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Polymorphisms of metabolic enzyme genes, living habits and prostate cancer susceptibility

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1. ABSTRACT

In this report, genetic polymorphism of phase I and II metabolic enzyme (CYP2E1, CYP17, GSTM1 and GSTT1) genes, living habits, and risk of prostate cancer (PCa) was studied in 163 patients with prostate carcinoma of Han nationality in Southern China and 202 age-matched controls. The genotypic polymorphism of CYP2E1, CYP17, GSTM1 and GSTT1 genes was analyzed by PCR-RFLP assay using genomic DNA isolated from peripheral blood lymphocytes. The significant risk factors for PCa included long-term exposure to toxicant (OR=2.27, 95%CI: 1.26-4.09), the tumor history of lineal consanguinity (OR=2.19, 95%CI: 1.30-3.67), sexual history before age 30 of no more than 8 times per month (OR=1.85, 95%CI: 1.22-2.81), deep inhalation of cigarette smoke (OR=2.01, 95%CI: 1.20-3.37) or heavy smoking (OR=1.67,95%CI: 1.01-2.76). Among individuals with long-term heavy smoking without tea-drinking habit, the risk increased significantly (OR=4.27, 95%CI: 1.62-11.24 and OR), 2.76, 95%CI: 1.20-6.32). CYP2E1 C1/C1 genotype significantly increased the risk for PCa (OR=1.61, 95%CI: 1.04-2.49) with an apparent interaction with alcohol (OR=2.07, 95%CI: 1.07-4.00). However, stratification by the amount of accumulative smoking revealed that among people with a heavy smoking history, the individuals with the CYP2E1 C1/C1 genotype (OR=2.55, 95%CI: 1.20-5.43) and the individuals with GSTT1 null genotype (OR=2.23, 95%CI: 1.09-4.57) showed a significantly increased risk. Any other significant results with GSTM1 or CYP17 genes were not observed in this research. Individuals with more sensitive genotypes (from one to four) were at an increased risk. The data show that, in the development of PCa, there are many interactions among predisposing genotypes and genetic polymorphisms and unhealthy living habits. Individuals with more susceptible genotypes and unhealthy habits such as prolonged exposure to smoking are at an increased risk.

2. INTRODUCTION

The incidence of prostate cancer (PCa), one of the most common neoplasm in the Western world, has obvious ethnic differences and varies greatly with race and geography. For example, the incidence in the African-American population is 60 times that of the Chinese population of Han nationality, so the research of pathogenesis of PCa from a genetic aspect has important significance (1-3). At present, the research on the relationship between genotypic polymorphism of phase I or II metabolic enzymes and tumor susceptibility has become a focus, because differences in metabolic activity have been considered to exert an influence on tumor susceptibility. Phase I enzymes (mainly the cytochrome P450 family) activate carcinogenic compounds and produce DNA adducts. In contrast, phase II enzymes, such as the family of glutathione-S-transferases (GSTs), induce the binding effect between bio-molecules, such as glutathione and carcinogenic compounds, and make the compounds easily excreted (4-5). Consequently, an individual who has high metabolic activity, but low detoxification (i.e., high responsive activities of phase I enzymes, but low activities of phase II enzymes) might have higher risk for PCa (6).

In order to identify the genetic markers of PCa susceptibility and to explain the relations among the genetic polymorphisms of metabolic enzymes, living habits, and PCa susceptibility, our research was performed in Chinese population of Han nationality in Southern China.

3. MATERIALS AND METHODS

3.1. Study Subjects

Between September 2003 and April 2005, a total of 163 PCa patients, who were diagnosed with PCa through biopsy sampling, and 202 male control patients with other urological diseases, were included in this study. Consent of all patients was obtained. The mean age of patients was 71.2 years versus 71.6 years in the controls. All subjects were ethnic Han Chinese, were able to answer questions clearly, and were permanently residing in Jiangsu province of China. Serological (prostate specific antigen, prostatic acid phosphatase), physical and other auxiliary examinations were conducted on all controls to exclude the possibility of PCa, and any control would be excluded from this study if he ever had an abnormal prostate-specific antigen test (*i.e.,* ≥4ng/dl), an abnormal digital rectal examination, or any previous cancer diagnosis.

