Systematic Review and Meta-Analysis of the Relationship between EPHX1 Polymorphisms and Colorectal Cancer Risk

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Abstract

Background: Microsomal epoxide hydrolase (EPHX1) plays an important role in both the activation and detoxification of PAHs, which are carcinogens found in cooked meat and tobacco smoking. Polymorphisms at exons 3 and 4 of the EPHX1 gene have been reported to be associated with variations in EPHX1 activity. The aim of this study is to quantitatively summarize the relationship between EPHX1 polymorphisms and colorectal cancer (CRC) risk.

Methods: Two investigators independently searched the Medline, Embase, CNKI, and Chinese Biomedicine Databases for studies published before June 2012. Summary odds ratios (ORs) and 95% confidence intervals (CIs) for EPHX1 Tyr113His (rs1051740) and His139Arg (rs2234922) polymorphisms and CRC were calculated in a fixed-effects model and a random-effects model when appropriate.

Results: This meta-analysis yielded 14 case-control studies, which included 13 studies for Tyr113His (6395 cases and 7893 controls) and 13 studies for His139Arg polymorphisms (5375 cases and 6962 controls). Overall, the pooled results indicated that EPHX1 Tyr113His polymorphism was not associated with CRC risk; while the His139Arg polymorphism was significantly associated with decreased CRC risk (Arg/His vs. His/His, OR = 0.90, 95%CI = 0.83–0.98; dominant model, OR = 0.92, 95%CI = 0.85–0.99). The statistically significant association between EPHX1 His139Arg polymorphism and CRC was observed among Caucasians and population-based case-control studies. This association showed little heterogeneity and remained consistently strong when analyses were limited to studies in which genotype frequencies were in Hardy–Weinberg equilibrium, or limited to studies with matched controls. When cumulative meta-analyses of the two associations were conducted by studies’ publication time, the results were persistent and robust.

Conclusion: This meta-analysis suggests that EPHX1 Tyr113His polymorphism may be not associated with CRC development; while the EPHX1 His139Arg polymorphism may have a potential protective effect on CRC.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females worldwide, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008 [1]. In the United States, CRC is the third most common cancer and third leading cause of cancer death for both men and women [2]. In Europe, CRC represents one of the primary causes of cancer deaths [3] and in Asia, CRC is the fourth leading cause of mortality by cancer, and its incidence is increasing [4]. In recent years, the incidence of CRC is increasing in China, which accounts for about 6.5% of total cancers in urban areas and 4.6% in rural areas [3]. However, the mechanism of colorectal carcinogenesis is still not fully understood. As with other complex diseases, CRC is caused by both genetic and environmental factors [6]. Because well-recognized genetic predisposition syndromes account for less than 3% of CRC, low-penetration genetic factors alone or in combination with environmental factors probably contribute to CRC development [7].

Red meat consumption has frequently shown an association with an increased risk of CRC. It has been proposed that this risk may be due to carcinogenic polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines produced when meat is cooked at high temperatures [8]. Microsomal epoxide hydrolase (mEH) (EPHX1) is an enzyme found on the endoplasmic reticulum of many tissues and is responsible for the hydrolysis of various epoxides, including PAHs [9]. Epoxides are often the most toxicologically active form of a drug or environmental chemical, because they are highly reactive oxidative metabolites. EPHX1 breaks the three-membered epoxide ring structure by the
transaddition of water to form a less-reactive diol that can be conjugated and more readily excreted. Nevertheless, EPHX1 plays a dual role in the detoxification and activation of procarcinogens, and its role in carcinogenesis may depend on exposures to different environmental substrates [10].

The human EPHX1 gene is 35.48 kb with nine exons and eight introns on chromosome 1q42.1. There are more than 110 validated single nucleotide polymorphisms (SNPs) in EPHX1 gene reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP), two of which are common and the two alleles of EPHX1 in codons 113 (site T337G, amino acid change Tyr113His, dbSNP: rs1051740) and 139 (A415G, His139Arg, rs2234922) affect enzyme activity [11]. The tyrosine to histidine substitution in exon 3 (Tyr113His) of the EPHX1 gene decreases in vitro enzyme activity by 40%, whereas the histidine to arginine substitution in exon 4 (His139Arg) increases in vitro enzyme activity by 25% [11].

Given the known differential effect of EPHX1 alleles in the detoxification of procarcinogens, it has been proposed that the two functional polymorphisms may affect cancer risk.

Over the last two decades, a number of studies were conducted to investigate the association between EPHX1 polymorphisms and CRC risk in different populations. However, the results of these studies are conflicting rather than conclusive. Until recently, few studies had been conducted to examine association between EPHX1 Tyr113His and His139Arg polymorphisms and CRC risk by the systematic review or meta-analysis. In order to derive a comprehensive estimation of the associations between EPHX1 polymorphisms and CRC risk, we conducted a meta-analysis to assess the association between Tyr113His and His139Arg polymorphisms of the EPHX1 gene and CRC susceptibility.

Materials and Methods

Literature Search Strategy

We searched the PubMed, Embase, CNKI (China National Knowledge Infrastructure) and Chinese Biomedicine databases for all articles on the association between EPHX1 polymorphisms and CRC risk (last search update 5th June 2012). The following key words were used: “microsomal epoxide hydrolase” or “EPHX1” or “mEH”, “colorectal” or “colo*”, “cancer” or “tumor” or “carcinoma”, and “polymorphism” or “variant” or “allele” or “genotype”. The search was without restriction to the language and on studies conducted on human subjects. The reference lists of reviews and retrieved articles were hand searched at the same time. We did not consider abstracts or unpublished reports. If more than one article was published by the same author using the same case series, we selected the study where the most individuals were investigated.

