RESEARCH ARTICLE

# Association Between the (GT)n Polymorphism of the *HO-1* Gene Promoter Region and Cancer Risk: a Meta-analysis

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## Abstract

**Background: Several studies have previously focused on associations between the (GT)n repeat polymorphism of the heme oxygenase-1 (HO-1) gene promoter region and risk of cancers, but results are complex. We conducted the present meta-analysis to integrate relevant findings and evaluate the association between HO-1 (GT)n repeat polymorphism and cancer susceptibility. Materials and Methods: Published literature was retrieved from the PubMed/MEDLINE, EMBASE and ISI Web of Science databases before November 2013. For all alleles and genotypes, odds ratios were pooled to assess the strength of the associations using either fixed-effects or random-effects models according to heterogeneity. Subgroup analysis was conducted according to ethnicity and histopathology. Results: A total of 10 studies involving 2,367 cases and 2,870 controls were identified. The results showed there was no association between HO-1 (GT)n repeat polymorphism and the cancer risk both at the allelic and genotypic level. However, in the stratified analysis, we observed an increased risk of squamous cell carcinoma in persons carrying the LL genotype and the LL+LS genotype as compared with those carrying the SS genotype. When the LS and SS genotypes were combined, the odds ratio for squamous cell carcinoma in LL-genotype carriers, were also significantly increased. No publication bias was observed. Conclusions: The LL genotype and L-allele carrying genotypes (LL+LS) of HO-1 (GT)n repeat polymorphism are potential genetic factors for developing squamous cell carcinoma. More large and well-designed studies are required for further validations.**

**Keywords:** Heme oxygenase-1 - polymorphism - cancer - meta-analysis

*Asian Pac J Cancer Prev,* **15 (11)**, 4617-4622

## Introduction

Cancer is a major public health problem worldwide presently. Transition from normal to pre-cancer and cancer cells is a result of multi-step accumulation of genetic and epigenetic modifications. Oxidative stress induced by reactive oxygen species (ROS) that can modulate all steps of the process has been proposed to be one important aspect of tumorigenesis by causing apoptotic/necrotic cell death or accumulation of DNA damage (oxidative stress theory) (Davies, 1995; Aruoma et al., 2006; Li et al., 2013; Luo et al., 2014). ROS levels are precisely regulated by endogenous defense systems, among which heme oxygenase-1 (*HO-1*; also called HMOX1) has drawn much attention with its potent antioxidant, anti-inflammatory, and anti-proliferative effects (Deshane et al., 2005).

*HO-1*, also known as heat shock protein-32 (Hsp32), is the inducible isoform of heme oxygenase that catalyzes the degradation of heme to carbon monoxide, ferrous iron, and biliverdin, the latter is rapidly converted to bilirubin by biliverdin reductase (Maines, 1997) . The induction of *HO-1* has been considered an adaptive cellular defense response protecting cells or tissues against injuries in pathophysiological states (Song et al., 2009), ranging from Alzheimer's disease to cancer. Lines of evidence have demonstrated elevated *HO-1* expression and activity in various malignant tumors including lymphosarcomas, pancreatic cancer, prostate tumors, human renal cell carcinoma etc (Hirai et al., 2003; Berberat et al., 2005; Sacca et al., 2007; Datta et al., 2010). However, a dual role of *HO-1* with both tumor-promoting and anti-tumor properties in solid neoplasms has been reported.

The *HO-1* gene is localized to chromosome 22 (refined in 22q12), a chromosomal region clearly implicated in development and progression of several malignancies (Nuhn et al., 2009). Recently, Exner et al. (2004) proposed that, the basal transcriptional activity of *HO-1* is dependent on (GT)n repeats in its promoter region, with longer (GT) n repeats being associated with lower transcription of the *HO-1* gene. Up to date, the (GT)n repeat polymorphism has been reported to be associated with oral squamous cell carcinoma, lung, melanoma and gastric cancer, as well as gastrointestinal stromal tumors previously (Chang et al., 2004; Kikuchi et al., 2005; Okamoto et

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al., 2006; Lo et al., 2007; Vashist et al., 2009). However, there is no uniformity concerning this (GT)n pattern with biological tumor behaviors. In several cancers such as lung adenocarcinoma, postmenopausal breast cancer, malignant mesothelioma, the long (GT)n repeat in the *HO-1* gene promoter is associated with a higher cancer risk (Kikuchi et al., 2005; Hong et al., 2007; Murakami et al., 2012), whereas in malignant melanoma and pancreatic cancer, the short (GT)n allele has been correlated with the tumorigenesis and tumor progression (Okamoto et al., 2006; Vashist et al., 2011). Similar contradictory results are also observed for the role of (GT)n polymorphism in gastric cancer (Lo et al., 2007). Even more complicated, risk of lung squamous cell carcinoma and sporadic colorectal cancer seems not be influenced by the (GT)n polymorphism in the *HO-1* gene promoter (Kikuchi et al., 2005; Jiraskova et al., 2012).

