

Original Article

A meta-analysis of glutathione S-transferase M1 and T1 genetic polymorphism in relation to susceptibility to nasopharyngeal carcinoma

Rong-Rong Liu, Ji-Chuan Chen, Ming-Dong Li, Te Li, Yun Tan, Min Zhang

Department of Otolaryngology Head and Neck Surgery, Institute of Surgery Research & Daping Hospital, Third Military Medical University, Chongqing 400042, China

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Abstract: Objective: To investigate the relationship between glutathione S-transferase M1 (GSTM1), and T1 (GSTT1) genetic polymorphism and susceptibility to nasopharyngeal carcinoma (NPC) using meta-analysis method. Methods: Data of published case-control studies on the relationship between GSTT1, GSTM1 genetic polymorphism and susceptibility to NPC were collected from EMBASE, PubMed, Web of Science, China Academic Journals Full-text Database, Chinese Biomedical Literature Database, and Wanfang Database. Meta-analysis was conducted using Revman 5.2 software. Results: Nine studies were included for meta-analysis with a total of 1295 cases of NPC patients and 1967 control individuals. Meta-analysis showed that the risk of NPC was significantly higher in population with GSTM1 gene deletion (OR=1.43, 95% CI: 1.42-1.65; P<0.001). Similarly, the risk of NPC was significantly higher in Chinese population with GSTM1 gene deletion (OR=1.38, 95% CI: 1.18-1.62; P<0.001). We did not find association between GSTT1 gene deletion and NPC risk not only in total population (OR=1.32, 95% CI: 0.92-1.87; P=0.12), but in Chinese population (OR=1.41, 95% CI: 0.97-2.04; P=0.07). Conclusion: GSTM1 genetic polymorphism, but GSTT1, is associated with susceptibility to NPC.

Keywords: Glutathione S-transferase, polymorphism, nasopharyngeal carcinoma, genetic susceptibility

Introduction

Nasopharyngeal carcinoma (NPC) is a highly malignant tumor, and EB virus is closely related to its onset. Additionally, NPC is also affected by genetic, environmental, dietary and other factors [1, 2]. Mutations in glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1), cytochrome p450, HLA I and type II may increase the risk of NPC [3, 4]. GSTT1 and GSTM1, glutathione S-transferase (GST) supergene family members, are involved in the body's metabolism and detoxification of poisons and belong to phase II metabolic enzymes. The genetic polymorphism is characterized by the increase or decrease in GST activity which contributes to changes of susceptibility of the body to poisons and carcinogens [5]. Currently, there have been quite a lot studies exploring GSTT1, GSTM1 genetic polymorphism and NPC [6-14]. However, the interactions of small sample size, regional difference, tumor location,

genetics, diet and environmental factors lead to controversial results. This article meta-analyzed the published literatures and aimed to explore NPC-specific screening and diagnostic markers.

Materials and methods

Literature search

EMBASE, Pubmed, ISI Web of Science, China Academic Journals Full-text Database, Chinese Biomedical Literature Database, Wanfang Database and VIP Full-Text Database were searched and the published case-control studies on GSTT1, GSTM1 genetic polymorphism and susceptibility to nasopharyngeal carcinoma were collected. A second search using Google Scholar was performed to avoid omission. Glutathione S-transferase M1, GSTM1, glutathione S-transferase T1, GSTT1, polymorphism, polymorphisms, mutation, variation,

GSTT1, GSTM1 and laryngeal cancer

Table 1. The characteristics of included studies involved GSTM1 gene polymorphism

First author	Publication Year	Case	Control	Country	Ethnicity	Case		Control	
						Null	Present	Null	Present
Nazar-Stewart V	1999	83	142	USA	Multiethnicity	45	38	63	79
Da SJ	2002	80	80	China	Asian	48	32	36	44
Cheng YJ	2003	314	337	China	Asian	173	141	168	169
Deng ZL	2004	91	135	China	Asian	56	35	64	71
Liao ZL	2005	80	72	China	Asian	50	30	32	40
Tiwawech D	2005	78	145	Thailand	Asian	50	28	74	71
Bendjemara K	2006	45	100	France	Caucasian	23	22	33	67
Guo X	2008	341	590	China	Aasian	204	137	328	262
Jiang Y	2011	182	366	China	Asian	97	85	157	209