3.2. Data collection

By using a uniform questionnaire, an individual interview with reviewing of medical records was conducted by study coordinators. The data collected included general characteristics, personal medical history, life style, job history, sexual life history, dietary factors, family cancer history and history of smoking, alcohol drinking and tea drinking (Table 1) (2). Smoking habit was defined as smoking more than 5 cigarettes per day for more than 5 years. Deep inhalation or regular inhalation was defined as deep breath or regular breath when smoking. The unit cigarette/year for evaluating the amount of accumulative smoking was defined as 1 cigarette/day for 1 year. Light smoking was defined when the amount of accumulative smoking was less than 700 cigarettes/year (the average) and heavy smoking was defined when the amount of accumulative smoking was more than 700 cigarettes/year. Alcohol drinking habit was defined as drinking at least 3 times per week lasting more than 10 years. Tea-drinking habit was defined as at least drinking once per day for more than 10 years (Table 1).

3.3. Collection for blood specimens

With consent from patients, 5ml of periphery vein blood was collected. After anticoagulated with EDTA, the specimens were immediately stored at - 70^{o}C for genotyping.

3.4. DNA purification

Genomic DNA was isolated and purified from leukomonocytes of anticoagulated blood (5ml) by the traditional phenol/chloroform extraction and ethanol precipitation (2), dissolved in TE buffer (pH=7.4) and stored at -20°C.

3.5. Analysis of genotype

Primers were synthesized by Bioasia Co. (Shanghai, China) (5) and 10×PCR reaction buffers, 25 mmol/ L $MgCl_2$, 10mmol/L dNTPs, Taq DNA polymerase, and three kinds of restriction endonucleases (Rsa I, Pst I and MspA1 I) were all purchased from Promega Co. (Madison, WI, USA) (7).

3.5.1. Determination of CYP2E1 genotypic polymorphism

CYP2E1 genotype of each sample was detected using the PCR-RFLP technique. Referring to the report (7) and Primer3 software, the oligonucleotide primer sequences were 5'-TTCATTCTGTCTTCTAACTGG-3' and 5' -CCAGTCGAGTCTACATTGTCA-3'. The length of PCR-amplified fragment was 410 bp. The total volume of PCR reaction system was 30 μl, containing 3.0 μl of 10 × PCR buffer, 2.5 μl of 25mmol/L $MgCl_2$, 0.5 μl of 10 mM/L dNTPs, 0.3 μl of 25 μM/L each primer, 0.25 μl of 5U /μl Taq DNA polymerase, and 100ng of genomic DNA. The PCR amplification consisted of initially 5-min incubation at 94°C, followed by 35 cycles of denaturing at 94°C for 30 sec and annealing at 55°C for 40 sec, with an extension at 72°C for 50 sec. The reaction was terminated after a final extension of 10 min at 72°C and kept at 12°C. PCR-amplified DNA fragments including the polymorphic site were firstly electrophoresed through 1.5% agarose gels and visualized under UV light by ethidium bromide staining in order to find whether the amplification of sample was well done. Successfully amplified sample was digested with Rsa I and Pst I, the restriction endonucleases, separately. The volume of enzyme reaction system was 12 μl, including 1 μl of 10×buffer, 5 U of restriction endonuclease, 0.1 μl BSA (bovine serum albumin), and 10μl of PCR products. After the reaction system of enzymatic digestion was kept in 37°C water for 3-6 hours, the digested products were allowed to electrophorese at 3% agarose gels in order to analyze the genotypes of samples. Wild type was denoted

Table 1. Comparisons of general characteristics and living-habits between cases and controls

