P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2003;9(12):2654-2657
World Journal of Gastroenterology
 ISSN 1007-9327

• ESOPHAGEAL CANCER •

Epoxide hydrolase *Tyr*113*His* polymorphism is not associated with susceptibility to esophageal squamous cell carcinoma in population of North China

Jian-Hui Zhang, Xia Jin, Yan Li, Rui Wang, Wei Guo, Na Wang, Deng-Gui Wen, Zhi-Feng Chen, Gang Kuang, Li-Zhen Wei, Shi-Jie Wang

Jian-Hui Zhang, Xia Jin, Yan Li, Wei Guo, Na Wang, Deng-Gui Wen, Zhi-Feng Chen, Gang Kuang, Li-Zhen Wei, Shi-Jie Wang, Hebei Cancer Institute, Hebei Medical University, Shijiazhuang 050011, Hebei Province, China
Rui Wang, Shi-Jie Wang, The Fourth Affiliated Hospital, Hebei Medical University, Shijiazhuang 050011, Hebei Province, China
Supported by the Scientific Grant of Educational Department of Hebei Province, China, No. 2001150
Correspondence to: Professor Shi-Jie Wang, The Fourth Affiliated Hospital, Hebei Medical University, Jiankanglu 12, Shijiazhuang 050011, China. wang.sj@hbmu.edu
Telephone: +86-311-6085231 **Fax:** +86-311-6077634
Received: 2003-06-16 **Accepted:** 2003-08-25

Abstract

AIM: To investigate the possible association of *microsomal epoxide hydrolase* (*mEH*) *Tyr*113*His* polymorphism with susceptibility to esophageal squamous cell carcinoma (ESCC) in a population of North China.

METHODS: The *mEH Tyr*113*His* genotypes were determined by polymerase-chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis in 257 patients with esophageal squamous cell carcinoma (ESCC) and 252 healthy subjects as a control group.

RESULTS: The frequencies for *Tyr* and *His* alleles were 44.2 %, 55.8 % in ESCC patients, and 44.0 % and 56.0 % in healthy subjects, respectively. No statistic difference in allele distribution was observed between ESCC patients and controls (χ^2=0.008, *P*=0.929). The overall genotype distribution difference was not observed between cancer cases and controls (χ^2=2.116, *P*=0.347). Compared with *Tyr/Tyr* genotype, neither *His/His* genotype nor in combination with *Tyr/His* genotype significantly modified the risk of the development of ESCC, the adjusted odds ratio was 1.076 (95 % CI=0.850-1.361) and 0.756 (95 % CI=0.493-1.157), respectively. When stratified for sex, age, smoking status and family history of upper gastrointestinal cancer, *His/His* genotype alone or in combination with *Tyr/His* genotype also did not show any significant influence on the risk of developing ESCC.

CONCLUSION: *MEH Tyr113His* polymorphism may not be used as a stratification marker in screening individuals at a high risk of ESCC.

Zhang JH, Jin X, Li Y, Wang R, Guo W, Wang N, Wen DG, Chen ZF, Kuang G, Wei LZ, Wang SJ. Epoxide hydrolase *Tyr*113*His* polymorphism is not associated with susceptibility to esophageal squamous cell carcinoma in population of North China. *World J Gastroenterol* 2003; 9(12): 2654-2657
http://www.wjgnet.com/1007-9327/9/2654.asp

INTRODUCTION

China is one of the prevalent areas of esophageal squamous cell cancer (ESCC). Exposure to environmental carcinogens is considered as the main risk factors of ESCC[1,2]. Among them, chemical carcinogens such as polycyclic aromatic hydrocarbons (PAHs) in consumed tobacco or ingested food may contribute to the high incidence of ESCC in China[3,4]. Metabolization of PAHs involves a complex enzymatic mechanism. Microsomal epoxide hydrolase (mEH) is an enzyme that hydrolyzes epoxides such as PHA, yielding corresponding *trans*-dihydrodiols. Usually, this hydrolysis acts as a detoxifying step, although in some instances, *trans*-dihydrodiols generated from PAHs are highly toxic and mutagenic. Therefore, mEH plays a dual role in the detoxification and activation of procarcinogens, and its role in carcinogenesis may depend on exposures to different environmental substrates[5].

