Original Article

Human GSTs Polymorphisms in the Hakka Population of South China and Their Associations with Family History of Several Chronic Diseases*

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Abstract

Objective To investigate the associations of genetic polymorphisms in GSTs genes of the Hakka population of south China with family histories of certain chronic diseases.

Methods Five hundred and thirty-nine healthy Hakka natives of Meizhou city of Guangdong province in south China were involved. The genotypes of *GSTM1, GSTT1, GSTP1, GSTM3,* and *GSTA1* were determined using PCR and restriction fragment length polymorphism analysis. The observed polymorphisms were analyzed by Chi-square and Hardy-Weinberg equilibrium tests. Logistic regression analysis was used to determine the associations of the distributions of *GST* genotypes with family history of certain chronic diseases.

Results The distributions of polymorphisms in GSTP1, GSTM3, and GSTA1 conformed to the Hardy-Weinberg equilibrium. Compared to the Cantonese, the Hakka had a lower distribution of the GSTM3 deletion genotype (3.15% vs. 11.9%). A weak association was observed between the GSTM1 genetic polymorphism and family history of hypertension. Alcohol drinkers had a higher frequency of the null-*GSTM1* genotype, while smokers had a higher frequency of a variant GSTP1 genotype.

Conclusion The results suggest that the Hakka is a special and distinctive Han Chinese ethnic group with different GSTs genetic polymorphisms. Smoking and drinking might be related to the distribution of *GST* genotypes.

Key words: Genetic polymorphism; Glutathione-S- transferases; The Hakka

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INTRODUCTION

The Hakka is a distinctive Han Chinese ethnic group speaking the unique Hakka dialect, and possessing its own civilization. The Hakka originated from ancient Han residents in middle China and migrated to the south because of war, starvation, and poor living conditions. After five long-distance migrations from the Eastern Jin Dynasty to the late Qing Dynasty, they settled in several global regions[1]. In China, they live mainly in south Jiangxi, west Fujian, and east Guangdong provinces. These three areas are the primary residence of the Hakka, particularly northeast Guangdong. In Meizhou city, located in northeast Guangdong, over 95% of the 4 850 000 inhabitants

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are Hakka, and the dialect they speak is a particular Chinese dialect, which has been certified as the standardized Hakka dialect[2].

The human glutathione S-transferases (*GSTs*) belong to a supergene family of detoxification enzymes. To date, *GSTs* have been grouped into eight classes: alpha, mu, theta, pi, zeta, sigma, kappa, and omega[3], and most of them contain genetic polymorphisms. GSTs are involved in the metabolism of drugs and detoxification of a wide range of xenobiotic compounds, including carcinogens. Inherited genetic polymorphisms in the xenobiotic metabolizing enzymes play an important role in individual susceptibility to various diseases. Recent data have shown that *GST* genetic polymorphisms are closely correlated to drug effects, several cancers, and the outcomes of therapy for asthma, coronary heart disease, and atherosclerosis[4-9].

A wealth of research on *GST* genetic polymorphisms has been carried out recently, showing that the distributions of *GST* polymorphisms vary among different ethnic, national, and regional populations[10-14]. Although many studies on *GST* polymorphisms in north and middle China have been reported, polymorphisms in the Hakka population of south China have not yet been studied. Therefore, this study investigated the genetic polymorphisms of *GSTT1, GSTM1, GSTM3, GSTP*, and *GSTA1* in the Hakka of Guangdong province and their possible associations with family histories of several chronic diseases were analyzed.

MATERIALS AND METHODS

Study Population

Five hundred and thirty-nine participants, 328 males and 211 females, were randomly selected by a multi-stage stratified random sampling method from five towns (Sanjiao, Chengjiang, Chengdong, Chengbei, and Yanyang) in Meizhou in northeast Guangong province from May to July of 2003, where the majority of residents are Hakka. All participants were healthy residents without ties of kindred to each other and most of them were married and had senior high schooling or above. The mean age of the participants was 38.4±13.5 years (ranging between 15 and 80 years). Each participant independently completed a structured questionnaire concerning drinking, smoking, dietary habits, and family history of special and chronic diseases, including esophageal cancer, nasopharyngeal cancer, favism, and hypertension. The involvement of these chronic diseases was determined by medical certification. Alcohol drinking was defined as drinking at least once a week and smoking was defined as smoking one cigarette a day for at least six months.