General characteristics	Controls (%)	Cases (%)	P value	OR	95% CI
Number of subjects	202	163			
Mean age (M±SD)	71.2±6.4	71.6±7.3	0.540		
Age group					
≤70 years	94 (46.5)	74 (45.4)	1[Δ]	-	-
>70 years	108 (53.5)	89 (54.6)	0.829	1.05	0.69-1.58
The history of long-term toxicant exposure					
No	181 (89.6)	129 (79.1)	1[Δ]	-	-
Yes	21 (10.4)	34 (20.9)	0.005	2.27	1.26-4.09
The tumor history of lineal consanguinity					
No	172 (85.1)	118 (72.4)	1[Δ]	-	-
Yes	30 (14.9)	45 (27.6)	0.003	2.19	1.30-3.67
The sexual life history before 30 years old					
> 8 times per month	110 (54.5)	64 (39.3)	1[Δ]	-	-
≤8 times per month	92 (45.5)	99 (60.7)	0.004	1.85	1.22-2.81
Smoking history					
No	69 (34.2)	46 (28.2)	1[Δ]	-	-
Yes	133 (65.8)	117 (71.8)	0.225	1.32	0.84-2.07
Smoking method					
Never smoking	69 (34.2)	46 (28.2)	1[Δ]	-	-
Regular inhalation	80 (39.6)	46 (28.2)	0.577	0.86	0.51-1.45
Deep inhalation	53 (26.2)	71 (43.6)	0.008	2.01	1.20-3.37
Age when beginning to smoke					
Never smoking	69 (34.2)	46 (28.2)	1[Δ]	-	-
≥20 years	104 (51.5)	93 (57.1)	0.217	1.34	0.84-2.14
< 20 years	29 (14.4)	24 (14.7)	0.519	1.24	0.64-2.40
Accumulative smoking amount					
Never smoking	69 (34.2)	46 (28.2)	1[Δ]	-	-
≤700 cigarette/year	70 (34.7)	47 (28.8)	0.979	1.01	0.60-1.70
>700 cigarette/year	63 (31.2)	70 (42.9)	0.047	1.67	1.01-2.76
The long-term habit of tea drinking					
No	76 (37.6)	69 (42.3)	1[Δ]	-	-
Yes	126 (62.4)	94 (57.7)	0.361	0.82	0.54-1.25
The long-term habit of alcohol drinking					
No	156 (77.2)	118 (72.4)	1[Δ]	-	-
Yes	46 (22.8)	45 (27.6)	0.288	1.29	0.80-2.08

[Δ]: Baseline for analysis

"C1/C1", there was only one band of 360 bp on Rsa I cut mapping, and also one band of 410 bp on Pst I digestion mapping. Heterozygous type, denoted "C1/C2", showed two bands of 410 bp and 360 bp on Rsa I mapping and 410-, 290-, 120-bp band on Pst I mapping. Mutant homozygous type was marked as "C2/C2", only one band of 410 bp on Rsa I mapping, and 290-, 120-bp band on Pst I mapping (Figure 1).

3.5.2. Determination of CYP17 genotypic polymorphism

A previous study by Stanford *et al* (8) used the PCR-RFLP technique. We also adopted the PCR-RFLP technique for this study. The primer sequences were 5'-CATTCGCACTCTGGAGTC-3' and 5'-AGGCTCTTGGGGTACTTG-3', and the length of PCR-amplified fragment was 414bp. Among the PCR's reaction system, PCR cycle parameters enzymatic digestion system and reaction conditions, all were the same as the determination of CYP2E1 polymorphism except for annealing at 56°C and the endonuclease (MspA1 I). The digested products were analyzed with genotype by 3% agarose gel electrophoresis. Wild type was denoted "A1/A1", and had only one 414-bp band on MspA1 I mapping. Heterozygous type (A1/A2) had three bands of 414, 290 and 124 bp, and mutant homozygous type (A2/A2) had two bands of 290 and 124 bp (Figure 2).

3.5.3. Determination of GSTM1 and GSTT1 genotypic polymorphism

Referred to in previous studies (9, 10), multiple PCR methods were adopted to amplify the genes of GSTM1 and GSTT1 with β-globulin as a positive reference. The primer sequences of GSTM1 were 5'-CTGGATTGTAGCAGATCATGC-3' and 5'-CTCCTGATTATGACAGAAGCC-3', the length of amplified fragment was 648bp; the primers of GSTT1 were 5'-TGACCTCGTAGCCATCACGG-3' and 5'-

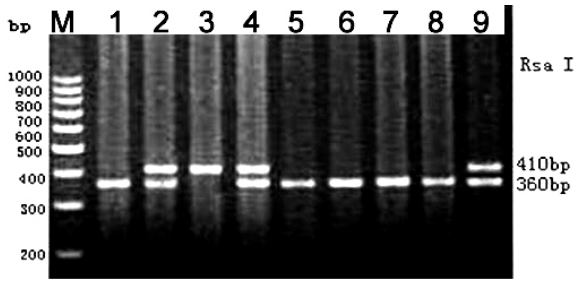

Figure 1. Determination of the CYP2E1 Rsa I (upper panel) and Pst I (lower panel) genotype from RFLP results M: Marker; 1,5,6,7,8: Wild-type Homozygous; 2,4,9: Heterozygous; 3: Mutant Homozygous.

