

RESEARCH ARTICLE

No Association Between the GSTM1 Null Genotype and Risk of Renal Cell Carcinoma: A Meta-analysis

Rui Liu, Xiao-Hua Wang, Li Liu, Qiang Zhou*

Abstract

Background: Many studies have focused on possible associations between the glutathione S-transferase M1 (GSTM1) null genotype and risk of renal cell carcinoma (RCC), but the impact remains unclear owing to obvious inconsistencies among the findings. The present study aimed to quantify the strength of any association in a meta-analysis. **Methods:** We searched the PubMed, Embase and CBM databases for studies concerning the association between the GSTM1 null genotype and risk of RCC. We estimated the summary odds ratio (OR) with its 95% confidence intervals (95% CI) to assess the association. **Results:** The meta-analysis showed the GSTM1 null genotype was not associated with risk of RCC overall (OR = 1.04, 95% CI 0.92-1.18, P = 0.501). For Caucasians, the GSTM1 null genotype was also not associated with risk of RCC (OR=1.02, 95% CI 0.90-1.16, P = 0.761). The cumulative meta-analyses showed a trend of no obvious association between GSTM1 null genotype and risk of RCC as information accumulated. Sensitivity analyses by omitting those studies also did not materially alter the overall combined ORs. No evidence of publication bias was observed. **Conclusion:** Meta-analyses of available data show that the GSTM1 null genotype is not significantly associated with risk of renal cell carcinoma.

Keywords: Renal cell carcinoma - GSTM1 - meta-analysis - polymorphism

Asian Pacific J Cancer Prev, 13, 3109-3112

Introduction

The incidence of renal cell carcinoma (RCC) has been increasing not only in the United States, other Western countries and Japan, but also worldwide (Siegel et al., 2012). RCC are emerging as a complex set of diseases with major socioeconomic impact and a continued rise in incidence throughout the world. The incidence rates of RCC vary more than 10-fold around the world, and the rates are higher in Western countries than in Asia (Murai and Oya, 2004). However, the causes of RCC are still largely unknown. As the field of urologic oncology faces these trends, the genetic factors may play an important role in renal carcinogenesis, and several major genomic and mechanistic discoveries have altered our core understanding of this multitude of cancers, including several new rare subtypes of renal cancers (Jonasch et al., 2012). Glutathione S-Transferases (GSTs) are the most important family of phase II isoenzymes known to detoxify a variety of electrophilic compounds and carcinogens, chiefly by conjugating them with glutathione (Hayes et al., 2005). The GSTM1 is one of the genes encoding the Mu class of GSTs, which is located on chromosome 1p13.3 and contains 10 exons (Hayes and Strange, 2000). The most common variants of GSTM1 genes is homozygous deletion (null genotype), which has been suggested to be associated with the loss of enzyme activity. GSTM1

null genotype is associated with increased vulnerability to cytogenetic damage and oxidative DNA damage, and may result in the susceptibility to cancers (Hayes and Strange, 2000). Numerous studies have investigated the relationships between the GSTM1 null genotype and risk of RCC, but the conclusions from those studies were conflicting. We present herein the results of a meta-analysis of published data investigating the association between GSTM1 null genotype and risk of RCC to shed some light on these contradictory results and to decrease the uncertainty of the effect size of the estimated risk.

Materials and Methods

Search strategy

We conducted a comprehensive search of the PubMed, Embase and CBM databases from its inception through May 2012. We combined search terms for GSTM1 polymorphism and RCC. Search terms included GST, GSTM1, glutathione S-transferase; and kidney carcinoma, kidney cancer, renal cell carcinoma, renal cell cancer, renal carcinoma or renal cancer. There was no language limitation. The retrieved studies were manually screened in their entirety to assess their appropriateness for eligibility criteria. All references cited in the studies were also reviewed to identify additional published articles not indexed in the common database.