Genotyping

Genomic DNA was extracted from peripheral blood by a classic phenol/chloroform extraction method. All polymerase chain reactions (PCR) were in a volume of 20 μL containing target DNA, 0.2 mmoL/L $d(NTP)_4$, 10× PCR buffer, 0.1-0.4 moL/L primers (Shanghai Shenggong Ltd. Co.), 1.5 mmoL/L $MgCL_2$, and 1U Taq DNA polymerase (American Promega Ltd. Co., Shanghai). Different genetic *GST* polymorphisms required different PCR conditions (Table 1), and gene-specific *GST* primers were designed as previously described[15-17] (Table 2). *GSTM1* and *GSTT1* had a null-allele variant in which the entire gene was absent (null genotype), so the absence or presence of *GSTM1* or *GSTT1* was detected by co-amplifying *GSTM1* or *GSTT1* and the β-globin gene. The results were visualized on 2% agarose gels stained with ethidium bromide (Figure1 and Figure 2). For *GSTP1*, the wild-type *GSTP1*A* and variant *GSTP1*G* alleles were detected by PCR-RFLP (restriction fragment length polymorphism), with PCR products digested with 5U *BsmA1* (Shanghai Shenggong Ltd. Co.) for 16 h at 37 °C in 20 l after PCR amplification. Fragments were visualized on 4% agarose gels stained with ethidium bromide (Figure 3 and Figure 4). For *GSTA1*, the same method was used to determine wild-type *GSTA1*A* and variant *GSTA1*B* alleles, but digestion was with *Ear1* (New England Biolabs) for RFLP detection (Figure5). For *GSTM3*, the *GSTM3*B* allele had a 3 bp deletion in intron 6 that was absent in the GSTM3*A allele; therefore, PCR products were subjected to electrophoresis on a 12% polyacrylamide gel to distinguish the genotypes (Figure 6). DNA samples were kept at 4 °C for short-term storage and at -80 °C for longer storage.

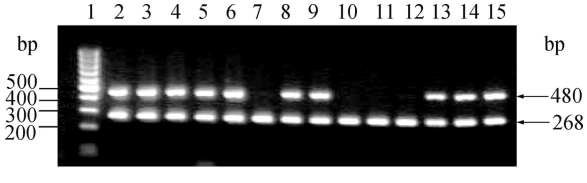

Figure 1. Co-amplification products of *GSTM1* (480 bp) and *β-Globin* (268 bp), Lanes 2, 3, 4, 5, 6, 8, 9, 13-15:*GSTM1*+ samples. lane 7, 10-12:*GSTM1*-null genotype samples. lane 1: 100 bp markers.

Table 1. PCR Conditions for *GST* Genes

Gene	PCR Conditions					
	Initial Denaturation	Denaturation	Annealing	Elongation	Cycles	Final Elongation
GSTT1 +ß-Globin	95 °C 10min	95 °C 45min	59 °C 45min	72 °C 45min	37	72 °C 10min
GSTM1 +ß-Globin	95 °C 10min	95 °C 45min	58 °C 45min	72 °C 45min	37	72 °C 10min
GSTP1	95 °C 5min	95 °C 45min	60 °C 45min	72 °C 45min	45	72 °C 10min
GSTM3	95 °C 10min	95 °C 50min	62 °C 45min	72 °C 45min	37	72 °C 10min
GSTA1	95 °C 5min	95 °C 45min	52 °C 45min	72 °C 45min	45	72 °C 10min

Table 2. Primes Used to Identify Polymorphic Gene Frequencies of *GSTs*

Gene	Polymorphism	Primers	Products (bp)
GSTT1	gene deletion	5'TCT CCT TAC TGG TCC TCA CAT CTC3' 5'TCA CCG GAT CAT GGC CAG CA3'	480
GSTM1	gene deletion	5'GAA CTC CCT GAA AAG CTA AAG C3' 5'GTT GGG CTC AAA TAT ACG GTG G3'	215
GSTP1	codon +313 A>G	5'ACC CCA GGG CTC TAT GGG AA3' 5'TGA GGG CAC AAG AAG CCC CT3'	176
GSTM3	3 bp deletion in intron 6	5'AAG GGA AGA AGG ATG GAA AAG GGG3' 5'ATG ATG AGG AGT CTG GAT TCG TAG3'	79/76
GSTA1	codon -69 C>T	5'TGT TGA TTG TTT GCC TGA AAT T3' 5'GTT AAA CGC TGT CAC CGT CCT3'	480
ß-Globin[*]	—	5'GAA GAG CCA AGG ACA GGT AC3' 5'CAA CTT CAT CCA CGT TCA CC3'	260

Note. [*] *ß-Globin* was a control that was co-amplified in *GSTT1* or *GSTM1* PCR reactions.

