Correlation of polymorphism of the coding region of glutathione S-transferase M1 to susceptibility of nasopharyngeal carcinoma in South China population

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Background and Objective: Glutathione S-transferase M1 (GSTM1) deficiency may increase the risk of nasopharyngeal carcinoma (NPC). This study was to evaluate the correlation of the single nucleotide polymorphism (SNP) in the coding region of GSTM1 gene to NPC susceptibility in southern China population. Methods: In total 239 NPC patients and 286 age-matched healthy controls were entered into the study. Among them, 225 out of 239 NPC patients and 273 out of 286 controls were used for statistical analysis. SNP screening of all exons, relevant intron-exon boundaries, and the promoter region of GSTM1, in total 4739bp, was performed by PCR direct sequencing. The loci T1270533G and C1256088C were selected for the case-control study using the tetra-Primer ARMS-PCR, as well as the sequencing method. Results: In total 29 SNPs of GSTM1 were identified by sequencing. Missense mutation occurred in the polymorphic loci of T1270533G and C1256088C. However, no evident relationships between the variants of T1270533G and clinical phenotypes of NPC were observed in the NPC group and healthy control group (OR = 0.170, 95%CI = 0.95–0.306 for homozygote TT). The deletion frequency of C1256088C was 45% (45/100) for NPC patients and 42% (42/100) for controls. Conclusions: The polymorphism of T1270533G does not affect the detoxification function of GSTM1. The T1270533G locus has no apparent association with genetic susceptibility to NPC in the southern China population. The loss rate of C1256088C is high in this study.

Nasopharyngeal carcinoma (NPC) is a malignant tumor of the head and neck with a relatively high incidence in South China and Southeast Asia. Because NPC seldom happens in other areas of the world, it is also called “Guangdong Cancer”. Epidemiologic evidence shows that NPC is associated with ethnicity and family aggregation. The NPC incidences of people who migrate away from high risk areas are similar to those of people who live in those areas, and 10% of the NPC patients have a family history of cancer.1 It is suggested that hereditary susceptibility or genetic predisposition may be a significant factor of NPC. Currently, although Epstein-Barr virus (EBV) infection and chemical carcinogen are considered to play important roles in the process of NPC,2,3 the molecular mechanism of NPC remains unclear.

Glutathione S-transferase (GST) belongs to the multifunctional poly-2-protein family, and is mainly responsible for catalyzing the interaction between reduced glutathione hormone (GSH) and electrophile substances. GST deprives the active DNA binding character of GSTM1. The T1270533G locus has no apparent association with genetic susceptibility to NPC in the southern China population. The loss rate of C1256088C is high in this study.
polymorphism (SNP) loci. The polymorphism of T1270533G in the coding region was genotyped to explore the correlation of the locus to NPC susceptibility in Guangdong region.

Materials and Methods

Patients and blood samples. Peripheral blood samples were collected from 239 histopathologically confirmed primary NPC patients (163 males and 76 females, mean age 46.9 years) who did not undergo radiotherapy and chemotherapy at the Nanfang Hospital, Jiangmen Central Hospital, and Guangzhou Tumor Hospital in Guangdong province, China. Among them, four cases were classified as keratinizing squamous cell carcinomas, 34 as non-keratinizing differentiated carcinomas, and 201 as undifferentiated carcinomas. Two hundred and eighty-six healthy blood donors (151 males and 135 females, mean age 47.2 years) were used as control. More men were entered into the NPC group than in the control group (68.2% vs. 52.8%, \( \chi^2 = 12.2, p = 0.01 \)), and the age distribution in the two groups was not significantly different.

SNP discovery. The study protocol was described previously. In brief, SNP screening of all exons, relevant intron-exon boundaries, and the promoter region of GSTM1 (GenBank accession number NT_029860) was performed by PCR direct sequencing. The primers for the target sequences were designed by the special software Primer 3.0 (http://www-genome.wi.mit.edu). DNA samples from 27 individuals were amplified, purified, and re-sequenced. SNPs candidates were identified by the PolyPhred program (http://droog.mbt.washington.edu/PolyPhred.html). Linkage disequilibrium (LD) analysis was performed for these SNPs whose frequency was larger than 0.01.

Genotyping of the coding region of polymorphic loci. Genomic DNA was isolated from peripheral blood leukocytes using phenol/chloroform or a DNA Extraction Kit. The coding region of T1270533G polymorphic locus was selected for genotyping using the tetra-Primer amplification refractory mutation system PCR (ARMS-PCR) method or PCR direct sequencing analysis (Table 1).

The polymorphic locus of C1256088C was genotyped by PCR direct sequencing. The primers 5’-GGG ACC CTA AGA AGC TGT GTG-3’ and 5’-CGA AGG ATA GTG GGT AGC TGA-3’ were used to amplify and sequence the target region. PCR conditions identified in 12 pairs of sites (p < 0.05). Although the polymorphic loci were not concentrated, close linkage was still identified in 12 pairs of sites (p < 0.05).

The tetra-Primer ARMS-PCR method was used to evaluate T1270533G polymorphism and was applied to genotype the locus T1270533G T→G successfully. Genotypes determined by this method were consistent with those determined by PCR sequencing. The lengths of PCR products were 206bp (GG), 226bp (TT) and 377bp (GT), respectively.

In the study, 225 out of 239 NPC patients and 273 out of 286 controls were used for statistical analysis. The data of 13 controls and 14 patients were not complete. The frequency distribution of GSTM1 genotype and its association with NPC risk are shown in Table 2.

Human β-globin was used as an internal control to rule out the failure of PCR. The primer sequences of β-globin were 5’-AAC TTC ATC CAC GTT CAC C-3’ and 5’-GAA GAG CCA AGG ACA GGT AC-3’.