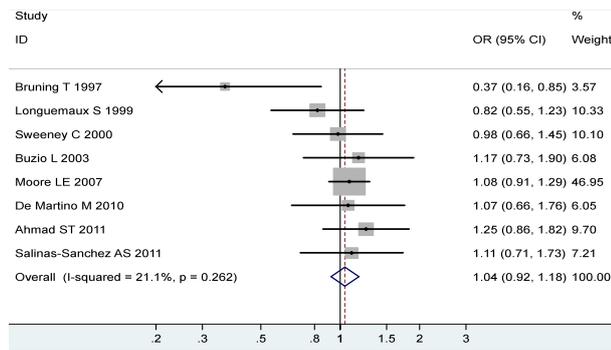


Figure 1. Forest Plots Showed No Association Between GSTM1 Null Genotype and Risk of RCC

Study eligibility

Eligibility criteria included the following: (i) Case-control design with the genotyping of men with and without RCC; (ii) provided information on genotype frequency or odds ratio (OR) with their 95% confidence interval (95% CI) to assess the association. In studies with overlapping cases or controls, the most recent and/or the largest study with extractable data was included in the meta-analysis. Studies investigating progression, severity, phenotype modification, response to treatment, or survival were excluded from this review. Genome scans investigate linkages and were also excluded. In addition, family-based association studies were excluded because they use different study designs.

Data extraction

Two investigators independently extracted data, and disagreements were resolved through consensus. The extracted data included the year of publication, ethnicity of the study population, definition of RCC, inclusion criteria for RCC patients and normal controls, demographics, matching, clinical status of controls, genotyping method, and the genotype distribution of cases and controls for the GSTM1 polymorphism. The frequencies of GSTM1 null genotype were extracted or calculated for cases and controls. All data were extracted from published articles, and we did not contact individual authors for further information.

Statistical analysis

The association between GSTM1 null genotype and RCC risk was estimated by calculating pooled OR with its 95% CI. Heterogeneity across studies was estimated using the I^2 statistic to quantify the proportion of the total variation due to heterogeneity (Higgins et al., 2003), and a $I^2 < 50\%$ suggested a lack of obvious heterogeneity. Meta-analysis was carried out by using random-effects (DerSimonian and Laird, 1986) or fixed effects (Mantel and Haenszel, 1959) models based on the pooled effect estimates in the presence ($I^2 > 50\%$) or absence ($I^2 \leq 50\%$) of obvious heterogeneity. We also performed a cumulative meta-analysis to provide a framework for updating a genetic effect from all studies and to measure how much the genetic effect changes as evidence accumulates and find the trend in estimated risk effect (Muellerleile and Mullen, 2006; Zintzaras and Lau, 2008). In cumulative meta-analysis, studies were chronologically ordered by

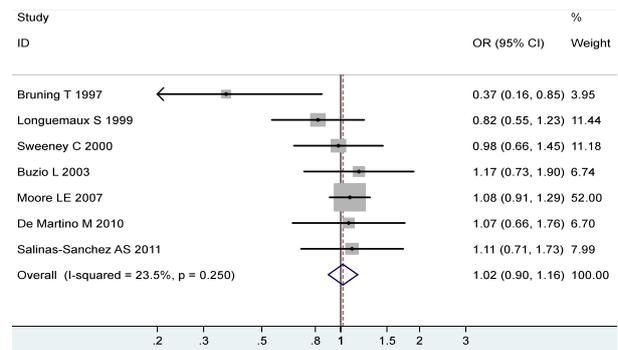


Figure 2. Forest Plots Showed No Association Between GSTM1 Null Genotype and Risk of RCC in Caucasians

publication year, then the pooled ORs were obtained at the end of each year (ie, at each information step). Potential publication bias was estimated by constructing funnel plots and asymmetric funnel plot indicated a relationship between effect and study size, which suggested the possibility of either publication bias or a systematic difference between smaller and larger studies. In addition, funnel-plot's asymmetry was assessed by the method of Egger's linear regression test (Egger et al., 1997). The data analysis was performed (STATA, version 12, StataCorp LP, College Station, TX).

Results

Characteristics of included studies

With our search criterion, 361 individual records were found, and 15 full-text publications were preliminarily identified for further detailed evaluation. According to the exclusion criteria, seven publications were excluded for lack of available data. Finally, 8 case-control studies with 4,460 subjects were included into this meta-analysis (Bruning et al., 1997; Longuemaux et al., 1999; Sweeney et al., 2000; Buzio et al., 2003; Moore et al., 2007; De Martino et al., 2010; Salinas-Sanchez et al., 2011; Ahmad et al., 2012). All included studies were English language literature. Among these 8 case-control studies, 7 (87.5%) were from Caucasian population and 1 (12.5%) were from India (Ahmad et al., 2012). The number of cases varied from 45 to 855, with a mean of 222, and the numbers of controls varied from 48 to 1166, with a mean of 336. All studies were published in English.