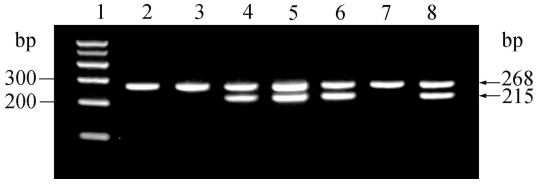

Figure 2. Co-amplification products of *GSTT1* (215 bp) and *ß-Globin* (268 bp), Lanes 2,3, and 7 :*GSTT1*-null genotype samples. Lanes 4, 5, 6, and 8: *GSTT1*+ samples. lane 1: 100 bp markers.

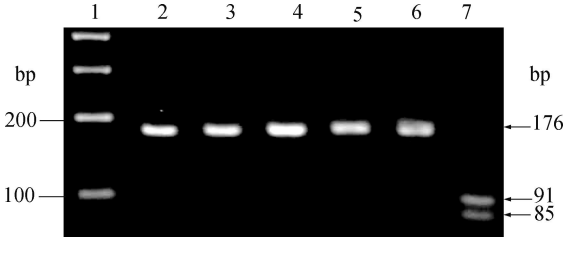

Figure 3. RFLP fragments of *GSTP1* after digestion of PCR products. Amplified products (176 bp) were cut into two fragments(91 bp and 85 bp) by *BsmA1.* Lane 2, 3, 4, 5, 6: homozygous *GSTP1*A/*A* genotype samples. Lane 7: homozygous *GSTP1*G/*G* genotype sample. lane 1: 100 bp markers.

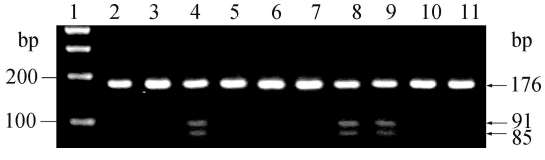

Figure 4. RFLP fragments of *GSTP1* after digestion of PCR products. Amplified products (176 bp) were cut into two fragments (91 bp and 85 bp) by *BsmA1*. Lanes 2, 3, 5,6,7, 10,11: homozygous *GSTP1*A/*A* genotype samples. Lanes 4, 8, 9: heterozygous *GSTP1*A/*G* genotype samples. lane 1: 100 bp markers.

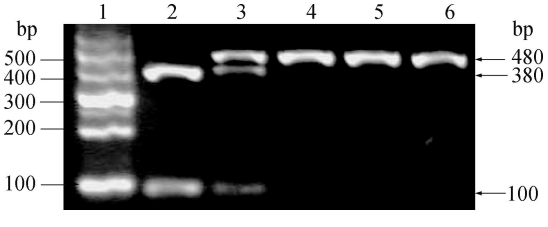

Figure 5. RFLP fragments of GSTA1 after digestion of PCR products. Amplified products (480 bp) were cut into two fragments (380 bp and 100 bp) by Ear1. Lane 2: homozygous GSTA1*B/ *B genotype samples. Lane3:heterozygous GSTA1 *A/*B genotype sample. Lanes 4-6: homozygous GSTA1*B/*B genotype samples. lane 1: 100 bp markers.

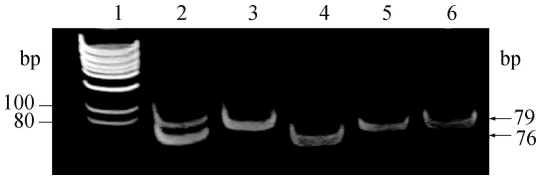

Figure 6. PCR products of *GSTM3* separated into 79 bp and 76 bp fragments on a 12% polyacrylamide gel.Lane 2: heterozygous *GSTM3*A/*B* genotype sample. Lanes 3, 5, 6: homozygous *GSTM3*A/*A* genotype samples. Lane 4: homozygous *GSTA1*B/*B* genotype sample. lane 1: 100 bp markers.

Statistical Analysis

Data were analyzed with SPSS software (ver. 10.0 SPSS In., Chicago, IL, USA). The statistical differences of *GST* polymorphism frequencies were determined by a Chi-squared test. Logistic regression analysis was used to determine the associations of *GST* genetic polymorphisms with family history of coronary heart disease, hypertension, stroke, lung cancer, esophageal cancer, nasopharyngeal cancer, favism, and thalassemia. A Chi-squared test for Hardy-Weinberg equilibrium was performed on *GSTP1, GSTM3,* and *GSTA1* polymorphisms.

