RESEARCH COMMUNICATION

Meta-analysis of the Association Between GSTM1 and GSTT1 Gene Polymorphisms and Cervical Cancer

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Abstract

Aim: We conducted a meta-analysis to analyze the influence of GSTM1 and GSTT1 gene polymorphisms on cervical cancer risk, and explore gene-environment interactions. Methods: Identification of relevant studies was carried out through a search of Medline and the EMbase up to Oct. 2011. All case-control studies that investigated the association between GSTM1 and GSTT1 gene polymorphisms and risk of cervical cancer were included. The pooled odds ratio (OR) was used for analyses of results and the corresponding 95% confidence intervals (CI) were estimated. Results: A total of 21 case-control studies were included in the meta-analysis of GSTM1 (2,378 cases and 2,639 controls) and GSTT1 (1,229 cases and 1,223 controls) genotypes. The overall results showed that the GSTM1 null was related to an increased risk of cervical cancer (OR=1.50, 95% CI=1.21-1.85). Subgroup analysis were performed based on smoking and ethnicity. Our results showed that smokers with null GSTM1 genotype had a moderate increased risk of cervical cancer (OR=1.85, 95% CI=1.07-3.20). For the ethnicity stratification, moderate significantly increased risk of null GSTM1 genotype was found in Chinese (OR=2.12, 95% CI=1.43-3.15) and Indian populations (OR=2.07, 95% CI=1.49-2.88), but no increased risk was noted in others. Conclusion: This meta-analysis provided strong evidence that the GSTM1 genotype is associated with the development of cervical cancer, especially in smokers, and Chinese and Indian populations. However, no association was found for GSTT1 null genotype carriers.

Keywords: GSTM1 - GSTT1 - polymorphism - cervical cancer - meta-analysis

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Introduction

Cervical cancer is the third most common cancer in women, and the seventh overall, with an estimated 530,000 new cases in 2008. More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers. High-risk regions are Eastern and Western Africa (ASR greater than 30 per 100,000), Southern Africa (26.8 per 100,000), South-Central Asia (24.6 per 100,000), South America and Middle Africa (ASRs 23.9 and 23.0 per 100,000 respectively). Rates are lowest in Western Asia, Northern America and Australia/ New Zealand (ASRs less than 6 per 100,00) (IARC, 2011). The different incidence in different areas indicates the genetic factors and environmental factors play a role in the development of cervical cancer.

It is well established that human papilloma virus (HPV) infection is a necessary but insufficient event for the development of cervical cancer(Walboomers and Meijer, 1997; Herrington, 1999; Walboomers et al., 1999; Schiffman et al., 2007), because not all HPV-infected patients do develop cervical cancer. Therefore, there are other cofactors for cervical cancer development. Previous studies showed the glutathione S-transferases (GSTs) genetic variants is related to human phase II detoxification enzymes. Cytosolic GSTs (GSTM and GSTT) play a role in the detoxification of the carcinogenic electrophiles of aflatoxin and polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke. The mode of action of GSTs is considered to co-effect with activation and detoxification of tobacco carcinogens. Therefore, several studies found the association between the genetic polymorphisms of GSTs and the risk of cancer development (Carlsten et al., 2008; Mo et al., 2009; Zhuo et al., 2009).

GSTM1 facilitates the excretion of a wide range of carcinogens, reactive oxygen species and chemotherapeutic agents with a variety of substrate specificities (Rebbeck, 1997). The GSTT1 polymorphism is considered in the detoxification of environmental carcinogens, including 1,3 butadiene and ethylene oxide in tobacco smoke and ambient air (Landi, 2000). The null GST (GST-null genotype) results in a completely loss of enzyme activity to bind with genotoxic substrates, including epoxides derived from aflatoxin and PAHs (Hayes and Pulford, 1995). There are large number of epidemiological studies concerning the association between GSTM1 and GSTT1 and risk

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Table 1. Characteristics of Studies Included in the Meta-analysis

