Common polymorphisms in the *HIF-1α* gene confer susceptibility to digestive cancer: a meta-analysis

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ABSTRACT. Recent evidence suggests that common functional polymorphisms in the hypoxia inducible factor-1α (*HIF-1α*) gene may play an important role in the development and progression of digestive cancer, but individually published results are inconclusive. Our meta-analysis is aimed to derive a more precise estimation of the relationships between *HIF-1α* gene polymorphisms and digestive cancer risk. An extensive literature search for relevant studies was conducted on Pubmed, Embase, Web of Science, Cochrane Library, and CBM databases from their inception through May 1, 2013. This meta-analysis was performed using the STATA 12.0 software. The crude odds ratios (OR) with 95% confidence interval (CI) were calculated. Eight case-control studies were included with a total of 1276 digestive cancer patients and 3392 healthy controls. Our meta-analysis revealed that the A variant of *HIF-1α* G1790A polymorphism might be associated with increased risk of colorectal, esophageal, gastric, and liver cancers, especially among Asian populations. However, no statistically significant associations were found between *HIF-1α* C1772T polymorphism and susceptibility

to digestive cancer. No publication bias was detected in this meta-analysis. The current meta-analysis suggests that the *HIF-1α* G1790A polymorphism may increase the risk of colorectal, esophageal, gastric, and liver cancers, especially among Asian populations.

Key words: Digestive cancer; Hypoxia inducible factor-1α; Polymorphism; Susceptibility; Meta-analysis

INTRODUCTION

Hypoxia inducible factor-1α (*HIF-1α*) is one of basic helix-loop-helix transcription factor expressed uniquely in response to physiologically relevant levels of hypoxia (Semenza, 2012a; Semenza and Prabhakar, 2012). *HIF-1α* can control cellular response to hypoxia by activating transcription of genes to enhance oxygen availability and to allow metabolic adaptation to hypoxia (Nguyen et al., 2013). Up-regulated *HIF-1α* is involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism, and invasion (Semenza, 2012b). Intratumoral hypoxia and genetic alterations can lead to *HIF-1α* overexpression, which has been associated with increased susceptibility to several types of cancer (Semenza, 2012b,c). Therefore, it was hypothesized that *HIF-1α* gene variations can be used as biomarkers and primary chemotherapeutic targets for the detection and treatment of cancer (Watanabe, 2012).

The human *HIF-1α* gene is located on the chromosome 14q21-q24 and consists of 14 exons and 13 introns (Semenza et al., 1996). Several single-nucleotide polymorphisms (SNPs) have been identified in the *HIF-1α* gene. Among these SNPs, C1772T (rs11549465 C>T) and G1790A (rs11549467 G>A) are the most common and widely investigated polymorphisms. However, the exact mechanism of these polymorphisms in the development of digestive cancer is not clearly understood (Huang et al., 2012). Recent evidence has suggested that *HIF-1α* gene polymorphisms may be associated with increased digestive cancer risk (Ling et al., 2005; Fransen et al., 2006; Li et al., 2009; Hsiao et al., 2010; Kang et al., 2011; Wang et al., 2011); however, individually published results are inconclusive (Kuwai et al., 2004; Knechtel et al., 2010). Therefore, we attempt to perform a meta-analysis of all eligible case-control studies to provide insights into these associations, which may promote our understanding of the exact role of the *HIF-1α* gene in the development of carcinogenesis in the digestive organs.

MATERIAL AND METHODS

Literature search strategy

An extensive literature search for relevant studies was conducted on Pubmed, Embase, Web of Science, Cochrane Library, and CBM databases from inception through May 1, 2013. We used the following keywords and MeSH terms: ('genetic polymorphism' or 'polymorphism' or 'SNP' or 'mutation' or 'variation' or 'variant') and ('digestive system neoplasms' or 'cancer of digestive system' or 'digestive cancer' or 'gastric cancer' or 'esophageal cancer' or 'colorectal cancer' or 'intestinal cancer' or 'liver cancer' or 'pancreatic cancer') and ('hypoxia inducible factor-1α' or 'HIF-1α'). There was no language restriction. Manual search of reference lists from potentially relevant articles was also performed to identify other potential studies.