Inclusion and Exclusion Criteria

We reviewed abstracts of all citations and retrieved studies. The following criteria were used to include published studies: (i) case–control studies were conducted to evaluate the association between at least one of these two polymorphisms (Tyr113His and His139Arg) and CRC risk; (ii) sufficient genotype data were presented to calculate the odds ratios (ORs) and 95% confidence intervals (CIs); (iii) The paper should clearly describe CRC diagnoses and the sources of cases and controls. Major reasons for exclusion of studies were (i) review, or editorial, or comment; (ii) duplicated studies; (iii) cell line studies.

Data Extraction

Two investigators (Fei Liu and Ding Yuan) extracted information from all eligible publications independently according to the inclusion criteria listed above. Disagreements were resolved by discussion between the two investigators. The following characteristics were collected from each study: the first author’s name, year of publication, the country of participants, ethnicity, source of control group (population- or hospital-based controls), number of cases and controls, genotypes, genotyping methods, minor allele frequency (MAF) in controls, and evidence of Hardy–Weinberg equilibrium (HWE) (Table 1). According to definitions in previous study [12], population-based case-control study (PCC) was defined as controls from healthy people, and hospital-based case-control study (HCC) were from hospitalized patients.

Statistical Analysis

We first assessed HWE in the controls for each study using goodness-of-fit test (chi-square or Fisher’s exact test) and a P<0.05 was considered as statistically significant. The strength of the association between CRC and the EPHX1 Tyr113His and His139Arg polymorphisms were estimated using ORs, with the corresponding 95% CIs. In addition, Z-test was also used, and the P value <0.05 indicated statistical significance for the association. The crude ORs and 95% CIs were calculated by several comparisons. Taking EPHX1 Tyr113His as an example: co-dominant model (His/His vs. Tyr/Tyr and Tyr/His vs. Tyr/Tyr), dominant model (His/His/Tyr/His vs. Tyr/Tyr) and recessive model (His/His vs. Tyr/His*Tyr/Tyr) respectively [13].

Both the Cochran’s Q statistic [14] to test for heterogeneity and the F statistic to quantify the proportion of the total variation due to heterogeneity [15] were calculated. A P value of more than the nominal level of 0.10 for the Q statistic indicated a lack of heterogeneity across studies, allowing for the use of a fixed-effects model (the Mantel–Haenszel method) [16];otherwise, the random-effects model (the DerSimonian and Laird method) was used [17]. All meta-analyses are presented as forest plots that include ORs and 95% CIs for all individual studies, as well as the pooled estimator. Shaded figures provided for all ORs have dimension proportional to study weight. The Galbraith plot was used to detect the potential sources of heterogeneity [18]. Heterogeneity was also explored using subgroup analysis with ethnicity, study sample size (≥1000/<1000 subjects), matched control (Yes/No), HWE in controls (Yes/No) and source of controls (HCC/PCC).

Sensitivity analyses were performed to assess the stability of the results, namely, a single case-control study in this meta-analysis was omitted each time to reflect the influence of the individual data set to the pooled OR. Several methods were used to assess the potential publication bias. Visual inspection of funnel plot asymmetry was conducted. The Begg’s rank correlation method [19] and the Egger’s weighted regression method [20] were used to statistically assess publication bias (P<0.05 was considered statistically significant). All analyses were done using STATA software, version 11.0 (STATA Corp., College Station, TX, USA). All the P values were two-sided.

Results

Characteristics of Studies

Through literature search and selection, a total of 15 case-control studies in 14 publications [7,21–33], which included 14 studies for Tyr113His and 13 studies for His139Arg polymorphisms, were found to examine the EPHX1 polymorphisms and CRC susceptibility. Because the populations in two studies [7,30] were partially overlapped, we selected the study with the most individuals [7]. As a result, a total of 14 case-control studies in 13 publications [7,21–29,31–33], which included 13 studies for Tyr113His (6395 cases and 7893 controls) and 13 studies for...
Table 1. Characteristics of studies included in this meta-analysis.