Table 2. The characteristics of included studies involved GSTT1 gene polymorphism

First author	Publication Year	Case	Control	Country	Ethnicity	Case		Control	
						Null	Present	Null	Present
Cheng YJ	2003	314	337	China	Asian	160	154	174	163
Deng ZL	2004	91	135	China	Asian	54	37	55	80
Bendjemara K	2006	45	100	France	Caucasian	7	38	20	80
Guo X	2008	341	590	China	Aasian	164	177	269	321
Yan J	2011	182	366	China	Asian	120	62	180	186

nasopharyngeal cancer, nasopharyngeal carcinoma and NPC were used as search keywords.

Literature inclusion and exclusion criteria

Inclusion criteria: (1) the studied subjects were NPC patients of definite diagnosis and normal control population; (2) case-control studies on the relationship between GSTT1, GSTM1 genetic polymorphism and susceptibility to NPC; (3) quantitative findings were described using the odds ratio (OR) and 95% confidence interval (95% CI); (4) original data were complete, and genotype frequencies were provided in the literatures; (5) written in Chinese or English. Literature exclusion criteria: (1) researches with duplicate data; (2) no control group established in the study; (3) literatures with unclear or ambiguous data or genotype frequencies.

Data extraction

Universal data extraction form and unblinded extraction method were used. The data included: name of the first author, publication year of the literature, country of the study, source of control, matching factors, genotype frequencies of the case and the control group, OR and

corresponding 95% CI. The data were evaluated independently by two researchers who established meta-analysis database and inconsistent part was tackled with discussion and consultation.

Statistical analysis

Statistical analysis was performed using the statistical software Revman 5.2. The comparison of genotype frequency distribution in case and control group was described as OR. Difference in heterogeneity of studies was determined by chi-square test. The included studies were tested for heterogeneity. If the heterogeneity was of good quality ($P > 0.1$, $I^2 < 25\%$) a fixed-effect model would be used. If not, random effect model was to be used. The results were presented as OR values and 95% confidence intervals, and $P < 0.05$ was considered statistically significant. Subgroup analysis was performed based on study countries or regions to explore possible sources of heterogeneity, and the included studies were removed out one by one for sensitivity analysis to assess the impact on the overall result of a single study. The existence of publication bias was analyzed using funnel plot.

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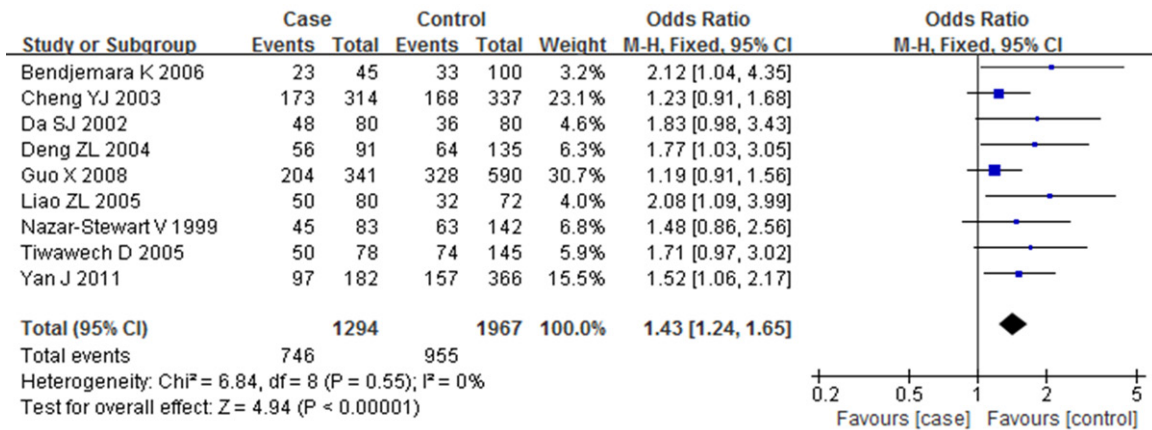


