Introduction

Nasopharyngeal carcinoma (NPC) is considered as one of the rarer cancer forms globally, the incidence of NPC has been quoted as 84,000 cases diagnosed annually, with an age standardized rate (ASR) of 1.2 per 100,000 for both sexes (Ferlay et al., 2008). NPC represents 24th most frequently diagnosed cancer form globally and 22nd within the developing countries (Jemal et al., 2011). However, the distribution of this cancer is highly skewed, the highest incidence rates seen in China and Southeast Asian region (Cheng et al., 2003). The NPC is most prevalent in Chinese and Malaya population and is a leading cause of death among Cantonese in Southern China (Guo et al., 2003).

The aetiology of NPC is majorly attributed to three risk factors namely infection with Epstein-Barr virus (EBV), genetic predisposition, and environmental pollutants like cigarette smoking, formaldehyde vapours, occupational exposure to products of combustion, and cotton dust (ICMR bulletin, 2003). Besides, Chinese foods such as salt-cured fish and smoke-dried meat which while cooking aerosolize carcinogenic nitrosamines that are subsequently inhaled may also pose a risk of developing NPC (Guo et al., 2003). Despite many individuals being exposed to these risk factors, only a minority of them develop NPC. This evidence suggests that the inter-individual differences in susceptibility can be attributed to the individual’s effectiveness in the detoxification of these chemicals which in turn is ascribed to genetic differences.

Glutathione S-transferases constitute a super-family of ubiquitous, multifunctional enzymes, which play a key role in cellular detoxification (Ye et al., 2004). GSTM1 and GSTT1 are known to be highly polymorphic. This genetic variation may change an individual’s susceptibility to carcinogens and toxins. Homozygous deletions of these genes, referred to as GSTM1 null and GSTT1 null, respectively, result in lack of enzyme activity and therefore have been associated with increased risk for a number of cancers including NPC. Though a number of studies have focussed on GSTM1 and GSTT1 genetic variation with respect to NPC, they have yielded contradictory results. Hence, an evidence based quantitative meta-analysis was conducted to address this controversy. In addition, the risks of developing NPC in relation to GSTM1 and GSTT1 null genotypes were also analysed.

Materials and Methods

Selection of studies

Studies with information on GSTM1 and GSTT1 deficiency and the risk of nasopharyngeal cancer were identified by bibliographic search in two electronic databases; MEDLINE and EMBASE, covering all papers published up to December 2012. The search strategy...
used was conducted using the combination of following search terms ‘GSTM1, GSTT1, nasopharyngeal cancers, polymorphisms, head and neck, neoplasm, carcinoma, glutathione’. A manual review of the references cited in the selected articles was conducted to retrieve additional articles. When several articles were identified for the same population, only the most updated source was referred. The following criteria were used for the selection of articles for the meta-analysis: 1) Articles explicitly describing studies in the association of nasopharyngeal cancer with GSTM1 / GSTT1 polymorphisms; 2) Case-control studies; 3) The nasopharyngeal cancer diagnoses and the sources of cases and controls should be stated and the studies in which individuals were genotyped by PCR technique only; 4) The size of the sample, odds ratios (ORs) and their 95% confidence intervals (CIs) or the information that can help deduce the results should also be stated; 5) Those publications that gave data to allow the calculation of such outcomes were also selected. Accordingly, the exclusion criteria used were: 1) Design and the definition of the experiments were obviously different from those of the selected papers; 2) The sample size, source of cases and controls and other essential information was not presented; 3) Reviews and studies where patients were overlapped.

**Extraction of data**

Data from the selected articles were extracted and entered into STATA, version 10.1 database. The extraction was performed by 2 investigators independently. For conflicting evaluations, an agreement was reached following a discussion. For each study, the author, year of publication, country where the study was carried out, number, race, and gender of patients and controls, control source (hospital based or population based), tumour site, and matching of cases and controls were rigorously tabulated.

**Statistical analysis**

The study-specific crude odds ratio of GSTM1 and GSTT1 null polymorphisms and nasopharyngeal cancers were recalculated for each study along with their corresponding 95% confidence intervals. To take into account the possibility of heterogeneity across the studies, a Chi-square based Q statistic test was performed. If the result of the heterogeneity test was p>0.05 indicating the absence of heterogeneity, ORs were pooled according to fixed – effect model by Mantel-Haenszel method, otherwise, the random effect model by DerSimonian and Laird Method was used (Cooper and Hedges., 1994). To identify publication bias, Egger Regression test was used (Egger et al., 1997).