There are two polymorphic sites that affect the enzyme activity in human *mEH* gene. One variant is characterized by substitution of histidine for tyrosine at the amino acid position 113, the other is characterized by substitution of arginine for histidine at the position 139. The proteins encoded by polymorphic alleles demonstrated different enzyme activities *in vitro*[6]. *MEH* polymorphism has been associated with chemical carcinogen-induced cancers occurring in lung[7,8], ovary[9], colorectum[10] and liver[11]. The correlation of *mEH* polymorphism with susceptibility to ESCC has not been reported so far. Therefore, the current study investigated the *Tyr*113*His* polymorphism in *mEH* exon 3 in ESCC patients and healthy individuals from North China.

MATERIALS AND METHODS

Subjects

This study included 257 patients with histologically confirmed esophageal squamous cell carcinomas and 252 healthy individuals without overt cancer. The cancer patients were hospitalized for surgery in the Fourth Affiliated hospital, Hebei Medical University between 2001 and 2003. The healthy subjects were from the same hospital for physical examination in the same period. All of the patients and control subjects were from Shijiazhuang city or its surrounding regions. Information of sex, age, smoking habits and family history was obtained from cancer patients and healthy controls by interview following sampling. The smokers were defined as former or current smoking 5 cigarettes per day for at least two years. The individuals with at least one first-degree relative or at least two second-degree relatives having esophageal/cardiac/gastric cancer were defined as having family history of upper gastrointestinal cancers (UGIC). The smoking status and family history were available from some of the cases and controls. Informed consent was obtained from all the recruited subjects. The study was approved by the Ethics Committee of Hebei Cancer Institute.

DNA extraction

Five ml of venous blood from each subject was drawn in Vacutainer tubes containing EDTA and stored at 4 ℃. Genomic DNA was extracted within one week after sampling by using proteinase K digestion followed by a salting out procedure.

MEH genotyping by PCR and restriction fragment analysis

The exon 3 T to C variant in *mEH* gene, changing tyrosine 113 to histidine, creates an *Eco*R restriction site (GATATC), which can be exploited for genotyping by PCR and subsequent RFLP analysis[12]. PCR was performed in a 25 μl volume containing 100 ng of DNA template, 2.5 μl of 10×buffer, 1 U of *Taq*-DNA-polymerase (BioDev-Tech., Beijing, China), 200 μmol of dNTPs and 200 nmol of sense primer (5'-GATCGATAAGTTCCGTTTCACC-3') and antisense primer (5'-ATCCTTAGTCTTGAAGTGAGGAT-3'). Initial denaturation at 94 ℃ for 5 min was followed by 35 cycles at 94 ℃ for 30 sec, at 56 ℃ for 30 sec and at 72 ℃ for 1 min. Subsequently, the PCR products were digested with 10 units of *Eco*R V (TakaRa Biotechnology Co., Ltd, Dalian, China) overnight at 37 ℃ and separated on a 3 % agarose gel. RFLP bands were visualized through ethidium bromide staining under UV light. *Tyr*113 wild-type homozygote was characterized by two bands at the position of 140 bp and 22 bp, while *His*113 homozygotes were identified by a single band (162 bp) and the heterozygotes by three bands (162 bp, 140 bp and 22 bp). For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system. For 10 % of the samples, the reaction was repeated once.

Statistical analysis

Statistical analysis was performed using the SPSS10.0 software package (SPSS Company, Chicago, Illinois, USA). Comparison of *mEH* genotype distribution in the study groups was performed by means of two-sided contingency tables using Chi-square test. A probability level of 5 % was considered significant. The odds ratio (OR) and 95 % confidence interval (CI) were calculated using an unconditional logistic regression model and adjusted by age and sex accordingly.