Inclusion and exclusion criteria

To be included in the analysis, these studies must meet the following criteria: a) case-control studies focus on the associations between *HIF-1α* gene polymorphisms and digestive cancer risk; b) all patients diagnosed with digestive cancer should be confirmed by histopathologic examination; c) published data about genotype frequencies of SNPs must be sufficient; d) genotype distribution in healthy controls should conform to Hardy-Weinberg equilibrium (HWE). Studies were excluded if they do not meet all of these inclusion criteria. Any disagreements were resolved by discussions and subsequent consensus.

Data extraction

Two authors independently extracted data from eligible studies by using a standardized form. The following information was collected prospectively: surname of first author, year of publication, source of publication, country of origin, ethnicity, language of publication, study type, total number of subjects, source of cases and controls, pathological type, SNP type, DNA sample, SNP-detection method, genotype frequencies, and evidence of HWE in controls. In cases of conflicting evaluations, disagreements on inconsistent data from the eligible studies were resolved through discussion and careful reexamination of the full text by the authors.

Quality assessment of included studies

The quality of included studies was assessed independently by 2 authors based on the STROBE quality score systems (da Costa et al., 2011). Forty assessment items related to quality appraisal were used in this meta-analysis with scores ranging from 0 to 40. The included studies were classified into 3 levels based on their scores: low quality (0-19), moderate quality (20-29), and high quality (30-40), respectively. Disagreements on STROBE scores of the included studies were resolved through a comprehensive reassessment by the authors.
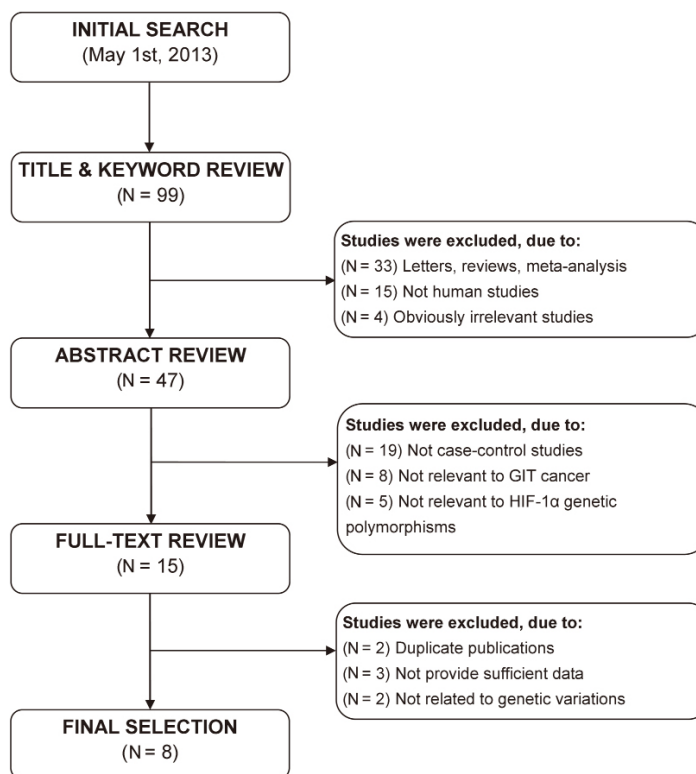
Statistical analysis

