selected the largest and/or the latest sample size in the familiar or overlapping studies.

Inclusion and exclusion criteria

The included studies have to meet the following criteria: i). The diagnosis was confirmed pathologically for the patients with prostate, esophageal, or lung cancer and the controls were confirmed as free from any cancer, ii). The study was an independent case-control or cohort study that quantitatively evaluated the association between the cancer risk and MnSOD gene polymorphism, iii). The study had sufficient data to calculate odds ratio (OR) with 95% confidence interval (CI), and iv). Enough subgroup information or data for further analysis. Major exclusion criteria were as follows: i). the study had no control population, ii). no available genotype frequency data, iii). or it was a duplication of a previous study.

Data extraction

We followed a standard protocol for data extraction (Swerissen et al., 2006). Two independent investigators extracted data from the original studies and then reached a consensus on all of the items by discussing all disagreements. In the event that no consensus could be reached, the third investigator would be consulted to resolve the dispute, and a final decision would be formulated by a majority of the authors. The information was sought from each study as follows: the first author, publication date, country, characteristics of matching criteria in controls, total number of cases and controls, genotype frequencies for cases and controls with Val/Val, Val/Ala, Ala/Ala, respectively, and the result of the Hardy-Weinberg equilibrium test.

Statistical analysis

Crude ORs with 95% CIs were applied to estimate the relative risk of the cancer associated with MnSOD polymorphisms. For all subjects, we evaluated the risk of Val/Ala versus Val/Val, Ala/Ala versus Val/Val, Val/Val versus Val/Ala+Val/Val, Val/Ala+Val/Val versus Val/Val, assuming the co-dominant model (heterozygote model and homozygote model), the recessive model and the dominant model, respectively. A chi-square test using a web-based program (http://ihg2.helmholtzmuenchen.de/cgi-bin/hw/hwa1.pl) was performed to determine if distribution of these genotypes in the control population conformed to Hardy-Weinberg equilibrium (P<0.05 was considered significant). After that, subgroup analysis was further conducted by factors related to disease status, history of tobacco smoke, histological type, and clinical stage whenever possible. Heterogeneity assumption was determined by the χ²-based Q test using the formula: Q=Σ(weight ×(lnOR -lnOR)²), where weight = 1/variance. There was a lack of heterogeneity among studies at P values greater than 0.10 for the Q test. We also used the fixed-effects model (Mantel-Haenszel et al., 1959) and random-effects model (DerSimonian et al., 1986) to combine values from each of the studies. The random-effects model was more appropriate when heterogeneity was present. If the controls in the study were found not to be in Hardy-Weinberg equilibrium, sensitivity analysis was performed with and without these studies to test the robustness of the findings. Similarly, sensitivity analysis was also conducted by omitting each study in turn to identify potential outliers. Potential publication bias was estimated by the Begg’s funnel plots and by the method of Egger’s test, in which an asymmetric plot suggested a possible publication bias. If P<0.05, publication bias was considered statistically significant, which was determined by the Egger’s test. If publication bias existed, the Dual and Tweedie nonparametric “trim and fill” method was used to adjust for it. All the statistical analyses were performed by using STATA version 11.0 (Stata Corporation, College Station, TX). All P values were two-sided.
Table 1. Odds Ratios for MnSOD Genotypes and Prostate Cancer, According to Disease Status