| First author | Reference | Year | Country | Ethnicity | SNPs studied               | Source of Controls | Sample size (case/control) | Genotyping Methods | MAF in Controls | HWE     |
|--------------|-----------|------|---------|-----------|---------------------------|--------------------|--------------------------|-------------------|----------------|---------|
| Harrison     | [21]      | 1999 | UK      | Caucasian | Tyr113His; His139Arg      | PCC                | 101/203                  | PCR-RFLP          | 0.31; 0.15   | 0.04; 0.47|
| Sachse       | [22]      | 2002 | UK      | Caucasian | Tyr113His; His139Arg      | PCC                | 490/593                  | PCR-RFLP          | 0.38; 0.19   | 0.00; 0.06|
| Yu           | [23]      | 2004 | China   | Asian     | His139Arg                 | PCC                | 140/340                  | PCR-RFLP          | 0.10          | 0.37     |
| Landi        | [24]      | 2005 | Spain   | Caucasian | Tyr113His; His139Arg      | HCC                | 363/323; 36/321          | ASO-PCR            | 0.29; 0.17   | 0.45; 0.40|
| Robien       | [25]      | 2005 | USA     | Mixed     | Tyr113His; His139Arg      | PCC                | 1593/1960               | Taqman            | 0.29; 0.20   | 0.42; 0.15|
| Tranah       | [26]      | 2005 | USA     | Caucasian | Tyr113His; His139Arg      | PCC                | 197/490                  | Taqman            | 0.32; 0.18   | 0.69; 0.83|
| Tranah1      | [26]      | 2005 | USA     | Caucasian | Tyr113His; His139Arg      | PCC                | 273/453                  | Taqman            | 0.28; 0.19   | 0.49; 0.83|
| Van der Logt | [27]      | 2006 | Netherlands | Caucasian | Tyr113His; His139Arg      | PCC                | 365/391; 371/414         | DCAS-PCR          | 0.29; 0.20   | 0.71; 0.72|
| Kiss         | [28]      | 2007 | Hungary | Caucasian | Tyr113His; His139Arg      | HCC                | 500/500                  | PCR-RFLP          | 0.28; 0.18   | 0.05; 0.05|
| Skjelbred    | [29]      | 2007 | Norway  | Caucasian | Tyr113His; His139Arg      | PCC                | 102/299                  | Taqman; PCR-RFLP  | 0.33; 0.21   | 0.91; 0.07|
| Hlavata      | [31]      | 2010 | Czech   | Caucasian | Tyr113His; His139Arg      | HCC                | 495/495                  | Taqman            | 0.32; 0.23   | 0.75; 0.31|
| Cleary       | [32]      | 2010 | Canada  | Caucasian | Tyr113His                 | PCC                | 1163/1292                | Taqman            | 0.30          | 0.87     |
| Nisa         | [32]      | 2012 | Japan   | Asian     | Tyr113His; His139Arg      | PCC                | 685/778                  | Taqman; PCR-RFLP  | 0.44; 0.18   | 0.35; 0.41|
| Sahin        | [33]      | 2012 | Turkey  | Caucasian | Tyr113His; His139Arg      | HCC                | 68/116                   | PCR-RFLP          | 0.35; 0.19   | 0.02; 0.01|

Abbreviations: SNPs- single nucleotide polymorphisms; HCC, hospital-based case-control; PCC, population-based case-control; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; ASO-PCR, allele-specific oligonucleotide-polymerase chain reaction; DCAS-PCR, dual-colour allele-specific polymerase chain reaction; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.
His139Arg polymorphisms (5375 cases and 6962 controls), were identified based on MOOSE (Meta-analysis Of Observational Studies in Epidemiology) guidelines [34]. One article [26] mentioned two independent case-control studies (NHS and PHS), and the study was thus treated as two separate estimates.

The literature search and study selection procedures are shown in Figure 1.

The characteristics of selected studies are summarized in Table 1. There were two studies of subjects of Asian descent, 11 studies of subjects of Caucasian descent and one of subjects of Mixed descent. Studies had been carried out in China, UK, USA, Spain,
Canada, Czech, Japan, Turkey, Norway, Hungary, and Netherlands. The cases definition used in the individual studies were pathologically or histologically diagnosed with CRC. Controls were mainly from healthy populations and matched for age and/or sex, of which 10 were population-based and four were hospital-based. Most of studies extracted DNA from peripheral blood and the classic PCR-RFLP assay and Taqman PCR were mainly used for genotyping. The genotype distributions among the controls of all studies followed HWE except for four studies [21,22,20,33] for the Tyr113His polymorphism and one study [33] for the His139Arg polymorphism.

Quantitative Synthesis

Association of the EPHX1 Tyr113His polymorphism with CRC susceptibility. 13 case-control studies [7,21,22,24–29,31–33] with 6395 cases and 7893 controls for EPHX1 Tyr113His were included eventually. Table 2 listed the main results of this pooled analysis and Figure 2A showed the association of CRC risk with EPHX1 Tyr113His polymorphism in the form of forest plots. Overall, the genotypes including at least one variant allele (His/His and Tyr/His) of the Tyr113His were not associated with CRC risk when compared with the wild-type Tyr/Tyr homozygote (His/His vs. Tyr/Tyr, OR = 1.08, 95%CI = 0.88–1.31; Tyr/His vs. Tyr/Tyr, OR = 1.03, 95%CI = 0.96–1.10). Similarly, no associations were observed in the dominant models (dominant model, OR = 1.02, 95%CI = 0.96–1.10). In the recessive models (homozygote comparison model, OR = 1.02, 95%CI = 0.96–1.09; recessive model, OR = 1.08, 95%CI = 0.88–1.33).

On the basis of the potential underestimation of the true effect of the polymorphism on the CRC risk, we stratified these studies according to ethnicity, source of controls, study sample size, matched control, and HWE in controls. Different ethnicities were categorized as Caucasians and others; while different source of controls were defined as HCC and PCC. In stratified analyses, the variant genotypes (His/His and Tyr/His) had no significant relationship with CRC in all of the subgroups except that a significantly increased CRC risk was observed among the HCC populations in the homozygote comparison. Also, no significant associations were found in the dominant and recessive models in any subgroup (Table 2).

Association of the EPHX1 His139Arg polymorphism with CRC susceptibility. 13 case-control studies [21–29,31–33] with 5375 cases and 6962 controls for EPHX1 His139Arg were included eventually. Table 3 listed the main results of this pooled analysis and Figure 2B showed the association of CRC risk with EPHX1 His139Arg polymorphism in the form of forest plots. Overall, the results of combined analyses of all studies suggested that the His139Arg polymorphism was significantly associated with decreased CRC risk (Arg/His vs. His/His, OR = 0.90, 95% CI = 0.83–0.98; dominant model, OR = 0.92, 95% CI = 0.85–0.99), without any between-study heterogeneity. However, the association was not observed in the homozygote comparison and recessive genetic models (homozygote comparison model, OR = 1.14, 95% CI = 0.86–1.52; recessive model, OR = 1.18, 95% CI = 0.89–1.57).