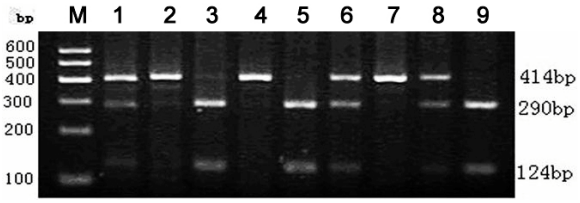

Figure 2. Determination of the CYP17 MspA1 I genotype from RFLP results 2,4,7: Wild-type Homozygous; 1,6,8: Heterozygous; 3,5,9: Mutant Homozygous.

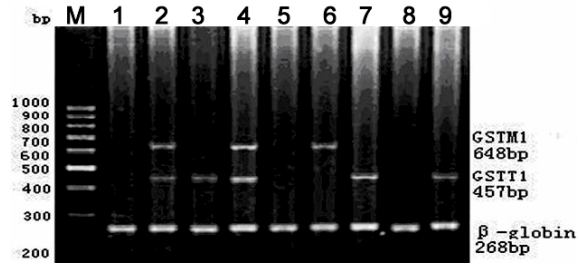

Figure 3. Determination of the GSTM1 and GSTT1 genotype 1,3,5,7,8,9:GSTM1 (+); 2,4,6:GSTM1 (-); 2,3,4,7,9:GSTT1 (+); 1,5,6,8:GSTT1 (-).

ACGGTGCAAGGGTGAGGTT-3'□and the length of amplified fragment was 457bp; the primers of β–globulin were 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3', the length of amplified fragment was 268bp.

The total volume of the PCR reaction system was 25μl, containing 2.5μl of 10 × PCR buffer, 2.5μl of 25 mmol/L $MgCl_2$, 0.5 μl of 10 mM/L dNTPs, 0.3μl of 25μM/L each primer, 0.25μl of 5U /μl Taq DNA polymerase, 100ng of genomic DNA. The cycle parameters for PCR amplification were all the same as the determination of CYP2E1 polymorphism. The amplified fragments were directly electrophoresed through 2% agarose gels containing 0.5□g/mL ethidium bromide, the lack of band with blank genotype was marked as (-); non-deletion of band with positive genotype was marked as (+). In GSTM1 (+) genotype sample, one band of 648bp and one band of 457bp was found in GSTT1 (+) genotype sample (Figure 3).

3.6. Statistical analysis

Epidata3.0 software was used to input the data and SPSS12.0 software was used for the statistical analysis. T-test was used to evaluate mean difference between cases and controls, Hardy-Weinberg test for assessing heredity equilibrium, X^2-test for enumeration data and the analysis of their reciprocity. The risk of PCa was evaluated with odds ratio (OR) and 95% confidence interval (CI). For all statistical procedures performed, $P < 0.05$ was considered significant.

3.7. Quality control

Investigators were trained uniformly and direct person interviews were conducted at the hospital. Results of investigation and experiments were checked randomly. Biolong Gene Limited Company (7) sequenced the PCR amplified products. Data input and processing were double tracked and logic check was adopted.

4. RESULTS

4.1. Comparisons of essential features and living habits between cases and controls

There was no difference in mean age (P=0.586), age group (grouping by 70 years old, the integer of mean age, P=464) and gender (all male) between cases and controls. There were significant differences in terms of history of long-term exposure of toxicants, such as dimethyl benzene, pesticide or radioisotopes (P=0.005, OR =2.27), and the tumor history of lineal consanguinity (P=0.003, OR =2.19) (2, 6). Compared with the sexual life history that was no more than 8 times per month before 30 years old, the sexual life history of more than 8 times per month raised the risk of PCa (P=0.004, OR=1.85). Though there was no significant difference with long-term smoking history between cases and controls (P =0.225, OR =1.32), however, deep inhalation (P=0.008, OR=2.01), and heavy smoking (P=0.047, OR=1.67) significantly increased the susceptibility of PCa after stratification by smoking method and amount. There was also no significant difference between cases and controls by stratification of age when beginning to smoke. Moreover, no evidence proved that the habit of long-term tea drinking (P=0.361) or alcohol drinking (P=0.288) significantly affected the risk for PCa (Table 1).

