Association of heme oxygenase-1 polymorphisms with cancer risk: A systematic review and meta-analysis

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Summary

Purpose: Observational studies have recently focused on the association between heme oxygenase-1 (HMOX1) gene promoter polymorphisms and cancer risk. However, conflicting results have been obtained. To derive a precise estimate of the association, a systematic review and meta-analysis were conducted.

Methods: This study followed the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses. PubMed, Medline, Embase and Web of Knowledge were systematically searched for relevant studies. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the allelic and genotypic comparisons according to the homozygous, heterozygous, dominant, and recessive genetic models. Between-study heterogeneity was quantified through I² statistics, and publication bias was appraised by using funnel plots. Sensitivity analyses were conducted to evaluate the robustness of the meta-analysis findings.

Results: Meta-analysis of 9 studies involving 2491 cases and 3380 controls did not reveal any significant association of the HMOX-1 (GT)n and 413A>T polymorphisms with cancer risk. Stratified analysis by ethnicity showed a statistically significant association between (GT)n repeat length variant and susceptibility to cancer for the heterozygous genetic model among Asian populations (OR=1.42, 95% CI: 1.04-1.95, P heterogeneity=0.218), which is a robust finding according to sensitivity analysis. Funnel plot inspection did not reveal any publication bias.

Conclusion: In conclusion, this study comprehensively examined the available literature on the association of HMOX-1 (GT)n and 413A>T polymorphisms with cancer risk. Meta-analysis results suggest (GT)n repeat length polymorphism as a potential susceptibility variant for cancer in Asians. Additional large-scale and well-designed studies are needed to confirm these results.

Key words: cancer, genetics, heme oxygenase-1, meta-analysis, risk, single nucleotide polymorphism

Introduction

Heme oxygenase (comprising three isoforms, namely, HO-1, -2, and -3) was originally identified as a rate-limiting enzyme in the degradation of heme to biliverdin, which results in the generation of free iron and carbon monoxide [1,2]. Recent studies have shown that HO-1 (also known as HMOX-1) is highly induced by various forms of oxidative stress and provides protection against oxidant-mediated chronic diseases, such as emphysema [3], asthma [4], diabetes mellitus [5], and cardiovascular diseases [6]. Accordingly, the amount of emerging evidence has increased to support the idea of HO-1 as an important protective antiapoptotic factor for malignant tumors [7]. Various studies have reported that HO-1 expression is associated with cellular proliferation and
angiogenesis, which are both important for tumor growth in vivo [8]. Moreover, several single-nucleotide polymorphisms, all of which are localized on chromosome 22q12, have been identified in the promoter of the HO-1 gene [9]. Among these polymorphisms, microsatellite polymorphism is the most commonly studied and is characterized by a varying number of \( (GT)n \) repeats ranging from 15 to 40 in the promoter region of the HO-1 gene. The \( (GT)n \) variable length polymorphism has been shown to be associated with the differential transcriptional activity of the gene. Short fragments of repeats (S alleles) are related to high transcription rates and increased transcriptional upregulation of HO-1 in response to various oxidant stimuli, such as hydrogen peroxide and reactive oxygen species (ROS), whereas long \( (GT)n \) repeats (L alleles) are associated with low transcription of the HO-1 gene [10]. Another commonly studied polymorphism is 413A>T, which is an A to T transition at nucleotide -413 in the promoter region that results in increased HO-1 promoter activity involved in ROS scavenging [11]. Therefore, HO-1 is a crucial enzyme responsible for the metabolic deletion of oxidative stimuli and may serve an important function in the pathogenesis of various cancers.

To date, several studies have focused on the association of the HO-1 polymorphisms with cancer risk. However, results of recent studies are conflicting because of their limited sample size or genuine heterogeneity. Until recently, neither meta-analysis nor genome-wide association studies has been performed to assess the association between the HO-1 polymorphisms and susceptibility to cancer. Considering that a single study is inadequate to detect the overall effects, a quantitative synthesis of accumulated data from different studies is important to provide evidence of the association of this polymorphism with cancer risk. Therefore, we performed this meta-analysis on all published case-control studies to obtain a more precise estimate of the overall effects, quantify heterogeneity between the individual studies, and investigate potential publication bias.