<table>
<thead>
<tr>
<th>MnSOD genotypes</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>95% CI</th>
<th>χ²</th>
<th>P</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val/Ala vs Val/Val</td>
<td>Non aggressive¹</td>
<td>305/155</td>
<td>1009/533</td>
<td>1.04</td>
<td>0.83-1.30</td>
<td>2.78</td>
<td>0.60</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Aggressive²</td>
<td>412/181</td>
<td>1009/533</td>
<td>1.21</td>
<td>0.98-1.48</td>
<td>4.40</td>
<td>0.36</td>
<td>1.76</td>
</tr>
<tr>
<td>Ala/Ala vs Val/Val</td>
<td>Non aggressive³</td>
<td>171/155</td>
<td>496/1542</td>
<td>1.18</td>
<td>0.92-1.45</td>
<td>3.23</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Aggressive⁴</td>
<td>183/155</td>
<td>496/1542</td>
<td>1.17</td>
<td>0.99-1.29</td>
<td>3.23</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Ala/Ala vs Val/Val + Val/Ala</td>
<td>Non aggressive</td>
<td>171/460</td>
<td>496/1542</td>
<td>1.27</td>
<td>0.99-1.43</td>
<td>4.08</td>
<td>0.40</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>Aggressive⁵</td>
<td>183/593</td>
<td>496/1542</td>
<td>0.98</td>
<td>0.81-1.10</td>
<td>5.57</td>
<td>0.23</td>
<td>0.15</td>
</tr>
<tr>
<td>Val/Ala + Ala/Ala vs Val/Val</td>
<td>Non aggressive⁶</td>
<td>476/155</td>
<td>1505/533</td>
<td>1.09</td>
<td>0.89-1.33</td>
<td>3.01</td>
<td>0.36</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Aggressive⁷</td>
<td>595/181</td>
<td>1505/533</td>
<td>1.18</td>
<td>0.97-1.44</td>
<td>6.47</td>
<td>0.17</td>
<td>1.68</td>
</tr>
</tbody>
</table>

¹Test for heterogeneity; ²Test for overall effect; ³Aggressive prostate cancer, stage ≥3, or Gleason grade ≥7; ⁴Non aggressive prostate cancer, stage < 3, or Gleason grade < 7

Results

Study characteristics

In this meta-analysis, we have identified 22 eligible studies from 2003 to 2009, including 8,181 cancer cases and 11,844 controls using our inclusion and exclusion criteria. Briefly, of the 22 studies (Wang et al., 2001; Woodson et al., 2001; Lin et al., 2003; Liu et al., 2004; Wang et al., 2004; Li et al., 2005; Tauffer et al., 2005; Ho et al., 2006; Choi et al., 2007; di Martino et al., 2007; Ergen et al., 2007; Kang et al., 2007; Arsova-Sarafinovska et al., 2008; Iguchi et al., 2008; Mikhak et al., 2008; Bica et al., 2009; Cheng et al., 2009; Iguchi et al., 2009; Sun et al., 2009; Zejinilovic et al., 2009), the sample size ranged between 100 and 3,030, and there were twelve studies on prostate cancer, four studies on esophageal cancer, and six studies on lung cancer. All studies, except for five of them (Tauffer et al., 2005; Bica et al., 2009; Sun et al., 2009; Zejinilovic et al., 2009), indicated that the distribution of genotypes in the controls was consistent with Hardy-Weinberg equilibrium, while only one study conducted by Lin et al. (2003) contained no data for Hardy-Weinberg equilibrium.

