Relationship of tobacco smoking, CYP1A1, GSTM1 gene polymorphism and esophageal cancer in Xi’an

An-Hui Wang, Chang-Sheng Sun, Liang-Shou Li, Jiu-Yi Huang, Qing-Shu Chen

An-Hui Wang, Chang-Sheng Sun, Liang-Shou Li, Jiu-Yi Huang, Qing-Shu Chen, Department of Thoracic Surgery, Tangdu Hospital, Fourth Military Medical University Xi’an 710038, Shaanxi Province, China

AIM: To analyze the association of tobacco smoking, polymorphism of CYP1A1 (7th exon) and GSTM1 genotype and esophageal cancer (EC) in Xi’an.

METHODS: A hospital based case-control study, with molecular epidemiological method, was carried out. Polymorphism of CYP1A1 and GSTM1 of samples from 127 EC cases and 101 controls were detected by PCR method.

RESULTS: There were no significant difference of age and gender between cases and controls. Tobacco smoking was the main risk factor (OR=1.97; 95% CI=1.12-3.48) for EC in Xi’an. The proportions of CYP1A1 Ile/Ile, Ile/Val and Val/Val gene types in cases and controls was 19.7%, 45.7%, 34.6% and 30.7%, 47.5%, 21.8% respectively (P=0.04). Individuals with CYP1A1 Val/Val genotype compared to those with CYP1A1 Ile/Ile genotype had higher risk for EC increased (OR=2.48, 95% CI=1.12-5.54). The proportions of GSTM1 deletion genotype in cases and controls were 58.3% and 43.6% (OR=1.81, 95% CI=1.03-3.18, P=0.028). Analysis of gene-environment interaction showed that tobacco smoking and CYP1A1 Val/Val genotype; tobacco smoking and GSTM1 deletion genotype had synergistic interaction respectively. Analysis of gene-gene interaction did not find synergistic interaction between these two genes. But in GSTM1 deletion group, there was significant difference of distribution of CYP1A1 genotype between cases and controls (P=0.011).

CONCLUSION: CYP1A1 Val/Val and GSTM1 deletion genotypes are genetic susceptibility biomarkers for EC. The risk increases, when person with CYP1A1 Val/Val and/or GSTM1 deletion genotype. And these two metabolic enzymes seem to have interactions with tobacco smoking, in which the mechanism still needs further study.

Wang AH, Sun CS, Li LS, Huang JY, Chen QS. Relationship of tobacco smoking, CYP1A1, GSTM1 genotype and esophageal cancer in Xi’an. World J Gastroenterol 2002;8(1):49-53

INTRODUCTION

Esophageal cancer (EC) is one of the most common malignant tumors of human being. The incidence of EC varies in different countries. China is the country with highest incidence and mortality rate of EC. Research showed that risks for EC in different countries or different places were different[1-4]. In western countries alcohol intake and tobacco smoking were studied deeply[5-12]. It was thought that besides tobacco smoking and alcohol drinking, nutrition factors, life style, viruses infection, heredity or exposure to nitrosamines, fungi or AFB1 maybe involved in the process of EC[1,3,13-19]. In China, researches showed risks for EC were different in areas with different incidence[1-3,16,18,20,21]. The mortality rate of EC of Xi’an city in Shaanxi province is about 24 per 100,000, which ranks first in all cancer mortalities. Previous studies showed that both of tobacco smoking and family history of EC were main risk factors for EC in Xi’an city[1,2,22,23].

EC is a multi-etiology disease; environmental risks exposures and genetic susceptibility may take the role part[12,22-24]. Almost all of the environmental carcinogens (procarcinogens) are activated to be ultimate carcinogens before initiate the process of carcinogenesis. Some metabolic enzymes are closely related to the activation and detoxification of procarcinogens. Alterations of the key oncogene or tumor suppress gene can disturb the cycle of cell proliferation, which can also initiate the process of carcinogenesis[23,25,26]. Susceptibility of cancer is associated with the genetics polymorphism of related metabolic enzymes. Both certain susceptibility related biomarkers and certain environmental carcinogens perhaps are indispensable factors for EC[20,23,27,28]. To explore the bio-basis of genetic susceptibility of EC in Xi’an, we carried out a hospital based case-control study to analyze the associations of tobacco smoking, CYP1A1, GSTM1 gene polymorphism and EC.