**Results**

A total of 14 studies regarding GSTM1 and GSTT1 were identified. Based on the inclusion and exclusion criteria, 5 studies were excluded and finally 9 studies pertaining to GSTM1 and 5 studies regarding GSTT1 were selected. A database was established according to the extracted information from each article and has been listed in Tables 1 and 2.

Of the included 9 studies, 7 were carried out in Asian countries, 1 in America and 1 in Europe. General population was used as source of controls in 3 studies whereas hospital patients were controls in 2 studies and 4 did not mention the source of controls. In 3 studies, the controls were age and sex-matched with cases and in 2 studies, controls were matched with cases according to the geographical location. In the other 4 studies, matching was not mentioned.

**Population frequencies**

For GSTM1 polymorphism, the data from the 9 included case-control studies showed 1294 cases and 1967 controls, of which 747 cases and 956 controls had the null genotype. The frequencies of GSTM1 deficiencies ranged from 51.1-64.1% among the cases and 33-55.6% among the controls.

For GSTT1 polymorphism, total study subjects were

| SL. No. | Author (Year) | Country | Control Source | Matching of controls | Cases | %GSTM1 | Controls | %GSTM1 | OR (95% CI) |
|--------|---------------|---------|----------------|----------------------|-------|--------|----------|--------|-------------|
| 1      | Nazar-Stewart (1999) | USA | Population healthy | Geographical area, age and sex | 45/83 | 54.2 | 63/142 | 44.4 | 1.48 (0.86, 2.56) |
| 2      | Da (2002) | China | Not available | None | 48/80 | 60 | 36/80 | 45 | 1.83 (0.98, 3.43) |
| 3      | Cheng (2003) | Taiwan | Population healthy | Age, sex and residence | 173/314 | 55.1 | 169/337 | 50.1 | 1.22 (0.90, 1.66) |
| 4      | Deng (2004) | China | Not available | None | 56/91 | 61.5 | 64/135 | 47.4 | 1.77 (1.03, 3.05) |
| 5      | Liao (2005) | China | Not available | None | 50/80 | 62.5 | 32/72 | 44.4 | 2.08 (1.19, 3.59) |
| 6      | Tiwawech (2005) | Thailand | Hospital | Age | 50/78 | 64.1 | 74/145 | 51 | 1.71 (0.97, 3.02) |
| 7      | Bendjemana (2006) | France | Not available | None | 24/45 | 51.1 | 33/100 | 33 | 2.32 (1.13, 4.76) |
| 8      | Guo (2008) | China | Population healthy | Geographic region | 204/341 | 59.8 | 328/590 | 55.6 | 1.19 (0.91, 1.56) |
| 9      | Jiang (2011) | China | Hospital | Age and sex | 97/182 | 53.3 | 157/366 | 42.9 | 1.52 (1.05, 2.17) |

| SL. No. | Author (Year) | Country | Control Source | Matching of controls | Cases | %GSTM1 | Controls | %GSTM1 | OR (95% CI) |
|--------|---------------|---------|----------------|----------------------|-------|--------|----------|--------|-------------|
| 1      | Cheng (2003) | Taiwan | Population healthy | Age, sex and residence | 160/316 | 50.6 | 174/336 | 51.8 | 0.96 (0.7, 1.3) |
| 2      | Deng (2004) | China | Not available | None | 54/91 | 59.3 | 55/135 | 40.7 | 2.12 (1.24, 3.65) |
| 3      | Bendjemana (2006) | France | Not available | None | 9/45 | 20 | 16/100 | 15.5 | 1.31 (0.53, 3.25) |
| 4      | Guo (2008) | China | Population healthy | Geographic region | 164/338 | 48.5 | 269/585 | 46 | 1.11 (0.85, 1.45) |
| 5      | Jiang (2011) | China | Hospital | Age and sex | 120/182 | 65.0 | 180/366 | 49.2 | 2.00 (1.38, 2.89) |
Asian Pacific Journal of Cancer Prevention, Vol 14, 2013

Meta-analysis of GSTM1 and GSTT1 Polymorphisms and Risk of Nasopharyngeal Cancer

972 cases and 1522 controls of 48.9% and 40.5% of cases and controls respectively had null genotype.

Test of heterogeneity
The analysis of heterogeneity for all the 9 studies of GSTM1 gave the Chi square Q value of 7.57 with 8 degree of freedom (df) and p=0.477 indicating lack of heterogeneity and hence the fixed effect model was used. Similarly, the association of GSTT1 null genotype and NPC risk, the Chi square Q value was 23.6 with 4 df and p=0.100 also suggesting the absence of heterogeneity. Therefore the fixed effect model was used for the analysis.

