Null Genotype of GSTT1 Contributes to Colorectal Cancer Risk in Asian Populations: Evidence from a Meta-analysis

Donghua Xu1&*, Shushan Yan2&, Jie Yin1, Pengjun Zhang3

Abstract

Background/Aims: Studies of associations between genetic polymorphism of glutathione S-transferase T1 (GSTT1) and risk of colorectal cancer (CRC) in Asian populations have reported controversial results. Thus, a meta-analysis was performed to clarify the effects of GSTT1 polymorphism on the risk of developing colorectal cancer. Methods: A literature search of PubMed and EMBASE up to June 7, 2011 was conducted and 13 eligible papers were finally selected, involving totals of 4,832 CRC cases and 7,045 controls. Subgroup analyses were performed according to the sample size and the research design with the software programs Review Manager (version 5.0.10) and STATA (version 9.2). Results: Analyses of all relevant studies showed an increased CRC risk was significantly associated with the null genotypes of GSTT1 (OR=1.09, 95%CI=1.01-1.17, P=0.027; I²=40.2%). Besides, a more obvious association was observed after heterogeneity was eliminated (OR=1.13, 95%CI 1.04-1.23, P=0.002; I²=0.0%). Subgroup analyses and sensitivity analysis further identified an association in Asians. Conclusions: This meta-analysis demonstrated the GSTT1 null genotype to be associated with an increased risk of CRC in Asian populations.

Key words: Colorectal cancer - gene polymorphism - glutathione S-transferase T1 - meta-analysis

Introduction

Colorectal cancer (CRC) is one of the most frequent malignant tumors and the most spread gastrointestinal tract cancer on account of its incidence ranking fourth in frequency in men and third in women in Asian countries (Parkin et al., 2005). Some Asian countries have been experiencing a rapidly increasing incidence at present (Sung et al., 2005), which may be due to both environmental factors (Slattery, 2000; Giovannucci, 2001) and genetic susceptibility. Epidemiological researches have revealed that smoking, diets and obesity may play important roles in the risk of developing colorectal cancer. However, not all of those who have been exposed to the similar risk factors will develop CRC, suggesting other causes including genetic susceptibility, might contribute to the variation in individual CRC risk (Naccarati et al., 2007).

To our knowledge, glutathione S-transferase is related to the sensitivity of CRC, mainly including mu(GSTM), theta (GSTT), pi (GSTP) and alpha (GSTA) classes (Zhang et al., 2007; Eppelein et al., 2009; Hlavata et al., 2010). They inactivate carcinogens by catalyzing the conjugation of electrophiles to detoxicate glutathione. GSTT1, a significant candidate gene implicated in colorectal cancers, is located on 22q11.23 with 8146 base pairs, 5 exons and 4 introns totally (Jemth and Mannervik, 1997; Mcllwain et al., 2006). It has lower glutathione binding activity, along with increased catalytic efficiency. It is conceivable that individuals with GSTT1 null genotype may become susceptible to chemical carcinogens and thus develop CRC at a high risk. Over the last two decades, several studies on the association between the GSTT1 polymorphism and CRC susceptibility have been published. However, they have shown inconclusive or contradictory results probably due to limited sample sizes, ethnic difference and publication bias.

Under the light of the above, the need for a comprehensive, up-to-date meta-analysis on GSTT1 genotype seems evident. Meta-analysis is a statistical procedure for combining results from several published studies, in order to acquire a precise estimation of the major effect. One of the major advantages of meta-analysis is to increase the sample size, which may reduce the probability of false-positive or false-negative associations. Therefore, meta-analysis is an ideal and powerful tool for summarizing the inconsistent results from different studies. We carried out a meta-analysis using published data from June 1996 to June 2010 to...
obtain more precise estimates of risk, in order to clarify
the association between GSTT1 null genotype and the
genetic susceptibility of CRC in Asian populations.

Materials and Methods

Search strategy and selection criteria
We conducted a literature search in PubMed, Embase and CBM database (up to June 7, 2011) using the following search strategy: (‘Colorectal carcinoma’, ‘Colorectal cancer’ or ‘CRC’) and (‘Glutathione S-Transferase’, ‘GST’, ‘GSTT’ or ‘GSTT1’) and (‘Polymorphism’, ‘Polymorphisms’ or ‘Genetic polymorphism’). All eligible articles were retrieved and their references were checked for other relevant studies. There was no restriction on time period, sample size, language, or type of report. The inclusion criteria were: (1) Case-control studies which evaluated associations between GST polymorphisms and CRC risk in Asian population; (2) Used an unrelated case-control design; (3) Had an odds ratio (OR) with 95% confidence interval (CI) or other available data for estimating OR(95%CI); (4) Control population didn’t contain malignant tumor patients. When multiple reports were available for a single unique study population, we included only the most recent or largest report (Petitti,2000). Besides, interim analyses and comparisons of laboratory methods were all excluded.