ID	Ethnicity	Study design	Mean age of cases	Mean age of controls	Cases	Controls	Null GSTM1 genotype Cases	Null GSTM1 genotype Controls	Adjusted OR	Null GSTT1 genotype Cases	Null GSTT1 genotype Controls	Adjusted OR
Song 2006	China	Hospital-based	49.1	47.2	130	130	77	57	NA	-	-	-
Zhou 2006	China	Hospital-based	40.7	50.5	125	125	73	54	1.85(1.12-3.05)	24	43	1.47(0.89-2.42)
Singh 2008	India	Population-based	45.2	50.3	150	168	64	40	1.52(1.1-2.0)	40	18	2.4(1.4-4.0)
Sobti 2006	India	Hospital-based	48.6	48	103	103	42	38	NA	16	26	NA
Niwa 2005	Japan	Hospital-based	47.2	56.2	131	320	70	184	0.85(0.56-1.2)	-	-	-
Lee 2004	Korea	Hospital-based	NA	NA	81	86	42	42	1.2(0.6-2.1)	38	54	NA
Kim 2000	Korea	Population-based	46.5	46.5	181	181	95	96	2.40(1.53-3.78)	120	92	1.9(1.2-2.9)
Sharma 2004	India	Hospital-based	NA	NA	142	96	81	33	2.5(1.4-4.5)	28	12	1.7(0.8-3.8)
Chen 1999	America	Hospital-based	NA	NA	190	206	101	118	0.8(0.6-1.3)	-	-	-
Joseph 2006	India	Population-based	46	47	147	165	79	54	2.40(1.53-3.78)	24	16	1.84(0.95-3.57)
Palma 2010	Italy	Population-based	NA	NA	81	111	49	58	1.93(0.96-3.88)	23	66	1.6(0.82-3.14)
Nishino 2008	Japan	Population-based	41.6	40.6	124	125	77	59	1.92(1.04-3.54)	56	58	NA
Ueda 2008	Japan	Population-based	NA	NA	144	54	75	28	NA	-	-	-
Settheetham-Ishida 2009	Thailand	Population-based	NA	NA	69	72	54	56	0.62(0.30-2.03)	42	38	0.72(0.29-1.80)
Agodi 2010	Italy	Hospital-based	NA	NA	27	162	15	17	NA	16	92	NA
Ma 2009	China	Hospital-based	46.7	48.8	43	45	29	15	NA	-	-	-
Sierra-Torres 2003	America	Population-based	38.3	34.8	69	72	35	29	3.3 (1.0-10.8)	39	43	NA
Agorastos 2007	Greece	Hospital-based	NA	NA	176	114	33	60	0.96(0.45-2.06)	62	86	0.68(0.32-1.45)
Sierra-Torres 2006	America	Population-based	44.5	42.3	91	92	36	38	0.7 (0.31-1.54)	25	26	1.4(0.57-3.44)
Goodman 2001	America	Population-based	32.3	39.1	131	180	74	98	1.6(0.8-3.0)	44	56	1.0(0.5-1.9)
de Carvalho 2008	Brazil	Hospital-based	NA	NA	43	86	28	49	NA	22	16	4.58(2.04-10.28)
Total					2378	2639	1229	1223	1.14(0.95-1.32)	619	742	1.35(0.97-1.73)
P for heterogeneity									<0.05			<0.05

of cervical cancer in different populations, however, the results is inconsistent (Singh et al., 2006; Song et al., 2006; Agodi et al., 2010; Palma et al., 2010). Although there is meta-analysis regarding on the two gene polymorphism and cervical cancer, no gene-environment interaction was explored, especially for smoking and ethnicity. Therefore, we conducted a meta-analysis regarding the effect of GSTM1 and GSTT1 gene polymorphisms on cervical cancer risk, and explore the gene-environment interaction on cervical cancer risk.

Materials and Methods

Selection criteria and search strategy

Identification of relevant studies was to carried out through a search of Medline and EMbase up to Oct. 2011 using the following terms without any restriction on language, including 'cervical cancer', 'cervical tumor', 'cervical neoplasm' , 'cervical adenocarcinoma', 'glutathione S-transferase', 'GST', 'GSTM' and 'GSTT'.

All studies that examined the association/non association of the GST gene polymorphisms with cervical cancer were identified. 215 potentially relavant studies were searched. Of the 215 literatures, 187 literatures were irrelevant, and 7 studies were excluded were excluded because of various reasons (3 studies were conducted on overlapping population, and 4 studies did not include controls in analysis). Finally, 21 literatures were met the inclusion criteria and included.

The literature search was performed up to Oct. 2011. The inclusion criteria were as following: case-control studies that investigated the association between GSTM1 and GSTT1 gene polymorphisms and risk of cervical cancer; Studies presented original data and the number of null genotype of GSTM1 and GSTT1 in cases and controls. For each study, the following information were excluded: author, publication year, country of origin, average years of cases and controls, number of cases and controls, number of null genotype for GSTM1 and GSTT1 in cases and controls and the adjusted ORs of selected studies. Two authors independently assessed the articles for inclusion/exclusion, resolved disagreements, and reached consistency.