Thus, we carried out this meta-analysis to integrate previous studies and for the first time to elucidate the potential association between *HO-1* gene (GT)n promoter polymorphism and cancer risk.

## Materials and Methods

We conducted this systematic review according to MOOSE guideline (Stroup et al., 2000).

*Literature and search strategy*

An electronic literature search was performed with PubMed/MEDLINE, EMBASE and ISI Web of Science for all relevant reports up to Nov 2, 2013, which had examined the association between *HO-1* gene (GT)n microsatellite polymorphisms and cancer. The searching strategy was performed using three groups of key words ''heme oxygenase-1 or HMOX1 or *HO-1*'', ''polymorphism or susceptibility'' in combination with ''cancer or carcinoma or tumor or neoplasm or malignancy or malignant''. In the PubMed database, all keywords were used with Medical Subject Headings (Mesh). The publication language was restricted to English. Reference lists in retrieved articles were also reviewed.

*Inclusion criteria*

Included studies should met the following criteria: (1) case-control study or cohort study, (2) be in accord with Hardy-Weinberg equilibrium in control groups, (3) focus on the association between *HO-1* (GT)n polymorphism and cancer risk, and (4) providing an odds ratio (OR) with 95% confidence interval (CI) or sufficient data for the calculation of OR and 95%CI.

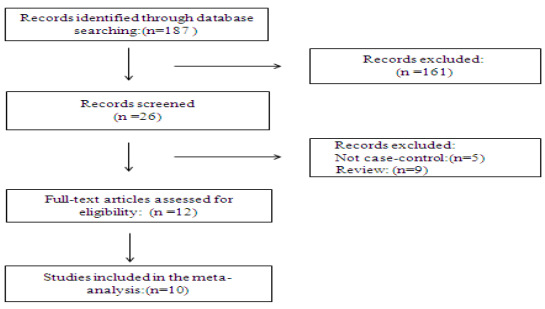


**Figure 1. Flow Chart of Inclusion/Exclusion of the Individual Studies**

*Data extraction*

Data extraction from the included studies was conducted as previously described (Marcos et al., 2009). The following information was collected from each eligible study: the first author's last name, year of publication, country of origin, ethnicity, sample size of cases and controls, cancer types, and allele and genotype frequency of (GT)n repeat length polymorphism for both cases and controls. The literature search, selection and data extraction were performed independently by two authors and disagreements were resolved by consensus for all data.

*Statistical analysis*

We calculated OR and 95%CI, in accordance with the method described by Woolf (Woolf, 1955), as the metrics of effect size for each study and overall studies, to evaluate the association between the *HO-1* (GT)n repeat polymorphism and cancer. Further stratified assessments were carried out for *HO-1* (GT)n repeat polymorphism by ethnicity and histopathology separately. Heterogeneity across all the eligible studies was estimated by two methods: the Cochran's Q statistic and the $I^2$ metric, which quantify between-study heterogeneity irrespective of the number of studies. For the Q statistic, heterogeneity was considered significant at $p<0.10$, while for the $I^2$ metric, heterogeneity was ranked as "no-" ($0\%\leq I^2<25\%$), "moderate-" ($25\%\leq I^2<50\%$), "large-" ($50\%\leq I^2<75\%$) and "extreme-" ($75\%\leq I^2\leq 100\%$) heterogeneity groups according to the quartile cutoff points (Marcos et al., 2009). A random-effects model (DerSimonian and Laird method) and fixed-effects model (Mantel-Hansel method) were used to calculate the pooled OR in the presence ($p\leq 0.10$) or absence ($p>0.10$) of heterogeneity, respectively (Mantel et al., 1959; DerSimonian et al., 1986). The statistical significance of the pooled OR was determined with the Z test and visualized by the forest plot. Publication bias was investigated with the Begg's funnel plot, and further evaluated by Egger's linear regression method (Egger et al., 1997). Analyses were conducted using RevMan 5.1 and Stata 11.0. All *P* values presented are two-tailed with a significance of 0.05.