MATERIALS AND METHODS

Selection of patients and controls

All cases with esophageal cancer (confirmed by pathological diagnosis) came from inpatients of Tandu Hospital during half a year period (December, 1999 to April, 2000). All controls were stratified randomly selected from non-cancer inpatients from different department of the same hospital during the same period. Both cases and controls were confined to residents with long-term living in Xi’an with similar proportion of gender and age.

Collected data

Trained interviewers using a structured questionnaire interviewed cases and controls in the hospital. The questionnaires obtained detailed information on residence, occupation, tobacco smoking habit and so on. Here tobacco smoking was defined as smoking at least one cigarette per day and persisting for more than one year. 127 cases (male 97, female 30) and 101 (male 78, female 23) controls were included. Blood samples were also collected for extraction of DNA genome. All blood samples had been stored at -70°C before started DNA extraction.

PCR methods to detect polymorphism of CYP1A1 and GSTM1

Digested by Proteinase K, DNA genomes were extracted from blood...
clot of cases and controls with hydroxybenzene, chloroform method in a uninterrupted period. CYP1A1 and GSTM1 polymorphisms were identified by polymerase chain reaction (PCR) before which DNA samples were stored at 4°C. Primers for GSTM1 (P1: 5’-GTA CCC TAC TTG ATT GAT GGG-3’; P2: 5’-CTG GAT TGT AGC AGA TCA TGC-3’) and for CYP1A1 (P3: 5’-CGG TCG TAT GAG ACC A-3’; P4: 5’-CGG AAG TGT ATC GGT GAG ACC G-3’; P5: 5’-GTA GAC AGA GTC TAG GCC TCA-3’) were synthesized by Shenggong bio-technical company of Shanghai. PCR condition for GSTM1 as follows, 50 µL solution including 10xbuffer 5µL, MgCl2 2µL, P1, P2 1µL respectively, template DNA 1.5µL, dNTPs 1µL and Taq DNA polymerase 3U. After denaturation at 94°C for 10 min, Taq DNA polymerase was added followed by 30 cycles with 94°C 1min, 60°C 1min, and 72°C 1min.20g·L⁻¹ agar was used to electrophoresis PCR production, then observed under the violate light. GSTM1 exist genotype was characterized as had a 273bp fragment; while GSTM1 deletion genotype had no fragment (Figure 1).

We used two pairs of primers to detect the polymorphism of CYP1A1 (7th exon). For each DNA sample two sets of PCR were carried out using P3, P5 (marked as tube A) and P4, P5 (marked as tube B) respectively. PCR conditions were the same: 50µL solution including 10xbuffer 5µL, MgCl2 2µL, P3, P5 (or P4, P5) 1µL, template DNA 1.5µL, dNTPs 1µL and Taq DNA polymerase 3U. The genotypes of CYP1A1 were identified by polymerase chain reaction method in an uninterrupted period. CYP1A1 and GSTM1 clot of cases and controls were characterized as had a 273bp fragment; while GSTM1 deletion genotype had no fragment (Figure 1).

Figure 1 Identify the GSTM1 genotype
M: 100bp DNA ladder, 3, 4 were GSTM1 exist, 2, 5 were GSTM1 deletion; 1 positive control, 6 negative control, 7 was blank control (without DNA template).

We used two pairs of primers to detect the polymorphism of CYP1A1 (7th exon). For each DNA sample two sets of PCR were carried out using P3, P5 (marked as tube A) and P4, P5 (marked as tube B) respectively. PCR conditions were the same: 50µL solution including 10xbuffer 5µL, MgCl2 2µL, P3, P5 (or P4, P5) 1µL, template DNA 1.5µL, dNTPs 1µL and Taq DNA polymerase 3U. After denaturation at 94°C for 10 min, Taq DNA polymerase was added. Followed by 30 cycles with 94°C 1min, 60°C 1min, and 72°C 1min. 20g·L⁻¹ agar was used to electrophoresis PCR production, then observed under the violate light. GSTM1 exist genotype was characterized as had a 273bp fragment; while GSTM1 deletion genotype had no fragment (Figure 1).