**Methods**

**Search strategy**

This systematic review and meta-analysis followed the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The included studies were retrieved from electronic databases, which included PubMed, Embase and Web of Knowledge. Combinations of words were used for search terms as follows: (“heme oxygenase-1” OR HMOX-1 OR HO-1) AND (polymorphism OR allele OR variant OR susceptibility OR mutation) AND (cancer OR tumor OR neoplasm OR carcinoma). Additional studies were retrieved through manual search from references of the original studies or review articles about this subject. The last search was performed on April 20, 2014.

**Identification of eligible studies**

The studies were selected according to the following inclusion criteria: (1) used a case-control design, (2) contained an evaluation of the HMOX-1 polymorphism and cancer risk, (3) gave information on allelic/genotypic frequencies of HMOX-1 in both cases and corresponding controls, and (4) achieved Hardy-Weinberg equilibrium (HWE) in control groups. We excluded studies in which the genotypic frequencies in controls exhibited significant deviation from the HWE. When the study populations overlapped, we generally retained only the study with the most extensive data to avoid duplication in the meta-analysis. When the eligible studies were without adequate reporting of genotype frequency, we contacted the corresponding authors for missing information, and if no response by the time of analysis and writing had been received, then these papers were ultimately excluded. Conference abstracts, case reports, editorials, review articles, letters, and animal/basic science/clinical research were also excluded. The study identification and data extraction process were independently conducted by two researchers (N.Y. and L.H.), and any discrepancy was resolved through discussion and consensus.

**Quality score evaluation**

In this meta-analysis, quality assessment of the case-control studies was performed using the Newcastle Ottawa scale (NOS) recommended by the Cochrane Non-randomized Study Methods Working Group [12,13]. As described in detail previously [14], NOS contains 8 items that are divided into 3 categories: selection (4 items, 1 star each), comparability (1 item, up to 2 stars), and exposure (3 items, 1 star each). A “star” presents a “high quality” choice of an individual study. Two independent reviewers (N.Y. and L.H.) discussed their evaluations, and any disagreement was resolved through discussion and consultation. Given the variability in quality of the case-control studies from our initial literature search, we considered studies as high quality if they met 6 or more of the NOS criteria [15].

**Statistics**

The pooled odds ratio (OR), including the corresponding 95% confidence interval (CI), was used to calculate and assess the association of HMOX-1 polymorphisms with cancer risk. The allelic and genotypic comparisons of heterozygous, homozygous, dominant,
and recessive models were estimated [16]. The statistical significance of the summarized OR was determined by the Z-test with p<0.05 indicating statistical significance.

Heterogeneity assumption was examined using the chi-square test based on a Q test [17]. The combined OR estimation of each study was calculated using a random-effects model (DerSimonian and Laird method) when p<0.10, otherwise a fixed-effects model was used (Mantel-Haenszel method) [18]. To determine the causes of heterogeneity, subgroup analyses were performed by grouping the studies with similar characteristics, such as ethnicity, sample size, and genotyping method. The ethnic subgroups were categorized into two ethnic groups: Caucasian and Asian. Sensitivity analysis was also performed by omitting each individual study to reflect the influence of the individual dataset on the pooled OR by using the “metaninf” STATA command. The appropriate chi-square goodness-of-fit test [19] was performed using the “genhwcci” STATA command to assess the deviation from HWE, but only in the control groups. For the interpretation of the chi-square test, statistical significance was defined as p<0.05.

As described in our previously published paper [14], publication bias was evaluated by using Begg’s and Egger’s Asymmetry tests [20] and through visual inspection of the funnel plots, in which the standard error was plotted against the log (OR) to form a simple scatterplot. Statistical significance for the interpretation of the Egger’s test was defined as p<0.10.

STATA version 11.0 (STATA Corporation, College Station, Texas) was used for all statistical analyses. All statistical tests were two-sided.