Statistical analysis

The association between GSTM1 and GSTT1 gene polymorphisms and cervical cancer was estimated by calculating pooled ORs and 95% CIs. Odds ratio (OR) was used for analyses of results and their corresponding 95% confidence intervals(CI) were estimated. Heterogeneity across studies was estimated using the Q statistic, and a $p>0.05$ suggested a lack of heterogeneity. Meta analysis was carried out by using random-effects or fixed effects methods (Der and Laird, 1986; Mantel and Haenszel, 1959) model based on the pooled effect estimates in the presence ($p\leq0.1$) or absence ($p>0.1$) of heterogeneity. Potential publication bias was estimated by constructing funnel plots (Begg and Mazumdar, 1994). As 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 (small study effects). Furthermore, publication bias was assessed by Egger's test (Egger et al., 1997). Studies were categorized into subgroups based on ethnicity and smoking status. The data analysis was performed (STATA, version 10, StataCorp LP, College Station, TX).

Results

A total of 21 case-control studies were included in the meta-analysis of GSTM1 (2,378 cases and 2,639 controls)

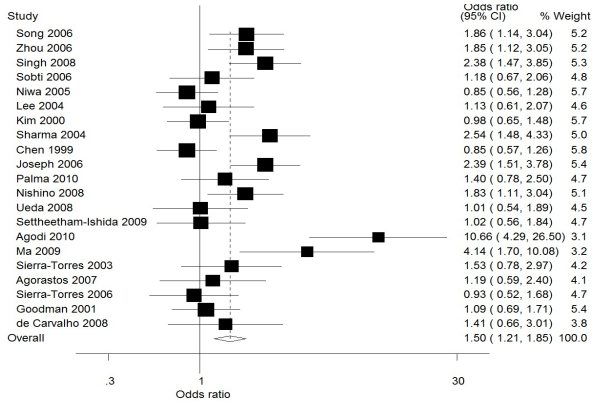

Figure 1. Forest Plot for the Overall Association Between GSTM1 Gene Polymorphism and Cervical Cancer Risk

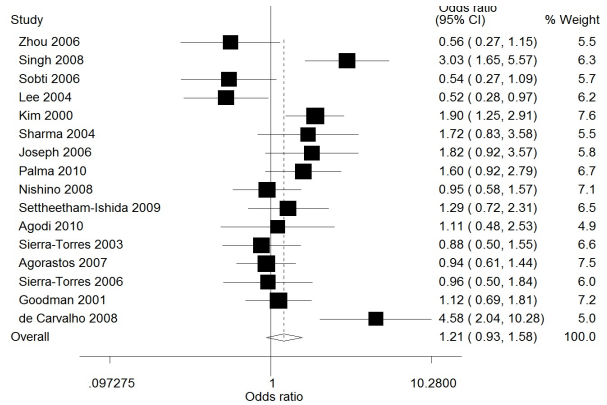

Figure 2. Forest Plot for the Overall Association Between GSTT1 Gene Polymorphism and Cervical Cancer Risk

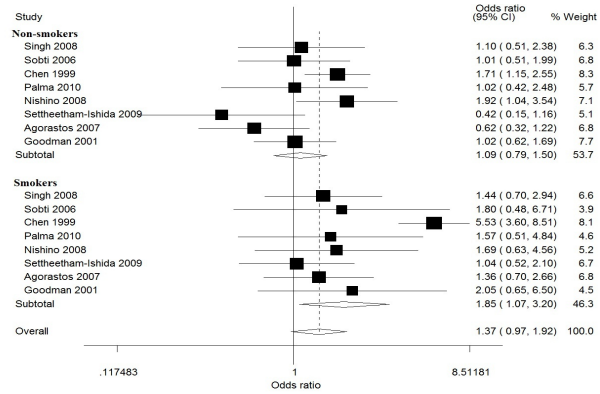

Figure 3. Relationship Between GSTM1 Gene Polymorphism and Cervical Cancer Risk by Smoking Status

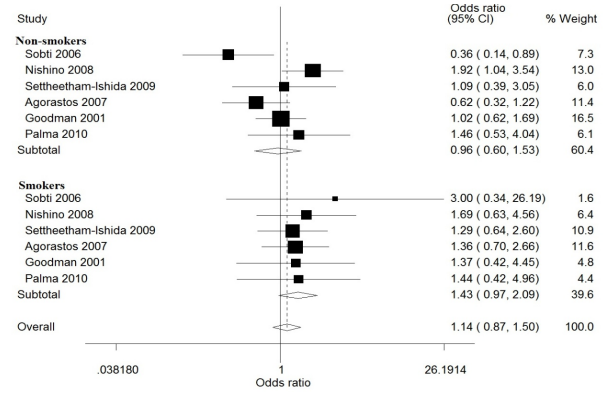

Figure 4. Relationship Between GSTT1 Gene Polymorphism and Cervical Cancer Risk by Smoking Status