Quality control
DNA extraction and PCR were conducted in different period and places. The genotypes of DNA samples were identified blindly. Every PCR had set controls as blank control (without DNA template), positive control and negative control, and when any one of these controls was failure, PCR was re-conducted.

Statistical analysis
Data were input into computer, then the values of χ²; odds ratio (OR) and OR95% CI (confidence intermediate) were calculated. And ORs of gene-environment and gene-gene interaction were also estimated.

RESULTS

Comparability between cases and controls
The age and gender in cases and controls were comparable (Table 1).

| Factor          | Case | Control | χ² | P       |
|-----------------|------|---------|----|---------|
| Age(year)       |      |         |    |         |
| <50             | 28   | 16      | 1.97| 0.162   |
| 50~              | 38   | 44      | 4.85| 0.028   |
| ≥60             | 61   | 41      | 4.73| 0.094   |
| Gender          |      |         |    |         |
| Male            | 97   | 78      | 0.02| 0.901   |
| Female          | 30   | 23      | 0.02| 0.901   |

Risk factors for EC
The proportions of tobacco smoking, GSTM1 deletion genotype and CYP1A1 genotype (Val/Val) in cases and controls were significantly different (P<0.05) (Table 2).

| Factors         | Case | Control | OR | OR95%CI | χ² | P       |
|-----------------|------|---------|----|---------|----|---------|
| Tobacco smoking |      |         |    |         |
| Yes             | 69   | 38      | 1.97| 1.12-3.48| 6.28| 0.012   |
| No              | 58   | 63      |    |         |
| GSTM1 deletion  |      |         |    |         |
| Yes             | 74   | 44      | 1.81| 1.30-2.48| 4.85| 0.028   |
| No              | 53   | 57      |    |         |
| CYP1A1 genotype |      |         |    |         |
| Ile/Ile         | 25   | 31      | 1.00|         |
| Ile/Val         | 58   | 48      | 1.54| 0.74-3.03| 1.00| 0.22    |
| Val/Val         | 44   | 22      | 2.48| 1.12-5.34| 5.93| 0.015   |

Interaction of tobacco smoking and GSTM1 deletion genotype or CYP1A1 Val/Val genotypes
Analysis showed that there was synergistic interaction between tobacco smoking and GSTM1 deletion genotype (Table 3).

| Factors         | Case | Control | OR | OR95%CI | χ² | P       |
|-----------------|------|---------|----|---------|----|---------|
| Tobacco smoking |      |         |    |         |
| Yes             | No   | 25      | 37 | 1.00    |
| Yes             | Yes  | 28      | 20 | 2.07    | 0.90-4.80| 3.48| 0.062   |
| No              | 33   | 26      | 1.88| 0.86-4.13| 2.93| 0.087   |
| Yes             | Yes  | 41      | 18 | 3.37    | 1.49-7.69| 10.29| 0.0013  |

Interaction of tobacco smoking and GSTM1 deletion genotype or CYP1A1 Val/Val genotypes
Analysis showed that there was synergistic interaction between tobacco smoking and GSTM1 deletion genotype (Table 3).

| Factors         | Case | Control | OR | OR95%CI | χ² | P       |
|-----------------|------|---------|----|---------|----|---------|
| Tobacco smoking |      |         |    |         |
| Yes             | No   | 25      | 37 | 1.00    |
| Yes             | Yes  | 28      | 20 | 2.07    | 0.90-4.80| 3.48| 0.062   |
| No              | 33   | 26      | 1.88| 0.86-4.13| 2.93| 0.087   |
| Yes             | Yes  | 41      | 18 | 3.37    | 1.49-7.69| 10.29| 0.0013  |

SIA=3.37/(1.88+2.07-1.00)=1.14
Tobacco smoking and CYP1A1 Val/Val genotype also appeared synergistic interaction (Table 4).

| Smoking | CYP1A1(Val/Val) | Case | Control | OR | 95%CI | χ² | P |
|---------|----------------|------|---------|----|-------|----|---|
| No      | No             | 36   | 47      | 1.00 |       | 0.98-3.76 | 4.18 | 0.04 |
| Yes     | No             | 47   | 32      | 1.92 |       | 0.98-3.76 | 4.18 | 0.04 |
| No      | Yes            | 22   | 16      | 1.80 |       | 0.77-4.20 | 2.18 | 0.14 |
| Yes     | Yes            | 26   | 6       | 4.79 |       | 1.62-14.83 | 10.30 | 0.0013 |