The ORs and 95%CIs were calculated under 5 genetic models: allele model (mutant [M] allele versus wild [W] allele), dominant model (WM+MM versus WW), recessive model (MM versus WW+WM), homozygous model (MM versus WW), and heterozygous model (MM versus WM). The significance of the pooled estimate was determined using the Z-test. Genotype distributions in the control subjects were tested for HWE by the chi-square test. We estimated the degree of heterogeneity among studies by using Cochran's Q-statistic, which is considered to be significant at $P < 0.05$ (Jackson et al., 2012). The I^2 test was also used to quantify the heterogeneity (ranges from 0 to 100%) (Zintzaras and Ioannidis, 2005). When a significant Q-test with $P_h < 0.05$ or $I^2 > 50\%$ indicated that heterogeneity among studies existed, the random-effect model (DerSimonian Laird method) was conducted for the meta-analysis; otherwise, the fixed-effect model (Mantel-Haenszel method) was used. In order to explore sources of heterogeneity, subgroup analyses were performed based on ethnicity, cancer type, source of control, and genotype method (Ioannidis et al., 2008). To evaluate the influence of single studies on overall risk estimate, we conducted a sensitivity analysis by omitting each

study in turn. Funnel plots and the Egger linear regression test were used to assess potential publication bias of included studies (Peters et al., 2006). All tests were two-sided and a P value of < 0.05 was considered to be statistically significant. All analyses were calculated using the STATA software, version 12.0 (Stata Corp., College Station, TX, USA).

RESULTS

Baseline characteristics of included studies

In accordance with the inclusion criteria, 8 case-control studies (Kuwai et al., 2004; Ling et al., 2005; Fransen et al., 2006; Li et al., 2009; Hsiao et al., 2010; Knechtel et al., 2010; Kang et al., 2011; Wang et al., 2011) were included in this meta-analysis and 91 articles were excluded. The flow chart of the study selection process is shown in Figure 1. The publication year of involved studies ranged from 2004 to 2011. A total of 1276 digestive cancer patients and 3392 healthy controls were included. All patients diagnosed with digestive cancers were confirmed by pathological examinations. Two common polymorphisms in the *HIF-1α* gene, C1772T (rs11549465 C>T) and G1790A (rs11549467 G>A), were assessed. The HWE test was conducted on the genotype distribution of the controls in all 12 studies. Each study did not deviate from the HWE (P > 0.05). The characteristics and methodological quality of the included studies are summarized in Table 1.

INITIAL SEARCH
(May 1st, 2013)

↓

TITLE & KEYWORD REVIEW
(N = 99)

Studies were excluded, due to:
(N = 33) Letters, reviews, meta-analysis
(N = 15) Not human studies
(N = 4) Obviously irrelevant studies

↓

ABSTRACT REVIEW
(N = 47)

Studies were excluded, due to:
(N = 19) Not case-control studies
(N = 8) Not relevant to GIT cancer
(N = 5) Not relevant to HIF-1α genetic polymorphisms

↓

FULL-TEXT REVIEW
(N = 15)

Studies were excluded, due to:
(N = 2) Duplicate publications
(N = 3) Not provide sufficient data
(N = 2) Not related to genetic variations

↓

FINAL SELECTION
(N = 8)

Figure 1. Flow chart of literature search and study selection. Eight case-control studies were included in this meta-analysis.

Table 1. Main characteristics and methodological quality of all eligible studies.

First author	Year	Country	Ethnicity	Number		Source		Cancer type	Sample	Genotyping method	SNP ID	STROBE score
				Case	Control	Case	Control					
Kuwai et al.	2004	Japan	Asian	100	100	HB	PB	Colorectal cancer	Blood	PCR-RFLP	rs11549465	25
Ling et al.	2005	China	Asian	95	104	HB	PB	Esophageal cancer	Blood	PCR-RFLP	rs11549465	25
Fransén et al.	2006	Sweden	Caucasian	198	258	HB	PB	Colorectal cancer	Tissue	PCR-RFLP	rs11549465 rs11549467	25
Li et al.	2009	China	Asian	87	106	HB	PB	Gastric cancer	Blood	MicroArray	rs11549465 rs11549467	27
Hsiao et al.	2010	China	Asian	102	347	HB	HB	Liver cancer	Blood	PCR-RFLP	rs11549465 rs11549467	27
Knechtel et al.	2010	Austria	Caucasian	381	2156	HB	HB	Colorectal cancer	Tissue	TaqMan	rs11549465 rs11549467	23
Kang et al.	2011	Korea	Asian	50	50	HB	PB	Colorectal cancer	Tissue	PCR-RFLP	rs11549465	22
Wang et al.	2011	China	Asian	263	271	HB	PB	Pancreatic cancer	Blood	PCR-RFLP	rs11549465 rs11549467	22