Figure 1. Forest plot of comparison of the nasopharyngeal carcinoma and control subjects in GSTM1 gene polymorphism in total population. The horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

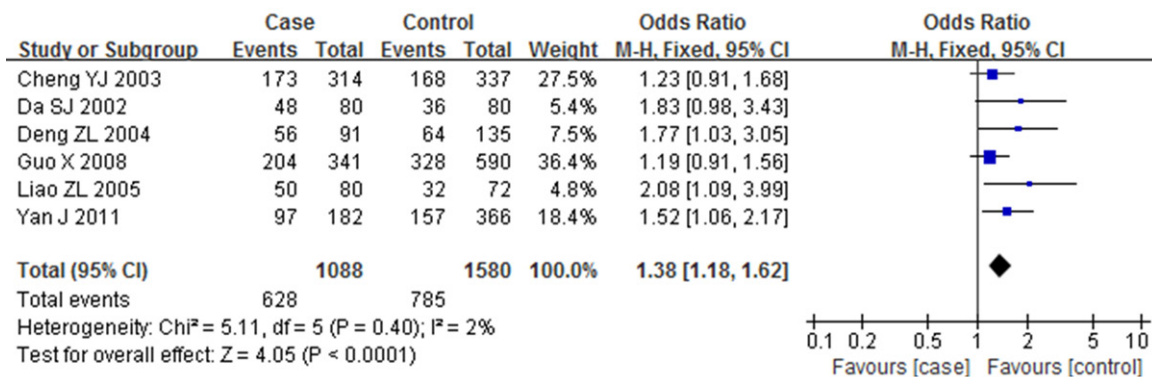


Figure 2. Forest plot of comparison of the nasopharyngeal carcinoma and control subjects in GSTM1 gene polymorphism in Chinese population. The horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

Results

Search results

A total of 143 articles were preliminarily retrieved as per the search protocol. One hundred and twenty-three were excluded due to duplication, poor quality, and non-clinical property after reading the title and abstract. The remaining twenty were further extensively read. According to the inclusion criteria of the study, nine literatures were eventually included for meta-analysis, with a total of 1294 cases of NPC patients and 1967 controls. Among the included literatures, there were six papers of Chinese subjects [6, 7, 9, 12, 13, 15], 9 on GSTM1 genetic polymorphism [6-15] and 5 on GSTT1 genetic polymorphism [6, 9, 10, 12, 13]. See **Tables 1** and **2**.

Correlation between GSTM1, GSTT1 genetic polymorphism and susceptibility to NPC

Heterogeneity test showed no heterogeneity ($I^2=0\%$, $P=0.55$) among GSTM1 genetic polymorphism studies, thus the fixed effect model was used for meta-analysis. The results showed that compared with control groups, the risk of NPC among the population with GSTM1 gene deletion was significantly higher (OR=1.43, 95% CI: 1.42-1.65; $P<0.001$), as is shown in **Figure 1**. Further subgroup analysis showed that GSTM1 genetic polymorphism was closely related to NPC susceptibility in Chinese population (OR=1.38, 95% CI: 1.18-1.62; $P<0.001$, **Figure 2**). Meanwhile, there was heterogeneity among the results of various studies about GSTT1 genetic polymorphism ($I^2=72\%$,

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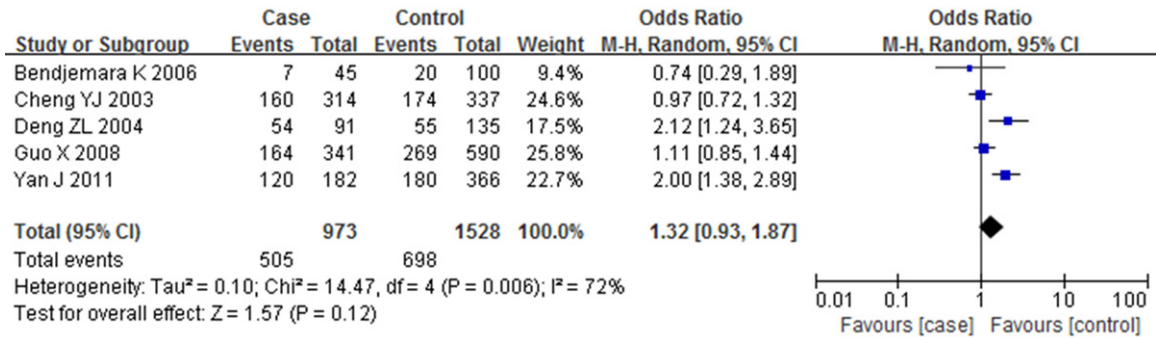