RESULTS

The mean age of all ESCC cases was 58.5±9.39 years (range 32-85) and that of controls was 49.4±8.56 years (range 29-79 years). The gender distribution in ESCC patients (66.5 % men) was comparable to that in healthy controls (58.3 % men). Moreover, the proportion of smokers in ESCC patients (52.0 %) was also not significantly different from that in healthy controls (49.5 %) (χ^2=0.283, P=0.595). In addition, the frequency of the positive family history of UGIC in ESCC patients (44.1 %) was significantly higher than that in healthy controls (14.0 %) (χ^2=49.87, P<0.0001). Thus, the positive family history of UGIC significantly increased the relative risk to develop ESCC in this population, with an age and sex adjusted odds ratio of 4.06 (95 % CI=2.46-6.69). The demographic distribution of ESCC patients and healthy controls is shown in Table 1.

MEH Tyr113His genotyping was successfully performed in all study subjects. The *mEH* genotype distribution was not

Table 2 Influence of *mEH Tyr113His* polymorphism on ESCC development

	Tyr/Tyr	Tyr/His+His/His	His/His	aOR(95%CI)[c]	aOR (95%CI)[d]
Overall					
Normal	76 (30.2)	176 (69.8)	105 (41.7)		
ESCC	84 (32.7)	173 (67.3)	115 (44.7)	0.756 (0.493~1.157)	1.076 (0.850~1.361)
Male					
Normal	44 (30.6)	100 (69.4)	60 (41.7)		
ESCC	61 (35.7)	110 (64.3)	73 (42.7)	0.724 (0.427~1.225)	1.087 (0.811~1.458)
Female					
Normal	32 (29.6)	76 (70.4)	45 (41.7)		
ESCC	23 (26.8)	63 (73.2)	42 (48.8)	0.825 (0.398~1.710)	1.047 (0.704~1.558)
Age≤50					
Normal	46 (32.6)	95 (67.4)	60 (42.6)		
ESCC	19 (33.9)	37 (66.1)	22 (39.3)	0.867 (0.450~1.671)	1.064 (0.738~1.534)
Age>50					
Normal	30 (27.0)	81 (73.0)	45 (40.6)		
ESCC	65 (32.3)	136 (67.7)	93 (46.3)	0.790 (0.472~1.323)	1.018 (0.768~1.348)
Nonsmoker[a]					
Normal	31 (27.7)	81 (72.3)	49 (43.7)		
ESCC	34 (28.3)	86 (71.7)	60 (50.0)	0.659 (0.338~1.286)	1.135 (0.790~1.631)
Smoker					
Normal	35 (31.8)	75 (68.2)	41 (37.3)		
ESCC	46 (35.4)	84 (64.6)	52 (40.0)	0.901 (0.494~1.644)	1.022 (0.728~1.433)
Negative family history[b]					
Normal	59 (30.9)	132 (69.1)	80 (41.9)		
ESCC	49 (37.1)	83 (62.9)	51 (38.7)	0.660 (0.385~1.134)	1.237 (0.912~1.678)
Positive family history					
Normal	7 (22.6)	24 (77.4)	10 (32.2)		
ESCC	29 (27.9)	75 (72.1)	52 (50.0)	0.638 (0.241~1.689)	0.946 (0.546~1.639)

ESCC: esophageal squamous cell carcinoma. a,b. information of smoking status and family history was available from some of subjects. c,d. the age and sex adjusted odds ratio of Tyr/His+His/His (c) and His/His genotype (d) against Tyr/Tyr genotype.

correlated with gender, age and smoking status both in ESCC patients and in healthy controls (data not shown). The *Tyr* and *His* allele frequencies were 44.0 %, 56.0 % in ESCC patients and 44.2 %, 55.8 % in healthy controls, respectively. There was no statistic difference in allele distribution between ESCC patients and controls (χ^2=0.008, *P*=0.929). The frequencies of *Tyr/Tyr*, *Tyr/His* and *His/His* genotype were 30.2 %, 28.2 % and 41.6 % in healthy controls, respectively. The overall *mEH* genotype distribution in ESCC patients was not significantly different from that in healthy controls (χ^2=2.116, *P*=0.347) (Table 1).