Data extraction and study design
The following information was extracted from included studies: first author, year of publication, ethnicity, study design, number of cases and controls, characteristics of cases and controls, genotype frequency of cases and controls. To ensure the accuracy of extracted information, two investigators extracted information independently and difference was settled by reaching an agreement between all investigators. Subgroup analyses were mainly performed according to the sample size and the research design.

Statistical analysis
The strength of the associations between GSTT1 polymorphism and CRC risk was measured by odds ratio (OR) with 95% confidence interval (CI). The significance of the pooled OR was determined by the Z test and a p value of less than 0.05 was considered significant. We examined the associations of null genotype of GSTT1 with CRC risk on genetic comparison model (Null genotype vs. Present genotype). In our study, two models of meta-analysis for dichotomous outcomes were conducted: the random-effects model and the fixed-effects model. The random-effects model was conducted using the DerSimonian and Laird’s method (DerSimonian and Laird,1986), which assumed that studies were taken from populations with varying effect sizes and calculated the study weights both from in-study and between-study variances. The fixed-effects model was conducted using the Mantel-Haenszel’s method(Mantel and Haenszel,1959), which assumed that studies were sampled from populations with the same effect size and made an adjustment to the study weights according to the in-study variance. To assess the between-study heterogeneity more precisely, both the chi-square based Q statistic test (Cochran’s Q statistic)(Cochran,1954) to test for heterogeneity and the I2 statistic (Higgins et al.,2003) to quantify the proportion of the total variation due to heterogeneity were calculated. Because of the low power of Cochran’s Q statistic, heterogeneity was considered significant for P Cochran’s Q statistic < 0.05, and the random-effects model was used to pool the results; on the contrary, the fixed-effects model was used to pool the results when P value of Cochran’s Q statistic was more than 0.05. Besides, Galbraith plot was also used to spot the outliers as the possibly major sources of heterogeneity (Galbraith,1988). To validate the credibility of outcomes in this meta-analysis, sensitivity analysis was performed by sequential omission of individual studies or by omitting studies potted by Galbraith plot’s method as the possibly major source of heterogeneity. Publication bias was investigated by funnel plot, in which the standard error of logor of each study was plotted against its logor. An asymmetric plot suggested possible publication bias. In addition, funnel-plot’s asymmetry was assessed by the method of Egger’s linear regression test (Egger et al.,1997).

Statistical analyses were performed with the software programs Review Manager (version 5.0.10) and STATA (version 9.2). All P values were two-sided. To ensure the reliability and the accuracy of the results, two authors inputted the data into the statistic software programs independently and got the same results.

Results

Characteristics of included studies
A database was established according to the extracted information from each article. 13 eligible papers that described the association between the GSTT1 polymorphism and colorectal cancer were finally selected, involving 4,832 CRC cases and 7,045 controls totally. The first author, research region, publishing year, the number of cases and controls, ratios of null GSTT1 genotype in case group and control group respectively were showed in Table 1 in great details.

Meta-analysis results
The main results of this meta-analysis were listed in table 2. For GSTT1 polymorphism, the between-study heterogeneity was also large but not significant when all 13 eligible studies were pooled into meta-analysis (I²=40.2%, P =0.066), thus the fixed-effects model was used to pool the results. The combined results showed that null genotype of GSTT1 was associated with
Null Genotype of GSTT1 Contributes to Colorectal Cancer Risk in Asians

Table 1. Characteristics of Included Studies in this Meta-analysis Evaluating the Effect of GSTT1 Polymorphism on Risk of CRC