Figure 3. Forest plot of comparison of the nasopharyngeal carcinoma and control subjects in GSTT1 gene polymorphism in total population. The horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

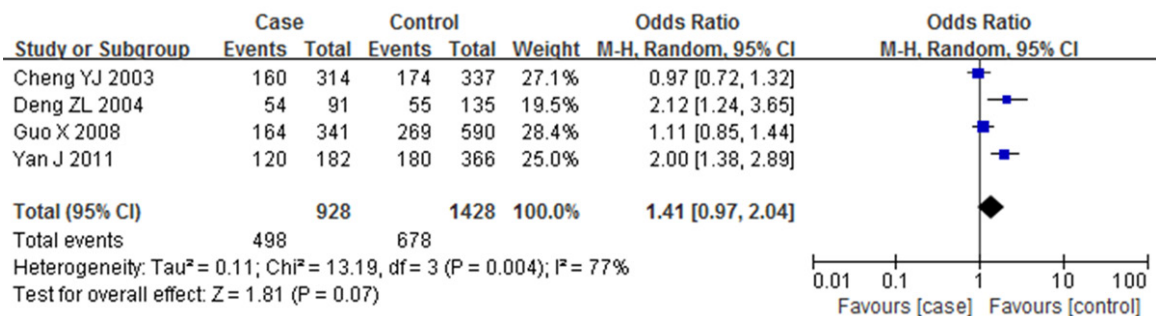


Figure 4. Forest plot of comparison of the nasopharyngeal carcinoma and control subjects in GSTT1 gene polymorphism in Chinese population. The horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

P=0.006), and a random-effect model was hence used for meta-analysis. We did not find association between GSTT1 gene deletion and NPC risk not only in total population (OR=1.32, 95% CI: 0.92-1.87; P=0.12, **Figure 3**), but in Chinese population (OR=1.41, 95% CI: 0.97-2.04; P=0.07, **Figure 4**).

Sensitivity and publication bias analysis

The included studies were removed one by one for sensitivity analysis to assess the impact of a single study on the correlation between GSTM1 and GSTT1 genetic polymorphism and susceptibility to NPC. The results showed no single study could markedly affect the statistical significance of the original analysis results. When publication bias of the 9 studies on GSTM1 genetic polymorphism and susceptibility to NPC included for meta-analysis was assessed, the funnel plot showed a symmetrical distribution (**Figure 5**).

Discussion

Glutathione S-transferase enzymes (GSTs), including GSTM, GSTT and GSTA and other isoenzymes, may protect cells from effects of cytotoxic and carcinogenic agents through an endogenous detoxification reaction. Molecular genetic polymorphism of GSTs can cause changes in enzyme molecular structure, energy level, thereby affecting the detoxification ability of cells [16-18]. It has been confirmed that GSTM1 and GSTT1 genetic polymorphism includes functional (positive) and deletion (null) genotype. Functional genotype may reduce cancer risk, while deletion genotype promote the expression of certain oncogenes by decreasing detoxification. For example, functional GSTM1 can metabolically deactivate carcinogens and lipid peroxidation products in the body, so as to prevent and repair DNA damage. GSTM1 gene deletion can cause inactivation of GST μ enzyme of the GST family, thereby affect-

