



Correlation of CYP1A1 and GSTM1 gene polymorphisms and environmental factors to familial aggregation of esophageal cancer among the Kazakh ethnic group in Xinjiang

M. Zeng, Y. Lv, H.F. Wang, H.A. Yiguli, J.R. Zhang and A. Yisikandaer

Department of Radiotherapy of the Chest and Abdomen,
Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, China

Corresponding author: H.F. Wang
E-mail: wanghf2016@126.com

Genet. Mol. Res. 14 (4): 19102-19109 (2015)
Received September 2, 2015
Accepted November 3, 2015
Published December 29, 2015
DOI <http://dx.doi.org/10.4238/2015.December.29.19>

ABSTRACT. This study aimed to investigate the correlation of CYP1A1 and GSTM1 gene polymorphisms and environmental factors to familial aggregation of esophageal cancer (EC) among the Kazakh ethnic group in Xinjiang. CYP1A1 and GSTM1 gene polymorphisms were detected using peripheral blood from 86 subjects belonging to families with EC and 82 control subjects. Additionally, a questionnaire survey was conducted to ascertain environmental risk factors. Combined effects of CYP1A1 and GSTM1 gene polymorphisms and environmental factors in familial aggregation of EC were evaluated. Distribution frequencies of CYP1A1 MspI and GSTM1 genotypes between EC and control families showed significant differences ($P = 0.002$, $P = 0.001$). Contribution of interaction between CYP1A1 MspI mutant and GSTM1 deletion polymorphisms to familial aggregation of EC was significant, with OR = 3.571 (95%CI = 1.738-3.346). Logistic multivariate analysis indicated that familial aggregation of EC is correlated with 3 factors: drinking water, intake of fresh vegetables and fruits, and CYP1A1 MspI polymorphism ($P = 0.005$, $P = 0.013$, and

P = 0.001). Sufficient intake of fresh vegetables and fruits (OR = 0.278, 95%CI = 0.137-0.551) protected against familial aggregation of EC, while drinking water (OR = 3.468, 95%CI = 1.562-6.551) and CYP1A1 MspI polymorphism (OR = 2.732, 95%CI = 1.741-3.886) were the risk factors. In conclusion, CYP1A1 and GSTM1 gene polymorphisms affect familial aggregation of EC among the Kazakh ethnic group in Xinjiang. River water intake and CYP1A1 MspI polymorphism were risk factors that likely contributed to high incidence of EC among families.

Key words: Kazakh ethnic group; Esophageal cancer family; CYP1A1 gene; GSTM1 gene

INTRODUCTION

Esophageal cancer (EC) is the most common malignancy among the Kazakh ethnic group in Xinjiang, which is believed to be the result of interactions between genetic and environmental factors. Disordered metabolism of chemical carcinogens contained in food and the environment may contribute to EC. Cytochrome P450 (CYP450) enzymes are essential for the metabolism of compounds and are involved in the activation of carcinogens. Glutathione-S-transferase (GST) is an enzyme involved in catalyzing detoxification of a variety of carcinogens. We detected CYP1A1 and GSTM1 gene polymorphisms in the peripheral blood sampled from EC families and control families of the Kazakh ethnic group. Combining this clinical data with a questionnaire survey related to environmental risk factors, we studied the role of CYP1A1 and GSTM1 gene polymorphisms and environmental factors in familial aggregation of EC among the Kazakh ethnic group in Xinjiang.

MATERIAL AND METHODS

Subjects

EC families and control families of the Kazakh ethnic group in North Xinjiang were studied. The following inclusion criteria for EC families were determined based on relevant literature (Zhao et al., 2004): 1) the family consisted of at least two generations, 2) the family had at least two patients with EC, and 3) the family contained first-degree relatives. The inclusion criteria for control families were as follows: the control families lived in the same place and belonged to the same ethnic group as the EC families and the families had similar size and sporadic occurrence of EC. In total, 86 subjects satisfying the criteria for EC families and 82 subjects satisfying the criteria for control families from the Kazakh ethnic group were enrolled from North Xinjiang (Table 1). Signed informed consent was obtained from all subjects.