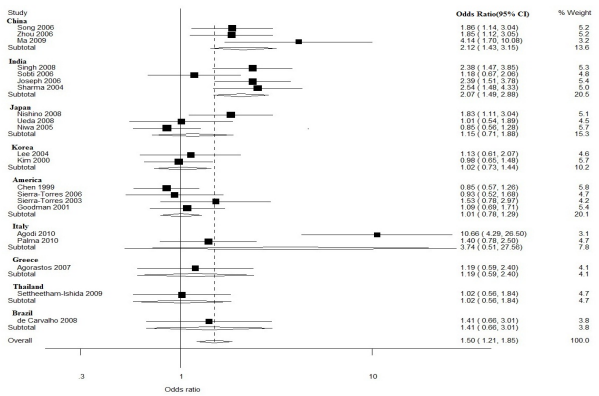

Figure 5. Relationship Between GSTM1 Gene Polymorphism and Cervical Cancer Risk by Ethnicity

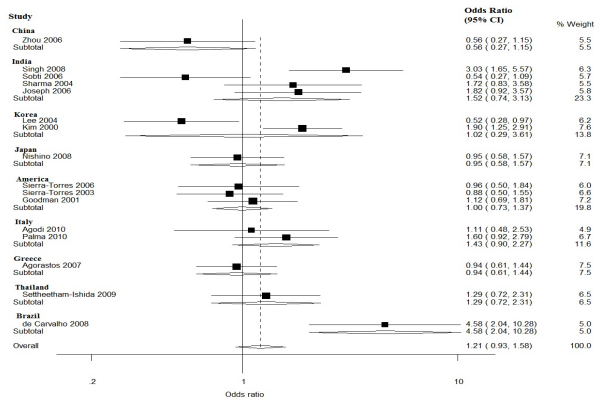

Figure 6. Relationship Between GSTT1 Gene Polymorphism and Cervical Cancer Risk by Ethnicity

and GSTT1(1,229 cases and 1,223 controls) genotypes. Most of the included studies were among young adults with the average year of 30 to 50 years old. For the meta-analysis of GSTM1 and GSTT1, studies were from on China, India, Japan, Korea, Italy, America, Greece, Brazil and Thailand. Study characteristics included in the meta-analysis are presented in Table 1. The pooled adjusted ORs of GSTM1 and GSTT1 were 1.14 (0.95-1.32) and 1.35 (0.97-1.73), respectively.

The forest plot of the meta-analysis of GSTM1 and GSTT1 is shown in Figure 1. There was heterogeneity in studies on GSTM1 and GSTT1 (P_Q<0.001, I^2=74.5% for GSTM1 and P^Q<0.001, I^2=68.4% for GSTT1), a random-effects model was used. The overall results showed that the GSTM1 null was related to the increased risk of cervical cancer (OR=1.50, 95% CI=1.21-1.85). Moreover, an non-significant increased risk of cervical cancer was found in individual carrying GSTT1 null genotype (1.21-1.58).

Subgroup analysis were performed based on smoking and ethnicity (Figure 2-6). The results showed that smokers with null GSTM1 genotype had a moderate increased risk of cervical cancer (OR=1.85, 95% CI=1.07-3.20), while no significant increased risk was found in non-smokers. However, we only found a non-significant increased risk of cervical cancer in null GSTT1 genotype carriers (OR=1.43, 95% CI=0.97-2.09).

After stratification by smoking, the heterogeneity was significantly decreased (P=0.14 and P=0.54 for