4.2. Analysis of interactions between living habits and environmental factors at risk of PCa

Compared with individuals who had tea-drinking habit without history of long-term toxicant exposure, the persons with a history of long-term toxicant exposure but without tea drinking habit, had a significantly increased susceptibility of PCa (P=0.002, OR=4.27). Similarly, the risk of PCa markedly increased (P =0.015, OR =2.76) in individuals who had a history of heavy smoking (accumulative smoking amount >700 cigarettes/year) but without tea-drinking habit, when compared with the individuals who had a tea-drinking habit but never smoked (Table 2).

Table 2. Influence of interactions between living habits and environmental factors at the risk of PCa

Living habit/Environmental factor		Controls (%)	Cases (%)	P value	OR	95% CI
History exposed to toxicants	**Tea drinking habit**					
No	Yes	111 (55.0)	78 (47.9)	1^{Δ}	-	-
No	No	70 (34.7)	51 (31.3)	0.878	1.04	0.65-1.65
Yes	Yes	15 (7.4)	16 (9.8)	0.281	1.52	0.71-3.25
Yes	No	6 (3.0)	18 (11.0)	0.002	4.27	1.62-11.24
Total smoking amount	Tea drinking habit					
Never smoking	Yes	35 (17.3)	22 (13.5)	1^{Δ}	-	-
Never smoking	No	34 (16.8)	24 (14.7)	0.761	1.12	0.53-2.37
≤700 cigarette*year	Yes	43 (21.3)	28 (17.2)	0.923	1.04	0.51-2.12
≤700 cigarette*year	No	27 (13.4)	19 (11.7)	0.780	1.12	0.51-2.47
>700 cigarette*year	Yes	48 (23.8)	44 (27.0)	0.270	1.46	0.75-2.86
>700 cigarette*year	No	15 (7.4)	26 (16.0)	0.015	2.76	1.20-6.32

$^{\Delta}$: Baseline for analysis

4.3. Comparisons of genotypic polymorphisms between cases and controls

After analysis by the Hardy-Weinberg test, the genotypic frequencies of Rsa I/Pst I site of CYP2E1 gene and MspA1 site of CYP17 gene were ascertained in a balanced state in the Chinese population of Han nationality in Southern China, according to the heredity balance rule (P=0.32 and 0.54, respectively). The distribution of GSTM1 or GSTT1 null genotype between cases and controls had no significant difference (P=0.824 and 0.672, respectively). We did not find any significant results with the CYP17 gene after stratification, according to genotype. The genotype of Rsa I site of CYP2E1 gene was completely unanimous with the genotype of Pst I site in every sample. Compared to the individuals carrying CYP2E1 C1/C2 or C2/C2 genotype, the subjects with C1/C1 genotype had a significantly higher risk for PCa (P =0.032, OR =1.61) (Table 3).

4.4. Reciprocities among genes at the risk of PCa

According to various researchers (4, 5, 7, 8, 10), GSTM1 (-), GSTT1 (-), CYP2E1 C1/C1, and CYP17 A2/A2 genotypes were defined as the 'in risk' genotype for PCa (Table 3, OR value). We discovered an increasing tendency for higher risk of PCa (OR value from 1.31 to 2.83) in individuals with more 'in risk' genotypes (from 1 to 4) when compared with individuals without any 'in risk' genotypes, although there did not exist any significant results (Table 4).

4.5. Interactions between living habits and genotypes at PCa risk

After stratification, according to the accumulative smoking habit of 700 cigarettes/year, the risk of PCa increased significantly (P =0.014, OR =2.55) in the subjects who carried the CYP2E1 C1/C1 genotype and had a history of heavy smoking (>700 cigarettes/year) in comparison with the subjects who were C1/C2 or C2/C2 genotype and never smoked. Compared with the individuals who carried GSTT1 (-) genotype and had a history of no smoking, the subjects who were GSTT1 (+) genotype and had a history of heavy smoking, had a significantly increased risk of PCa (P =0.027, OR =2.23). The significant interactions between GSTM1 or CYP17 genotypes and accumulative smoking amount were not found. In addition, the subjects who carried the CYP2E1 C1/C1 genotype and had an alcohol-drinking habit had an obviously higher risk for PCa in comparison to the subjects who carried the C1/C2 or C2/C2 genotype and had no alcohol-drinking habit (P =0.029, OR =2.07) (Table 5).