**Results**

**Eligible studies**

The study identification and selection process is presented in Figure 1. A total of 365 citations were identified during the initial search. Then 266 duplicate records were excluded. Of the 99 abstracts retrieved through the search criteria, 80 were irrelevant reasons for exclusion described in Table S1. One study [21] had overlapping populations with an eligible study [22], 3 articles [23–25] did not have a case-control design, 3 articles [26-28] were reviews, and 3 articles [29-31] did not report the relevant genotype frequencies. Overall, 9 articles [22,32-38] involving 2,491 cases and 3,380 controls were eligible for this meta-analysis. Among the eligible articles, 2 [35,37] investigated (GT)n repeats and 413A>T polymorphism, 6 [22,32-34,36,38] investigated (GT)n repeat polymorphism, and 1 [39] investigated 413A>T polymorphism. Therefore, 8 articles [22,32-38] provid-
### Table 1. Distribution of genotypes of the heme oxygenase-1 (HMOX1) polymorphisms and susceptibility to cancer

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Control source</th>
<th>Genotyping method</th>
<th>Cases / controls</th>
<th>Genotype data (cases)</th>
<th>Genotype data (controls)</th>
<th>HWE (P)</th>
<th>Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang, 2004</td>
<td>Taiwan</td>
<td>Asian</td>
<td>HB</td>
<td>Sequencing</td>
<td>147/83</td>
<td>122 172 29 64 54 74 92 17 40 26</td>
<td>0.82 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okamoto, 2006</td>
<td>Austria</td>
<td>Caucasian</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>152/398</td>
<td>114 190 32 50 70 269 527 46 177 175</td>
<td>0.9 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hong, 2007</td>
<td>USA</td>
<td>mixed</td>
<td>PB</td>
<td>Taqman</td>
<td>478/492</td>
<td>287 669 52 183 243 311 675 47 217 228</td>
<td>0.65 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lo, 2007</td>
<td>Taiwan</td>
<td>Asian</td>
<td>HB</td>
<td>Sequencing</td>
<td>185/250</td>
<td>169 197 34 101 48 210 290 47 116 87</td>
<td>0.45 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hu, 2010</td>
<td>China</td>
<td>Asian</td>
<td>HB</td>
<td>PCR-RFLP</td>
<td>143/264</td>
<td>127 159 29 69 45 297 231 90 117 57</td>
<td>0.11 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vashist, 2011</td>
<td>Germany</td>
<td>Caucasian</td>
<td>HB</td>
<td>PCR-RFLP</td>
<td>150/100</td>
<td>162 138 45 72 33 100 100 25 50 25</td>
<td>0.99 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jiraskova, 2012</td>
<td>Czech</td>
<td>Caucasian</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>777/986</td>
<td>586 968 100 386 291 720 1252 133 454 399</td>
<td>0.83 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murakami, 2012</td>
<td>Japan</td>
<td>Asian</td>
<td>HB</td>
<td>Sequencing</td>
<td>78/44</td>
<td>33 125 2 29 47 31 57 6 19 19</td>
<td>0.74 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>413A&gt;T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hu, 2010</td>
<td>China</td>
<td>Asian</td>
<td>HB</td>
<td>Taqman</td>
<td>143/264</td>
<td>137 149 37 63 43 242 286 51 140 73</td>
<td>0.27 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andersen, 2011</td>
<td>Denmark</td>
<td>Caucasian</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>385/763</td>
<td>426 340 118 190 75 901 625 260 381 122</td>
<td>0.37 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jiraskova, 2012</td>
<td>Czech</td>
<td>Caucasian</td>
<td>PB</td>
<td>PCR-RFLP</td>
<td>777/986</td>
<td>878 676 253 372 152 1119 851 311 497 177</td>
<td>0.38 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X/x for S/L of (GT)n, A/T of 413A>T; HB: hospital-based; PB: population-based; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; HWE: Hardy-Weinberg equilibrium.

aP value for HWE in the control group.
ed data on the association between (GT)n repeat polymorphism and cancer risk, whereas 3 articles [35,37,39] provided data on 415A>T. Studies were conducted covering 3 ethnicities. Four studies [32,36,37,39] involved Caucasians, another 4 studies [22,34,35,38] involved Asians, and 1 study [33] involved mixed populations. Three genotyping methods were used: Taqman [33], sequencing [22,34,38], and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [32,35-37,39]. In 5 studies, controls were from hospital-based subjects, whereas population-based subjects were used in the other eligible studies. No consensus on the optimum cutpoint for the (GT)n repeat length polymorphism in the HMOX1 gene promoter has been set. Thus, the harmonization of

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**Figure 2.** Forest plot for subgroup analysis of association between (GT)n repeat length polymorphism of HMOX-1 and cancer risk among Asians under the heterozygous model.