SNP = single nucleotide polymorphism; HB = hospital-based study; PB = population-based study; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism.
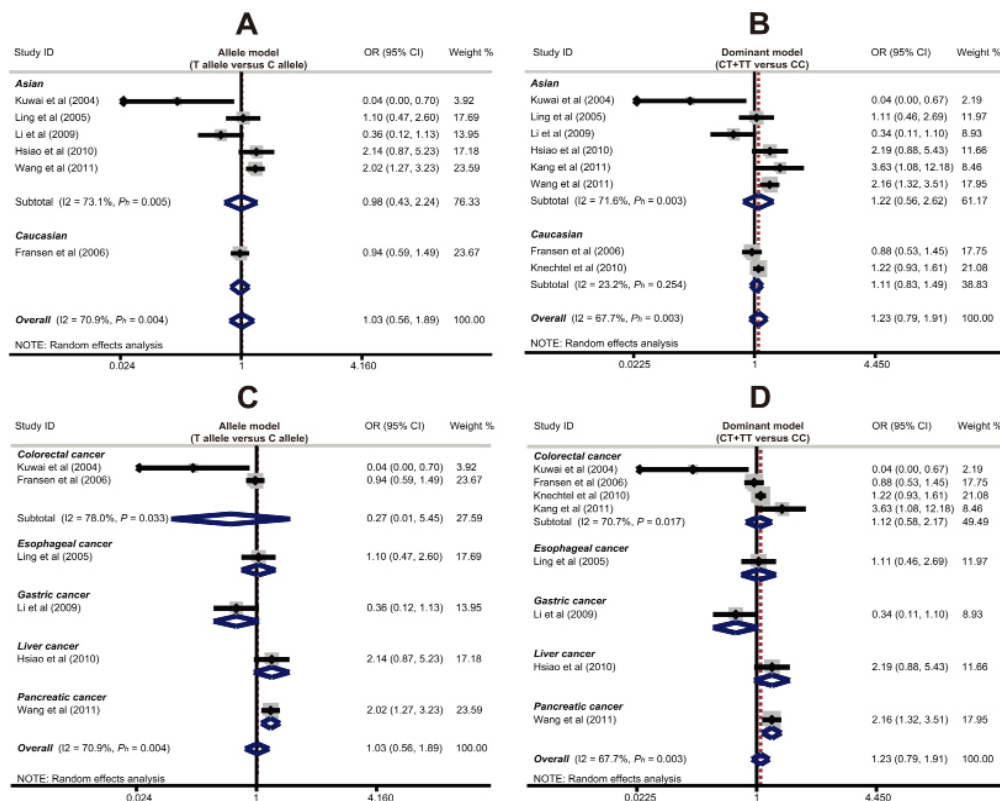
HIF-1α C1772T polymorphism and digestive cancer

The association between *HIF-1α* C1772T (rs11549465 C>T) polymorphism and digestive cancer risk is discussed in all 8 studies. Since significant heterogeneity existed, the random-effect model was conducted to pool the results. The meta-analysis results showed that the *HIF-1α* C1772T polymorphism was not associated with digestive cancer risk (T *vs* C: OR = 1.03, 95%CI = 0.56-1.89, P = 0.920; CT+TT *vs* CC: OR = 1.23, 95%CI = 0.79-1.91, P = 0.367; TT *vs* CC+CT: OR = 1.97, 95%CI = 0.33-11.9, P = 0.460; TT *vs* CC: OR = 1.91, 95%CI = 0.32-11.58, P = 0.480; TT *vs* CT: OR = 2.30, 95%CI = 0.36-14.67, P = 0.377) (Figure 2).
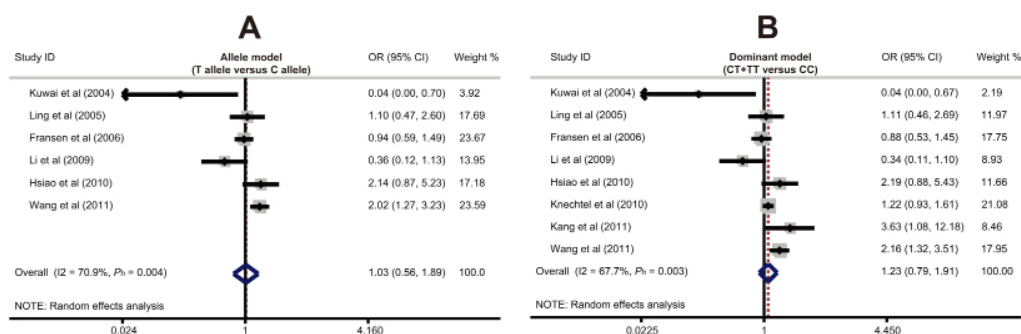
Figure 2. Forest plots for the association between *HIF-1α* C1772T polymorphism and digestive cancer risk under the allele (**A**) and dominant (**B**) models.