5. DISCUSSION

According to the epidemiological analyses of questionnaire data, we found that the PCa risk of subjects with tumor history of lineal consanguinity increased significantly (OR=2.19, 95%CI: 1.30-3.67), which might prove that the tumor sensitive genotype played an important role in occurrence of PCa. The deep-smoking method (OR=2.01, 95% CI: 1.20-3.37) and heavy smoking history (OR =1.67, 95% CI: 1.01-2.76) obviously increased the susceptibility of PCa, in which there is a dosage-response relation. It might be due to the increased intake of nitrated compounds, as well as the antagonistic effect of estrogen caused by smoking, which could increase the level of androgen *in vivo* (11). In addition, there was a significant difference (OR =2.27, 95%CI: 1.26-4.09) between cases and controls in terms of the long-term exposure history of toxicants, such as dimethyl benzene, pesticide, and radioisotopes, which showed the influence of environmental factors to PCa susceptibility (6, 12, 13).

The cytochrome P450 2E1 (CYP2E1) gene encoded the N-nitrosodimethylamine demethylase, which mainly participated in the metabolism of nitrosamine and ethanol, and played an important role in the metabolism and activation of carcinogens, such as benzene, chlorine ethylene and butadiene (13).

Many experiments have demonstrated that the CYP2E1 Pst I and Rsa I sites showed complete linkage heredity in the upstream regulatory sequence. In our experiments, the genotypes reflected by the two sites were unanimous in every sample, which confirmed the results reported in 1990 by Watanabe *et al.* (14), and also endorsed the reliability of our experimental data. There are few

Table 3. Comparisons of genotypes of phase I and II enzymes between cases and controls.

Genotype	Controls (%)	Cases (%)	P value	OR	95% CI
GSTM1 gene					
+	90 (44.6)	64 (39.3)	1[Δ]	-	-
-	112 (55.4)	99 (60.7)	0.309	1.24	0.82-1.89
GSTT1 gene					
+	107 (53.0)	74 (45.4)	1[Δ]	-	-
-	95 (47.0)	89 (54.6)	0.150	1.36	0.90-2.05
CYP2E1 gene					
C1/C2+C2/C2	84 (41.6)	50 (30.7)	1[Δ]	-	-
C1/C1	118 (58.4)	113 (69.3)	0.032	1.61	1.04-2.49
CYP17 gene					
A1/A1	32 (15.8)	30 (17.8)	1[Δ]	-	-
A1/A2	102 (50.5)	68 (41.7)	0.252	0.71	0.40-1.28
A2/A2	68 (33.7)	65 (39.9)	0.950	1.02	0.56-1.86
A1/A2+A2/A2	170 (84.2)	133 (81.6)	0.517	0.84	0.48-1.44

[Δ]: Baseline for analysis

Table 4. Influence of interaction among genes on the overall risk of PCa.

In risk genotype	Controls (%)	Cases (%)	P value	OR	95% CI
0	17 (8.4)	8 (4.9)	1[Δ]	-	-
1	47 (23.3)	29 (17.8)	0.579	1.31	0.50-3.42
2	80 (39.6)	57 (35.0)	0.368	1.51	0.61-3.75
3	46 (22.8)	53 (32.5)	0.054	2.45	0.97-6.20
4	12 (5.9)	16 (9.8)	0.066	2.83	0.92-8.73

[Δ]: Baseline for analysis

Table 5. Influence of interaction between living habits and genotypes on PCa risk.