Meta-analysis results

There was no obvious between study heterogeneity among those 8 studies ($I^2 = 21.1\%$), thus the fixed-effects model was used to pool the ORs. Meta-analysis showed GSTM1 null genotype was not associated with risk of RCC (OR = 1.04, 95% CI 0.92-1.18, $p = 0.501$) (Figure 1). Sensitivity analyses by omitting those studies also did not materially alter the overall combined ORs.

For meta-analysis of Caucasian studies, there was no obvious between study heterogeneity among those 7 studies ($I^2 = 23.5\%$), thus the fixed-effects model was used to pool the ORs. Meta-analysis showed GSTM1 null genotype was not associated with risk of RCC (OR=1.02, 95% CI 0.90-1.16, $p = 0.761$) (Figure 2). Sensitivity analyses by omitting those studies also did not materially

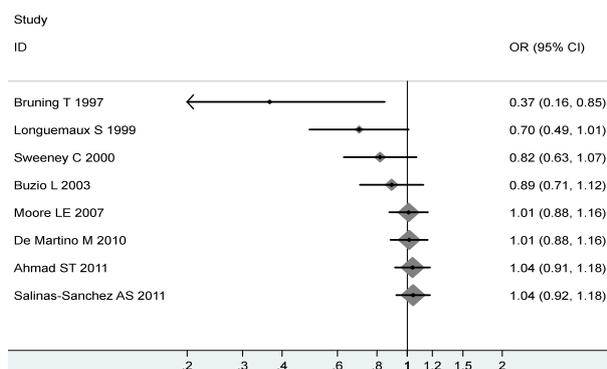


Figure 3. Forest Plots Showed Results of the Cumulative Meta-analysis (The fixed effects pooled odds ratio with the corresponding 95% confidence interval at the end of each information step was shown)

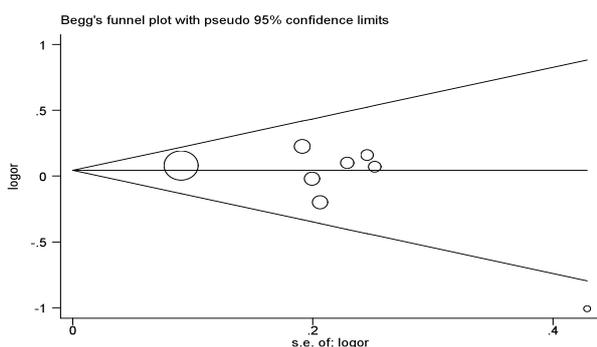


Figure 4. Begg's Funnel Plots to Athe Publication Bias Risk in Present Meta-analysis (P Egger = 0.224)

alter the overall combined ORs.

The cumulative meta-analyses showed a trend of no obvious association between *GSTM1* null genotype and risk of RCC as information accumulated (Figure 3).

Publication bias

The effect of publication bias on the overall estimate was determined, and each study was excluded one at a time, but no change was found in the pooled results, which showed the robust of the results. For analysis of publication bias, Begg's funnel plot were generated to assess potential publication bias (Figure 4), and the symmetry of the funnel plot showed no evidence of publication bias. Besides, the P value of the Egger's test was 0.224, providing statistical evidence of funnel plots' symmetry. Thus, the results above suggested that publication bias was not evident in this meta-analysis.

Discussion

Many studies have investigated the association between *GSTM1* null genotype and risk of RCC, but the impact of *GSTM1* null genotype on RCC risk is unclear owing to the obvious inconsistency among those studies. Each of these studies typically involved a few cases and controls and failed to confirm a strong and consistent association. Small genetic association studies have various designs, different methodology and insufficient power, and could inevitably increase the risk that chance could be responsible for their conclusions, while combining data from all eligible studies by meta-analysis has the advantage of reducing random error and obtaining precise estimates for some potential

genetic associations (Petitti, 2000; Attia et al., 2003). We searched the PubMed, Embase and CBM databases for studies relating the association between *GSTM1* null genotype and risk of RCC, and 8 case-control studies with a total of 4,460 subjects were included into this meta-analysis. Meta-analysis showed *GSTM1* null genotype was not associated with risk of RCC (OR = 1.04, 95% CI 0.92-1.18, P = 0.501). For Caucasians, meta-analysis showed *GSTM1* null genotype was also not associated with risk of RCC (OR = 1.02, 95% CI 0.90-1.16, P = 0.761). The cumulative meta-analyses showed a trend of no obvious association between *GSTM1* null genotype and risk of RCC as information accumulated by year. Sensitivity analyses by omitting those studies also did not materially alter the overall combined ORs. Thus, meta-analyses of available data show *GSTM1* null genotype is not associated with risk of RCC in Caucasians.