When searching for factors that might have impacted the results, we also performed further subgroup analyses by ethnicity and cancer type. Subgroup analysis by ethnicity indicated the lack of significant associations between *HIF-1α* C1772T polymorphism and the risk of digestive cancers in both Asian and Caucasian populations (P > 0.05) (Figure 3). Further subgroup analysis based on cancer type showed that the *HIF-1α* C1772T polymorphism was

not associated with the risks of colorectal, esophageal, gastric, and liver cancers (all P > 0.05). Although the *HIF-1α* C1772T polymorphism showed a significant association with increased risk of pancreatic cancer (T *vs* C: OR = 2.02, 95%CI = 1.27-3.23, P = 0.003; CT+TT *vs* CC: OR = 2.16, 95%CI = 1.32-3.51, P = 0.002), this result might have lacked sufficient reliability due to the estimation error from the effect size of a single study.
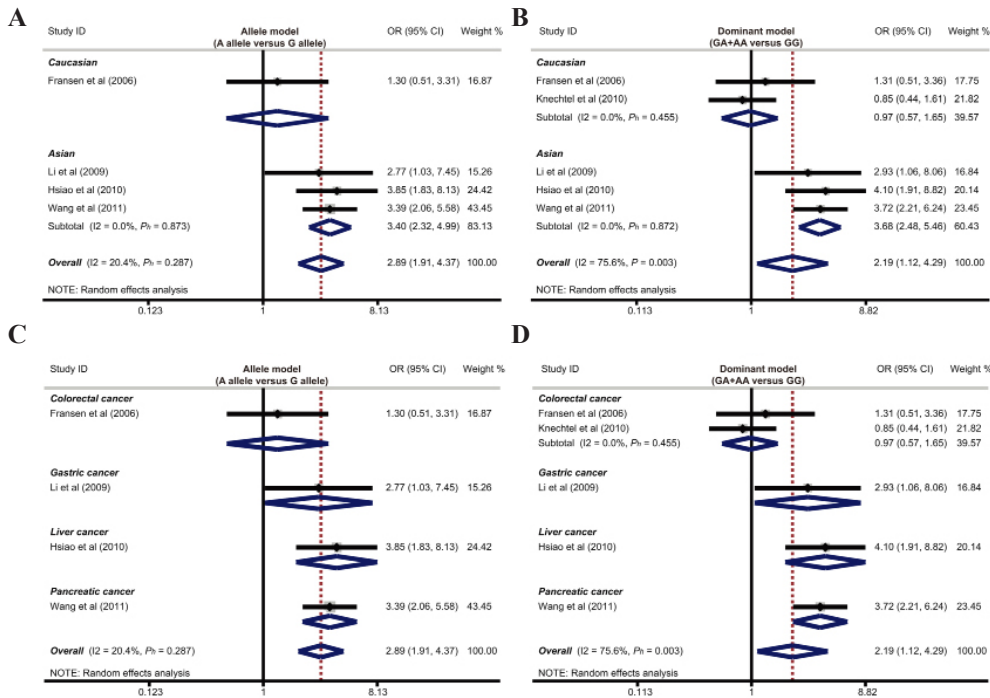

Figure 3. Subgroup analyses based on ethnicity and cancer type for the association between *HIF-1α* C1772T polymorphism and digestive cancer risk under the allele (**A**, **C**) and dominant (**B**, **D**) models.