SIM=4.79/(1.92×1.80)=1.39

But CYP1A1 mutation genotypes (Val/Val, Ile/Val) and GSTM1 deletion genotype did not show significant interaction (Table 5).

| GSTM1 deletion | CYP1A1 mutation | Case | Control | OR | 95%CI |
|----------------|-----------------|------|---------|----|------|
| No             | No              | 10   | 20      | 1.00 |       |
| No             | Yes             | 43   | 37      | 2.32 | 0.89-6.14 | 3.61 | 0.057 |
| Yes            | No              | 15   | 11      | 2.73 | 0.81-9.42 | 3.28 | 0.070 |
| Yes            | Yes             | 59   | 33      | 3.58 | 1.39-9.38 | 8.66 | 0.0033 |

OR of individuals with CYP1A1 mutation genotype and GSTM1 deletion genotype was greater than those with any other forms of the two genotypes. But there did not show any synergistic interaction between CYP1A1 mutation genotypes and GSTM1 deletion genotype.

Stratified with GSTM1 deletion genotype to analyze the distributions of CYP1A1 genotypes in cases and controls. Results showed that there were significant different in cases and controls (P=0.011) in GSTM1 exist genotype CYP1A1 genotypes, whereas there were no significant difference (P=0.83) between cases and controls in GSTM1 deletion genotype (Table 6).

| CYP1A1 genotype | GSTM1 deletion | existing GSTM1 |
|-----------------|----------------|---------------|
|                 | Case | Control | Case | Control |
| Ile/Ile         | 15   | 11      | 10   | 20      |
| Ile/Val         | 35   | 19      | 23   | 29      |
| Val/Val         | 24   | 14      | 20   | 8       |
| χ²              | 0.39 |         | 9.04 |         |
| P               | 0.83 |         | 0.011|         |

DISCUSSION

Under similar environmental carcinogens exposure only a few of individuals get neoplasm, for there were individual difference to environmental exposure. The different liability to cancer was called genetic susceptibility of cancer. Genetic susceptibility can affect on every step of carcinogenesis, including modify the effect of environmental carcinogens[24-29]. Oncogenes and tumor suppressor genes can also affect individual’s susceptibility to cancer. Cancer susceptibility genes include typeI, typeII metabolism enzyme gene, DNA repair gene and those affect cell proliferation rate gene. In recent years evidence has accumulated to support the hypothesis that cancer susceptibility gene may be of importance in determining individual susceptibility to cancer[30-36].

EC is a multi-factor determined disease; including environmental risk factors and genetic factors. In recent years, more and more researches considered environmental and genetic susceptibility factors and their interactions in evaluating the risks of cancer[12,17,43,47-50]. Investigations showed the mortality rate of EC in Shaanxi province did not decreased during the late 20 years, and risks factors for EC in Xi’an city were discussed in several researches[22,23]. In this hospital based case- control study, the results showed that tobacco smoking was a risk factor and tobacco smoking had interactions with GSTM1 deletion genotype and CYP1A1 Val/Val genotype.

Most chemical carcinogens in environment are pro-carcinogens. And aromatic hydrocarbons (AHs) in tobacco smoking are pro-carcinogens, they need to be activated to reactive electrophilic forms by type II metabolic enzymes (CYP450s), then initiate the carcinogenesis. On the other hand the reactive electrophilic forms of carcinogen can be detoxified and excreted by typeI metabolic enzymes such as GSTM1. Although theoretically the increase of activity of type II metabolic enzymes and/or decrease of activity of typeI metabolic enzymes can increase the risk for cancer, there were different results in different researches, some supported this hypothesis and others did not[27,34,35,37,40-42,51-56]. Our results showed that individuals with the GSTM1 deletion genotype or/and CYP1A1 Val/Val genotype had increased risks for EC.