Questionnaire survey

The survey included the following details: 1) drinking water (well water or river water intake); 2) history of pasteurization; 3) history of smoking (for 1-10 years, 11-20 years, >20 years); 4) alcohol consumption (yes or no); and 5) diet (eating speed 10-30 min, <10 min; occasional intake of spicy food, frequent intake of spicy food; occasional overeating, frequent overeating; occasional intake of green vegetables, frequent intake of green vegetables; occasional intake of fresh fruits, frequent intake of fresh fruits).

Gene polymorphism detection

Sample collection and DNA extraction

For all subjects, 4 mL peripheral venous blood was collected and EDTA was added. Blood samples were preserved at -20°C and total DNA was extracted. The tissues were lysed; RNaseA and Proteinase K were added for RNA and protein digestion, respectively. Extraction and precipitation were carried out to obtain genomic DNA, which was then dissolved in TE buffer and preserved at -20°C.

CYP1A1 and GSTM1 gene polymorphism detection

Reagents used were as follows: DNA Extraction Kit (SK1261, Sangon Biotech (Shanghai) Co., Ltd., China), TaqDNA polymerase [Sangon Biotech (Shanghai) Co., Ltd.], and PCR Purification Kit [SK1141, Sangon Biotech (Shanghai) Co., Ltd.].

The following method was used. CYP1A1 MspI polymorphism was detected by using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The upstream primer of the CYP1A1 MspI gene was 5'-CAGTGAAGAGGTGTAGCCGCT-3'; the downstream primer was 5'-TAGGAGTCT TGTCTCATGCCT-3'. The PCR reaction system (25 µL) consisted of: 2.0 µL 4X dNTP, 2.5 µL 10X buffer, 2.5 mM MgCl₂, 1 µL CYP1A1 primer, 1.0 µL TaqDNA polymerase, and 1.5 µL DNA template. PCR conditions were as follows: pre-denaturation at 94°C for 5 min; 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min for 35 cycles; and final extension at 72°C for 5 min. The length of the amplified product was 340 bp. After digestion with the restriction endonuclease MspI, genotype analysis was carried out using 2% agarose gel electrophoresis with ethidium bromide staining.

Homozygous deletion of the GSTM1 gene was detected using multiprimer PCR. The common GSTM1 gene primers were as follows: GSTM1-P1, 5'-CGCCATCTTGCTACATTGCCCG-3'; downstream primer, GSTM1-P2, 5'-ATCTTCTCCTCTTCTGTCTC-3' and GSTM1-P3, 5'-TTCTGG ATTGTAGCAGATCA-3'. β-globin was used as positive internal control. The 25 µL PCR reaction system consisted of 3.0 µL 4X dNTP, 2.5 µL 10X buffer, 3.0 mM MgCl₂, 1 µL each GSTM1 and β-globin primer, 1.5 U TaqDNA polymerase, and 1.0 µL DNA template. PCR conditions were as follows: pre-denaturation at 94°C for 4 min; 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min for 35 cycles; final extension at 72°C for 10 min. Genotype analysis was carried out using 2% agarose gel electrophoresis with ethidium bromide staining.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. Differences between the two groups was analyzed by the chi-square test and *t*-test. The expressions of the CYP1A1 MspI and GSTM1 genes were compared between the two groups by the chi-square test, measuring the odds ratio (OR) and 95%CI. All tests were two-sided with a significance $\alpha = 0.05$. Logistic regression analysis was used to analyze the correlation of environmental factors (smoking, alcohol consumption, intake of spicy, salted, and smoked food, source of drinking water, intake of fresh vegetables and fruits) and CYP1A1 and GSTM1 gene polymorphisms to familial aggregation of EC.

RESULTS

Balance of baseline data

The composition of the two groups did not differ significantly in terms of gender, occupation, and incidence of EC; the mean age of the two groups did not show any significant difference either. Thus, the baseline characteristics of the two groups were balanced (Table 1).

Table 1. Baseline characteristics of the two groups.