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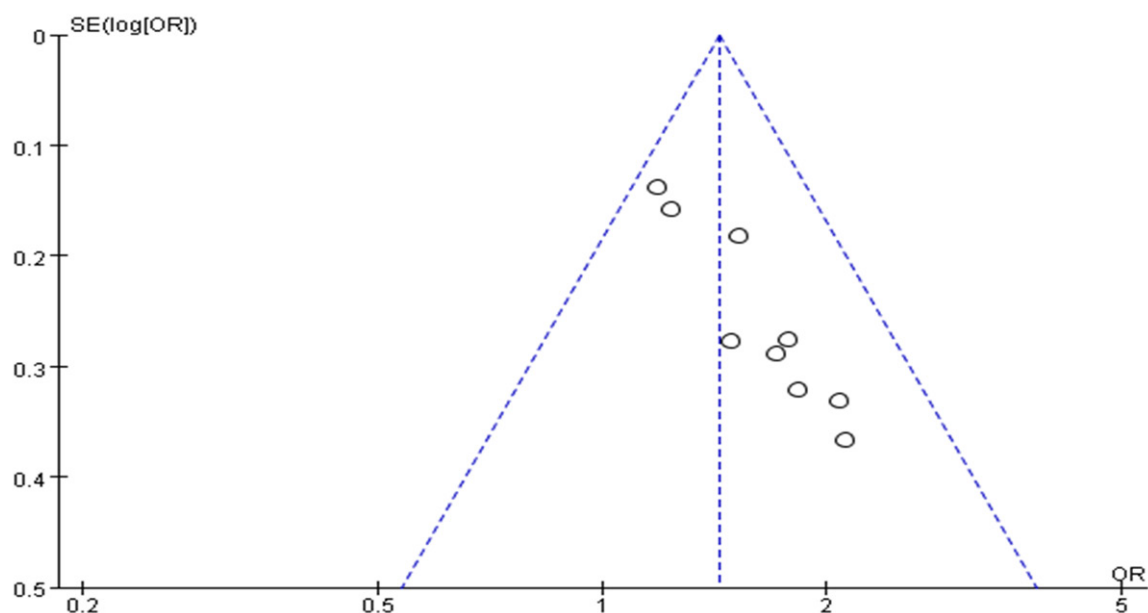


Figure 5. Funnel plot for publication bias tests. Each point represents a separate study for the indicated association. Log or represents natural logarithm of OR. Vertical line represents the mean effects size.

ing the change of susceptibility of an individual to some poisons and carcinogenic agents, resulting in loss of detoxification function and increasing the risk of cancer [13-15]. Functional GSTT1 genotype encodes GSR- θ isozymes and has a strong detoxification effect for halogenated hydrocarbons in epoxides and pesticides. GSTT1 gene deletion leads to missing of encoded isozymes, which may affect the body's detoxification function for these poisons and increase the sensitivity of the host to the effect of these carcinogens [18]. Meta-analysis of relevant clinical studies indicate that GSTM1 and GSTT1 gene deletion is closely related to genetic susceptibility to liver cancer, cervical cancer, breast cancer, head and neck cancer, oral cancer, esophageal cancer, NPC and lung cancer [19-27]. It is a risk factor facilitating the occurrence of a variety of cancers. These findings are consistent with the results on the correlation between GSTM1 genetic polymorphism and NPC susceptibility in this study, suggesting that this gene plays an important role in tumorigenesis.

This study remains several limitations: The meta-analysis included only those studies published in English, therefore the sample size was relatively small and the included literatures did not undergo quality assessment. A small sam-

ple size would have inevitably reduced the statistical power and adversely affected result stability. Hence, more studies with increased sample size are warranted. In terms of heterogeneity, presumably because the case groups, control groups and their corresponding sex ratios did not fully match, there was heterogeneity among the Chinese studies when performing GSTT1 genetic polymorphism subgroup analysis by countries. Moreover, there was no indication of gene-gene and gene-environment interactions in the meta-analysis. Specific environmental factors and lifestyle may also change the relationship between GSTM1, GSTT1 gene polymorphism and NPC susceptibility. Matching degree, environment and diet factors may be sources of heterogeneity. The studies included in this meta-analysis were all case-control ones. The existence of potential publication bias in the included case-control studies on GSTM1 genetic polymorphism could affect the results of the analysis. The possible reasons may be as follows: First, it may be related to sample size. About 40% of the studies had relatively small sample size, and the studies with large sample size were at the bottom of the funnel plot effect values. Second, it may be related to effect value OR and 95% CI. Statistically significant studies were located at the top of effect values. Third, it may be associated with

unreachable relevant gray literatures. Unpublished gray literatures may lead to publication bias. In addition, case-control study itself was vulnerable to selection bias, implementation bias, confounding bias and other biases.