GSTs are considered to be involved in the conjugation reaction of phase II metabolism of xenobiotics, catalyzing reactions between glutathione and a variety of potentially toxic and carcinogenic electrophilic compounds. Moreover, GSTs also play an important role in modulating the induction of other enzymes and proteins for cellular functions, such as DNA repair. *GSTM1* null genotype may promote the development of cancer by inhibiting the detoxification of polycyclic hydrocarbons and other compounds that influence oxidative stress and DNA adduct formation (Simic et al., 2009). The relationship between *GSTM1* polymorphism and cancer risk has been investigated in various studies (Wang et al., 2010; Liu and Xu, 2012; Kumar et al., 2011; Zhang et al., 2011; Zhu et al., 2012). Previous studies suggested *GSTM1* null genotype was associated with risks of laryngeal cancer, cervical cancer, hepatocellular carcinoma and gastric cancer, but a race-specific effect may exist in those associations and a more obvious association was found in Asians (Wang et al., 2010; Kumar et al., 2011; Zhang et al., 2011; Liu and Xu, 2012; Zhu et al., 2012). Many studies have reported on the effect of ethnic differences on genetic predisposition to human diseases. Our meta-analysis showed *GSTM1* null genotype is not associated with risk of RCC in Caucasians, but this possible association in Asians is still unclear and need further studies. These differences in cancer susceptibility by ethnicities might be due to different of lifestyle, nutrition, environmental factors, and genetic factors.

Some limitations of this meta-analysis should be addressed. Firstly, the design of some studies in evaluating *GSTM1* null genotype as risk for RCC was unsatisfactory. To identify a relationship between the genotype and cancer risk, it is critical to examine large samples in the design of the population-based case-control studies. As is evident in this study, about 50% of studies used hospital based controls for comparison with cancer cases and cases and controls were not matched in half of the studies. These findings suggest caution in the interpretation of such studies. Secondly, all eligible studies were published papers written in English. It is possible that some relevant unpublished studies written in English or published papers in other languages that may have met the inclusion criteria were missed. Thus, some inevitable publication bias may

exist in the results, although the funnel plots as well as Egger's linear regression tests indicated no remarkable publication bias in the meta-analysis. Thirdly, in the subgroup analyses by ethnicity, no study was conducted in Africans and in Asians respectively. Therefore, to conduct a more precise analysis of this functional polymorphism on RCC risk, additional studies with larger sample size and involving different ethnicities (especially African and Asians) are warranted. Finally, gene-gene or gene-environment interactions on RCC risk were not analyzed in present meta-analysis. Several gene polymorphisms and environment risk factors such as tobacco use and alcohol have been indentified, and an analysis of gene-gene or gene-environment interactions may be helpful to full understanding of the pathogenesis of RCC (Chen et al., 2011; Bex et al., 2012; Jonasch et al., 2012). Hence, it is important to evaluate the gene-environment interactions and also gene-gene interactions in further studies.

In conclusion, meta-analyses of available data show GSTM1 null genotype is not associated with risk of renal cell carcinoma in Caucasians. This possible association in Asians and African need analyzing in further studies and studies investigating the effects of gene-gene or gene-environment interactions on RCC risk are required.

Acknowledgements

The author(s) declare that they have no competing interests.