Statistical analysis. Odds ratios (OR) was estimated by the odds ratio method to determine the correlation between GSTM1 genotype and NPC development. Additionally, the OR was used to estimate the association of the genetic inheritance pattern of GSTM1 gene with the NPC phenotype. The 95% confidence interval (95% CI) was calculated to determine the statistical significance of the results. The OR and 95%CI were calculated using statistical software SPSS 11.0.

Results

A total length of approximately 4739bp of the target region of GSTM1 was re-sequenced in 27 samples, among which 29 SNPs were discovered. There were 13 SNPs presenting high LD among 18 SNPs, whose frequency was larger than 0.01 (p < 0.001). Although the polymorphic loci were not concentrated, close linkage was still identified in 12 pairs of sites (p < 0.05).

The tetra-Primer ARMS-PCR method was used to evaluate T1270533G polymorphism and was applied to genotype the locus T1270533G T→G successfully. Genotypes determined by this method were consistent with those determined by PCR sequencing. The lengths of PCR products were 206bp (GG), 226bp (TT) and 377bp (GT), respectively.

In the study, 225 out of 239 NPC patients and 273 out of 286 controls were used for statistical analysis. The data of 13 controls and 14 patients were not complete. The frequency distribution of GSTM1 genotype and its association with NPC risk are shown in Table 2. The OR of genotype TT and GT was 0.170 and 0.605, respectively. 95% CI for homozygote TT was 0.095–0.306, and 95% CI for heterozygote GT was 0.168–2.178 (Table 2).

The null genotype frequency of polymorphic locus C1256088C was higher in NPC patients (45%, 45/100) than in controls (42%, 42/100).

Discussion

It is known that the development of tumor is associated with heredity and environment. The primary reason of tumor development is due to differences in metabolism activation and receptors, which are affected by inheritance and environmental agents. The process of chemical carcinogenesis includes initiation, induction and evolving, and formation of the tumors. When exogenous chemical substances are absorbed into organs via different ways, a series of chemical changes occur to form some breakdown products of the substance or derivates. This process of biotransformation involves two critical enzyme systems: cytochrome P450 (CYP450) and glutathione S-transferase (GST). Many Phase I and Phase II enzymes are
involved in the metabolism of carcinogens. Phase I enzymes activate carcinogens and phase II enzymes, such as GSTM1 (which is present in all human tissues), detoxify active epoxide carcinogens formed from benzo(a)pyrene and other procarcinogens found in cigarette smoke. Therefore, the cancer incidence and category are usually dependent on the polymorphism of phase I and phase II metabolic enzymes.5 Gene mutation may happen in biological cells during the process of duplication, and may induce polymorphism and different metabolic activation induced by exogenous chemicals. Those changes could cause different susceptibility of the body towards various tumors.6

The GSTM1 null genotype exists in all races, of which the frequency ranges from 23–48% in African population, from 33–63% in Asian population, and from 39–62% in European population.10,12,13 In our study, we detected a basic exchange [a T to A substitution replacing serine (Ser) with threonine (Thr)] on the polymorphic locus C1256088C, which caused a missense mutation. The frequency of C1256088C null genotype was 45% in the NPC group and 42% in the control group, similar to previous reports. GSTM1 genetic polymorphism and various carcinogen exposures, as well as the pathogenetic multiplicity genotype determine the individual risk toward cancer.14 Epidemiologic evidence suggests that differences in environment or lifestyle reflected by geographical variation may result in genetic variation. Loss of homozygosity or heterozygosity (LOH) in GSTM1 gene could cause deficiency of the antibiotic function, thus increase the risk of cancer. In this regards, individuals with GSTM1 and GSTT1 null genotypes are more prone to cancer caused by chemical carcinogens.15,16 GSTM1 deficiency is reported to likely increase the risk of lung cancer, especially in the smoking population.17 GSTM1 gene offers protection for cytogenetic lung injury caused by chemicals and for the formation of DNA bulky adducts. Loss of GSTM1 also increases the risk of colorectal cancer, bladder cancer, breast cancer and head-neck tumors, squamous cell carcinoma in particular, whose null gene frequency (-/-) is remarkably elevated in the smoking population.18,19 Jahnke et al.20 find that susceptibility of throat squamous cell carcinoma is in relation with GSTM1(-/-) genotype in Caucasian, Germany. In 1999, Nazar-Stewart et al.21 reported that GSTM1 deficiency may increase the risk of NPC (OR = 1.9). In comparison to drinking, long-term smoking does more harm to human health and is also correlated to age and gender to some extent. There are more reports on the relationship between GSTM1 deletion or mutation and NPC.22-24

The polymorphic locus of T1270533G was discovered for the first time in this study. The frequency of the rare allele was 22.2%. The second genetic code of T1270533G in the 96th amino acid occurred a basic exchange and presented a missense mutation [a G to T substitution replacing arginine (Arg) with leucine (Leu)]. Arg and Leu are both aliphatic amino acids. Arg is a basic amino acid with positive charges, and Leu is a nonpolar amino acid. It was noticed that genotype variation at T1270533G did not have any apparent association with the clinical phenotype of NPC, implying that even though T1270533G variation occurs in the coding area of GSTM1, it does not influence the detoxication function of GSTM1, thus the variation has no obvious correlation to NPC susceptibility in Guangdong areas. This was further confirmed by different genotyping screening methods (the tetra-Primer ARMS-PCR and direct sequencing). Our finding is contradictory to the accepted knowledge that the protein function is affected if the genetic codes present a missense mutation. Further studies with a large sample size are needed to confirm this discovery.

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