## Results

*Eligible studies*

A schematic representation of the literature search and selection procedures is presented in Figure 1. A total of 26 relevant literatures were identified to evaluate the relationship between *HO-1* (GT)n repeat polymorphism and cancer risk, among which 9 reviews were excluded. In addition, 5 papers were discarded without setting control group. Thus, 12 eligible studies matching the search criteria were retrieved from the public databases and reviewed independently by 2 investigators. Among them, there were 2 literatures employing the same subjects (Hong et al., 2007; Li et al., 2009), thereafter we selected the study with the most detailed data (Hong et al., 2007). For studies that did not provide raw data in the initial

publication, we attempted to obtain this information by correspondence with the authors (Kikuchi et al., 2005; Lin et al., 2006; Sawa et al., 2008). For one study investigated two cancers concomitantly, we treated it as two studies in the following analysis (Kikuchi et al., 2005). Finally, 10 studies comprised over 2,367 cases and 2,870 controls were included in our final meta-analysis.

*(GT)n repeat length polymorphism*

After reviewing these 10 included literatures, we had not find a consensus on the optimum cutpoint for the (GT) n repeat length polymorphism of the *HO-1* gene promoter, so the uniformity of cutpoints was taken into consideration (Table 1). We divided the allelic repeats into 2 subclasses: <25 or<27 (GT)n repeats defined as class S (short), and≥25 or≥27 (GT)n repeats defined as class L (long), and then 3 genotypes of SS, SL and LL were assigned. The detailed characteristics and genotype distribution of the *HO-1* (GT) n repeat polymorphism of the included studies are showed in Table 2.

**Table 1. Cutpoints for (GT)n Repeat Length Polymorphism of HO-1 Gene in the Included Studies**

| First Author | Year | Cutpoint Values | Categories ofthe Alleles |
|---|---|---|---|
| Chang | 2004 | 25 and 30 | Class S: ≤25 (GT) repeats |
| | | | Class M: 26-30 (GT) repeats |
| | | | Class L: ≥31 (GT) repeats |
| Kikuchi | 2005a | 27 and 32 | Class S: <27 (GT) repeats |
| | | | Class M: 27-32 (GT) repeats |
| | | | Class L: ≥33 (GT) repeats |
| Kikuchi | 2005b | 27 and 32 | Class S: <27 (GT) repeats |
| | | | Class M: 27-32 (GT) repeats |
| | | | Class L: ≥33 (GT) repeats |
| Okamoto | 2006 | 25 | Class S: <25 (GT) repeats |
| | | | Class L: ≥25 (GT) repeats |
| Hong | 2007 | 25 and 30 | Class S: <25 (GT) repeats |
| | | | Class M: 25-29 (GT) repeats |
| | | | Class L: ≥30 (GT) repeats |
| Lo | 2007 | 25 and 31 | Class S: ≤25 (GT) repeats |
| | | | Class M: 26-30 (GT) repeats |
| | | | Class L: ≥31 (GT) repeats |
| Hu | 2010 | 25 | Class S: <25 (GT) repeats |
| | | | Class L: ≥25 (GT) repeats |
| Vashist | 2011 | 25 | Class S: <25 (GT) repeats |
| | | | Class L: ≥25 (GT) repeats |
| Jirásková | 2012 | 27and 32 | Class S: <27 (GT) repeats |
| | | | Class M: 27-32 (GT) repeats |
| | | | Class L: ≥33 (GT) repeats |
| Murakami | 2012 | 24 | Class S: <24 (GT) repeats |
| | | | Class L: ≥24 (GT) repeats |

*Meta-analysis results*

For overall comparisons (Figure 2 and Table 3), there was no association between *HO-1* (GT)n repeat polymorphism and cancer risk at the allelic level (OR=1.03, 95%CI: 0.89-1.18), as well as the genotypic level (LL *vs* SS: OR=1.05, 95%CI: 0.74-1.49; LL *vs* LS + SS: OR=1.08, 95%CI: 0.87-1.33; LL+ LS *vs* SS: OR=1.02, 95%CI: 0.74-1.41). Due to the presence of significant heterogeneities with overall analyses, stratified analyses of this (GT)n repeat polymorphism were further carried out by ethnicity, and histopathology (Table 3).