P450 CYP1A1 gene located in chromosome 15q22 mainly metabolizes pro-carcinogens. There are three kinds of polymorphism of CYP1A1: MspI site, 7th exon (Ile/Val) and AA polymorphism. MspI polymorphism include three genotypes: without MspI enzyme cleavage site allele gene m1(m1/m1) as A genotype; having MspI cleavage site allele (m2/m2) as C genotype and m1/m2 as B genotype. In different populations the distribution of these three genotypes were different. CYP1A1 Ile-Val polymorphism caused by 7th exon 4889th base difference (A or G), transition of A to G results in 462th amino turned from isoleucine to valine[57,58], then form three kinds of genotypes: homozygote wild genotype(Ile/Ile), mutation genotype(Val/Val) and heterozygote Ile/Val genotype. Polymorphism of 7th exon correlated with polymorphism of MspI in Asia and Caucasian populations, and in Americans from Africa these two kind of CYP1A1 polymorphism were independent. CYP1A1 7th exon polymorphism and MspI site were incomplete linkage. Research showed CYP1A1 Val/Val genotype have higher ability to activate pro-carcinogen than CYP1A1 Ile/Ile genotype. PAH-DNA adducts in leukocyte were higher in heavy smoking population with CYP1A1 Val/Val genotype than those with CYP1A1 Ile/Val or Ile/Ile genotype. AA polymorphism was new special MspI polymorphism, which still under discussion.

Although evidence showed that CYP1A1 mutation genotype (Val/Val) had the strongest ability to activate pro-carcinogens, the associations between CYP1A1 genotype and susceptibility to cancers were varied[30-33,37,57,58]. Data from Guandong province in China showed that MspI C correlated with no-smoking population’s lung cancer susceptibility[52]. Study in Shanghai and Haerbin no significant relation was discovered between CYP1A1 (Ile-Val) polymorphism and lung cancer susceptibility in non-smoking female patients[51]. CYP1A1 Val/Val genotype only appear about 3.2%~5% in white population, while in Japanese it was about 19.8%, in Chinese it was 22.3%. Our study showed that distributions of CYP1A1 genotypes in cases and controls were different (P=0.049). CYP1A1 Val/Val genotype was associated with EC (OR=2.48,95%CI=1.12-5.54) and there was interaction of tobacco smoking and CYP1A1 Val/Val genotype.

GSTM1 can detoxify a number of reactive electrophilic compound substances, including the carcinogens PAHs. If
individuals with GSTM1 deletion genotype, the ability to detoxify the carcinogens decreased. Individuals with GSTM1 deletion can have the increased risk of cancers[24,43,61]. In China there were similar research on GSTM1 deletion genotype and the risks of lung cancer(OR=2.56)[31] and stomach cancer(OR=1, 90, 95% CI=1.01-3.56)[42]. Researches showed that in Henan province, high incidence of EC in China, GSTM1 deletion gene polymorphisms had not significant relation with EC susceptibility[20]. Results of our study indicated GSTM1 deletion genotype was significant different in cases and controls (P=0.028) and the OR was 1.81(95%CI=1.03-3.18). GSTM1 deletion genotype had synergistic interaction with tobacco smoking.

In summary, we found tobacco smoking, CYP1A1 Val/Val genotype; GSTM1 deletion genotype had associations with EC in Xi’an area. Gene-environment interaction analysis showed that tobacco smoking had synergistic interactions with CYP1A1 Val/Val genotype and with GSTM1 deletion genotype. Gene-gene interaction analysis did not find synergistic interaction between CYP1A1 mutation genotypes and GSTM1 deletion genotype, though individuals with these two genotypes had increased risk for EC. The synergistic interactions and their mechanisms of tobacco smoking with these two metabolic enzymes gene polymorphisms still need further study with large (population-based) samples and modified designs.

Acknowledgment We would like to thank Bing-Quan Gu for their help in collecting blood sample.