<table>
<thead>
<tr>
<th>Study(ref.)</th>
<th>Country</th>
<th>Study date</th>
<th>Study design</th>
<th>Cases</th>
<th>Controls</th>
<th>Null GSTT1 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisa et al. (2010)</td>
<td>Japan</td>
<td>2010</td>
<td>Population-based</td>
<td>685</td>
<td>778</td>
<td>338 (49.3)</td>
</tr>
<tr>
<td>Yang et al. (2010)</td>
<td>China</td>
<td>2010</td>
<td>Population-based</td>
<td>322</td>
<td>1247</td>
<td>164 (50.9)</td>
</tr>
<tr>
<td>Yeh et al. (2010)</td>
<td>Taiwan</td>
<td>2010</td>
<td>Hospital-based</td>
<td>722</td>
<td>733</td>
<td>396 (54.8)</td>
</tr>
<tr>
<td>Piao et al. (2009)</td>
<td>South Korea</td>
<td>2009</td>
<td>Hospital-based</td>
<td>1829</td>
<td>1699</td>
<td>950 (51.9)</td>
</tr>
<tr>
<td>Chen et al. (2009)</td>
<td>China</td>
<td>2009</td>
<td>Hospital-based</td>
<td>57</td>
<td>68</td>
<td>24 (42.1)</td>
</tr>
<tr>
<td>Lin et al. (2008)</td>
<td>China</td>
<td>2008</td>
<td>Hospital-based</td>
<td>120</td>
<td>204</td>
<td>64 (53.3)</td>
</tr>
<tr>
<td>Fu et al. (2006)</td>
<td>China</td>
<td>2006</td>
<td>Hospital-based</td>
<td>315</td>
<td>438</td>
<td>174 (55.2)</td>
</tr>
<tr>
<td>Probst-H et al. (2006)</td>
<td>Singapore</td>
<td>2006</td>
<td>Population-based</td>
<td>300</td>
<td>1168</td>
<td>100 (33.3)</td>
</tr>
<tr>
<td>Chen et al. (2009)</td>
<td>China</td>
<td>2009</td>
<td>Hospital-based</td>
<td>1829</td>
<td>1699</td>
<td>950 (50.5)</td>
</tr>
<tr>
<td>Lin et al. (2008)</td>
<td>China</td>
<td>2008</td>
<td>Hospital-based</td>
<td>120</td>
<td>204</td>
<td>64 (53.3)</td>
</tr>
<tr>
<td>Fu et al. (2006)</td>
<td>China</td>
<td>2006</td>
<td>Hospital-based</td>
<td>315</td>
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<td>2006</td>
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<td>315</td>
<td>438</td>
<td>174 (55.2)</td>
</tr>
</tbody>
</table>

Study time was not provided in original studies; McGlynn KA’s study didn’t report genotype frequency of cases and controls but provided OR with 95% CI

Table 2. Summary of Pooled Odds Ratios (OR) with Confidence Interval (CI) in the Meta-analysis

<table>
<thead>
<tr>
<th>Null vs. Present*</th>
<th>Studies (No. of cases / No. of controls)</th>
<th>Odds Ratio</th>
<th>M*</th>
<th>Heterogeneity</th>
<th>P H</th>
<th>P Egger's test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total studies</td>
<td>13 (4,832/7,045)</td>
<td>1.09</td>
<td>0.027</td>
<td>F</td>
<td>0.066</td>
<td>0.424</td>
</tr>
<tr>
<td>Total studies†</td>
<td>12 (4,532/5,877)</td>
<td>1.13</td>
<td>0.002</td>
<td>F</td>
<td>0.486</td>
<td>0.273</td>
</tr>
<tr>
<td>Subgroup analyses by sample size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case sample size≥500</td>
<td>3 (3,236/3,210)</td>
<td>1.14</td>
<td>0.009</td>
<td>F</td>
<td>24.6</td>
<td>0.265</td>
</tr>
<tr>
<td>Case sample size&lt;500</td>
<td>10 (1,596/3,835)</td>
<td>1.02</td>
<td>0.129</td>
<td>F</td>
<td>41.7</td>
<td>0.080</td>
</tr>
<tr>
<td>Studies (case sample size&lt;500†)</td>
<td>4 (1,145/2,364)</td>
<td>1.14</td>
<td>0.124</td>
<td>F</td>
<td>0.0</td>
<td>0.454</td>
</tr>
<tr>
<td>Subgroup analyses by study design</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital-based study</td>
<td>9 (3,387/3,513)</td>
<td>1.13</td>
<td>0.012</td>
<td>F</td>
<td>7.2</td>
<td>0.376</td>
</tr>
<tr>
<td>Population-based study</td>
<td>4 (1,445/3,532)</td>
<td>0.98</td>
<td>0.880</td>
<td>R</td>
<td>70.0</td>
<td>0.018</td>
</tr>
<tr>
<td>Population-based study†</td>
<td>4 (1,145/2,364)</td>
<td>1.14</td>
<td>0.090</td>
<td>F</td>
<td>0.0</td>
<td>0.391</td>
</tr>
</tbody>
</table>