Table 1 Demographic characteristics and *mEH Tyr113His* polymorphism in ESCC patients and healthy individuals

Groups	Control *n* (%)	ESCC *n* (%)
Sex		
Male	147 (58.3)	171 (66.5)
Female	105 (41.7)	86 (33.5)
Age (mean±SD)	49.4±8.56	58.5±9.39
Smoking status[a]		
Ex-or current smoker	110 (49.5)	130 (52.0)
Non-smoker	112 (50.5)	120 (48.0)
Family history of UGIC[b]		
Positive	31 (14.0)	104 (44.1)
Negative	191 (86.0)	132 (55.9)
Genotype		
Tyr/Tyr	76 (30.2)	49 (37.1)
Tyr/His	71 (28.2)	32 (24.2)
His/His	105 (41.6)	51 (38.7)
Allele type		
T	223 (44.2)	226 (44.0)
C	281 (55.8)	288 (56.0)

ESCC: esophageal squamous cell carcinoma, UGIC: upper gastrointestinal cancer. a. Information of smoking status was available from some of subjects, b. Positive family history of UGIC significantly increased the risk to develop ESCC. Age and sex adjusted OR=4.06 (95 % CI=2.46-6.69), χ^2=49.87, *P*<0.0001.

By using *Tyr/Tyr* as the reference genotype, neither *His/His* genotype alone nor in combination with *Tyr/His* genotype significantly modified the risk of ESCC, the adjusted odds ratio was 1.076 (95 % CI=0.850-1.361) and 0.756 (95 % CI=0.493-1.157), respectively. When stratified for sex, age, smoking status and family history of upper gastrointestinal cancer, the frequency of *His/His* and *Tyr/His* genotype in ESCC patients was not significantly different from healthy controls. Consistently, *His/His* alone, or in combination with *Tyr/His* genotype, did not show any significant influence on the risk of ESCC (Table 2).

DISCUSSION

Chemical carcinogens in consumed alcohol and tobacco, polluted water, ingested food, are in general considered as the main risk factors of ESCC in China. However, not all individuals exposed to the above exogenous risk factors will develop ESCC, indicating that the host susceptibility factors may play an important role in cancer development. In recent years, many polymorphic carcinogen metabolic enzymes, such as aldehyde dehydrogenase-2 (ALDH2)[13], cytochrome P450(CYP)[14,15], glutathione S-transferase(GST)[15,16], methylenetetrahydrofolate reductase (MTHFR)[17], NAD(P)H, quinone oxidoreductase 1 (NQO1)[18,19] have been found to be able to modify the susceptibility to chemically induced cancers including esophageal and gastric cancer. Therefore, these polymorphic genes, alone or in combination with each other or with other newly developed genetic markers, may be used as predicative parameters for screening individuals at a high risk of ESCC.

MEH is involved in the metabolism of environmental carcinogens. Polymorphisms in *mEH* gene might affect the enzyme expression and lead to different phenotypes, probably by the alteration of protein stability[6]. *Tyr113His* substitution in exon 3 could reduce the enzyme expression by about 40 %, producing a slow phenotype with a low epoxide hydrolase activity. In contrast, *Arg139 His* in exon 4 could increase the expression by about 25 %, producing a fast phenotype with an increased enzyme activity[6]. The relationship between *mEH* gene polymorphisms and susceptibility to cancers studied had inconsistent conclusions due to different cancer types and populations. The *Tyr* allele in exon 3 was reported to increase the risk of several cancer types including ovarian cancer[9], oropharyngeal carcer[20] and acute leukaemia[21], whereas the *His* allele was associated with increased susceptibility to cancers occurring in colon[10], liver[11] and cervix[22]. In addition, gene-environment interaction was strongly suggested by some investigations, thus, cumulative cigarette smoking might play a pivotal role in association of *His* homozygous genotype with lung cancer development, altering the direction of risk from a risk factor in nonsmokers to a relatively protective factor in heavy smokers[8].