When stratifying by ethnicity and source of controls, the significantly decreased CRC risk was observed among Caucasians (Arg/His vs. His/His, OR = 0.88, 95%CI = 0.79–0.98) and PCC studies (Arg/His vs. His/His, OR = 0.90, 95%CI = 0.82–0.98). This association remained consistently strong when analyses were limited to studies in which genotype frequencies were in HWE (Arg/His vs. His/His, OR = 0.91, 95%CI = 0.84–0.99), or limited to studies with matched controls (Arg/His vs. His/His, OR = 0.85, 95%CI = 0.76–0.98).

Table 2. Quantitative analyses of the EPHX1 Tyr113His polymorphism on the colorectal cancer (CRC) risk.

| Genetic model | Sample size | Homozygote | Heterozygote | Dominant model | Recessive model |
|---------------|-------------|------------|--------------|----------------|-----------------|
|                | N* | Case/control | OR(95%CI) | P value | OR(95%CI) | P value | OR(95%CI) | P value |
| Total          | 13 | 6395/7893 | 1.08(0.88,1.31) | 0.004 | 1.03(0.96,1.10) | 0.704 | 1.02(0.96,1.09) | 0.684 | 1.08(0.88,1.33) | <0.001 |
| Ethnicity      |                |            |            |        |            |        |            |        |            |        |
| Caucasians     | 11 | 4117/5155 | 1.13(0.87,1.47) | 0.002 | 1.04(0.95,1.14) | 0.652 | 1.04(0.95,1.13) | 0.678 | 1.14(0.86,1.50) | <0.001 |
| Others         | 2  | 2278/2738 | 0.98(0.81,1.18) | 0.217 | 1.00(0.89,1.13) | 0.333 | 1.00(0.89,1.12) | 0.224 | 0.99(0.83,1.18) | 0.355 |
| Source of controls |       |            |            |        |            |        |            |        |            |        |
| HCC           | 4  | 1426/1434 | 1.33(1.02,1.73) | 0.117 | 1.11(0.95,1.29) | 0.644 | 1.14(0.99,1.33) | 0.770 | 1.36(0.88,2.08) | 0.056 |
| PCC           | 9  | 4969/6459 | 0.98(0.79,1.21) | 0.017 | 1.00(0.93,1.09) | 0.640 | 0.99(0.92,1.07) | 0.729 | 0.99(0.79,1.25) | 0.003 |
| Study sample size |       |            |            |        |            |        |            |        |            |        |
| ≥1000          | 5  | 4431/5123 | 0.99(0.76,1.29) | 0.008 | 1.04(0.95,1.13) | 0.626 | 1.02(0.94,1.11) | 0.343 | 0.97(0.76,1.25) | 0.006 |
| <1000          | 8  | 1964/2770 | 1.18(0.87,1.62) | 0.054 | 1.00(0.89,1.14) | 0.513 | 1.03(0.91,1.16) | 0.695 | 1.22(0.87,1.72) | 0.011 |
| Matched control |       |            |            |        |            |        |            |        |            |        |
| Yes           | 8  | 3871/4717 | 1.00(0.78,1.27) | 0.019 | 1.04(0.95,1.14) | 0.583 | 1.02(0.93,1.11) | 0.475 | 0.99(0.77,1.26) | 0.008 |
| No            | 5  | 2524/3176 | 1.24(0.86,1.78) | 0.043 | 1.00(0.90,1.12) | 0.545 | 1.03(0.93,1.14) | 0.621 | 1.28(0.87,1.90) | 0.016 |
| HWE in controls |       |            |            |        |            |        |            |        |            |        |
| Yes           | 9  | 5236/6481 | 0.98(0.86,1.11) | 0.843 | 1.01(0.94,1.10) | 0.671 | 1.01(0.94,1.09) | 0.650 | 0.98(0.86,1.10) | 0.002 |
| No            | 4  | 1159/1412 | 1.73(0.77,3.90) | <0.001 | 1.08(0.91,1.28) | 0.432 | 1.09(0.93,1.27) | 0.463 | 1.79(0.75,4.28) | <0.001 |

*Number of comparisons.

**P value of Q-test for heterogeneity test. Random-effects model was used when P value for heterogeneity test <0.05; otherwise, fixed-effects model was used.

aHCC, hospital-based case-control; PCC, population-based case-control.

bHWE, Hardy–Weinberg equilibrium.

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95% CI = 0.76–0.96; dominant model, OR = 0.86, 95% CI = 0.77–0.96. When stratifying by study sample size, this association was not observed neither among large sample studies (≥1000 subjects) nor among small sample studies (<1000 subjects) (Table 3).

Heterogeneity Analysis
For Tyr113His polymorphism, there was substantial heterogeneity among these studies for homozygote comparison (His/His vs. Tyr/Tyr: \( P_{\text{heterogeneity}} = 0.004 \)), and recessive model comparison

Table 3. Quantitative analyses of the EPHX1 His139Arg polymorphism on the colorectal cancer (CRC) risk.