In summary, based on the current evidence and this meta-analysis, it was shown that GSTM1 genetic polymorphism was significantly correlated to NPC risk. GSTM1 deletion genotype may be a risk factor for the occurrence of NPC in the Chinese population. However, the current studies could not provide evidence of GSTM1 genetic polymorphism as an independent risk factor of NPC. In future, research selection method of control group and the interaction between genes and the environment should be carefully considered. The previous research findings are yet to be confirmed by multi-center, large sample size, homogeneous clinical studies or well-designed epidemiological studies in order to more scientifically and accurately evaluate the correlation between GSTT1, GSTM1 genetic polymorphism and susceptibility to NPC, so as to provide more reliable evidence for basic research and clinical treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Rong-Rong Liu, Department of Otolaryngology Head and Neck Surgery, Institute of Surgery Researping Hospital, Third Military Medical University, Chongqing 400042, China. Tel: +86-029-6691999; Fax: +86-029-6691999; E-mail: rongronnl@yeah.net

References

- [1] Qiu F, Sun R, Deng N, Guo T, Cao Y, Yu Y, Wang X, Zou B, Zhang S, Jing T, Ling T, Xie J, Zhang Q. miR-29a/b Enhances Cell Migration and Invasion in Nasopharyngeal Carcinoma Progression by Regulating SPARC and COL3A1 Gene Expression. *PLoS One* 2015; 10: e0120969.
- [2] Chen T, Long B, Ren G, Xiang T, Li L, Wang Z, He Y, Zeng Q, Hong S, Hu G. Protocadherin20 acts as a tumor suppressor gene: epigenetic inactivation in nasopharyngeal carcinoma. *J Cell Biochem* 2015; 116: 1766-75.
- [3] He Y, Zhou GQ, Li X, Dong XJ, Chai XQ, Yao KT. Correlation of polymorphism of the coding region of glutathione S-transferase M1 to susceptibility of nasopharyngeal carcinoma in South China population. *Ai Zheng* 2009; 28: 5-7.
- [4] He Y, Zhou GQ, Li X, Dong XJ, Yao KT. Association of the T1270533G polymorphism in GSTM1 gene coding region with susceptibility to nasopharyngeal carcinoma in a Chinese population. *Nan Fang Yi Ke Da Xue Xue Bao* 2009; 29: 36-9.
- [5] Ghosh SK, Singh AS, Mondal R, Kapfo W, Khamo V, Singh YI. Dysfunction of mitochondria due to environmental carcinogens in nasopharyngeal carcinoma in the ethnic group of Northeast Indian population. *Tumour Biol* 2014; 35: 6715-24.
- [6] Jiang Y, Li N, Dong P, Zhang N, Sun Y, Han M, Wen J, Chen M. Polymorphisms in GSTM1, GSTT1 and GSTP1 and nasopharyngeal cancer in the east of China: a case-control study. *Asian Pac J Cancer Prev* 2011; 12: 3097-100.
- [7] Zhuo X, Cai L, Xiang Z, Li Q, Zhang X. GSTM1 and GSTT1 polymorphisms and nasopharyngeal cancer risk: an evidence-based meta-analysis. *J Exp Clin Cancer Res* 2009; 28: 46.
- [8] Tiwawech D, Chindavijak S, Karalak A, Ishida T. Real-time PCR assay for rapid detection of GSTM1 polymorphism in nasopharyngeal carcinoma patients. *Asian Pac J Cancer Prev* 2008; 9: 233-7.
- [9] Guo X, O'Brien SJ, Zeng Y, Nelson GW, Winkler CA. GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 1760-3.
- [10] Bendjemana K, Abdennebi M, Gara S, Jmal A, Ghanem A, Touati S, Boussen H, Ladgham A, Guemira F. Genetic polymorphism of glutathione-S transferases and N-acetyl transferases 2 and nasopharyngeal carcinoma: the Tunisia experience. *Bull Cancer* 2006; 93: 297-302.
- [11] Tiwawech D, Srivatanakul P, Karalak A, Ishida T. Glutathione S-transferase M1 gene polymorphism in Thai nasopharyngeal carcinoma. *Asian Pac J Cancer Prev* 2005; 6: 270-5.
- [12] Deng ZL, Wei YP, Ma Y. Frequent genetic deletion of detoxifying enzyme GSTM1 and GSTT1 genes in nasopharyngeal carcinoma patients in Guangxi Province, China. *Zhonghua Zhong Liu Za Zhi* 2004; 26: 598-600.
- [13] Cheng YJ, Chien YC, Hildesheim A, Hsu MM, Chen IH, Chuang J, Chang J, Ma YD, Luo CT, Hsu WL, Hsu HH, Huang H, Chang JF, Chen CJ, Yang CS. No association between genetic polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, NAT2, and nasopharyngeal carcinoma in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 179-80.
- [14] Nazar-Stewart V, Vaughan TL, Burt RD, Chen C, Berwick M, Swanson GM. Glutathione S-transferase M1 and susceptibility to nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev* 1999; 8: 547-51.