As shown in the subgroup analysis of ethnicity (Table 3), no significant associations with cancer risk were found respectively in Asians (OR=1.18, 95%CI: 0.87-1.61), Europeans (OR=0.92, 95%CI: 0.82-1.03) and Americans (OR=1.08, 95%CI: 0.89-1.31) for the comparison of L allele with S allele. The stratified analysis among different histological types of cancer also showed no significant change in the risk of cancer conferred by the

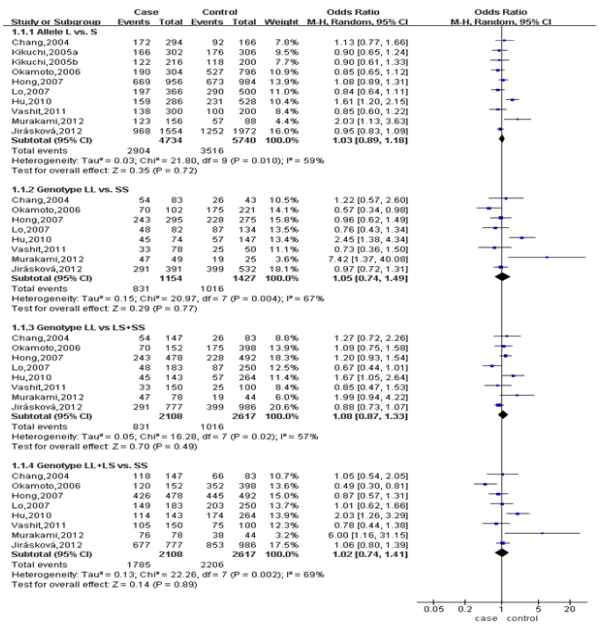


**Figure 2. Forest Plots Describing the Association between the HO-1 (GT)n Repeat Length Polymorphism and Risk of Cancer**

**Table 2. Characteristics and Genotype Distribution of the (GT)n Repeat Polymorphism of the Included Studies of the HO-1 Gene and Susceptibility to Cancer**

| First Author | Year | Ethnic Origin | Cancer Type | No. of Eligible Subjects | | Frequency of Class L Allele, % | | Genotype(n) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | SS | | LS | | LL | |
| | | | | Ca | Co | Ca | Co | Ca | Co | Ca | Co | Ca | Co |
| Chang | 2004 | Asian | Oral Squamous cell carcinoma | 147 | 83 | 58.5 | 55.4 | 29 | 17 | 64 | 40 | 54 | 26 |
| Kikuchi | 2005a | Asian | Lung Adenocarcinoma | 151 | 153 | 55 | 57.5 | N/A | N/A | N/A | N/A | N/A | N/A |
| Kikuchi | 2005b | Asian | Lung Squamous cell carcinoma | 108 | 100 | 56.5 | 59 | N/A | N/A | N/A | N/A | N/A | N/A |
| Okamoto | 2006 | Europe | Melanoma | 152 | 398 | 62.5 | 66.2 | 32 | 46 | 50 | 177 | 70 | 175 |
| Hong | 2007 | American | Postmenopausal Breast cancer | 478 | 492 | 70 | 68.4 | 52 | 47 | 183 | 217 | 243 | 228 |
| Lo | 2007 | Asian | Gastric Adenocarcinoma | 183 | 250 | 53.8 | 58 | 34 | 47 | 101 | 116 | 48 | 87 |
| Hu | 2010 | Asian | Esophageal Squamous cell carcinoma | 143 | 263 | 55.6 | 43.8 | 29 | 90 | 69 | 117 | 45 | 57 |
| Vashist | 2011 | Europe | Pancreatic Cancer | 150 | 100 | 46 | 50 | 45 | 25 | 72 | 50 | 33 | 25 |
| Jirásková | 2012 | Europe | Sporadic Colorectal cancer | 777 | 986 | 62.3 | 63.5 | 100 | 133 | 386 | 454 | 291 | 399 |
| Murakami | 2012 | Asian | Malignant Mesothelioma | 78 | 44 | 78.8 | 64.8 | 2 | 6 | 29 | 19 | 47 | 19 |

*Abbreviation: Ca, indicates case; Co, indicates control; N/A, indicates not available