| Genetic model          | Sample size | Homozygote | Heterozygote | Dominant model | Recessive model |
|------------------------|-------------|------------|--------------|----------------|----------------|
|                        | Variables   | Arg/Arg vs. His/His | Arg/His vs. His/His | Arg/Arg+Arg/His vs. His/His | Arg/Arg vs. Arg/His+His/His |
|                        | N\(^a\) | Case/control | OR(95%CI) | \(P_{\text{value}}^b\) | OR(95%CI) | \(P_{\text{value}}^b\) | OR(95%CI) | \(P_{\text{value}}^b\) |
| Total                  | 13        | 5375/6962 | 1.14(0.86,1.52) | 0.067 | 0.90(0.83,0.98) | 0.763 | 0.92(0.85,0.99) | 0.559 | 1.18(0.89,1.57) | 0.057 |
| Ethnicity              | Caucasians | 10 | 4949/6404 | 1.31(0.94,1.81) | 0.166 | 0.88(0.79,0.98) | 0.594 | 0.92(0.83,1.02) | 0.344 | 1.35(0.98,1.88) | 0.162 |
|                        | Asian      | 2 | 2278/2738 | 0.74(0.42,1.32) | 0.224 | 0.89(0.72,1.10) | 0.848 | 0.87(0.71,1.07) | 0.643 | 0.91(0.33,2.47) | 0.231 |
| Source of controls     | HCC\(^c\) | 4 | 1426/1434 | 1.00(0.65,1.54) | 0.586 | 0.92(0.79,1.08) | 0.243 | 0.93(0.79,1.08) | 0.302 | 1.03(0.67,1.57) | 0.531 |
|                        | PCC\(^c\)  | 9 | 5801/7708 | 1.22(0.84,1.78) | 0.022 | 0.90(0.82,0.98) | 0.857 | 0.92(0.84,1.00) | 0.538 | 1.27(0.87,1.85) | 0.020 |
| Study sample size      | ≥1000      | 4 | 5263/6372 | 0.86(0.67,1.12) | 0.334 | 0.92(0.83,1.02) | 0.737 | 0.91(0.82,1.00) | 0.859 | 1.00(0.71,1.41) | 0.287 |
|                        | <1000      | 9 | 1964/2770 | 1.41(0.99,2.02) | 0.227 | 0.88(0.77,1.00) | 0.558 | 0.93(0.82,1.05) | 0.279 | 1.43(0.93,2.20) | 0.234 |
| Matched control        | Yes        | 7 | 4703/5966 | 0.92(0.68,1.24) | 0.556 | 0.85(0.76,0.96) | 0.460 | 0.86(0.77,0.96) | 0.559 | 0.96(0.72,1.29) | 0.497 |
|                        | No         | 6 | 2524/3176 | 1.53(0.91,2.58) | 0.020 | 0.95(0.85,1.07) | 0.971 | 0.98(0.88,1.09) | 0.701 | 1.56(0.92,2.63) | 0.017 |
| HWE\(^d\) in controls | Yes        | 12 | 6068/7730 | 1.13(0.85,1.51) | 0.055 | 0.91(0.84,0.99) | 0.940 | 0.93(0.86,1.00) | 0.753 | 1.17(0.88,1.56) | 0.050 |
|                        | No         | 1 | 1159/1412 | 4.14(1.07,13.71) | NA\(^e\) | 0.47(0.24,0.94) | NA\(^e\) | 0.50(0.26,0.99) | NA\(^e\) | 5.18(0.21,128.89) | NA\(^e\) |

\(^a\)Number of comparisons.  
\(^b\)\(P\) value of \(Q\)-test for heterogeneity test. Random-effects model was used when \(P\) value for heterogeneity test < 0.1; otherwise, fixed-effects model was used.  
\(^c\)HCC, hospital-based case-control; PCC, population-based case-control.  
\(^d\)HWE, Hardy–Weinberg equilibrium.  
\(^e\)Not applicable.  

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(His/His vs. Tyr/His; Tyr/Tyr: \(P_{\text{heterogeneity}} < 0.001\)). For His139Arg polymorphism, mild between-study heterogeneity was also detected. The homozygote comparison, and recessive model comparison. Galbraith plot analyses of all included studies were performed. The association after excluding these three outlier studies with reduced heterogeneity (His/His vs. Tyr/Tyr: \(P_{\text{heterogeneity}} = 0.614\); His/His vs. Tyr/Tyr vs. His/His: \(P_{\text{heterogeneity}} = 0.495\)). Only one study was found to be a contributor of heterogeneity for Tyr113His polymorphism (Figure S1A). We re-evaluated the association after excluding these three outlier studies with reduced heterogeneity (His/His vs. Tyr/Tyr: \(P_{\text{heterogeneity}} = 0.521\); Arg/Arg vs. Arg/His: \(P_{\text{heterogeneity}} = 0.212\).

**Sensitivity Analysis**

In the sensitivity analysis, the influence of each study on the pooled OR was examined by repeating the meta-analysis while omitting each study, one at a time. As for the association of the EPHX1 Tyr113His with CRC risk, the study that had the most influence on the overall pooled estimates (Figure S2A) seemed to be the one conducted by Kiss et al. [28]; however, the sensitivity analysis showed that the ORs were 1.02 (95% CI: 0.96, 1.09) and 1.01 (95% CI: 0.94, 1.08) before and after the removal of that study, respectively, indicating high stability of the results. Because there is known methodological issue with PCR-RFLP analysis of Tyr113His SNP [27], we performed analysis without studies using the biased method. When excluding the studies using PCR-RFLP analysis of Tyr113His SNP, the estimated pooled OR still did not change at all (Table S1). As for the association of the EPHX1 His139Arg with CRC risk, the study that had the most influence on the overall pooled estimates (Figure S2B) seemed to be the one conducted by Robien et al. [25]; however, the sensitivity analysis showed that the ORs were 0.92 (95% CI: 0.85, 0.99) and 0.91 (95% CI: 0.83, 0.99) before and after the removal of that study, respectively, indicating high stability of the results. When excluding the studies that were not in HWI, the estimated pooled OR still did not change at all (Table 2 and Table 3). This procedure proved that our results were reliable and robust.