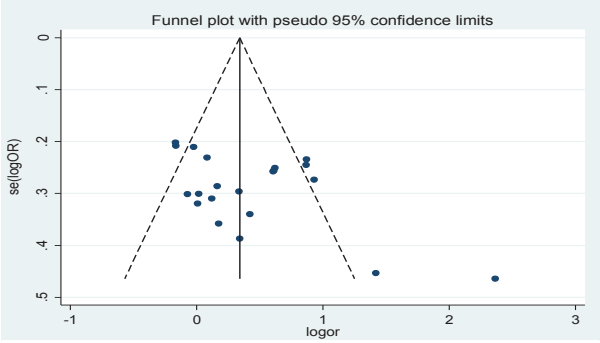

Figure 7. Funnel Plot for GSTM1 Gene Polymorphism and Cervical Cancer Risk

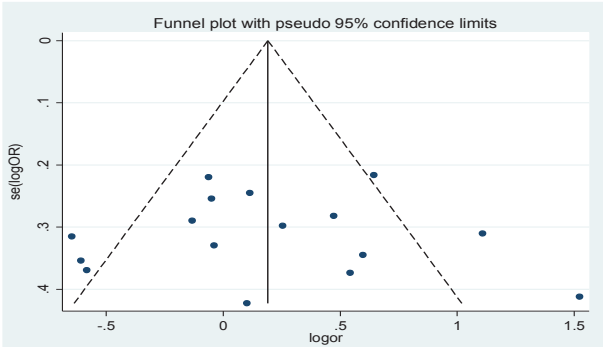

Figure 8. Funnel Plot for GSTT1 Gene Polymorphism and Cervical Cancer Risk

GSTM1 or GSTT1 non-smokers, respectively; P=0.38 and P=0.57 for GSTM1 or GSTT1 smokers). For the ethnicity stratification, moderate significantly increased risk of null GSTM1 genotype was found in Chinese (OR=2.12, 95% CI=1.43-3.15) and Indian population (OR=2.07, 95% CI=1.49-2.88), but no increased risk in other population. However, the non-significant risk was found in other populations. However, for GSTT1 genotype, no significant increased risk was found in each population.

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 for GSTM1 and GSTT1 (Figure 7 and figure 8), and the symmetry of the funnel plot showed no evidence of publication bias. Also. the P values of the Egger's test for GSTM1 and GSTT1 were 0.15 and 0.42, respectively.

Discussion

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 (Der Simonian and Laird, 1986; Hayes and Pulford, 1995). Moreover, GSTs also play an important role in modulating the induction of other enzymes and proteins for cellular functions, such as DNA repair(Der Simonian and Laird, 1986). The relationship between GST gene polymorphisms and cervical cancer has been investigated in various studies (Singh et al., 2006; Songet al., 2006; Agodi et al., 2010; Palma et al., 2010). However, the association between them has been controversial, and these discrepancies could have been due to limited sample numbers and ethnic differences. Our meta-analysis showed the role of GSTM1 polymorphism may promote the development of cervical cancer, and have interaction with smoking. This indicated the GSTM1 and GSTT1 gene deletions may promote the development of cervical dysplasia by inhibiting the detoxification of polycyclic hydrocarbons and other compounds that influence oxidative stree and DNA adduct formation (Parl, 2005).

Many studies have reported on the effect of ethnic differences on genetic predisposition to human diseases. For example, the incidence of cervical cancer is high in southern Africa, and is almost five-folds higher than in the Northern America and Australia (IARC, 2011). Our studies showed the GSTT1 and GSTM1 null genotypes had an increased risk of Chinese and Indian populations, and no risk in Japanese, European and American. These differences showed variations in cancer susceptibility by ethnicities. In addition, the data showed that the allele frequency of GSTM1 null genotype was higher in American and Japanese than in Chinese and India populations, which showed variation in the effect of the genotype might be due to different of lifestyle, nutrition, environmental factors, and genetic factors.

Our study showed tobacco constituents are modified by metabolizing enzymes and may promote malignant cellular growth (Prokopczyk et al., 1997). The mode of action is through the activation and detoxification of tobacco carcinogens, thus, one might expect the polymorphism of GSTs may alter the risk of cancer among smokers. The lack of GST activities caused by an inherited deletion of the GST have been reported to increase the risk of several tobacco-related cancers (Kietthubthew et al., 2001; Spurdle et al., 2001; Lee et al., 2002; van der Hel et al., 2003; Sweeney et al., 2003). It was therefore hypothesized that smoking and GST genotype may synergistically influence the cervical cancer development. Our study showed the null GSTM1 genotype may increased the cervical cancer risk among smokers, which provide strong evidences for the association between GSTs and cervical cancer risk.

A limitation of this study is that the environment and lifestyle of populations were not included in the influencing factors. The pathways of carcinogen metabolism are complex. Cervical cancer have major environmental determinations such as HPV infection, age and reproductive health. Secondly, the sample size reported in the literature is still relatively small and might not provide sufficient power to estimate the association between the null GSTM1 and GSTM1 polymorphism and cervical cancer risk.

In conclusion, this meta-analysis provided strong evidence that the GSTM1 genotype are associated with the development of cervical cancer, and especially in Chinese and Indian population, and smoking showed a modification on the association between GSTM1 null genotype and cervical cancer. However, no significant increased risk of cervical cancer was found in GSTT1 null genotype carriers. Further studies investigating the

effect of gene-environment interactions on cervical cancer risk are required.

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