HIF-1α G1790A polymorphism and digestive cancer

Five studies referred to the association between *HIF-1α* G1790A polymorphism and susceptibility to digestive cancer. No heterogeneity was observed, and therefore, the fixed-effect model was used. Meta-analysis of these studies showed significant associations between *HIF-1α* G1790A polymorphism and digestive cancer risk under allele and dominant models (A allele *vs* G allele: OR = 2.89, 95%CI = 1.91-4.37, P < 0.001; GA+AA *vs* GG: OR = 2.19, 95%CI = 1.12-4.29, P = 0.022) (Figure 4). Further subgroup analyses showed significant associations between *HIF-1α* rs11549467 polymorphism and digestive cancer risk among the Asian populations, especially in gastric, liver, and pancreatic cancers (Figure 5). However, no statistically significant associations were found between Caucasians and colorectal cancer (P > 0.05).
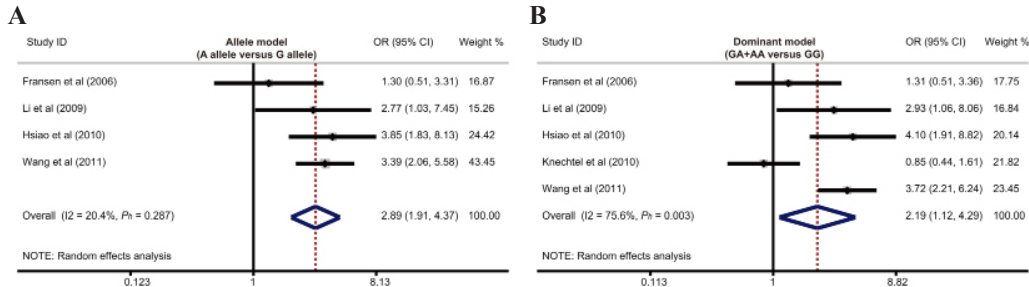
Figure 4. Forest plots for the association between *HIF-1α* G1790A polymorphism and digestive cancer risk under the allele (**A**) and dominant (**B**) models.


Figure 5. Subgroup analyses based on ethnicity and cancer type for the association between *HIF-1α* G1790A polymorphism and digestive cancer risk under the allele (**A**, **C**) and dominant (**B**, **D**) models.

Sensitivity analysis and publication bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by omitting individual studies in turn. The analysis results suggested that no individual study significantly affected the pooled ORs of *HIF-1α* C1772T and G1790A polymorphisms (Figure 6).