Recently, a slight decrease in *mEH Tyr*113 frequency was observed in esophageal adenocarcinoma (42 %) compared to controls (53 %, *P*=0.05)[23]. In the present study, the frequencies of *Tyr/Tyr*, *Tyr/His* and *His/His* genotype in healthy controls were in consistent with a recent report from a Chinese group[24]. The genotype distribution difference was not found in ESCC patients and healthy controls, as well as in stratification comparison according to the sex, age (>50 or ⩽50), smoking status (never smoking or current and ever smoking), and family history of UGIC. The result suggests that although *mEH Tyr113His* polymorphism is correlated with some cancer types, this genetic alteration may not be associated with susceptibility to ESCC in population of North China.

ACKNOWLEDGEMENTS

We greatly acknowledge Mr. Baoshan Zhao and Mr. Fanshu Meng for their assistance in recruiting study subjects.

REFERENCES

1 **Yokokawa Y,** Ohta S, Hou J, Zhang XL, Li SS, Ping YM, Nakajima T. Ecological study on the risks of esophageal cancer in Ci-Xian, China: the importance of nutritional status and the use of well water. *Int J Cancer* 1999; **83**: 620-624

2 **Launoy G,** Milan CH, Faivre J, Pienkowski P, Milan CI, Gignoux M. Alcohol, tobacco and oesophageal cancer: effects of the duration of consumption, mean intake and current and former consumption. *Br J Cancer* 1997; **75**: 1389-1396

3 **van Gijssel HE,** Divi RL, Olivero OA, Roth MJ, Wang GQ, Dawsey SM, Albert PS, Qiao YL, Taylor PR, Dong ZW, Schrager JA, Kleiner DE, Poirier MC. Semiquantitation of polycyclic aromatic hydrocarbon-DNA adducts in human esophagus by immunohistochemistry and the automated cellular imaging system. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1622-1629

4 **Roth MJ,** Dawsey SM, Wang G, Tangrea JA, Zhou B, Ratnasinghe D, Woodson KG, Olivero 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

5 **Omiecinski CJ,** Aicher L, Swenson L. Developmental expression of human microsomal epoxide hydrolase. *J Pharmacol Exp Ther* 1994; **269**: 417-423

6 **Hassett C,** Aicher L, Sidhu JS, Omiecinski CJ. Human microso-

mal epoxide hydrolase: genetic polymorphism and functional expression *in vitro* of amino acid variants. *Hum Mol Genet* 1994; **3**: 421-428

7 **Lee WJ,** Brennan P, Boffetta P, London SJ, Benhamou S, Rannug A, To-Figueras J, Ingelman-Sundberg M, Shields P, Gaspari L, Taioli E. Microsomal epoxide hydrolase polymorphisms and lung cancer risk: a quantitative review. *Biomarkers* 2002; **7**: 230-241

8 **Zhou W,** Thurston SW, Liu G, Xu LL, Miller DP, Wain JC, Lynch TJ, Su L, Christiani DC. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 461-466

9 **Lancaster JM,** Brownlee HA, Bell DA, Futreal PA, Marks JR, Berchuck A, Wiseman RW, Taylor JA. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol Carcinog* 1996; **17**: 160-162

10 **Harrison DJ,** Hubbard AL, MacMillan J, Wyllie AH, Smith CA. Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. *Br J Cancer* 1999; **79**: 168-171