**Figure 3.** Effect of individual studies on pooled odds ratios for (GT)n repeat length polymorphism of HMOX-1 and cancer risk among Asians under the heterozygous model.
Table 2. Results of meta-analysis for (GT)n and 413A>T polymorphisms of HO-1 and cancer risk

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. (study)</th>
<th>No. (cases/controls)</th>
<th>Allele model (x vs X) OR (95% CI)</th>
<th>Ph</th>
<th>Homozygous model (xx vs XX) OR (95% CI)</th>
<th>Ph</th>
<th>Heterozygous model (Xx vs XX) OR (95% CI)</th>
<th>Ph</th>
<th>Dominant model (xx + Xx vs XX) OR (95% CI)</th>
<th>Ph</th>
<th>Recessive model (xx vs Xx + XX) OR (95% CI)</th>
<th>Ph</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GT)n repeat length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>2108/2617</td>
<td>1.06(0.90,1.25)</td>
<td>0.004</td>
<td>1.05(0.74,1.49)</td>
<td>0.004</td>
<td>0.99(0.70,1.43)</td>
<td>0.003</td>
<td>1.02(0.74,1.41)</td>
<td>0.002</td>
<td>1.08(0.87,1.33)</td>
<td>0.023</td>
</tr>
<tr>
<td>Asian</td>
<td>4</td>
<td>551/641</td>
<td>1.28(0.88,1.87)</td>
<td>0.004</td>
<td>1.65(0.77,3.55)</td>
<td>0.007</td>
<td>1.42(1.04,1.95)</td>
<td>0.218</td>
<td>1.49(0.88,2.55)</td>
<td>0.055</td>
<td>1.25(0.75,2.09)</td>
<td>0.111</td>
</tr>
<tr>
<td>Caucasian</td>
<td>3</td>
<td>1079/1484</td>
<td>0.92(0.82,1.03)</td>
<td>0.706</td>
<td>0.84(0.66,1.07)</td>
<td>0.224</td>
<td>0.74(0.59,1.28)</td>
<td>0.005</td>
<td>0.76(0.47,1.23)</td>
<td>0.028</td>
<td>0.91(0.78,1.08)</td>
<td>0.597</td>
</tr>
<tr>
<td>HB</td>
<td>5</td>
<td>701/741</td>
<td>1.18(0.85,1.62)</td>
<td>0.003</td>
<td>1.37(0.72,2.61)</td>
<td>0.005</td>
<td>1.26(0.95,1.66)</td>
<td>0.132</td>
<td>1.29(0.80,2.08)</td>
<td>0.026</td>
<td>1.15(0.76,1.76)</td>
<td>0.175</td>
</tr>
<tr>
<td>PB</td>
<td>3</td>
<td>1407/1876</td>
<td>0.97(0.87,1.08)</td>
<td>0.350</td>
<td>0.88(0.71,1.11)</td>
<td>0.217</td>
<td>0.75(0.42,1.39)</td>
<td>0.004</td>
<td>0.80(0.52,1.21)</td>
<td>0.030</td>
<td>1.00(0.87,1.15)</td>
<td>0.149</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>4</td>
<td>1222/1478</td>
<td>1.03(0.79,1.33)</td>
<td>0.005</td>
<td>1.00(0.58,1.74)</td>
<td>0.002</td>
<td>0.92(0.53,1.62)</td>
<td>0.001</td>
<td>0.96(0.57,1.63)</td>
<td>0.001</td>
<td>1.06(0.80,1.40)</td>
<td>0.077</td>
</tr>
<tr>
<td>Sequencing</td>
<td>3</td>
<td>408/377</td>
<td>1.17(0.75,1.83)</td>
<td>0.024</td>
<td>1.40(0.56,3.53)</td>
<td>0.058</td>
<td>1.21(0.81,1.81)</td>
<td>0.242</td>
<td>1.15(0.79,1.68)</td>
<td>0.125</td>
<td>1.13(0.60,2.13)</td>
<td>0.023</td>
</tr>
<tr>
<td>Sample size &gt; 400</td>
<td>5</td>
<td>1735/2390</td>
<td>1.05(0.85,1.24)</td>
<td>0.006</td>
<td>0.99(0.67,1.47)</td>
<td>0.006</td>
<td>0.96(0.63,1.47)</td>
<td>0.001</td>
<td>0.99(0.67,1.45)</td>
<td>0.002</td>
<td>1.04(0.81,1.35)</td>
<td>0.016</td>
</tr>
<tr>
<td>Sample size &lt; 400</td>
<td>3</td>
<td>375/227</td>
<td>1.19(0.77,1.84)</td>
<td>0.045</td>
<td>1.43(0.54,3.78)</td>
<td>0.044</td>
<td>0.98(0.63,1.52)</td>
<td>0.166</td>
<td>1.22(0.56,2.66)</td>
<td>0.070</td>
<td>1.22(0.85,1.74)</td>
<td>0.209</td>
</tr>
<tr>
<td>413A&gt;T</td>
<td>All</td>
<td>3</td>
<td>1.04(0.94,1.15)</td>
<td>0.347</td>
<td>1.10(0.90,1.35)</td>
<td>0.291</td>
<td>0.94(0.80,1.10)</td>
<td>0.155</td>
<td>0.99(0.85,1.15)</td>
<td>0.155</td>
<td>1.16(0.97,1.39)</td>
<td>0.775</td>
</tr>
</tbody>
</table>