Meta-analysis
The overall OR for GSTM1 null genotype from the included 9 case-control studies was 1.43 (95%CI 1.24-1.66) and the test for overall effect Z value was 4.95 (p<0.05) using the fixed effect model (Figure 1). The results indicate that GSTM1 null status significantly increases the susceptibility to NPC.

With regard to GSTT1 null genotype, the overall OR for the 5 studies was 1.28 (95%CI 1.09-1.51) and the Z value was 2.95 (p<0.05) using fixed effect model (Figure 2). The data implied that GSTT1 null genotype also has significant association to NPC.

Publication bias
For the diagnosis of publication bias, the Egger’s test, when applied, showed an evidence of publication bias (p<0.05) for GSTM1 polymorphism. However, for GSTT1 polymorphism, p value of Egger’s test was more than 0.05 (p=0.415) Thus, the results above suggested that publication bias was not evident in this meta-analysis.

Discussion
In the present meta-analysis, risk of development of nasopharyngeal cancer in individuals with GSTM1 null and GSTT1 null genotype were tabulated and analyzed statistically. The outcome of this analysis showed GSTM1 null status significantly increases the susceptibility to NPC which was also true with the GSTT1 null status demonstrating significant association with NPC development.

Nasopharyngeal cancer (NPC) is an aggressive tumour with a high potential for nodal and distant metastasis. This tumour is relatively rare in most areas of the world but common in Southeast Asia (Lin et al., 2002). The risk factors include infection with Epstein-Barr virus (EBV), genetic predisposition, and environmental pollutants. However development of such a tumour is still not clear, presently hypothesised to metabolic activation of carcinogenic compounds by Phase I enzymes such as cytochrome P4502E1 (CYP2E1) to yield carcinogens such as an epoxide form of benzo(a)pyrene and aflatoxin that can further interact with host DNA. The epoxide thus formed may be detoxified by phase II enzymes, particularly GSTs, resulting in cancer inhibition. Therefore, NPC susceptibility to carcinogens is dependent on the metabolic balance between phase I and phase II enzymes customized to individual which meant that persons who carried genotypes for high activity of phase I enzymes and low activity of phase II enzymes were at high risk of developing NPC (Hayashi et al., 1991; Kihara et al., 1995). Further, human papilloma virus (HPV16) infection has been found to reduce GSTM1 enzyme activity and GSTM1 mRNA levels in human cervical keratinocytes in culture (Chen and Nirunsuksi, 1999). Hence, the present meta-analysis found that individuals with GSTM1 null and GSTT1 null genotype showed significant increase in the susceptibility to NPC with pooled OR being 1.43 and 1.28, respectively.

Over the past decades, a large number of meta-analyses have been done to investigate the association between GSTM1 and GSTT1 polymorphisms and various...
cancers. These include meta-analyses that suggest GSTM1 deficiency increases the risk of head and neck cancer (Hashibe et al., 2003; Ye et al., 2004; Tripathy and Roy, 2006), cervical cancers (Gao et al., 2011; Liu and Xu, 2012) and oral cancer (Zhuo et al., 2009). However, a number of meta-analyses indicated no marked association of GSTM1 null mutations with hepatocellular cancer (White et al., 2008), brain tumours (Sima et al., 2012), colorectal cancers (Zhao et al., 2012), ovarian cancers (Economopoulos et al., 2010), melanoma (Nie et al., 2011). In this study, the results indicate that null GSTM1 genotype might increase susceptibility to NPC which was in accordance with the evidence-based meta-analysis pertaining to GSTM1 and GSTT1 polymorphisms on nasopharyngeal cancer by Zhuo et al. (2009). Null genotype of GSTT1 has been suggested to associate with risks of number of cancers. Marked association of GSTT1 deletion with lung cancer (Hosgood et al., 2007), gastric cancer (Saadat, 2006), leukaemia (Ye and Song, 2005), cervical cancers (Gao et al., 2011), breast cancer (Chen et al., 2011), bladder cancer (Gong et al., 2012) and head and neck cancer (Hashibe et al., 2003) has been demonstrated. In this study, the results indicate that null GSTT1 genotype might increase susceptibility to NPC which was not in line with evidence-based meta-analysis of Zhuo et al. (2009) pertaining to GSTM1 and GSTT1 polymorphisms on nasopharyngeal cancer as the latter indicated no association.