RESULTS

The allele frequencies of GSTP1A and GSTP1G were 83.2% and 16.8%, respectively. The allele frequencies of GSTM3A and GSTM3B were 80.2% and 19.8%, respectively. The allele frequency of GSTA1A and GSTA1B were 87.9% and 12.1%, respectively.

Polymorphisms of *GSTT1, GSTM1, GSTP1,* and *GSTM3* were determined in our previous study on the Cantonese population in Guangdong province. Compared to this population, the Hakka had a significantly lower frequency of the *GSTM3B*/*B* genotype (3.1% vs. 11.9%, $P<0.05$). No statistical differences were observed between the Hakka and the Cantonese for *GSTT1, GSTM1*, or *GSTP1* genetic polymorphisms (Table 3). According to the results of the Chi-square test for Hardy-Weinberg equilibrium, *GSTP1, GSTM3,* and *GSTA1* polymorphisms in the Hakka population conformed to the Hardy-Weinberg equilibrium (Table 4, Table 5, and Table 6). To the best of our knowledge, this is the first report to determine genetic polymorphisms of *GSTA1* in the Hakka in China. The distributions of *GSTA1*A/*A, GSTA1*A/*B,* and *GSTA1*B/*B* were 77.1%, 21.7%, and 1.2%, respectively.

Table 3. GST Genotype Frequencies in the Hakka versus the Cantonese populations in Guangdong

Genotype	The Hakka, *n** (%)	Cantonese, *n** (%)	*P*- value
GSTT1	512	579	
+	269 (52.5%)	335 (57.9%)	＞0.05
null	243 (47.5%)	244 (42.1%)	
GSTM1	512	579	
+	194 (37.9%)	250 (43.2%)	＞0.05
null	318 (62.1%)	329 (56.8%)	
GSTP1	512	566	
A/A	354 (69.1%)	402 (71.0%)	＞0.05
A/G	144 (28.2%)	142 (25.1%)	
G/G	14 (2.7%)	22 (3.9%)	
GSTM3	482	570	
A/A	306 (63.5%)	355 (62.3%)	＜0.05
A/B	161 (33.4%)	147 (25.8%)	
B/B	15 (3.1%)	68 (11.9%)	
GSTA1	480	Blank**	
A/A	370 (77.1%)		
A/B	104 (21.7%)		
B/B	6 (1.2%)		

Note. *Some data are missing because of insufficient DNA or failure of PCR. **Blank: *GSTA1* was not included in the previous study.

Table 4. Hardy-Weinberg Equilibrium Test on *GSTP1*

	Genotype, % (*n*)			Allele, % (*n*)	
	*A/*A	*A/*G	*G/*G	A	G
Observed Frequencies	0.693	0.281	0.026	0.832	0.168
	(354)	(144)	(14)	(852)	(172)
Expected Frequencies	0.692	0.280	0.028		
χ^2		0.07*			

Note. *$P>0.05$.

Table 5. Hardy-Weinberg Equilibrium Test on *GSTA1*

	Genotype, % (*n*)			Allele, % (*n*)	
	*A/*A	*A/*B	*B/*B	A	B
Observed Frequencies	0.771	0.217	0.012	0.879	0.121
	(370)	(104)	(6)	(844)	(116)
Expected Frequencies	0.772	0.213	0.015		
χ^2		0.15*			

Note. *$P>0.05$.

Table 6. Hardy-Weinberg Equilibrium Test on *GSTM3*

	Genotype, % (*n*)			Allele, % (*n*)	
	*A/*A	*A/*B	*B/*B	A	B
Observed Frequencies	0.635	0.334	0.031	0.802	0.198
	(306)	(161)	(15)	(773)	(191)
Expected Frequencies	0.643	0.318	0.039		
χ^2		1.19*			

Note. *$P>0.05$.

Comparisons of GST genetic polymorphisms in the Hakka population with those reported for Han Chinese in other areas of China, as well as Caucasian and Indian populations are shown (Table 7). All these populations came from healthy volunteers and had similar social characteristics. Compared to Han Chinese in other areas of China, the Hakka had similar distributions of genetic polymorphisms in *GSTT1, GSTM1, GSTP1*, and *GSTM3,* whereas the Hakka and the Han Chinese from other areas of China tend to lack the *GSTT1* and *GSTM1* genes, when compared with Caucasians and Indians. Compared to Caucasians, the Hakka had a lower frequency of the homozygous *GSTP1*G/*G* genotype, which was similar to the Indian population.