REFERENCES
1. Zhang W, Bailey-Wilson JE, Li W, Wang X, Cao M, Xiu L, Zhou C, Wu M. Segregation analysis of esophageal cancer in a moderately high-incidence area of northern China. *Am J Hum Genet* 2000; 67: 110-119
2. Li LS, Sun C, Zhang XL, Qiao GB, Xu DZ, Han CL, Yang WX, Chang GS, Yan MX, Wang Y, Zhang HY. A comparative molecular epidemiological study on esophageal cancer between Xi’an and Lizhou. *Jiefangjun Yufangyixue Zazhi* 1999; 17: 255-259
3. Zhou XC, Watanabe S. Factor analysis of digestive cancer mortality and food consumption in 65 Chinese countries. *J Epidemiol* 1999; 9: 275-284
4. Wang LD, Zou JX, Hong JY, Zhou Q, Deng CJ, Xie DW, Holly C. Identification of a novel genetic polymorphism of human O-6-alkylguanine-alkyltransferase in patients with esophagus cancer. *Huren Xiaohua Zazhi* 1998; 6: 560-463
5. Lu JB, Lian SY, Sun XQ, Zhang ZK, Dai DX, Li BW, Cheng LP, Wei JR, Duan WJ. A case-control study on the risk factors for esophageal cancer in Linzhou. *Zhonghua Liusixingbingxue Zazhi* 2000; 21: 434-436
6. Li WD, Wang XQ, Zhang CL, Han XY, Chen DQ, Zhang T, Pan XF, Jia YT, Mao XQ, Zhang R. Esophageal carcinoma in part of population of yangquan city. *Zhonghua Yi Xue Za Zhi* 1998; 78: 203-206
7. Castellsague X, Munoz N, De Stefani E, Victoria CG, Quintana MJ, Castelletto R, Rolon PA, independent joint effects of tobacco smoking and alcohol drinking on the risk of esophageal cancer in men and women. *Int J Cancer* 1999; 82: 657-664
8. Castellsague X, Munoz N, De Stefani E, Victoria CG, Castelletto R, Rolon PA. Influence of mate drinking, hot beverages and diet on esophageal cancer risk in South America. *Int J Cancer* 2000; 88: 658-664
9. Dilhon PK, Farrow DC, Vaughan TL, Chow WH, Risch HA, Gammon MD, Mayne ST, Stanford JL, Schoenberg JB, Ahnian H, Dubrow R, West AB, Rotterdam H, Blot WJ, Fraumeni JJ Jr. Family history of cancer and risk of esophageal and gastric cancers in the United States. *Int J Cancer* 2001; 93: 148-152
10. Nayar D, Kapil U, Joshi YK, Sundra RA, Srivastava SP, Shukla NK, Tandon RK. Nutritional risk factors in esophageal cancer. *J Assoc Physicians India* 2000; 48: 781-787
11. Chang F, Syrjanen S, Shen Q, Cintorronio M, Santopietro R, Tosi P, Syrjanen K. Evaluation of HPV, CMV, HSV and EBV in esophageal squamous cell carcinomas from a high-incidence area of China. *Atalanta* 2000; 30:395-394
12. Li T, Lu ZM, Chen KN, Guo M, Xing HP, Mei Q, Yang HH, Lechner JF, Ke Y. Human papillomavirus type 16 is an important infectious factor in the high incidence of esophageal cancer in Anyang area of China. *Carcinogenesis* 2001; 22: 929-934
13. Shi QL, Xu DZ, Sun CS, Li LS. Study on family aggregation of esophageal cancer in Linzhou city. *Zhonghua Yufang Yi Xue Zazhi* 2000; 34: 269-270
14. Shen YP, Gao YT, Dai Q, Hu X, Xu TL, Xiang YB, Tang ZL, Li WL. A case-control study on esophageal cancer in Huaian city. *Jiansu province(5): role of the cigarette smoking and alcohol drinking* *Zhongliu* 1999; 19: 363-367
15. Laggergren J, Ye W, Lindgren A, Nyren O. Heredity and risk of cancer of the esophagus and GASTRIC cardia. *Cancer Epidemiol Biomarkers Pre* 2000; 9: 757-760
16. Lin DX, Tang YM, Lu SX, Kadlubar FF. Glutathione S-transferase M1, T1 genotypes and risks of esophageal cancer: a case-control study. *Zhonghua Liuxingbingxue Zazhi* 1998; 19: 195-199