Funnel plots and the Egger linear regression test were performed to assess publication bias of the included studies. The shapes of the funnel plots of *HIF-1α* C1772T and G1790A polymorphisms did not reveal any evidence of obvious asymmetry (Figure 7). The Egger test also revealed that there was no significantly statistical evidence of publication bias (P > 0.05).
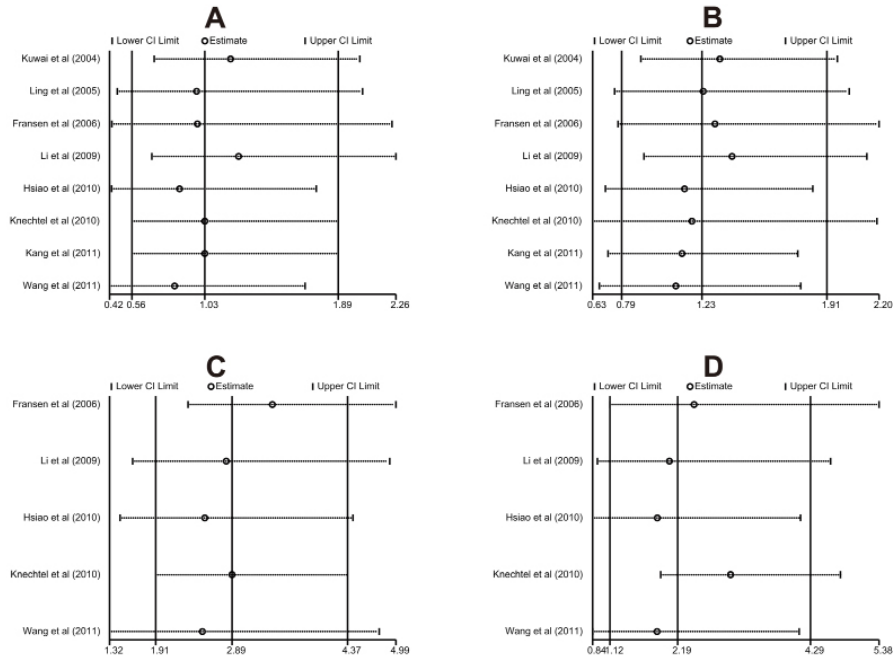
Figure 6. Sensitivity analyses for the associations of *HIF-1α* C1772T and G1790A polymorphisms with digestive cancer risk under the allele (**A**, **C**) and dominant (**B**, **D**) models. Results were computed by omitting each study in turn. Meta-analysis random-effect estimates (exponential form) were used. The two ends of the dotted lines represent the 95%CI.
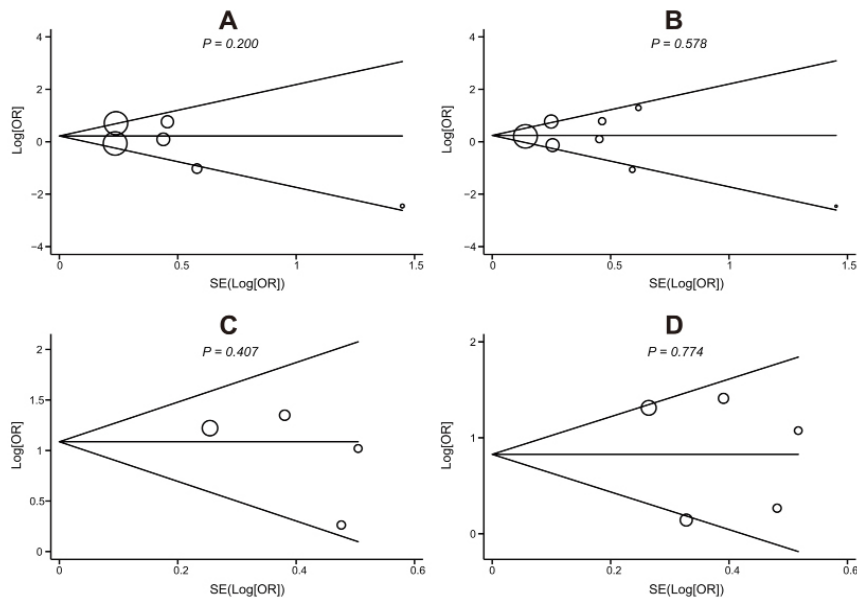
Figure 7. Begger's funnel plot for the associations of *HIF-1α* C1772T and G1790A polymorphisms with digestive cancer risk under the allele (**A**, **C**) and dominant (**B**, **D**) models. Each point represents a separate study for the indicated association. Log[OR] = natural logarithm of OR; SE = standard error. Horizontal lines mean magnitude of the effect.