**Cumulative Meta-analysis**

Cumulative meta-analyses of the 2 associations were also conducted via the assortment of studies by publication time. Figure S3A shows results from the cumulative meta-analysis of the association of the EPHX1 Tyr113His with overall CRC in chronologic order. Inclinations toward null significant associations were evident with each accumulation of more data over time. Figure S3B shows results from the cumulative meta-analysis of the association of the EPHX1 His139Arg with overall CRC in chronologic order. Inclinations toward decreased significant associations were evident with each accumulation of more data over time, although associations were initially null.

**Publication Bias**

Funnel plot, Begg’s and Egger’s tests were performed to evaluate publication bias of the literature on CRC. Figure S4 displayed funnel plots that examined the EPHX1 polymorphisms and overall CRC risk included in the meta-analysis in dominant comparison model. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry. The statistical results still did not show publication bias [1] EPHX1/Tyr113His, His/His vs. Tyr/Tyr: Begg’s test \(P = 0.50\), Egger’s test \(P = 0.16\); Tyr/Tyr vs. Tyr/Tyr: Begg’s test \(P = 0.43\), Egger’s test \(P = 0.33\); dominant model: Begg’s test \(P = 1.00\), Egger’s test \(P = 0.80\); recessive model: Begg’s test \(P = 0.43\), Egger’s test \(P = 0.12\). (2) EPHX1 His139Arg, Arg/Arg vs. His/His: Begg’s test \(P = 0.50\), Egger’s test \(P = 0.23\); Arg/His vs. His/His: Begg’s test \(P = 0.30\), Egger’s test \(P = 0.12\); dominant model: Begg’s test \(P = 0.96\), Egger’s test \(P = 0.65\); recessive model: Begg’s test \(P = 0.50\), Egger’s test \(P = 0.21\).

**Discussion**

The present meta-analysis, including 14 case–control studies, explored the association between the Tyr113His and His139Arg polymorphisms of the EPHX1 gene and CRC risk. We found that EPHX1 Tyr113His polymorphism was not associated with CRC risk (6395 cases and 7893 controls). When subgroup analyses were performed by ethnicity, source of controls, study sample size, matched control, and HWE in controls; significant association was still not observed in any subgroup except for among hospital-based studies. Nevertheless, we found that EPHX1 His139Arg polymorphism was associated with decreased CRC risk. When stratifying by ethnicity and source of controls, the significant association was observed among Caucasians and among PCC studies. Moreover, this association showed little heterogeneity (\(I^2 = 0\)) and remained consistently strong when analyses were limited to studies in which genotype frequencies were in HWE, or limited to studies with matched controls. When cumulative meta-analyses of the two associations were conducted by studies’ publication date, the results were persistent and robust.

EPHX1 is a critical enzyme in xenobiotic metabolism [35], which plays an important role in both the activation and detoxification of PAHs and aromatic amines. EPHX1 catalyzes the hydrolysis of arene, alkene, and aliphatic epoxides from PAHs and aromatic amines. This hydrolysis is generally a detoxification reaction because less reactive and more water-soluble trans-dihydrodiols are produced [36]. In a sense, EPHX1 is a protective enzyme involved in general oxidative defenses against a number of environmental substances, and its genetic polymorphisms, EPHX1 Tyr113His and His139Arg, may affect enzyme activity [11]. Previous in vitro study found that EPHX1 Tyr113His was associated with 40% of decreased enzyme activity, while His139Arg was associated with 25% of increased enzyme activity [11]. Based on the assumption that the Tyr allele at exon 3 and the His allele at exon 4 confer low activity, whereas the His allele at exon 3 confers low activity and the Arg allele at exon 4 confers high activity, Benhamou et al. [37] classified the predicted EPHX1 activity as low (113His/113His, 113Tyr/113His and 113His/113Arg), intermediate (113Tyr/113His, 113 His/113Arg, 113 His/113Arg and 139His/139Arg) or high (113Tyr/113Arg and 113Tyr/113Arg and 113Tyr/113Arg, 113Tyr/113Arg, 113Tyr/139Arg and 113Tyr/139Arg). Given the different enzyme activity of the EPHX11 protein activity which depends on the polymorphic form, it is biologically plausible that the EPHX1 His139Arg polymorphism may decrease the risk of CRC.

Interestingly, we found that the EPHX1 His139Arg homozygotes, but not the homozygotes, had a significantly decreased risk of CRC. The observed effect is due mostly to the presence of heterozygous genotype and homozygous variant genotype rather dilutes this effect (Table 3 - heterozygous vs. dominant model). From the functional view there is lack of dose-relationship where the highest activity should exert the most significant effect.
Although the reason for a significantly decreased risk associated with the His139Arg variant heterozygote remains unknown, it is possible that these heterozygotes may have impaired function because of the potential imbalance of the protein structure. Another possible explanation is that the heterozygous genotype may be in linkage disequilibrium with other susceptibility loci. Similar phenomenon was observed by Ma et al. [39], who studied the variant genotypes of CDKN1A and CDKN1B and breast cancer risk. They found that the CDKN1B C -79T heterozygotes, but not the homozygotes, had a significantly increased risk of breast cancer.

Our results were in part consistent with previous studies. For example, Li et al. [40] performed a comprehensive meta-analysis of published epidemiological studies aims to systematically evaluate putative EPHX1 enzyme activity and risk of cancers and found that putative EPHX1 enzyme activity is related with risk of lung and upper aerodigestive tract cancers. However, they did not find any association between EPHX1 Tyr113His and His139Arg polymorphism and CRC risk. In recent, Zhao et al. [41] published a meta-analysis for the relationships between five metabolic gene (including EPHX1) polymorphisms and colorectal adenoma risk and found that EPHX1 Tyr113His and His139Arg did not have any associations with colorectal adenoma risk. Although the reasons for this difference are as yet unknown, some possibilities should be considered. First, those gene-variant associations vary in different kinds of diseases and may result from the different mechanisms of carcinogenesis among different kinds of tumor. Second, different ethnic composition may contribute to the discrepancy. Different meta-analyses included different original studies which were performed in different races and the ethnic composition in different meta-analyses may be diversity. Third, some methodological diversity, such as inclusion criteria, the quality of original studies, selection bias, Type I error and study sample size, also can contribute to the discrepancy.