Association of MnSOD SNPs with cancer risk

We first calculated OR values and their corresponding 95% CI values of MnSOD polymorphisms with prostate cancer. We found statistically significant associations between MnSOD polymorphism and prostate cancer risk in the co-dominant models (Val/Ala versus Val/Val, OR = 1.11; 95% CI = 1.01-1.22; Ala/Ala versus Val/Val, OR = 1.25; 95% CI = 1.03-1.51) and the dominant model (Val/Ala+Ala/Ala versus Val/Val, OR = 1.15; 95% CI = 1.01-1.31; Figure 1), but no significant association in the recessive model (Ala/Ala versus Val/Ala+Val/Val, OR = 1.18; 95% CI = 0.99-1.39). In the subgroup analysis by disease stages (Table 1), there was no significant association of MnSOD with prostate cancer aggressiveness in the heterozygote model (Val/Ala versus Val/Val, OR = 1.21; 95% CI = 0.98-1.48), homozygote model (Ala/Ala versus Val/Val, OR = 1.27; 95% CI = 0.99-1.63), recessive model (Ala/Ala versus Val/Val, OR = 0.98; 95% CI = 0.81-1.20), or dominant model (Val/Ala+Ala/Ala versus Val/Val, OR = 1.18; 95% CI = 0.97-1.44; Figure 2B). Moreover, we found that MnSOD polymorphisms were significantly associated with esophageal cancer risk when four studies were pooled in this meta-analysis (Val/Ala versus Val/Val, OR = 1.58; 95% CI = 1.22-2.04; Ala/Ala versus Val/Val, OR = 2.25; 95% CI = 1.61-3.15; Ala/Ala versus Val/Ala+Val/Val, OR = 1.69; 95% CI = 1.07-2.67; Val/Ala+Ala/Ala versus Val/Val, OR = 1.74; 95% CI = 1.36-2.22; Figure 3).
conclusions of our analysis in all genetic models (data corresponding results, but did not materially alter the equilibrium. The data showed significant levels of the by disease stages, one study (Wang et al., 2001) was = 1.11; 95% CI = 1.01-1.22). In the subgroup analysis dominant model (Val/Ala+Ala versus Val/Val, OR = 1.13; 95% CI = 0.75-0.94; Table 2). However, there was no statistically significant association between MnSOD polymorphisms and risk of either lung adenocarcinoma (Val/Val+ measures of the control, the significance levels of the corresponding OR and 95% CI were not altered with or without inclusion of the current analyses in the homozygote model (Ala/Ala versus Val/Val, OR = 0.69; 95% CI = 0.60-0.80), recessive model (Ala/Ala versus Val/Ala+Val/Val, OR = 0.78; 95% CI = 0.70-0.88), or dominant model (Val/Ala+Ala versus Val/Val, OR = 0.82; 95% CI = 0.74-0.91). Sensitivity analysis stratified by tobacco smoke status, histological type, or clinical stage of lung cancer showed that the pooled estimation of all genetic models was not sensitive enough to remove the individual study, and the corresponding pooled ORs were not substantially altered (data not shown), indicating that our data were statistically robust.

Sensitivity analysis
In prostate cancer, we excluded three studies (Taufet et al., 2005; Ergen et al., 2007; Bica et al.; 2009) because their controls were not in Hardy-Weinberg equilibrium and we found that the data did not statistically alter the conclusions of our analysis in the homozygote model (Ala/Ala versus Val/Val, OR = 1.13; 95% CI = 1.01-1.27) or dominant model (Val/Ala+Ala versus Val/Val, OR = 1.11; 95% CI = 1.01-1.22). In the subgroup analysis by disease stages, one study (Wang et al., 2001) was excluded because the control was not in Hardy-Weinberg equilibrium. The data showed significant levels of the corresponding results, but did not materially alter the conclusions of our analysis in all genetic models (data not shown). Moreover, in esophageal cancer, sensitivity analysis indicated that one study (Sun et al., 2009), with the control not in Hardy-Weinberg equilibrium, affected the pooled OR value in the co-dominant models (Val/Ala versus Val/Val, OR = 1.54; 95% CI = 1.13-2.11; Ala/Ala versus Val/Val, OR = 1.66; 95% CI = 1.05-2.55) and dominant model (Val/Ala+Ala versus Val/Val, OR = 1.59; 95% CI = 1.18-2.14). However, in lung cancer, although one study (Zejinolovic et al., 2009) did not follow Hardy-Weinberg equilibrium in the genotype distribution of the control, the significance levels of the corresponding OR and 95% CI were not altered with or without inclusion of the current analyses in the homozygote model (Ala/Ala versus Val/Val, OR = 0.68; 95% CI = 0.49-0.72; Figure 4), but not in the heterozygote model (Ala/Val versus Val/Val, OR = 0.97; 95% CI = 0.74-1.25). In addition, when the data was stratified by tobacco smoke status (Table 2), statistically significant decrease in lung cancer risk was found in dominant model (Val/Ala+Ala versus Val/Val, OR = 0.77; 95% CI = 0.63-0.94). However, there was no statistically significant association between MnSOD polymorphisms and risk of either lung adenocarcinoma (Val/Ala+Ala versus Val/Val, OR = 0.78; 95% CI = 0.59-1.04) or lung squamous cell carcinoma (Val/Ala+Ala versus Val/Val, OR = 0.62; 95% CI = 0.34-1.13). Similarly, there was no statistically significant association in both early stage lung cancer (Val/Ala+Ala versus Val/Val, OR = 0.29; 95% CI = 0.04-2.46) or advanced stage lung cancer (Val/Ala+Ala versus Val/Val, OR = 0.28; 95% CI = 0.03-2.87; Table 2).