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- [15] Da SJ, Liang B, Wu HL, Guan LL. Relationship between GSTM1 gene polymorphism and genetic susceptibility in nasopharyngeal carcinoma. *The Practical Journal of Cancer (Chinese)* 2002; 17: 617-619.
- [16] Di Pietro G, Magno LA, Rios-Santos F. Glutathione S-transferases: an overview in cancer research. *Expert Opin Drug Metab Toxicol* 2010; 6: 153-70.
- [17] Lacko M, Oude Ophuis MB, Peters WH, Manni JJ. Genetic polymorphisms of smoking-related carcinogen detoxifying enzymes and head and neck cancer susceptibility. *Anticancer Res* 2009; 29: 753-61.
- [18] Mohr LC, Rodgers JK, Silvestri GA. Glutathione S-transferase M1 polymorphism and the risk of lung cancer. *Anticancer Res* 2003; 23: 2111-24.
- [19] Meng X, Liu Y, Liu B. Glutathione S-transferase M1 null genotype meta-analysis on gastric cancer risk. *Diagn Pathol* 2014; 9: 122.
- [20] He HR, Zhang XX, Sun JY, Hu SS, Ma Y, Dong YL, Lu J. Glutathione S-transferase gene polymorphisms and susceptibility to chronic myeloid leukemia. *Tumour Biol* 2014; 35: 6119-25.
- [21] Liu D, Liu Y, Ran L, Shang H, Li D. GSTT1 and GSTM1 polymorphisms and prostate cancer risk in Asians: a systematic review and meta-analysis. *Tumour Biol* 2013; 34: 2539-44.
- [22] Wang Z, Chen JQ, Liu JL, Qin XG, Huang Y. Polymorphisms in ERCC1, GSTs, TS and MTHFR predict clinical outcomes of gastric cancer patients treated with platinum/5-Fu-based chemotherapy: a systematic review. *BMC Gastroenterol* 2012; 12: 137.
- [23] Su ZY, Shu L, Khor TO, Lee JH, Fuentes F, Kong AN. A perspective on dietary phytochemicals and cancer chemoprevention: oxidative stress, nrf2, and epigenomics. *Top Curr Chem* 2013; 329: 133-62.
- [24] Li J, Long J, Hu Y, Tan A, Guo X, Zhang S. Glutathione S-transferase M1, T1, and P1 polymorphisms and thyroid cancer risk: a meta-analysis. *Cancer Epidemiol* 2012; 36: e333-40.
- [25] Bin Q, Luo J. Role of polymorphisms of GSTM1, GSTT1 and GSTP1 Ile105Val in Hodgkin and non-Hodgkin lymphoma risk: a Human Genome Epidemiology (HuGE) review. *Leuk Lymphoma* 2013; 54: 14-20.
- [26] Liu Y, Xu LZ. Meta-analysis of association between GSTM1 gene polymorphism and cervical cancer. *Asian Pac J Trop Med* 2012; 5: 480-4.
- [27] Zhao ZQ, Guan QK, Yang FY, Zhao P, Zhou B, Chen ZJ. System review and metaanalysis of the relationships between five metabolic gene polymorphisms and colorectal adenoma risk. *Tumour Biol* 2012; 33: 523-35.