Because the allele frequencies of polymorphisms and their effects on the cancer risk were diverse in the different ethnicities, we carried out subgroup analysis by ethnicity. The results demonstrated that EPHX1 His139Arg polymorphism was associated with a decreased CRC risk among Caucasians, while there was no association between EPHX1 His139Arg polymorphism and CRC risk among Asians. The null result in Asians may be due to the limited number of studies with only two studies from Asian available in this meta-analysis. It is critical that larger and well-designed multicentric studies based on Asian patients should be performed to re-evaluate the association. Moreover, results of meta-analyses often depend on control selection procedures [42]. Different controls source may be a confounding factor which may impact on the conclusion of our study because of case-control studies.

In conclusion, this meta-analysis evaluates the relationship between genetic polymorphisms and CRC risk and reveals that EPHX1 Tyr113His polymorphism may be not associated with CRC development; while the EPHX1 His139Arg polymorphism may have a potential protective effect on CRC. Since limited studies were from Asian populations, it is critical that larger and well-designed multicentric studies based on Asians should be performed to re-evaluate the association. Moreover, further studies estimating the effect of both single-SNP analysis and combination of two-SNPs analysis may provide insights into the relationship between EPHX1 enzyme activity and CRC risk. However, only limited studies in this meta-analysis reported combination of two-SNP analyses (Table S2), which prevented us to perform pooled analysis. Third, there was significant between-study heterogeneity from studies of the EPHX1 polymorphism, and the genotype distribution also showed deviation from HWE in some studies. In spite of these, our meta-analysis also had some advantages. First, we did not detect any publication bias indicating that the whole pooled result should be unbiased. Second, the quality of case-control studies included in current meta-analysis was satisfactory and met our inclusion criterion.

In conclusion, this meta-analysis evaluates the relationship between genetic polymorphisms and CRC risk and reveals that EPHX1 Tyr113His polymorphism may be not associated with CRC development; while the EPHX1 His139Arg polymorphism may have a potential protective effect on CRC. Since limited studies were from Asian populations, it is critical that larger and well-designed multicentric studies based on Asians should be performed to re-evaluate the association. Moreover, further studies estimating the effect of both single-SNP analysis and combination of two-SNPs analysis and gene-environment interactions may eventually provide a better, comprehensive understanding of the association between the EPHX1 polymorphisms and CRC risk.

Supporting Information

Figure S1 Galbraith plots for heterogeneity test of Tyr113His and His139Arg polymorphisms. (A) Galbraith
plot of the association between Tyr113His polymorphism and CRC risk (The studies outside the range between -2 and 2 were seen as the outliers and the major source of heterogeneity); (B) Galbraith plot of the correlation between His139Arg polymorphism and CRC risk. (TIF)

Figure S2 Influence analysis of the summary odds ratio coefficients on the association between EPHX1 polymorphisms and colorectal cancer risk. Results were computed by omitting each study (left column) in turn. Bars, 95% confidence interval. (A), For EPHX1 Tyr113His His/His -plus-Tyr/Tyr/His genotypes vs. Tyr/Tyr genotype; (B), For EPHX1 His139Arg Arg/Arg-plus-Arg/His genotypes vs. His/His genotype. (TIF)

Figure S3 Results from cumulative meta-analysis of associations between EPHX1 polymorphisms and colorectal cancer risk. The circles and horizontal lines show the accumulated results as each study was redrawn, rather than the estimate for each individual study. Studies sorted by publication time; Bars, 95% confidence interval. (A), For EPHX1 Tyr113His His/His-plus-Tyr/Tyr/His genotypes vs. Tyr/Tyr genotype; (B), For EPHX1 Tyr113His Tyr113His-plus-Tyr/His genotypes vs. Tyr/Tyr genotype. (TIF)