Sensitivity analysis
In prostate cancer, the shapes of the Begg’s funnel plots seemed to have no evidence of obvious asymmetry in all comparison models, and the results of Egger’s test still suggested no evidence of publication bias (P = 0.90 for Val/Ala versus Val/Val; P = 0.16 for Ala/Ala versus Val/Val; P = 0.09 for Ala/Ala versus Val/Ala+Val/Val; P = 0.60 for Val/Ala+Ala versus Val/Val). Similarly, the shape of the funnel plots was symmetrical for esophageal cancer. The Egger’s test provided evidence that there was no publication bias among different models (P = 0.75 for Val/Ala versus Val/Val; P = 0.30 for Ala/Ala versus Val/Val; P = 0.35 for Ala/Ala versus Val/Ala+Val/Val; P = 0.61 for Val/Ala+Ala versus Val/Va). Moreover, in lung cancer, Egger’s test showed that publication bias was not present in the homozygote model (P = 0.17) or recessive model (P = 0.62), but was present in the heterozygote model (P = 0.00) and dominant model (P = 0.00). The Dual and Tweedie nonparametric “trim and fill” method did not draw different conclusions of the dominant model (P = 0.00), indicating that our results were statistically robust. In subgroup analyses stratified by disease stages of prostate cancer and tobacco smoke status, histological type or clinical stage of lung cancer, Begg’s funnel plots...
and Egger’s test still did not find any publication bias among the studies included \((P > 0.05)\).

**Discussion**

In this study, we searched published case and control studies on MnSOD and cancer risk of the prostate, esophagus, and lung and then performed a meta-analysis. We found very interesting data, i.e., in prostate cancer, statistically significant associations were found between MnSOD polymorphisms and prostate cancer risk in the co-dominant or dominant model, but not in the recessive model. Moreover, we found that MnSOD polymorphisms are significantly associated with esophageal cancer risk. However, MnSOD polymorphisms are associated with decreased lung cancer risk in the homozygote, recessive, or dominant model, but not in the heterozygote model. In addition, when stratifying the data by tobacco smoke status, MnSOD polymorphisms were associated with statistically significant decreases in lung cancer risk in the dominant model. There was no statistically significant association between MnSOD polymorphisms and the risk of either lung adenocarcinoma or squamous cell carcinoma, or the risk of early stage or advanced stage lung cancer. Sensitivity analysis showed that after stratifying unqualified studies, the data did not statistically alter the conclusions of our analysis in most statistical models, such as the homozygote or dominant model. Publication bias analysis also confirmed that there was no evidence showing obvious asymmetry in all comparison models in these cancer sites. However, in lung cancer, publication bias was present in the heterozygote or dominant model, although the Dual and Tweedie nonparametric “trim and fill” method did not draw different conclusions of the dominant model, indicating that our results were statistically robust. In conclusion, our current study demonstrated that the Ala allele MnSOD polymorphism was associated with the risk of prostate and esophageal cancers, but with the decreased risk of lung cancer. Further study warrants investigation of the functional aspect of MnSOD and the development and progression of these cancers.