X’x for S/L of (GT)n, A/T of 413A>T; Ph: P value for heterogeneity based on Q test. All pooled ORs were derived from random-effects model except for cells marked with (fixed F). Bold values denote statistical significance.
points was considered. In the eligible studies, allelic categories were defined as class S (short) for <25 or <27 (GT)n repeats and class L (long) for ≥25 or ≥27 (GT)n repeats [40]. By examining the genotype frequencies in the controls, no significant deviation from HWE was found in all studies. The studies were published between 2004 and 2012, and all were of high quality with score ≥6. Table 1 shows a summary of the characteristics of the included studies.

(GT)n repeat length polymorphism

We conducted a meta-analysis of the (GT)n repeat length polymorphism overall and in subgroups under various genetic models. Table 2 presents the pooled ORs, along with their 95% CIs. Overall, no significant association was found in allele (L vs S, OR=1.06, 95% CI: 0.90-1.25), homozygous (LL vs SS, OR=1.05, 95% CI: 0.74-1.49), heterozygous (LS vs SS, OR=0.99, 95% CI: 0.70-1.39), dominant (LS + LL vs SS, OR=1.02, 95% CI: 0.74-1.41), and recessive models (LL vs LS + SS, OR=1.08, 95% CI: 0.87-1.33). Further analysis was performed on the data stratified by the genotyping method to determine the possible factors that might have influenced the results. Table 2 shows that no association was found in cancer patients with (GT)n repeat length polymorphism among all comparisons. Moreover, similar results in the subgroup analyses by control source and sample size were obtained on all genetic models because the associations were not significant. However, changes occurred in the pooled results after subgroup analysis by ethnicity, and the risk in Asians significantly increased under LS vs SS comparison (OR=1.42, 95% CI: 1.04-1.95). However, no association was found for the other genetic comparisons in Asians and all genetic comparisons in Caucasians. Figure 2 shows the forest plot for subgroup analysis of the association between the (GT)n repeat length polymorphism of HMOX-1 and cancer risk under the heterozygous model.

We analyzed the heterogeneity of the selected studies according to the p value for heterogeneity. Table 2 illustrates that significant heterogeneity was found in all genetic models (p<0.10). Therefore, a random-effects model was used for all comparisons to calculate the pooled OR estimates. After assessing the source of heterogeneity for all genetic models compared by subgroup analysis based on ethnicity, control source, genotyping method, or sample size, the heterogeneity partially decreased or was removed. Notably, the meta-analysis found a low level of between-study heterogeneity (P_heterogeneity=0.218) for the heterozygous genetic model in Asians. Sensitivity analysis demonstrated the robustness of the heterozygous model result in Asian populations, in which the exclusion of any individual study did not significantly affect the summarized effect estimate (Figure 3). Similarly, no significant influence was found when sensitivity analysis was conducted on the other models.