Logistic regression analysis results indicated a weak association of *GSTM1* genetic polymorphisms with family history of hypertension (odds ratio (OR) = 1.868, 95% confidence intervals (95% CI) = 1.119 - 3.119). In addition, Chi-squared tests showed that people with a family history of hypertension had higher frequencies of the null-genotype for *GSTM1* than those without such a family history (73.0% vs. 59.2%, $P<0.05$). No relationships were observed between other *GST* genotype distributions and family histories of coronary heart disease, stroke, lung cancer, esophageal cancer, nasopharyngeal cancer, favism, or thalassemia.

In addition, a significant difference was observed in *GSTM1* genetic polymorphisms between alcohol drinkers and non-drinkers (70.5% vs. 59.8%, $P<0.05$). Significant differences were also observed in *GSTP1* genetic polymorphisms between smoking and non-smoking groups (*GSTP1*A/*A, GSTP1*A/*G, GSTP1*G/*G*: 62.5%, 29.2%, 8.3% vs. 70.4%, 28.1%, 1.5%, $P<0.05$). However, no differences were seen in the frequency of the *GSTP1* genotypes with regard to tobacco consumption, pickles consumption (Table 8), and differences in GSTs genetic polymorphisms were not observed among different age groups or genders ($P>0.05$).

Table 7. *GST* Genotype Frequencies in the Hakka Population Compared to other Populations

Genotype	The Hakka	Han Chinese in other Areas	Caucasian	Indian
GSTT1				
Total number	512	450[c]	415[e]	517[g]
+	52.5%	51.0%	83.4%	83.2%
Null	47.5%	49.0%	16.6%	16.8%
GSTM1				
Total number	512	450	415	517
+	37.9%	43.0%	51.1%	69.6%
Null	62.1%	57.0%	48.9%	30.4%
GSTP1				
Total number	512	450	414	518
A/A	69.1%	64.6%	42.0%	73.0%
A/G	28.2%	33.4%	44.7%	23.0%
G/G	2.7%	2.0%	13.3%	4.0%
GSTM3				
Total number	482	NA[a]	350[f]	518
A/A	63.5%		73.2%	74.4%
A/B	33.4%		23.7%	26.0%[b]
B/B	3.1%		3.1%	
GSTA1				
Total number	480	140[d]	411[f]	NA[a]
A/A	77.1%	75.0%	40.9%	
A/B	21.7%	24.3%	44.8%	
B/B	1.2%	0.7%	14.4%	

Note. [a]NA: not available; [b]A combination of *GSTM3 A/B* and *GSTM3 B/B*; [c]reference 18; [d]reference 19; [e]reference 20; [f]reference 21; [g]reference 13.

DISCUSSION

Several studies have shown that *GST* genes are polymorphic, with variable regional, ethnic, and national distributions. In this study, genetic polymorphisms of the *GSTT1, GSTM1, GSTP1, GSTM3*, and *GSTA1* genes in a Hakka population were compared to other populations. In the Hakka, the higher frequencies of null-*GSTM1* and null-*GSTT1* in comparison with both Caucasians and Indians, suggests that the Hakka is distinct from both these

Table 8. *GST* Genotype Frequencies in the Hakka Population and Life Style Factors

Life style		*GSTT1*		*GSTM1*		*GSTP1*			*GSTM3*			*GSTA1*		
		+	null	+	null	A/A	A/G	G/G	A/A	A/B	B/B	A/A	A/B	B/B
Smoking	Non-smokers	220	196	160	256	291	116	6	247	137	10	304	82	5
	Smokers	49	45	32	61	60	28	8	58	22	5	64	21	1
	P- value	0.894		0.466		0.001*			0.1058			0.505		
Smoking exposure	<10 cigarettes per day	27	22	17	31	33	13	1	28	13	2	38	7	0
	10-20 cigarettes per day	13	14	13	13	12	11	5	18	7	1	17	8	1
	>20 cigarettes per day	12	10	6	18	14	8	1	13	6	2	13	7	0
	P -value	0.834		0.180		0.064			0.868			0.108		
Alcohol Drinking	Non-drinkers	209	193	161	239	280	110	9	239	130	12	291	79	6
	Drinkers	60	50	33	79	74	34	5	67	31	3	79	25	0
	P - value	0.643		0.038**		0.369			0.799			0.758		
Pickles c	Often	20	25	18	26	31	13	2	25	16	3	35	8	0
	Occasionally	229	200	164	267	297	123	8	260	131	11	310	85	6
	Infrequently	19	18	12	24	25	8	4	21	13	1	25	10	0
	P -value	0.516		0.782		0.941			0.530			0.578		