17. Gao YT, Den J, Xiang YB, Ruan ZX, Wang ZX, Hu BY, Guo MR, Wu TK, Han JJ, Zhang YS. Smoking, related cancers, and other diseases in Shanghai:A 10-year prospective study. *Zhonghua Yufang Yi Xue Zazhi* 1999; 33: 5-8
18. Zhang HY, Sun CS, Li LS, Yan MX. CytochromeP4501A1 and the genetic susceptibility to esophageal carcinoma. *Zhonghua Yufang Yi Xue Zazhi* 2000; 34: 69-71
19. Wang AH, Zhang HY, Wang Y, Yan MX, Sun CS, Li LS, Huang JY, Cheng QS, Zhu YF. Molecular epidemiological study on esophageal cancer in Xi’an. *Disi Junji Daaxue Xuebao* 2001; 21: 61-63
20. Tan W, Song N, Wang GQ, Liu Q, Tang HJ, Kadlubar FF, Lin DX. Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferases M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. *Cancer Epidemiol Biomarkers Pre* 2000; 9: 551-556
21. Hu N, Huang J, Emmert-buck MR, Tang ZZ, Roth MJ, Wang C, Dawsey SM, Li WJ, Wang QH, Han XY, Ding T, Giffen C, Goldstein AM, Taylor PR. Frequent amplification of the TP53 gene in esophageal squamous cell carcinoma from a high-risk population in China. *Cancer Res* 2001; 17: 883-891
22. Taniere P, Martel-Planche G, Puitawibul P, Casson A, Montesano R, Chavanitan A, Hainaut P. TP53 mutations and MDM2 gene amplification in squamous-cell carcinomas of the esophagus in south Tailand. *Int J Cancer* 2000; 88: 223-227
23. Zhao G, Xu Z, Li EM, Li J, Wen BG. Relationship between the GSTM1 genetic polymorphism and susceptibility to squamous cell carcinoma of the esophagus. *Shantou Daxue Yi Xue Zazhi* 1999; 20: 1-3
24. Mizobuchi S, Furihata M, Sonobe H, Ohtsuki Y, Ishikawa T, Murakami H, Kurabayashi A, Ogoshi S, Sasaguri S. Association between p53 immunostaining and cigarette smoking in squamous cell carcinoma of the esophagus. *Int J Cancer* 2000; 88: 423-428
25. van Lieshout EM, Roelfs HM, Dekker S, Mulder CJ, Wobbes T, Jansen JB, Peters WH. Polymorphic expression of the glutathione S-transferase P1 gene and its susceptibility to Barrett’s esophagus and esophageal carcinoma. *Cancer Res* 1999;59: 586-589
26. Roth MJ, Dawsey SM, Wang G, Tangrea JA, Zhou B, Ratnasrieng D, Woodson KG, Oliviero OA, Poirier MC, Frye
BL, Taylor PR, Weston A. Association between GSTM1*0 and squamous dysplasia of the esophagus in the high risk region of Linxian, China. Cancer Lett 2000; 156: 73-81
31 Morita S, Yano M, Tsujinaka T, Akiyama Y, Taniguchi M, Kaneko K, Miki H, Fuji J, Yoshino K, Kusuoka H, Monden M. Genetic polymorphisms of drug-metabolizing enzymes and susceptibility to head-and-neck squamous-cell carcinoma. Int J Cancer 1999; 80: 685-688
32 Butler WJ, Ryan P, Roberts-Thomson IC. Metabolic genotypes and risk for colorectal cancer. J Gastroenterol Hepatol 2001;16:631-635
33 Rojas M, Cascoy I, Alexandrov K, Krcek F, Auburtin G, Mayer L, Kopp-Schneider A, Roots I, Bartsch H. Modulation of benzo [a] pyrene diol epoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 1999; 20:267-671
34 Tanimoto K, Hayashi S, Yoshiga K, Ichikawa T. Polymorphisms of the CYP1A1 and GSTM1 gene involved in oral squamous cell carcinoma in association with a cigarette dose. Oral Oncol 1999; 35: 191-196
35 Sato M, Sato T, Izumo T, Amagasa T. Genetic polymorphism of drug-metabolizing enzymes and susceptibility to oral cancer. Carcinogenesis 1999; 20: 1927-1931
36 Xing DY, Tan W, Song N, Lin DX. Genetic polymorphism in hOGG1 and susceptibility to esophageal cancer in Chinese. Zhonghua Yu Se 2000; 17: 377-380