Living habit	Genotype	Controls (%)	Cases (%)	P value	OR	95%CI
Total smoking amount	GSTM1					
Never smoking	+	23 (11.4)	19 (11.7)	1[Δ]	-	-
≤700 cigarette*year	-	37 (18.3)	27 (16.6)	0.757	0.88	0.40-1.94
>700 cigarette*year	-	29 (14.4)	45 (27.6)	0.105	1.88	0.87-4.04
Total smoking amount	GSTT1					
Never smoking	+	45 (22.3)	19 (11.7)	1[Δ]	-	-
≤700 cigarette*year	-	36 (17.8)	29 (17.8)	0.079	1.91	0.92-3.94
>700 cigarette*year	-	35 (17.3)	33 (20.2)	0.027	2.23	1.09-4.57
Total smoking amount	CYP2E1					
Never smoking	C1/C2+C2/C2	28 (13.9)	15 (9.2)	1[Δ]	-	-
≤700 cigarette*year	C1/C1	39 (19.3)	30 (18.4)	0.367	1.44	0.65-3.16
>700 cigarette*year	C1/C1	38 (18.8)	52 (31.9)	0.014	2.55	1.20-5.43
Total smoking amount	CYP17					
Never smoking	A1/A1+A1/A2	46 (22.8)	27 (16.6)	1[Δ]	-	-
≤700 cigarette*year	A2/A2	18 (8.9)	18 (11.0)	0.194	1.70	0.76-3.82
>700 cigarette*year	A2/A2	27 (13.4)	28 (17.2)	0.115	1.77	0.87-3.60
Alcohol drinking habit	CYP2E1					
No	C1/C2+C2/C2	65 (32.2)	36 (22.1)	1[Δ]	-	-
No	C1/C1	91 (45.0)	82 (50.3)	0.058	1.63	0.98-2.70
Yes	C1/C2+C2/C2	19 (9.4)	14 (8.6)	0.484	1.33	0.60-2.97
Yes	C1/C1	27 (13.4)	31 (19.0)	0.029	2.07	1.07-4.00

[Δ]: Baseline for analysis

studies at present showing the relationship between CYP2E1 gene polymorphism and PCa susceptibility. In a case-controlled study, Ferreira *et al.* (7) did not find marked relativity between the genetic polymorphism of Rsa I site of CYP2E1 gene and the risk of PCa, but it was found that the individuals with DD genotype of CYP2E1 Dra I site had a significantly increased PCa susceptibility (OR =2.12, 95% CI: 1.11-4.05). Our results were not in

accordance with what was reported by Ferreira *et al.* [7]. Our results showed that compared with the individuals with CYP2E1 C1/C2 or C2/C2, individuals carrying C1/C1 genotype of Rsa I/Pst I sites had an increased risk of PCa (OR =1.61, 95%CI: 1.04-2.49). These inconsistent results might be the result of the difference of potential distribution frequency of alleles among the ethnical groups. Many studies suggest that it was more significant to consider the combined effect of smoking and CYP2E1 polymorphism because CYP2E1 enzyme could activate nitrosamine, a type of carcinogen produced by cigarette burning, which might be involved in the carcinogenesis caused by smoking (11-13). In our study, we analyzed the combined effect and found that in subjects with C1/C1 genotype, the PCa risk of light smokers did not increase significantly (OR =1.44, 95%CI: 0.65-3.16), whereas the risk of heavy smokers did increase significantly (OR =2.55, 95% CI: 1.20-5.43). This might exhibit that the risk of people carrying sensitive genotypes were positively correlated with exposure dose of carcinogen (12). In addition, the interaction between CYP2E1 polymorphism and alcohol-drinking habit was also evaluated. It showed a marked increased risk in individuals with C1/C1 genotype and alcohol-drinking habit (OR =2.07, 95% CI: 1.07-4.00), which might be due to the important role that CYP2E1 played in ethanol metabolism, as well as the reason that ethanol might induce the activation of CYP2E1 enzyme (13).

CYP450 17α -hydroxyl enzyme encoded by CYP17 gene with three genotypes (A1/A1, A1/A2 and A2/A2) plays an important role in biosynthesis of androgen. A2 allele was considered to increase the transcription efficiency of the gene and improve enzyme activity, which increased androgen synthesis, and finally resulted in an increased risk of PCa (15). However, the present results from various researchers were inconsistent. Lunn *et al.* (16) discovered that the appearance of A2 allele might be the signal of the increased risk of PCa (OR =1.7, 95% CI: 1.0-3.0). Gsur *et al.* (17) found that in the population carrying A2/A2 genotype, the risk increased (OR = 2.80, 95%CI = 1.02-77.76) and especially stratification by median age (66 years) at time of diagnosis, a more markedly increased risk was found in carriers older than 66 years of the A2/A2 genotype (OR = 8.93, 95%CI = 1.78-49.19). Above-mentioned results suggested that A2 allele might have close relations with the incidence of PCa in American and European countries. However, the research on the Japanese population showed that between A2/A2 genotype and the risk of PCa, there was not obvious relativity, but the individuals carrying the A1/A1 genotype had a significantly increased risk (OR =2.6, 95% CI 1.4-4.8) (18).