Note. *$P<0.05$ when non-smokers were compared with smokers for GSTP1 genotype frequencies. **$P<0.05$ when non-drinkers were compared with drinkers for GSTM1 genotype frequencies.

racial groups. However, when compared to the Cantonese population, the Hakka have statistically significant differences in *GSTM3* genotype frequency distributions, a significantly lower frequency of the *GSTM3B*/*B* genotype, but have similar frequency distributions for the *GSTT1, GSTM1, GSTP1,* and *GSTA1* genes. The Hakka population appears to be a special branch of the Han Chinese by origin, with some changes in genetic structure, possibly caused by exoteric factors from long-distance southward migrations more than one thousand years ago. This result is consistent with a genetic analysis of the origin of the Hakka by Hui, et al.[22].

Tobacco smoking is one of the most important risk factors for lung cancer, with cigarettes producing many confirmed or suspected human chemical carcinogens and oxygen free radicals, such as polycyclic aromatic hydrocarbon amines, hydrogen peroxide free radicals, which might cause damage to DNA[23-24]. However, not every smoker will develop lung cancer, possibly because of individual genetic susceptibility. *GSTP1* is a candidate gene for lung cancer and is widely expressed in normal human epithelial tissue, particularly in the lung[25]. GSTP1-catalysed glutathione (GSH) conjugation is an important enzymatic reaction for protecting respiratory cells against environmental pollutants. Individuals with decreased enzyme activity have lower detoxification ability and are predisposed to an increased risk of lung cancer. In the Hakka population, the smokers had higher distributions of variant *GSTP1*A/*G* and **G/*G* compared to non-smokers (29.2% and 8.3% vs. 28.1%, 1.4%, respectively). This suggested that the smokers might be at a higher risk of lung cancer, but further case-control studies and cohort studies are needed for confirmation.

Considering the high incidence of esophageal cancer, nasopharyngeal cancer, favism, and thalassemia among the Hakka, logistic regression analysis was carried out to find possible associations between *GST* genotype distributions and family history of these diseases, together with lung cancer, coronary heart disease, hypertension and coronary heart disease. The results indicated a weak association of the *GSTM1* genetic polymorphisms with family history of hypertension. Furthermore, Chi-squared tests showed that individuals with a family history of hypertension had a higher frequency of the *null-GSTM1* allele compared to those without such family history (73.0% vs. 59.2%). *GSTM1* has five subfamilies, *GSTM1-5*. Only the *GSTM1* gene is abundantly expressed in hepatic tissue. The *null-GSTM1* correlates with many diseases, including prostate cancer, colorectal cancer, gastric cancer, asthma, and heart rate variability (HRV)[26-30]. In our study, the alcohol drinkers had higher frequencies of null-*GSTM1* genotypes than the non-drinkers (70.5% vs. 59.8%). Drinking is known to be an important risk factor for

hypertension. Oxidative metabolism of ethanol in alcohol produces acetaldehyde and various free radicals, causing hepatic damage[31]. The *null-GSTM1* might decrease detoxification ability and increase individual susceptibility to related diseases. As far as we know, the relationship between *GSTM1* genetic polymorphisms and alcohol consumption are still unclear. A study showed that the correlation between the null-*GSTM1* genotype and alcohol consumption was a low exposure-gene effect[32]. Our results suggest that individuals with the null-*GSTM1* genotype are at an increased risk for hypertension, but further studies are needed, based on the information presented here on the normal Hakka population.

As part of the research project, the family histories of several chronic diseases were selected as the research outcomes, which might be the limitation of the study. However, our results have provided basic genetic information on the Hakka population for further case-control study.

In conclusion, we have reported the genotype distributions of *GSTM1, GSTT1, GSTP1, GSTM3*, and *GSTA1* in the normal Hakka population of South China. We have also uncovered a possible primary relationship between smoking and *GSTP1* genetic polymorphisms, as well as a relationship between drinking and *GSTM1* genetic polymorphisms. These results might be useful in case-controlled, or cohort studies on associations between *GST* gene polymorphisms and disease risks, drug effects, and disease prognosis in the Hakka.

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