Publication bias on the overall OR analysis was not detected in any comparison. In addition, neither Begg’s test nor Egger’s test provided any evident indication of publication bias (Table 3, p>0.10). A relatively symmetrical funnel plot indi-

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Comparison</th>
<th>Egger’s test</th>
<th>95% CI</th>
<th>Begg’s test</th>
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<tr>
<td></td>
<td></td>
<td>t</td>
<td>P</td>
<td>LCI</td>
</tr>
<tr>
<td>(GT)n repeat length</td>
<td>L vs S</td>
<td>1.08</td>
<td>0.525</td>
<td>-2.114</td>
</tr>
<tr>
<td></td>
<td>LL vs SS</td>
<td>1.00</td>
<td>0.358</td>
<td>-2.312</td>
</tr>
<tr>
<td></td>
<td>LS vs SS</td>
<td>0.21</td>
<td>0.842</td>
<td>-4.068</td>
</tr>
<tr>
<td>Dominant model</td>
<td></td>
<td>0.60</td>
<td>0.572</td>
<td>-5.331</td>
</tr>
<tr>
<td>Recessive model</td>
<td></td>
<td>1.07</td>
<td>0.326</td>
<td>-1.752</td>
</tr>
<tr>
<td>413A&gt;T</td>
<td>T vs A</td>
<td>-0.29</td>
<td>0.819</td>
<td>-37.158</td>
</tr>
<tr>
<td></td>
<td>TT vs AA</td>
<td>-0.41</td>
<td>0.752</td>
<td>-40.319</td>
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<td></td>
<td>AT vs AA</td>
<td>-0.82</td>
<td>0.564</td>
<td>-37.005</td>
</tr>
<tr>
<td>Dominant model</td>
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<td>-0.66</td>
<td>0.630</td>
<td>-39.825</td>
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<tr>
<td>Recessive model</td>
<td></td>
<td>0.28</td>
<td>0.829</td>
<td>-20.587</td>
</tr>
</tbody>
</table>

LCI: low confidence interval, UCI: upper confidence interval
cated lack of evident publication bias (see Figure 4 for the heterozygous model comparison). Our results were statistically robust.

413A>T polymorphism

Table 2 presents in detail the pooled ORs, including their 95% CIs. No significant association of cancer with the 413A>T polymorphism was found in all comparisons. Further subgroup analysis by ethnicity, control source, genotyping method, and sample size was unnecessary because only 3 studies were available. Figure 5 shows the forest plot for the overall association between the 413A>T variant and cancer susceptibility under L vs S comparison. We also analyzed the heterogeneity of the selected studies according to the p value for heterogeneity. Table 2 shows that no significant heterogeneity was found in all comparisons (all p values based on heterogeneity were >0.10). Therefore, a fixed-effects model was used.

Figure 4. Begg’s funnel plots with pseudo 95% CI for association between (GT)n repeat length polymorphism of HMOX-1 and cancer risk.

Figure 5. Forest plot for association between 413A>T variant and cancer susceptibility under T vs A comparison.
for all comparisons. No publication bias for the overall analysis was observed in any comparison. The results of Beggs’s and Egger’s tests, which are presented in detail in Table 3 (p>0.10), suggested no publication bias.

**Discussion**

HO-1 not only functions as an enzyme-degrading heme, but also as a stress-responsive protein, which is an apparent novel protective factor with potent anti-inflammatory, anti-oxidant, and anti-proliferative effects [41-43]. Two functional polymorphisms in the promoter region of the HO-1 gene have drawn the most attention from researchers, namely, the HO-1 (GT)n repeat polymorphism and the 413A>T polymorphism. HO-1 promoter polymorphisms have been linked to several human diseases, such as pulmonary diseases [44], cardiovascular diseases [45], renal transplantation [46], obstetrics [47], and neurological diseases [48]. Accordingly, high HO-1 expression levels have been detected in malignant tumors in several studies [49,50]. However, the relationship between the HO-1 polymorphisms and cancer risk is inconsistent. Considering the inconsistent results from previous studies, our meta-analysis was conducted to obtain a precise evaluation of the association between the HO-1 polymorphisms and cancer risk.