**Table 3. Overall and Stratified Analyses on the Association of the HO-1(GT)n Repeat Polymorphism with Cancer Risk and Heterogeneity Test**

| Variables | Included papers(n) | Allele L *vs* S | | Included papers (n) | Genotype LL *vs* SS | |
|---|---|---|---|---|---|---|
| | | OR(95%CI), $P^a$ | $P^b$, $I^2$ (%) | | OR(95%CI), $P^a$ | $P^b$, $I^2$ (%) |
| Total | 10 | 1.03 (0.89, 1.18), 0.72 | 0.01, 59 | 8 | 1.05 (0.74, 1.49), 0.77 | 0.004, 67 |
| Ethnic Origin | | | | | | |
| Asian | 6 | 1.13 (0.87, 1.47), 0.37 | 0.004, 71 | 4 | 1.65 (0.77, 3.55), 0.20 | 0.01, 75 |
| Europe | 3 | 0.92 (0.82, 1.03), 0.16 | 0.71, 0 | 3 | 0.84 (0.66, 1.07), 0.17 | 0.22, 33 |
| American | 1 | 1.08 (0.89, 1.31), 0.45 | N/A, N/A | 1 | 0.96 (0.62, 1.49), 0.87 | N/A, N/A |
| Cancer histological type | | | | | | |
| Squamous cell carcinoma | 3 | 1.20 (0.85, 1.71), 0.30 | 0.05, 66 | 2 | 1.90 (1.21, 2.99), 0.01 | 0.15, 52 |
| Adenocarcinoma | 3 | 0.86 (0.72, 1.03), 0.11 | 0.95, 0 | 2 | 0.75 (0.48, 1.17), 0.21 | 0.93, 0 |
| Other | 4 | 1.03 (0.85, 1.26), 0.76 | 0.04, 63 | 4 | 0.79 (0.26, 2.39), 0.68 | <0.00001,95 |
| Variables | Included papers(n) | Genotype LL *vs* LS+ SS | | Included papers(n) | Genotype LL+LS *vs* SS | |
| | | OR(95%CI), $P^a$ | $P^b$, $I^2$ (%) | | OR(95%CI), $P^a$ | $P^b$, $I^2$ (%) |
| Total | 8 | 1.08 (0.87, 1.33), 0.49 | 0.002, 57 | 8 | 1.02 (0.74, 1.41), 0.89 | 0.002, 69 |
| Ethnic Origin | | | | | | |
| Asian | 4 | 1.25 (0.75, 2.09), 0.40 | 0.01, 73 | 4 | 1.49 (0.88, 2.55), 0.14 | 0.05, 61 |
| Europe | 3 | 0.91 (0.78, 1.08), 0.29 | 0.60, 0 | 3 | 0.76 (0.47, 1.23), 0.27 | 0.03, 72 |
| American | 1 | 1.20 (0.93, 1.54), 0.16 | N/A, N/A | 1 | 0.87 (0.57, 1.31), 0.50 | N/A, N/A |
| Cancer histological type | | | | | | |
| Squamous cell carcinoma | 2 | 1.50 (1.05, 2.14), 0.03 | 0.47, 0 | 2 | 1.64 (1.12, 2.40), 0.01 | 0.12, 60 |
| Adenocarcinoma | 2 | 0.72 (0.51, 1.01), 0.06 | 0.52, 0 | 2 | 0.91 (0.63, 1.31), 0.60 | 0.49, 0 |
| Other | 4 | 1.02 (0.89, 1.18), 0.47 | 0.07, 57 | 4 | 0.91 (0.55, 1.52), 0.73 | 0.01, 75 |

*Abbreviation: N/A, not available; $^aP$ value of Z test for Random- or fixed-effects model; $^bP$ value of Q test for heterogeneity test

**Table 4. Tests for Publication Bias**

| Comparison | Begg's test | | Egger's test | |
|---|---|---|---|---|
| | z | *P* | t | *P* |
| Allele L *vs* S | 1.07 | 0.283 | 0.83 | 0.428 |
| Genotype LL *vs* SS | 1.11 | 0.266 | 0.98 | 0.364 |
| Genotype LL *vs* LS+SS | 0.62 | 0.536 | 1.1 | 0.313 |
| Genotype LL+LS *vs* SS | -0.12 | 1 | 0.58 | 0.582 |

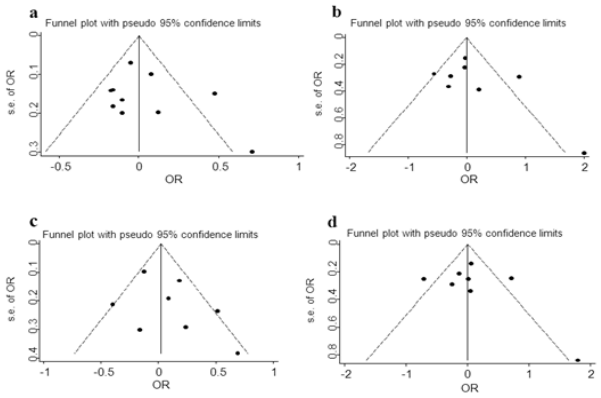


**Figure 3. Begg's Funnel Plots to Examine Publication Bias for Reported Comparisons of the HO-1 Polymorphism and Risk of Cancer. a**) Allele L *vs* S; **b)** Genotype LL *vs* SS; GT)n, **c)** Genotype LL *vs* LS+SS; **d)** Genotype LL+LS *vs* SS