11 **Tiemersma EW,** Omer RE, Bunschoten A, van't Veer P, Kok FJ, Idris MO, Kadaru AM, Fedail SS, Kampman E. Role of genetic polymorphism of glutathione-S-transferase T1 and microsomal epoxide hydrolase in aflatoxin-associated hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 785-791

12 **Cortessis V,** Siegmund K, Chen Q, Zhou N, Diep A, Frankl H, Lee E, Zhu QS, Haile R, Levy D. A case-control study of microsomal epoxide hydrolase, smoking, meat consumption, glutathione S-transferase M3, and risk of colorectal adenomas. *Cancer Res* 2001; **61**: 2381-2385

13 **Matsuo K,** Hamajima N, Shinoda M, Hatooka S, Inoue M, Takezaki T, Tajima K. Gene-environment interaction between an aldehyde dehydrogenase-2 (ALDH2) polymorphism and alcohol consumption for the risk of esophageal cancer. *Carcinogenesis* 2001; **22**: 913-916

14 **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 Prev* 2000; **9**: 551-556

15 **Cai L,** Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: a case-control study. *World J Gastroenterol* 2001; **7**: 506-509

16 **Cai L,** Yu SZ, Zhang ZF. Cytochrome P450 2E1 genetic polymorphism and gastric cancer in Changle, Fujian Province. *World J Gastroenterol* 2001; **7**: 792-795

17 **Song C,** Xing D, Tan W, Wei Q, Lin D. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Res* 2001; **61**: 3272-3275

18 **Zhang JH,** Li Y, Wang R, Geddert H, Guo W, Wen DG, Chen ZF, Wei LZ, Kuang G, He M, Zhang LW, Wu ML, Wang SJ. NQO1 C609T polymorphism associated with esophageal cancer and gastric cardiac carcinoma in North China. *World J Gastroenterol* 2003; **9**: 1390-1393

19 **Zhang J,** Schulz WA, Li Y, Wang R, Zotz R, Wen D, Siegel D, Ross D, Gabbert HE, Sarbia M. Association of NAD(P)H: quinone oxidoreductase 1 (NQO1) C609T polymorphism with esophageal squamous cell carcinoma in a German Caucasian and a northern Chinese population. *Carcinogenesis* 2003; **24**: 905-909

20 **Amador AG,** Righi PD, Radpour S, Everett ET, Weisberger E, Langer M, Eckert GJ, Christen AG, Campbell S Jr, Summerlin DJ, Reynolds N, Hartsfield JK Jr. Polymorphisms of xenobiotic metabolizing genes in oropharyngeal carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **93**: 440-445

21 **Lebailly P,** Willett EV, Moorman AV, Roman E, Cartwright R, Morgan GJ, Wild CP. Genetic polymorphisms in microsomal epoxide hydrolase and susceptibility to adult acute myeloid leukaemia with defined cytogenetic abnormalities. *Br J Haematol* 2002; **116**: 587-594

22 **Sierra-Torres CH,** Au WW, Arrastia CD, Cajas-Salazar N, Robazetti SC, Payne DA, Tyring SK. Polymorphisms for chemical metabolizing genes and risk for cervical neoplasia. *Environ Mol Mutagen* 2003; **41**: 69-76

23 **Casson AG,** Zheng Z, Chiasson D, MacDonald K, Riddell DC, Guernsey JR, Guernsey DL, McLaughlin J. Associations between genetic polymorphisms of Phase I and II metabolizing enzymes, p53 and susceptibility to esophageal adenocarcinoma. *Cancer Detect Prev* 2003; **27**: 139-146

24 **Yin L,** Pu Y, Liu TY, Tung YH, Chen KW, Lin P. Genetic polymorphisms of NAD(P)H quinone oxidoreductase, CYP1A1 and microsomal epoxide hydrolase and lung cancer risk in Nanjing, China. *Lung Cancer* 2001; **33**: 133-141

Edited by Su Q and Wang XL