References
1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90.
2. American Cancer Society (2010) Cancer facts and figures. Available: http://www.cancer.org/acs/groups/content/@abo/documents/document/acspc-024113.pdf.
3. Ferlay J, Parkin DM, Steliarova-Foucher E (2010) Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 46: 765–781.
4. Sung JJ, Lai YJ, God KL, Leung WK (2005) Asia Pacific Working Group on Colorectal Cancer. Increasing incidence of colorectal cancer in Asia: implications for screening. Lancet Oncol 6: 871–876.
5. Zhao P, Dai M, Chen W, Li N (2010) Cancer trends in China. Jpn J Clin Oncol 40: 281–295.
6. Lichtenstein P, Holm NV, Verkasalo PK,iao A, Kaprio J, et al. (2000) Environmental and heritable factors in the causation of cancer–analyses of cohorts from Sweden, Denmark, and Finland. N Engl J Med 343: 78–85.
7. Cleary SP, Cotterchio M, Shi E, Gallinger S, Harper P (2010) Cigarette smoking, genetic variants in carcinogen-metabolizing enzymes, and colorectal cancer risk. Am J Epidemiol 172: 1000–1014.
8. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, et al. (2005) Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst 97: 906–916.
9. Arand M, Cronin A, Adamska M, Oesch F (2005) Epoxide hydrolases: structure, function, mechanism, and assay. Methods Enzymol 400: 569–588.
10. Zhang JH, Jin X, Li Y, Wang R, Guo W, et al. (2003) Microsomal epoxide hydrolase polymorphisms are not associated with colon cancer risk. Cancer Epidemiol Biomarkers Prev 12: 665–669.
11. Hasselt C, Aicher L, Sidhu JS, Omiecinski CJ (1994) Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. Hum Mol Genet 3: 421–428.
12. Liu L, Liu L, Zeng F, Wang K, Huang J, et al. (2011) Meta-analysis of the association between VEGF-634 G>C and risk of malignancy based on 23 case-control studies. J Cancer Res Clin Oncol 137: 1027–1036.
13. Anttila J, Thakkinstian A, D'Este C (2005) Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. J Clin Epidemiol 58: 297–303.
14. Cochran WG (1954) The combination of estimates from several clinical trials. Brit Med J 2: 1101–1108.
15. Hedges IV, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557–560.
16. Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22: 719–748.
17. DeSimonean R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7: 177–188.
18. Galbraith RF (1988) A note on graphical presentation of estimated odds ratios from several clinical trials. Stat Med 7: 889–894.
19. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50: 1088–1101.
20. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634.
21. Harrison DJ, Hubbard AL, MacMillan J, Wollie AH, Smith CA (1999) Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. Br J Cancer 79: 168–171.
22. Sachse C, Smith G, Wilkie MJ, Barrett JH, Waxman R, et al. (2002) A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 23: 1839–1849.
23. Yu WP (2004) An epidemiological study on environmental exposure factors and genetic polymorphisms of colorectal cancer. Available at: http://dlfiles.edu.cnki.net/lans50/detail.aspx?dbname=CMFD2000&filename=200405020608.nh. [Article in Chinese].
24. Landi S, Gemignani F, Moreno V, Giapia-Patricola L, Chabrier A, et al. (2005) A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of colorectal cancer. Pharmacogenet Genomics 15: 535–546.
25. Robien K, Curtin K, Ulrich CM, Bigler J, Samowitz W, et al. (2005) Microsomal epoxide hydrolase polymorphisms are not associated with colon cancer risk. Cancer Epidemiol Biomarkers Prev 14: 1350–1352.
26. Tranah GJ, Chan AT, Giovannucci E, Ma J, Fuchs C, et al. (2005) Epoxide hydrolase and CYP2C9 polymorphisms, cigarette smoking, and risk of colorectal carcinoma in the Nurses’ Health Study and the Physicians’ Health Study. Mol Carcinog 44: 21–30.
27. van der Logt EM, Bergevoet SM, Roels HM, Te Morsche RH, Dijk Y, et al. (2006) Role of epoxide hydrolase, NADPH:quinone oxidoreductase, cytochrome P450 2E1 or alcohol dehydrogenase genotypes in susceptibility to colorectal cancer. Mutat Res 593: 39–49.
28. Kim I, Orosz Z, Gombos K, Bogusz B, Czejai A, et al. (2007) Association between allelic polymorphisms of metabolizing enzymes (CYP1A1, CYP1A2, CYP2E1, mEH) and occurrence of colorectal cancer in Hungary. Anticancer Res 27: 2931–2937.
29. Skjeldvogt CF, Zachos M, Hjartaker A, Gromov T, Hansteen IL, et al. (2007) Meat, vegetables and genetic polymorphisms and the risk of colorectal carcinomas and adenomas. BMC Cancer 7: 228.
30. Cotterchio M, Boucher BA, Manno M, Gallinger S, Okby AB, et al. (2008) Red meat intake, doneness, polymorphisms in genes that encode carcinogen-metabolizing enzymes, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 17: 3098–3107.
31. Hlavata I, Vrana D, Sumberovsk Z, Zdralini B, Naccarati A, et al. (2010) Association between exposure-relevant polymorphisms in CYP1B1, EPHX1, NQO1, GSTM1, GSTP1 and GSTT1 and risk of colorectal cancer in a Czech population. Oncol Rep 24: 1347–1353.
32. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, 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: 208–212.
35. Omiecinski CJ, Hassett C, Hosagrahara V (2000) Epoxide hydrolase–polymorphism and role in toxicology. Toxicol Lett 112–113: 365–370.
36. Oesch F (1973) Mammalian epoxide hydrases: inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica 3: 305–340.
37. Benhamou S, Reinikainen M, Bouchardy C, Dayer P, Hirvonen A (1998) Association between lung cancer and microsomal epoxide hydrolase genotypes. Cancer Res 58: 5291–5293.
38. Smith CA, Harrison DJ (1997) Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. Lancet 350: 630–633.
39. Ma H, Jin G, Hu Z, Zhai X, Chen W, et al. (2006) Variant genotypes of CDKN1A and CDKN1B are associated with an increased risk of breast cancer in Chinese women. Int J Cancer 119: 2173–2178.
40. Li X, Hu Z, Qu X, Zhai J, Li L, et al. (2011) Putative EPHX1 enzyme activity is related with risk of lung and upper aerodigestive tract cancers: a comprehensive meta-analysis. PLoS One 6: e14749.
41. Zhao ZQ, Guan QK, Yang FY, Zhao P, Zhou B, et al. (2012) System review and metaanalysis of the relationships between five metabolic gene polymorphisms and colorectal adenoma risk. Tumour Biol 33: 525–535.
42. Benhamou S, Lee WJ, Alexandrie AK, Boffetta P, Bouchardy C, et al. (2002) Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. Carcinogenesis 23: 1343–1350.
43. Hassett C, Lin J, Carty CI, Laurenzana EM, Omiecinski CJ (1997) Human hepatic microsomal epoxide hydrolase: comparative analysis of polymorphic expression. Arch Biochem Biophys 337: 273–283.