In the present meta-analysis, no evidence of heterogeneity is observed across studies. However, there existed publication bias which was evident in Egger’s test in relation to GSTM1 null genotype. In theory, publication bias could affect the results of the pooled analysis (Vogl et al., 2004). Publication bias can occur if studies with insignificant associations or null results are less likely to be published than studies with significant results, and subsequently the former will not be included in the pooled analysis. This would lead to biased results and hence the results must be interpreted with caution due to the fairly low power when applied to a few meta-analytically investigated studies. On the other hand, it a known fact that published studies are generally of greater quality than the unpublished ones (Boccia et al., 2006). Hence, if high-quality scored studies are more likely to yield valid information than low-quality studies, we can conclude that, on the basis of the currently available data, an additional slight risk of nasopharyngeal cancer for GSTM1 null individuals may exist.

Even though pooling and analyzing individual data from original studies has several advantages; the results of the present meta-analysis should be interpreted in light of a few limitations. Firstly, the study was a meta-analysis of case control studies, some of which were hospital-based; thus, selection bias might exist. Secondly, the present study was based on published articles only; therefore, publication bias exists, as suggested from the Egger’s test in relation to GSTM1 null genotype and nasopharyngeal cancer.

In conclusion, the meta-analysis suggests that GSTM1 null and GSTT1 null genotype may be associated with NPC susceptibility and they may be a potential risk factor for NPC. However, future studies with larger study populations and more rigorous designs are needed to investigate the gene effects and the potential effect of environmental factors on nasopharyngeal cancer.

References

Bendjelma K, Abdennebi M, Gara S, et al (2006). Genetic polymorphism of glutathion-S transferases and N-acetyl transferases 2 and nasopharyngeal carcinoma: the Tunisia experience. Bull Cancer, 93, 297-302.

Boccia S, La Torre G, Gianfagna F, Mannocci A, Ricciardi G (2006). Glutathione S-transferase T1 status and gastric cancer risk: a meta-analysis of the literature. Mutagenesis, 21, 115-23.

Chen C, Nirunsumkiri W (1999). Decreased expression of glutathione S-transferase M1 in HPV16-transfected human cervical keratinocytes in culture. Carcinogenesis, 20, 699-703.

Chen XX, Zhao RP, Qiu LX, et al (2011). Glutathione S-transferase T1 polymorphism is associated with breast cancer susceptibility. Cytokine, 56, 477-80.

Cheng YJ, Chien YC, Hildesheim A, et al (2003). No association between genetic polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, NAT2, and nasopharyngeal carcinoma in Taiwan. Cancer Epidemiol Biomarkers Prev, 12, 179-80.

Cooper HM, Hedges LV (1994). The Handbook of Research Synthesis. Vol 236. Russell Sage Foundation, New York.

Da SJ, Liang B, Wu HL, Guan LL (2002). Relationship between GSTM1 gene polymorphism and genetic susceptibility in nasopharyngeal carcinoma. Practical J Cancer (Chinese), 17, 617-9.

Deng ZL, Wei YP, Ma Y (2004). Frequent genetic deletion of detoxifying enzyme GSTM1 and GSTT1 genes in nasopharyngeal carcinoma patients in Guangxi Province, China. Zhonghua. Zhong Liu Za Zhi, 26, 598-600.

Economopoulos KP, Sergentanis TN, Vlahos NF (2010). Glutathione S-transferase M1, T1, and P1 polymorphisms and ovarian cancer risk: a meta-analysis. Int J Gynecol Cancer, 20, 732-7.

Egger M, Davey SG, Schneider M, Ciner (1997). Bias in meta-analysis detected by a simple, graphical test. BMJ, 315, 629-34.

Epidemiological and etiological factors associated with nasopharyngeal carcinoma (2003). ICMR bulletin, 33, 9.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer, 127, 2893-917.

Gao L-B, Pan X-M, Li L-J, et al (2011). Null Genotypes of GSTM1 and GSTT1 Contribute to Risk of Cervical Neoplasia: An Evidence-Based Meta-Analysis. PLoS ONE, 6, 20157.

Gong M, Dong W, An R (2012). Glutathione S-transferase T1 polymorphism contributes to bladder cancer risk: a meta-analysis involving 50 studies. DNA Cell Biol, 31, 1187-97.

Guo X, O’Brien SJ, Zeng Y, Nelson GW, Winkler CA (2003). GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. Cancer Epidemiol Biomarkers Prev, 17, 1760-3.

Hashibe M, Brennan P, Strange RC et al (2003). Meta- and pooled analyses of GSTM1, GSTT1, GSTP1 and CYP1A1 genotypes and risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev, 12, 1509-17.