To the best of our knowledge, this is the first study employing a meta-analysis to assess the association of HO-1 polymorphisms, including the (GT)n repeat polymorphism and the 413A>T polymorphism, with cancer risk systematically and comprehensively. The primary results showed that the (GT)n repeat and 413A>T polymorphisms were not associated with cancer risk in allelic and genotypic comparisons, following the homozygous, heterozygous, dominant, and recessive genetic models. However, the stratified analysis by ethnicity showed a statistically significant association between (GT)n repeat length variant and cancer risk for the heterozygous genetic model among Asian populations. In addition, Beggs’s test, Egger’s test, and funnel plots did not indicate publication bias and confirmed the conclusions. Thus, our findings indicated that the (GT)n repeat length polymorphism of HO-1 might increase cancer risk in Asian populations.

Previous studies have reported that the inducible subtypes of HO-1 polymorphisms are associated with the development of tumors in humans and experimental animal models [51-53]. In several studies, the (GT)n repeat length polymorphism was highly expressed in solid tumors among Asian populations. Therefore, Asian populations with (GT)n repeat length polymorphism might have increased tumor risk [55,54], which agrees with the results of the current meta-analysis. In addition, several studies also showed that the administration of an HO-1 inhibitor to an experimental model of solid tumor suppressed the tumor growth to a substantial extent [55,56]. Inhibition of HO-1 induced apoptosis of cancer cells and suppressed tumor growth. HO-1 might be an important target of cancer treatment. In the subtypes of HO-1 polymorphisms, a long (GT)n repeat polymorphism is associated with low transcription and expression of HO-1. A study has revealed that construction with lengths of <25 repeats increased the HO-1 basal promoter activity compared with >25 repeats [57]. A number of studies recently demonstrated that long (GT)n repeat polymorphism is associated with the development of tumors, which include lung cancer [58] and oral carcinogenesis [59], whereas short (GT)n repeat polymorphism is significantly associated with metastatic disease, high tumor recurrence rate, and low survival in gastrointestinal stromal tumors (GIST) and pancreatic cancer [23,36]. The mechanism may be explained by the hypothesis that long (GT)n repeats may alter the cytoprotective function through the inhibition of HO-1 expression [60]. A low M/M genotype frequency has been found in gastric cancer patients, and patients with moderate (GT)n repeat polymorphism showed a low frequency of lymphovascular invasion and nodal metastasis [54]. The result agrees with the hypothesis stating that a long (GT)n repeat may alter the cytoprotective function through the inhibition of HO-1 expression. Therefore, HO-1 polymorphism is an excellent potential prognostic marker for recurrence of cancer and patient survival.

Despite the conclusive results of the present meta-analysis, we should consider several limitations of this study. First, the relationship between 2 polymorphisms, including the (GT)n repeats and the 413A>T polymorphisms, and cancer risk was not simultaneously investigated in the 9 eligible articles included in our meta-analysis. The association of the (GT)n repeats with cancer risk was investigated by 8 articles, and the relationship between the 413A>T polymorphism and cancer risk was examined by 3 studies. As only 3 studies investigated the 413A>T polymorphism, further subgroup analysis in our study was not...
performed. Second, eligible studies included in our meta-analysis with common tumors were reported, however not all tumors were incorporated. Although the relationship between HO-1 polymorphisms and cancer risk has been recently studied, only a few works actually investigated their relationship. Third, the major ethnicities included in our study were only Caucasians and Asians. Fourth, the studies that were included in the meta-analysis used 3 genotyping methods, namely, Taqman, sequencing, and PCR-RFLP. Multiple genotyping methods could have confounded the results. Fifth, the differences in the definitions of class S and class L (GT)n repeats may slightly affect the relationship between the (GT)n repeat polymorphism and cancer risk.

Conclusion

Based on the available literature, the present study showed that the (GT)n repeat length polymorphism of HMOX-1 was associated with cancer risk in Asian populations. However, given the limitations of the studies included in the meta-analysis, larger well-designed case-control studies are necessary to draw comprehensive and true conclusions.

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References


47. Denschlag D, Marculescu R, Unfried G et al. The size of a microsatellite polymorphism of the haeme oxygenase-1 gene is associated with idiopathic recurrent