(GT)n L allele as compared with the (GT)n S allele. For genotypes, further stratification analysis indicated that genotype LL or genotypes carrying L allele (combined genotype LL and LS) had no effect on the cancer risk for each individual ethnic group (Table 3). In the subsets divided by histopathology, we observed an increased risk of squamous cell carcinoma in persons carrying the LL genotype as compared with those carrying the SS genotype (OR=1.90, 95%CI: 1.21-2.99) and the LS+SS genotypes (OR=1.50, 95%CI: 1.05-2.14), as well as in persons with the LL+LS genotypes when compared to those with the SS genotype (OR=1.64, 95%CI: 1.12-2.40). However, no statistical significance was reached in the risk of adenocarcinoma and other types of tumor within the genotype comparisons.

*Publication bias*

Begg's funnel plot and Egger's test were performed to determine whether a publication bias existed in the literature. The comparisons of the allelic and genotypic frequencies indicated that there was no obvious publication bias among the studies included in our meta-analysis, with all P values for Egger's test greater than 0.05. (Table 4 and Figure 3).

## Discussion

The current meta-analysis was based on ten studies with over 2, 367 cancer cases and 2, 870 controls to provide a comprehensive and systematic evaluation of the association between the *HO-1* gene promoter (GT)n repeat polymorphism and risk of cancer. As a whole, we did not observe a significant association between *HO-1* (GT)n repeat polymorphism and cancer risk both at the allelic and genotypic level. In the sub-group analysis, the LL genotype of *HO-1* (GT)n repeat polymorphism conferred a higher risk of squamous cell carcinoma than the LS and/or SS genotype. Similar results were found in analysis comparing the odds ratio for squamous cell carcinoma in persons with the LL/LS genotype and the SS genotype. The risk for squamous cell carcinoma in persons with the (GT)n L allele, compared with those with the (GT)n S allele was significantly increased, in a random-effects model (large between-study heterogeneity).

*HO-1* has been reported to play a crucial role in apoptosis, cell survival, and angiogenesis, acting as a target gene of transcription factor, like nuclear factor erythroid-2 p45-related factor 2 (Nrf2). Recently study showed that adaptive activation of Nrf2-*HO-1* pathway may contribute to the development of acquired drug-resistance in colorectal cancer, while inhibition of this pathway may be the mechanism for the recovered sensitivity to chemotherapeutics (Chian et al., 2014).

However, a consistency in the effect of *HO-1* on human malignancies has not been reached (Jozkowic et al., 2007). Up to now, two potentially functional polymorphisms in the promoter region of *HO-1* gene have been identified: a (GT)n microsatellite polymorphism and a single nucleotide polymorphism (SNP), T (-413)A (rs2071746) ( Exner et al., 2004). The basal transcriptional activity of the *HO-1* is dependent upon the (GT)n repeat polymorphism, usually classified as S (GT)n and L (GT)n (Exner et al., 2004). Cells with short (GT)n numbers has been reported with increased *HO-1* basal promoter activity and up-regulation in response to various stimuli and resistance to apoptosis induced by oxidative stress compared to the long (GT)n repeat harboring cells (Yamada et al., 2000; Exner et al., 2004). This may support a common attribute of gene transcription controlling mechanism for *HO-1*.