Findings from this study are consistent with that reported by Wang *et al.* (19). We did not find that individuals with A2/A2 genotype had an increased risk (OR =1.02; 95%CI: 0.56- 1.86). The results of studies in Asian populations differed greatly from American and European studies, which might explain partially the reason why PCa incidence was obviously an ethnic difference. Even stratification with total smoking amount, we still did not get any significant outcome. It showed that the polymorphism of CYP17 gene might not be the sensitive factor for PCa in the Chinese population of Han nationality in Southern China.

GSTs, a family of Phase II enzymes, can catalyze the conjugation of glutathione to numerous potentially genotoxic compounds, such as the metabolites of phase I enzyme, aliphatic aromatic heterocyclic radicals from lipid peroxidation, and DNA peroxide generated from oxidative stress, to facilitate excretion of them by increasing their water-solubility (20). GSTM1 and GSTT1 genes both have two kinds of different genotypes: deletion type and undeleted type (with one or two undeleted alleles), and the encoded enzymes by them were isozymes for each other. There has been no research about the relations of GST gene families' polymorphism and PCa susceptibility reported in the Chinese population. However, research results reported in other countries were inconsistent. Acevedo *et al.* (21) found that the frequency of GSTM1 deletion type in a case study of controls in Chile was 36.2% significantly higher than 22.7% of controls. Srivastava *et al.* (22) also discovered in a case study in Northern India that individuals with GSTM1 (-) genotype had an increased risk for PCa (P=0.001, 95%CI: 1.37-3.65). However, Murata *et al.* (23) found that compared with GSTM1(+) individuals, the risk of individuals with GSTM1 deletion type did not increase significantly (OR =1.3, 95%CI: 0.82- 2.04) in the Japanese population. Medeiros *et al.* (24) in a study of the Southern European population did not find any significant results with GSTM1 polymorphism (OR=1.20,95%CI: 0.75-1.90). Our results were consistent with Murata and Medeiros (OR =1.24, 95%CI: 0.82- 1.89). Even after stratification by the amount of total smoking, we did not get any significant consequence.

When GSTT1 acts on its substrates, it displays a dysphasic role, to detoxify ethylene oxide or to increase toxicity by metabolizing 2-halogenated methane (25). The conclusion of this research focus on the association of GSTT1 polymorphism and PCa risk were more inconsistent. In Medeiros' s research (24), no increase in PCa risk was observed for men carrying GSTT1 null genotypes (P = 0.550, 95% CI 0.50-1.51), but GSTT1 (-) was over represented in men with advanced PCa disease (P = 0.038). However, Srivastava *et al.* (22) found that the risk obviously increased in individuals with GSTT1 null genotype (P=0.026, 95%CI: 1.09-3.28).

In this study, no significant relation was found between GSTT1 (-) and the risk for PCa (OR =1.36, 95%CI: 0.90-2.05). However, with stratification by total smoking amount, a significant interaction of heavy smoking was observed, and GSTT1 (-) genotype on the risk of PCa (OR =2.23, 95% CI: 1.09- 4.57), which mechanism was still not clear; it might be that the individuals carrying GSTT1 null genotype could not effectively facilitate excretion of numerous carcinogens produced by burning of tobacco due to the lack of GSTT1 enzyme (20).

At present, only a few studies focus on the combined effect of PCa sensitive alleles because of

insufficient sample size or statistical power to adequately examine gene-gene interactions (27). We superficially analyzed the gene-gene interactions in this research and found that with an increase of sensitive genotypes (from 1 to 4), the risk for PCa exhibited an increasing trend. This demonstrated that there were extensive reciprocities among the sensitive genotypes. Further research is needed to explain whether the reciprocities were direct or indirect, as well as to find out the general rules among them.

In conclusion, there are extensive reciprocities in the risk of PCa among sensitive genotypes of phase I and II enzymes and between sensitive genotypes and unhealthy living-habits. Overall, individuals who carry more sensitive genotypes and have more unhealthy living-habits, have increased risk; also revealed was a positive correlation with exposure to tobacco.

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Abbreviations: PCa: prostate cancer, GSTs: glutathione-S-transferases, OR: odds ratio, 95% CI: 95% confidence interval, CYP2E1: cytochrome P450 2E1, CYP17: cytochrome P450 17

Key Words: Prostatic Neoplasm, Carcinoma, Smoking, Drinking, Genetic Polymorphism

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