Mitochondrial MnSOD functions to transform superoxide radical \(\text{O}_2^\cdot\) into \(\text{H}_2\text{O}_2\), and the latter is decomposed into water and oxygen by combined action of glutathione peroxidase (GPx) and catalase (CAT). This conversion removes superoxide radicals to protect cells from superoxide damage, indicating the importance of MnSOD in human cells. Lower MnSOD expression in the cells may lead to an increase in reactive oxygen species (ROS) in the mitochondria, and accumulated ROS will cause damage of DNA, protein, and lipids to increase genome instability and in turn contribute to cell transformation (Orrenius et al., 2007; Trachootham et al., 2009). However, previous studies on MnSOD polymorphism for association with cancer risk were inconclusive. Meta-analysis is a powerful tool to associate genetic polymorphisms with cancer risk because it potentially investigates a large number of samples and estimates the effect of a genetic factor with cancer risk. In this study, we conducted a meta-analysis to estimate the association between MnSOD polymorphism and the risk of three different human cancers. Our data showed that the Ala carriers had an increased risk of prostate and esophageal cancer. In support of this finding, the MnSOD Ala/Ala genotype was associated with a 1.7-fold increased risk for prostate cancer (Woodson et al., 2001), 1.9-fold increased risk for esophageal cancer (Murphy et al., 2007), and significantly elevated risk for breast cancer (Mitrune et al., 2001). Molecularly, the MnSOD Ala/Ala genotype causes an amino acid substitution from valine (Val) to alanine (Ala), which reduces MnSOD enzymatic activity and its ability to remove ROS (Sutton et al., 2003). The latter was confirmed to play an important role in regulating cell proliferation and carcinogenesis (Soini et al., 2001). Studies on human tissue specimens showed that MnSOD was absent or reduced in breast cancer (Soini et al., 2001) and prostate cancer (Wang et al., 2006). Nevertheless, MnSOD overexpression to decrease ROS levels in the cells was able to reduce cell doubling time and plating efficiency of osteosarcoma cells, thus reducing its transfection rate (Wang et al., 2005; Komatsu et al., 2005; Sun et al., 2012). MnSOD expression was also able to inhibit or reverse the malignant phenotype and growth of human fibroblast SV40 (Yan et al., 1996), malignant melanoma tumor (Zwacka et al., 1993), and glioma cells (Zhong et al., 2005). In addition, in our subgroup analysis based on disease stages, we did not find any significant associations between MnSOD genotypes (with at least one copy of the Ala allele) and prostate cancer aggressiveness. Thus, MnSOD polymorphisms associated with prostate cancer aggressiveness need further investigation and the mechanistic basis requires further exploration.

In contrast, our data showed a peculiar phenomenon that risk was obviously decreased in lung cancer with the MnSOD Ala variant, especially in tobacco smokers, although there were no statistically significant associations between the MnSOD Ala variant and risk of either a histological type or clinical stage of lung cancer. This contradicts data among different cancers, i.e., prostate, esophageal and lung cancers are reported for the very first time by this meta-analysis and the reason for this phenomenon remains unclear. Probably, however, it may be due to different mechanisms underlying tumorigenesis of different cell types and different roles of MnSOD in different pathological types of tumor. Technically, the differences might have been raised by chance from different studies with small sample size or selection bias. Thus, considering the limited studies included in this meta-analysis, our results should be interpreted with caution.

In addition, our current study also has several limitations to be taken into consideration when interpreting the data. First, theoretically, a majority of controls was chosen from healthy individuals, but a number of the control population may have been affected by benign diseases, such as esophagitis, pulmonary tuberculosis and prostatoplasia. Secondly, the overall sample size was sufficient, but it still was relatively small for some subgroup analyses. Thirdly, our data were based on unadjusted published estimates, and hence a more precise analysis should be performed if individual data were available to allow for the adjustment by other co-variants, including age, family
history, antioxidant diets, and environmental factors. In spite of these, our meta-analysis should have had some advantages. First, substantial numbers of cases and controls were pooled from 22 studies for a total of 20,025 subjects, which significantly increased the statistical power of analysis. Secondly, usually heterogeneity is a potential problem and may affect study conclusions. However, our data showed no statistically significant heterogeneity in this meta-analysis. Thirdly, majority of conclusions made in this study did not find obvious publication biases, indicating that the whole pooled results could be unbiased.

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