Since its first clinical description by Kimpara et al. in 1997 (Kimpara et al., 1997), the (GT)n dinucleotide repeat polymorphism has emerged as a potent genetic risk factor in various diseases, including chronic and degenerative diseases, inflammation, graft-survival in transplantation but also various malignant tumors. However, the effects of the (GT)n repeat polymorphism on tumor characteristics and clinical outcome show inconsistency among different types of tumors. It is likely that different genetic background, sample size and subject sources mainly account for the contradictory results. In this large meta-analysis, the results suggest that the *HO-1* (GT)n repeat polymorphism may not helpful to screen high-risk population suffering from cancer, which was mainly influenced by the study carried out in sporadic colorectal cancer with the largest sample size of all the include studies (Jiraskova et al., 2012). In this exploratory case-control study, the common genetic variations in promoter regions of *HO-1* were not associated with an increased risk of sporadic colorectal cancer. This can influence the assigned weight in meta-analysis (Higgins et al., 2012). Point of notice is that we found the longer (GT) n repeats in the *HO-1* gene promoter may contribute to the genetic susceptibility of squamous cell carcinoma. However, we cannot arbitrarily draw such a conclusion, due to the limitations of the meta-analysis. Firstly, the number and the sample size of included studies as well we related to the *HO-1* genetic polymorphisms and cancer was limited, especially for the stratification analysis. Only 1 study included more than 500 cases and 500 controls, and all the other studies had relatively small sample sizes which decreased their statistical power (Jiraskova et al., 2012). Secondly, the controls were not uniformly defined. Although all controls were healthy populations, it is possible that some of them were community-based, while others were hospital-based. Thirdly, even though we harmonized the cutpoints for the (GT)n repeat polymorphism at 25 or 27 in different studies, there is still subtle bias from the 2-repeat difference. Additionally, the vague cancer histological type in some studies and the potential errors in the genotype classification may also result in statistical biases in the current meta-analysis.

In conclusion, our meta-analysis suggested that persons carrying longer (GT)n repeats in the *HO-1* gene promoter may have a higher risk of squamous cell carcinoma. However, the overall cancer risk was not ascribed to the (GT)n repeats polymorphism for either allelic or genotypic frequencies. Further research with multi-centers, sufficient sample size and less heterogeneity will be needed for further clarification of the association between *HO-1* gene (GT)n repeats and cancers.

## Acknowledgements

This work was supported by grants from the Specialized Research Fund for the Doctoral Program of Higher Education (20101202120007), National Natural Science Foundation of China (no. 81302293).

## References

Aruoma OI, Grootveld M, Bahorun T (2006). Free radicals in biology and medicine: from inflammation to biotechnology. *Biofactors*, **27**, 1-3.

Berberat PO, Dambrauskas Z, Gulbinas A, et al (2005). Inhibition of heme oxygenase-1 increases responsiveness of pancreatic cancer cells to anticancer treatment. *Clin Cancer Res*, **11**, 3790-8.

Chang KW, Lee TC, Yeh WI, et al (2004). Polymorphism in heme oxygenase-1 (*HO-1*) promoter is related to the risk of oral squamous cell carcinoma occurring on male areca chewers. *Br J Cancer*, **91**, 1551-5.

Chian S, Li YY, Wang XJ, et al (2014). Luteolin sensitizes two oxaliplatin-resistant colorectal cancer cell lines to chemotherapeutic drugs via inhibition of the nrf2 pathway. *Asian Pac J Cancer Prev*, **15**, 2911-6.

Datta D, Banerjee P, Gasser M, Waaga-Gasser AM, Pal S (2010). CXCR3-B can mediate growth-inhibitory signals in human renal cancer cells by down-regulating the expression of heme oxygenase-1. *J Biol Chem*, **285**, 36842-8.

Davies KJ (1995). Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp*, **61**, 1-31.

DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials*, **7**, 177-88.

Deshane J, Wright M, Agarwal A (2005). Heme oxygenase-1 expression in disease states. *Acta Biochim Pol*, **52**, 273-84.

Egger M, Davey Smith G, Schneider M, Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, **315**, 629-34.

Exner M, Minar E, Wagner O, Schillinger M (2004). The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med*, **37**, 1097-104.

Higgins JPT, Green S, eds (2009). Cochrane Handbook for Systematic Reviews of Interventions, Version 5.0.2 [Updated September 2009]. The Cochrane Collaboration, Oxford.

Hirai H, Kubo H, Yamaya M, et al (2003). Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. *Blood*, **102**, 1619-24.

Hong CC, Ambrosone CB, Ahn J, et al (2007). Genetic variability in iron-related oxidative stress pathways (Nrf2, NQ01,

NOS3, and*HO-1*), ironintake, and risk of postmenopausal breast cancer. *Cancer Epidemiol Biomarkers Prev*, **16**, 1784-94.

Hu JL, Li ZY, Liu W, et al (2010). Polymorphism in heme oxygenase-1 (*HO-1*) promoter and alcohol are related to the risk of esophageal squamous cell carcinoma on Chinese males. *Neoplasma*, **57**, 86-92.

Jiraskova A, Novotny J, Novotny L, et al (2012). Association of serum bilirubin and promoter variations in HMOX1and UGT1A1 genes with sporadic colorectal cancer. *Int J Cancer*, **131**, 1549-55.