References

- Ahmad ST, Arjumand W, Seth A, et al (2012). Impact of glutathione transferase M1, T1, and P1 gene polymorphisms in the genetic susceptibility of North Indian population to renal cell carcinoma. *DNA Cell Biol*, **31**, 636-43.
- Attia J, Thakkestian A, D'Este C (2003). Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J Clin Epidemiol*, **56**, 297-303.
- Bex A, Gore M, Mulders P, et al (2012). Recent advances in the treatment of advanced renal cell carcinoma: towards multidisciplinary personalized care. *BJU Int*, [Publishing in progress].
- Bruning T, Lammert M, Kempkes M, et al (1997). Influence of polymorphisms of GSTM1 and GSTT1 for risk of renal cell cancer in workers with long-term high occupational exposure to trichloroethene. *Arch Toxicol*, **71**, 596-9.
- Buzio L, De Palma G, Mozzoni P, et al (2003). Glutathione S-transferases M1-1 and T1-1 as risk modifiers for renal cell cancer associated with occupational exposure to chemicals. *Occup Environ Med*, **60**, 789-93.
- Chen J, Cheng M, Yi L, et al (2011). Relationship between CYP1A1 genetic polymorphisms and renal cancer in China. *Asian Pac J Cancer Prev*, **12**, 2163-6.
- De Martino M, Klatte T, Schatzl G, et al (2010). Renal cell carcinoma Fuhrman grade and histological subtype correlate with complete polymorphic deletion of glutathione S-transferase M1 gene. *J Urol*, **183**, 878-83.
- DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials*, **7**, 177-88.
- Egger M, Davey Smith G, Schneider M, et al (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, **315**, 629-34.
- Hayes JD, Flanagan JU, Jowsey IR (2005). Glutathione transferases. *Annu Rev Pharmacol Toxicol*, **45**, 51-88.
- Hayes JD, Strange RC (2000). Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology*, **61**, 154-66.
- Higgins JP, Thompson SG, Deeks JJ, et al (2003). Measuring inconsistency in meta-analyses. *BMJ*, **327**, 557-60.
- Jonasch E, Futreal A, Davis I, et al (2012). State-of-the-science: An update on renal cell carcinoma. *Mol Cancer Res*, [Publishing in progress].
- Kumar V, Murthy AK, Suresh KP (2011). Glutathione S-transferase M1 and T1 status and the risk of laryngeal cancer: a meta-analysis. *Asian Pac J Cancer Prev*, **12**, 2221-6.
- Liu Y, Xu LZ (2012). Meta-analysis of association between GSTM1 gene polymorphism and cervical cancer. *Asian Pac J Trop Med*, **5**, 480-4.
- Longuemaux S, Delomenie C, Gallou C, et al (1999). Candidate genetic modifiers of individual susceptibility to renal cell carcinoma: a study of polymorphic human xenobiotic-metabolizing enzymes. *Cancer Res*, **59**, 2903-8.
- Mantel N, Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*, **22**, 719-48.
- Moore LE, Brennan P, Karami S, et al (2007). Glutathione S-transferase polymorphisms, cruciferous vegetable intake and cancer risk in the Central and Eastern European Kidney Cancer Study. *Carcinogenesis*, **28**, 1960-4.
- Muellerleile P, Mullen B (2006). Sufficiency and stability of evidence for public health interventions using cumulative meta-analysis. *Am J Public Health*, **96**, 515-22.
- Murai M, Oya M (2004). Renal cell carcinoma: etiology, incidence and epidemiology. *Curr Opin Urol*, **14**, 229-33.
- Petitti DB (2000). Meta-analysis, decision analysis, and cost effectiveness analysis: methods for quantitative synthesis in medicine(2nded). New York, NY: *Oxford University Press*.
- Salinas-Sanchez AS, Sanchez-Sanchez F, Donate-Moreno MJ, et al (2012). GSTT1, GSTM1, and CYP1B1 gene polymorphisms and susceptibility to sporadic renal cell cancer. *Urol Oncol*, [Publishing in progress].
- Siegel R, Naishadham D, Jemal A (2012). Cancer statistics, 2012. *CA Cancer J Clin*, **62**, 10-29.
- Simic T, Savic-Radojevic A, Pljesa-Ercegovac M, et al (2009). Glutathione S-transferases in kidney and urinary bladder tumors. *Nat Rev Urol*, **6**, 281-9.
- Sweeney C, Farrow DC, Schwartz SM, et al (2000). Glutathione S-transferase M1, T1, and P1 polymorphisms as risk factors for renal cell carcinoma: a case-control study. *Cancer Epidemiol Biomarkers Prev*, **9**, 449-54.
- Wang B, Huang G, Wang D, et al (2010). Null genotypes of GSTM1 and GSTT1 contribute to hepatocellular carcinoma risk: evidence from an updated meta-analysis. *J Hepatol*, **53**, 508-18.
- Zhang R, Xu G, Chen W, et al (2011). Genetic polymorphisms of glutathione S-transferase M1 and bladder cancer risk: a meta-analysis of 26 studies. *Mol Biol Rep*, **38**, 2491-7.
- Zhu Y, He Q, Wang J, et al (2012). The association between GSTM1 polymorphism and gastric cancer risk: a meta-analysis. *Mol Biol Rep*, **39**, 685-91.
- Zintzaras E, Lau J (2008). Synthesis of genetic association studies for pertinent gene-disease associations requires appropriate methodological and statistical approaches. *J Clin Epidemiol*, **61**, 634-45.