DISCUSSION

HIF-1α is a transcription factor that enhances many types of gene expression, including the expression of those involved in angiogenesis, cell proliferation, glucose metabolism, erythropoiesis, and cell survival (Tanaka et al., 2006). *HIF-1α* is an important mediator of the hypoxic response of tumor cells and controls the up-regulation of many factors important for tumor growth (Ryan et al., 2000). Overexpression of *HIF-1α* has been identified in multiple types of human cancer, including colorectal, breast, gastric, lung, skin, ovarian, pancreatic, prostate, and renal cancers (Zhong et al., 1999). Increased *HIF-1α* expression could occur in the early stages of carcinogenesis, before histological evidence of angiogenesis or invasion can be available (Mabjeesh and Amir, 2007). Therefore, it is biologically plausible that genetic variations of the *HIF-1α* gene may contribute to individual cancer susceptibility as genetic modifiers of cancer risk (Zhao et al., 2009). The *HIF-1α* gene has been mapped to locus 14q21-q24. Two common functional polymorphisms, C1772T (rs11549465 C>T) and G1790A (rs11549467 G>A), in the human *HIF-1α* gene that cause amino acid substitutions within the oxygen-dependent degradation domain may result in the overexpression of this protein and subsequent changes in the expression of downstream target genes, thus contributing to cancer development and progression (Chau et al., 2005). Several previous studies have suggested that *HIF-1α* C1772T and G1790A polymorphisms may play important roles in the risk of digestive cancer (Ling et al., 2005; Fransen et al., 2006; Li et al., 2009; Hsiao et al., 2010; Kang et al., 2011; Wang et al., 2011), while other studies found no convincing evidence of these polymorphisms in increasing susceptibility to digestive cancer (Kuwai et al., 2004; Knechtel et al., 2010). This controversy could be explained with several reasons such as the differences in study designs, sample size, ethnicity, source of controls, cancer types, and genotype methods. This meta-analysis aims to provide a more comprehensive and reliable conclusion on these associations.

This is the first meta-analysis of the relationship of *HIF-1α* gene polymorphisms with digestive cancer risk. In this meta-analysis, 8 independent case-control studies were included, with a total of 1276 digestive cancer patients and 3392 healthy controls. When all eligible studies were pooled into the meta-analysis, the results showed that the *HIF-1α* G1790A polymorphism was associated with increased risk of colorectal, esophageal, gastric, and liver cancers, especially among Asian populations. The G1790A induces an amino acid substitution from alanine to threonine at position 588 (Clifford et al., 2001). This amino acid substitution is located in the oxygen-dependent degradation domain of HIF-1α and is closely related to the N-terminal transactivation domain of *HIF-1α* (Huang et al., 1998). Our result suggests that the A588T amino acid substitution may increase *HIF-1α* stability and availability, thereby stimulating the development and progression of digestive cancer. For the *HIF-1α* C1772T polymorphism, however, no statistically significant associations were found with digestive cancer risk, indicating that the C1772T, which may result in an amino acid change from proline 582 to serine, could not change *HIF-1α* expression, and therefore, it was not the primary determinant of carcinogenesis in the digestive system. Previous studies have shown that the polymorphic allele of C1772T polymorphism was associated with increased breast cancer risk, but such association was not found in other cancers (Kim et al., 2008; Zhao et al., 2009). Our findings are partially consistent with the previous hypothesis that variability in the *HIF-1α* gene may alter the susceptibility to digestive cancers, and these polymorphisms may be useful as biomarkers in predicting cancer development.

Our meta-analysis has several limitations that should be acknowledged. The first limitation is that the sample size of this meta-analysis was relatively small and may not have sufficient statistical power in estimating the relationships between *HIF-1α* gene polymorphisms and digestive cancer risk. Therefore, further studies with a larger sample size are needed. On the other hand, a meta-analysis is a type of a retrospective study and may encounter recall or selection bias, thereby possibly influencing the reliability of our results. Most important of all, lack of access to the original study data limited further evaluation of the potential value of these polymorphisms in the *HIF-1α* gene.

In conclusion, this meta-analysis provides strong evidence that *HIF-1α* G1790A polymorphism may increase the risks of colorectal, esophageal, gastric, and liver cancers, especially among Asian populations. These findings increase the understanding of the mechanisms underlying the development of digestive cancer. However, no evidence indicates any correlations between *HIF-1α* C1772T polymorphism and susceptibility to digestive cancer. Based on the above-mentioned limitations, detailed studies are needed to confirm our findings.

ACKNOWLEDGMENTS

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