Jozkowicz A, Was H, Dulak J (2007). Heme oxygenase-1 in tumors: Is it a false friend? *Antioxid Redox Signal*, **9**, 2099-117.

Kikuchi A, Yamaya M, Suzuki S, et al (2005). Association of susceptibility to the development of lung adenocarcinoma with the heme oxygenase-1 gene promoter polymorphism. *Hum Genet*, **116**, 354-60.

Kimpara T, Takeda A, Watanabe K, et al (1997). Microsatellite polymorphism in the human heme oxygenase-1 gene promoter and its application in association studies with Alzheimer and Parkinson disease. *Hum Genet*, **100**, 145-7.

Li Q, Wang JM, Peng Y, et al (2013). Association of DNA base-excision repair XRCC1, OGG1 and APE1 gene polymorphisms with nasopharyngeal carcinoma susceptibility in a Chinese population. *Asian Pac J Cancer Prev*, **14**, 5145-51.

Li Y, Ambrosone CB, McCullough MJ, et al (2009). Oxidative stress-related genotypes, fruit and vegetable consumption and breast cancer risk. *Carcinogenesis*, **30**, 777-84.

Lin SC, Liu CJ, Yeh WI, et al (2006). Functional polymorphism in NFKB1 promoter is related to the risks of oral squamous cell carcinoma occurring on older male areca (betel) chewers. *Cancer Lett*, **243**, 47-54.

Lo SS, Lin SC, Wu CW, et al (2007). Heme Oxygenase-1 gene promoter polymorphism is associated with risk of gastric adenocarcinoma and lymphovascular tumor invasion. *Ann Surg Oncol*, **14**, 2250-6.

Luo H, Li Z, Qing Y, et al (2014). Single nucleotide polymorphisms of DNA base-excision repair genes (APE1, OGG1 and XRCC1) associated with breast cancer risk in a Chinese population. *Asian Pac J Cancer Prev*, **15**, 1133-40.

Maines MD (1997). The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol*, **37**, 517-54.

Mantel N, Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*, **22**, 719-48.

Marcos M, Gomez-Munuera M, Pastor I, Gonzalez-Sarmiento R, Laso FJ (2009). Tumor necrosis factor polymorphisms and alcoholic liver disease: a HuGE review and meta-analysis. *Am J Epidemiol*, **170**, 948-56.

Murakami A, Fujimori Y, Yoshikawa Y, et al (2012). Heme Oxygenase-1promoter polymorphism is associated with risk of malignant mesothelioma. *Lung*, **190**, 333-7.

Nuhn P, Künzli BM, Hennig R, et al (2009). Heme oxygenase-1 and its metabolites affect pancreatic tumor growth *in vivo*. *Mol Cancer*, **8**, 37

Okamoto I, Krögler J, Endler G, et al (2006). A microsatellite polymorphism in the heme oxygenase-1gene promoteris associated with risk for melanoma. *Int J Cancer*, **119**, 1312-5.

Sacca P, Meiss R, Casas G, et al (2007). Nuclear translocation of haeme oxygenase-1 is associated to prostate cancer. *Br J Cancer*, **97**, 1683-9.

Sawa T, Mounawar M, Tatemichi M, et al (2008). Increased risk of gastric cancer in Japanese subjects is associated with microsatellite polymorphisms in the heme oxygenase-1 and the inducible nitric oxide synthase gene promoters. *Cancer Lett*, **269**, 78-84.

Song F, Li X, Zhang M, et al (2009). Association between heme oxygenase-1 gene promoter polymorphisms and type 2 diabetes in a Chinese population. *Am J Epidemiol*, **170**, 747-56.

Stroup DF, Berlin JA, Morton SC, et al (2000). Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*, **283**, 2008-12.

Vashist YK, Uzunoglu G, Cataldegirmen G, et al (2009). Haeme oxygenase-1 promoter polymorphism is an independent prognostic marker of gastrointestinal stromal tumour. *Histopathology*, **54**, 303-8.

Vashist YK, Uzungolu G, Kutup A, et al (2011). Heme oxygenase-1 germ line GTn promoter polymorphism is an independent prognosticator of tumor recurrence and survival in pancreatic cancer. *J Surg Oncol*, **104**, 305-11

Woolf B (1955). On estimating the relation between blood group and disease. *Ann Hum Genet*, **19**, 251-3.

Yamada N, Yamaya M, Okinaga S, et al (2000). Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet*, **66**, 187-95.