*M, model of meta-analysis; R, random-effects model; F, Fixed-effects model; *P Egger’s test, the P value for Egger’s test; Values could not be calculated out; † Adjustment for Heterogeneity was performed by excluding Sun and Deng studies as the outliers and therefore possible major sources of heterogeneity

increased CRC risk in Asian population (OR=1.09, 95%CI 1.01-1.17, P OR=0.027) (Table 2). In subgroup analyses by study sample size, the pooled results of large studies showed that null genotype of GSTT1 was associated with increased CRC risk (OR=1.14, 95%CI 1.03-1.26, P OR=0.009) (Table 2). In subgroup analyses by study design, pooled analysis of hospital-based studies showed that null genotype of GSTT1 was associated with increased CRC risk (OR=1.13, 95%CI 1.03-1.24, P OR=0.012) (Table 2).

Heterogeneity analysis and sensitivity analysis

The between-study heterogeneity was large but not significant when all 13 eligible studies were pooled into meta-analysis (I²=40.2%, P H=0.066). The between-study heterogeneity was also significant in subgroup
analyses of Asians, population-based studies, but it wasn’t significant in the other subgroup analyses (Table 2). Galbraith plots spotted one study as the outliers and the possibly major sources of heterogeneity in the analysis pooling total available studies (Figure 2) and it was the same with subgroup analyses. All heterogeneity was eliminated (all $I^2$ values were 0%) after excluding the study (No.8) (Probst et al., 2006) as the possibly major source of heterogeneity.

Sensitivity analysis was performed by sequential omission of individual studies, and the significance of all ORs was not influenced excessively by omitting any single study (data not shown).

Publication bias

Funnel plot and Egger’s test were performed to access the publication bias in this meta-analysis. Funnel plots’ shape of all contrasts did not reveal obvious evidence of asymmetry, and all the P values of Egger’s tests were more than 0.05, providing statistical evidence of funnel plots’ symmetry (Figure 3, Table 2). Thus, the results above suggested that publication bias was not evident in this meta-analysis.

Discussion

GSTT1, as a detoxifying enzyme, is involved in the detoxification of several environmental carcinogens such as 1,3-butadiene and ethylene oxide in tobacco smoke and ambient air (Landi, 2000). It is well-known that over-expression of GSTT1 in rats fed with GSTT1-inducers can prevent them from some cancers, while other types of cancer are increased, depending on the carcinogen employed (Sherratt et al., 1998). Null genotypes of GSTT1 have already been found to be associated with increased genetic susceptibility to Gastric cancer (Saadat, 2006) and bladder cancer (Grando and Kuasne, 2009) in humans. In 1995, Chenevix-Trench first demonstrated that GSTT1 null genotype was likely to be a risk factor for susceptibility to colorectal cancer (Chenevix et al., 1995). Different ethnic studies investigating the association between genetic polymorphism of GSTT1 and risk of colorectal cancer (CRC) have given controversial results. In our study, the results of meta-analysis suggest a positive association between GSTT1 null genotype and risk of CRC in Asian population.

Although some previous studies of different ethnic populations suggested no significant association of CRC with GSTT1 null genotype, several studies have demonstrated a strong association between GSTT1 null genotype and increased risks of CRC (Butler et al., 2001; Ates et al., 2005; Economopoulos and Sergentanis, 2010; Liao et al., 2010). The discrepancies in the results of meta-analyses may be due to differences in genetic background and exposure history of the study populations. Our meta-analysis only focused on Asian populations, and we found null genotype of GSTT1 was significantly related to increased risk of CRC (OR=1.09, 95% CI=1.01-1.17, $P_{OR}=0.027$). When heterogeneity was eliminated, a more obvious association was observed (OR=1.13, 95% CI 1.04-1.23, $P_{OR}=0.002$; $I^2=0.0$%). Subgroup analyses and sensitivity analysis further identified this association in Asian population in the present study.

GSTs are multifunctional genes, so gene-environment interactions are numerous and may account for the increased risk of CRC. However, a potential limitation of this meta-analysis is the fact that no gene-environment interactions have been explored, which deserves to be estimated further. Although some analyses in the context of CRC risk did not point to any significant tobacco-genotype interaction for GSTT1, a non-significant positive interaction between GSTT1 null genotype and smoking has been found for CRC.
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