Hayashi S, Watanabe J, Kawajiri K (1991). Genetic polymorphisms in the 5’-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J Biochem, 110, 559-65.
Meta-analysis of GSTM1 and GSTT1 Polymorphisms and Risk of Nasopharyngeal Cancer

Zhuo X, Cai L, Xiang Z, Li Q, Zhang X (2009). GSTM1 and GSTT1 polymorphisms and nasopharyngeal cancer risk: an evidence-based meta-analysis. J Experimental & Clinical Cancer Res, 28, 46.

Hosgood HD, Berndt SI, Lan Q (2007). GST genotypes and lung cancer susceptibility in Asian populations with indoor air pollution exposures: a meta-analysis. Mutat Res, 636, 134-43.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. CA Cancer J Clin, 61, 69-90.

Jiang Y, Li N, Dong P, et al (2011). Polymorphisms in GSTM1, GSTT1 and GSTP1 and nasopharyngeal cancer in the East of China: a case-control study. Asian Pac J Cancer Prev, 12, 3097-100.

Kihara M, Kihara M, Noda K (1995). Risk of smoking for squamous and small-cell carcinomas of the lung modulated by combinations of CYP1A1 and GSTM1 gene polymorphisms in a Japanese population. Carcinogenesis, 16, 2331-6.

Liao ZL, Deng ZL, Wei YP, et al (2005). Associations of GSTM1 and GSTT1 polymorphisms with nasopharyngeal cancer risk. J Guangxi Medical University (Chinese), 22, 372-4.

Lin CL, Lo WF, Lee TH, et al (2002). Immunization with Epstein-Barr Virus (EBV) peptide-pulsed dendritic cells induces functional D8+ T-cell immunity and may lead to tumor regression in patients with EBV-positive nasopharyngeal carcinoma. Cancer Res, 62, 6952-8.

Liu Y, Xu LZ (2012). Meta-analysis of association between GSTM1 gene polymorphism and cervical cancer. Asian Pac J Trop Med, 5, 480-4.

Nazar-Stewart V, Vaughan TL, Burt RD, et al (1999). Glutathione S-transferase M1 and susceptibility to nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev, 8, 547-51.

Nie F, Chen Z, Cao C, Cen Y (2011). Absence of association between GSTM1 and GSTT1 polymorphisms and melanoma susceptibility: a meta-analysis. DNA Cell Biol, 30, 783-8.

Saadat M (2006). Genetic polymorphisms of glutathione S-transferase T1 (GSTT1) and susceptibility to gastric cancer: a meta-analysis. Cancer Sci, 97, 505-9.

Sima X-T, Zhong W-Y, Liu J-G, You C (2012). Lack of association between GSTM1 and GSTT1 polymorphisms and brain tumor risk. Asian Pac J Cancer Prev, 13, 325-8.

Tiwawech D, Srivatanakul P, Karalak A, Ishida T (2005). Glutathione S-transferase M1 gene polymorphism in Thai nasopharyngeal carcinoma. Asian Pac J Cancer Prev, 6, 270-5.

Tripathy CB, Roy N (2006). Meta-analysis of glutathione S-transferase M1 genotype and risk toward head and neck cancer. Head Neck, 28, 217-24.

Vogl FD, Taioli E, Maugard C et al (2004). Glutathione S-transferases M1, T1, and P1 and breast cancer: a pooled analysis. Cancer Epidemiol Biomarkers Prev, 13, 1473-9.

White DL, Li D, Nurgalieva Z, El-Serag HB (2008). Genetic variants of glutathione S-transferase as possible risk factors for hepatocellular carcinoma: a HuGE systematic review and metaanalysis. Am J Epidemiol, 167, 377-89.

Ye Z, Song H, Guo Y (2004). Glutathione S-transferase M1, T1 status and the risk of head and neck cancer: a meta-analysis. J Med Genet, 41, 360-5.

Ye Z, Song H (2005). Glutathione S-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukaemia: a systematic review and meta-analysis. Eur J Cancer, 41, 980-9.

Zhao ZQ, Guan QK, Yang FY, et al (2012). System review and metaanalysis of the relationships between five metabolic gene polymorphisms and colorectal adenoma risk. Tumour Biol, 33, 523-35.

Zhuo W, Wang Y, Zhuo X, et al (2009). CYP1A1 and GSTM1 polymorphisms and oral cancer risk: association studies via evidence-based meta-analyses. Cancer Investigation, 27, 86-95.