37 Chen S, Xue K, Xu L, Ma G, Wu J. Polymorphisms of the CYP1A1 and GSTM1 genes in relation to individual susceptibility to lung cancer in Chinese population. Mutat Res 2001;458:41-47
38 Song C, Xing D, Tan W, Wei Q, Lin D. Methylhydroxyethyldiol epoxide reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res 2001;61:3272-3275
39 Lee JM, Lee YC, Yang SY, Lan T, Chen SJ, Hsieh CY, Wu MT. Genetic polymorphisms of p53 and GSTP1, but not NAT2, are associated with susceptibility to squamous-cell carcinoma of the esophagus. Int J Cancer 2000;89: 485-497
40 Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiol Biomarkers 2000;9:3-28
41 Liu G, Zhou Q, Wang LD, Hong YJ, Deng C, Wang YY, Zou JX. Blood clot as a DNA source for studying genetic polymorphism of human carcinogen-metabolizing enzymes. World J Gastroenterol 1998; 4(Suppl 2): 108-109
42 Qu YH, Shi YB, Peter S, Zhong IJ, Sun L, Sun XW, Cheng JX, Lin YJ, Xian YB, Dai XD, Gao YT. The genotypes of cytochrome P4501A1 and GST M1 in non-smoking female lung cancer. Zhongliu 1998; 18: 80-82
43 Hu YL, Zhang Q. Genetic Polymorphisms of CYP1A1 and suscepti-
44 Butkiewicz D, Cole KJ, Phillips DH, Harris CC, Chorazy M, GSTM1, GSTP1, CYP1A1 and CYP2D6 polymorphisms in lung cancer patients from an environmentally polluted region of Poland: correlation with lung DNA adduct levels. Eur J Cancer Prev 1999;8: 315-323
45 Liu G, Zhou Q, Wang LD, Hong YJ, Deng C, Wang YY, Zou JX. Genetic polymorphism of CYP1A1 and GSTM1 and lung cancer risk. World J Gastroenterol 2000; 6:228-230
46 Cai L, Yu SZ, Zhang ZF. Glutathione S-transferase M1, T1 genotypes and the risk of gastric cancer: a case-control study. World J Gastroenterol 2001;7:506-509
47 Cai L, Yu SZ. A molecular epidemiologic study on gastric cancer in Jiangxi, Fujian province. Shijue Huaren Xinchao Zazhi 1999; 7: 652-655
48 Lee JM, Lee YC, Yang SY, Shi WL, Lee CJ, Hsieh CY, Wu MT. Genetic polymorphisms of p53 and GSTP1, but not NAT2, are associated with susceptibility to squamous-cell carcinoma of the esophagus. Int J Cancer 2000;89:485-497
49 Liu G, Zhou Q, Wang LD, Hong YJ, Deng C, Wang YY, Zou JX. Genetic polymorphism of CYP1A1 and GSTM1 and lung cancer susceptibility. Zhongliu 1998; 20: 185-186
50 Liu G, Zhou Q, Wang LD, Hong YJ, Deng C, Wang YY, Zou JX. Genetic polymorphism of CYP1A1 and GSTM1 and lung cancer susceptibility. Zhongliu 1998; 20: 185-186
51 Cai L, Yu SZ. Preliminary studies on cytochrome P4502E1 and glutathione S-transferase M1 polymorphisms and susceptibility to gastric cancer. Zhongguo Gongye Weisheng 1999; 15: 895-897
52 Olshan MK, Mark CW, Watson MA, Bell DA, GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 Polymorphisms, Tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomarkers 2000;9:185-186
53 London SJ, Yuan JM, Coeetze GA, Gao YT, Ross RK, Yu MC. CYP1A1 and NAT2, are associated with susceptibility to squamous-cell carcinoma. Int J Cancer 2000;9:3-28
54 Murata M, Watashima M, Yamanaka M, Kubota Y, Ito H, Nagao M, Kato T, Kamataki T, Kawamura J, Yatani R, Shiraiishi T. Genetic polymorphisms in cytochrome P450 and GST polymorphisms in the Japanese population. Cancer Epidemiol Biomarkers 2000; 9:185-186
55 Shen J, Wang RT, Xing XH, Wang L, Wang ZJ, Wang BY, Li MS, Wang JM, Hua ZL, Guo CH, Wang XR, Xu XP. Research on the interaction models of cytochrome P450 1A1 polymorphism(s) in the agents of stomach cancer. Zhonghua Yixue Yichuan Zazhi 2003;35:167-170

Edited by Wang JH and Xu XQ