	Gender		Age		Occupation			Incidence	
	Male	Female	Range	Mean	Herders	Farmers	Others	EC patients	Non-EC patients
EC family	40	46	21-81	42	27	29	30	36	50
Control family	42	40	20-79	47	26	27	29	38	44
P value	0.410			0.231	0.353			0.284	

Genotyping

Detection of CYP1A1 MspI polymorphism by electrophoresis

As shown in Figure 1, CYP1A1 wild-type homozygote (T/T) was amplified into one 340-bp fragment (genotype A); the CYP1A1 heterozygous mutant (T/C) was amplified into three fragments (340, 200, and 140 bp) (genotype B); and the CYP1A1 homozygous mutant (C/C) was amplified into two fragments (200 and 140 bp) (genotype C).

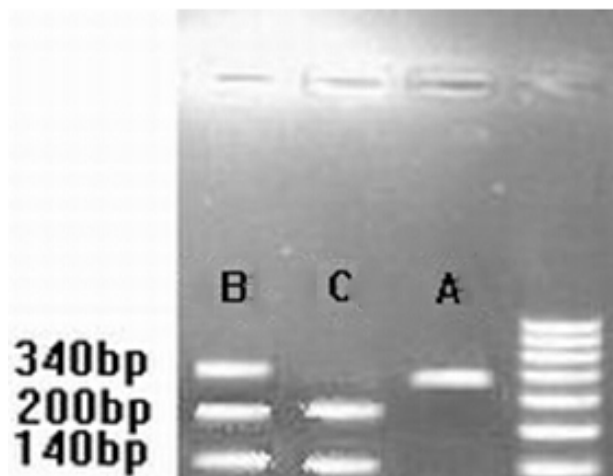


Figure 1. Electrophoretogram of different CYP1A1 MspI genotypes.

Detection of GSTM1 polymorphism by electrophoresis

As shown in Figure 2, amplification of both 157 and 230 bp fragments indicated the wild-type genotype (+), and amplification of the 157 bp fragment alone indicated the null genotype (-).

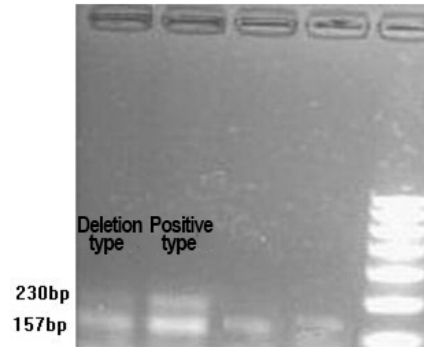


Figure 2. Electrophoretogram of different GSTM1 genotypes.

Correlation of CYP1A1 and GSTM1 gene polymorphisms to familial aggregation of EC

Among EC families, there were 13 subjects with CYP1A1 wild-type genotype (T/T) (7.7%) and 73 subjects with T/C or C/C genotype (43.5%). Among control families, there were 36 subjects with CYP1A1 wild-type genotype (T/T) (21.4%) and 46 subjects with T/C or C/C genotype (27.3%). The distribution frequencies of different CYP1A1 MspI genotypes were significantly different between the two groups ($P = 0.002$, OR = 5.050, 95%CI = 1.742-14.710). As to the GSTM1 genotype distribution, 16 subjects from EC families carried GSTM1 wild-type genotype (9.5%) and 40 subjects carried null genotype (23.8%). The distribution frequencies of different GSTM1 genotypes were also significantly different between the two groups ($P = 0.001$, OR = 4.831, and 95%CI = 1.802-12.987) (Table 2).

Table 2. Correlations between CYP1A1 and GSTM1 gene polymorphisms and familial aggregation of EC.

Group	CYP1A1 gene polymorphism		GSTM1 gene polymorphism	
	Genotype A	Genotype B+C	Wild-type genotype	Null genotype
EC family	13 (7.7%)	73 (43.5%)	16 (9.5%)	70 (41.7%)
Control family	36 (21.4%)	46 (27.3%)	42 (25.0%)	40 (23.8%)
OR	5.050	4.831		
95%CI	1.742-14.710	1.802-12.987		
P value	0.002	0.001		

Interaction between CYP1A1 gene polymorphism and GSTM1 null genotype in familial aggregation of EC

The interaction between CYP1A1 MspI genotypes (genotype B and C) and GSTM1 null genotype played a role in familial aggregation of EC with an OR value of significant level. However, the OR value representing the association between CYP1A1 MspI genotypes and GSTM1 non-null genotype was not significantly different from that representing the association between CYP1A1 MspI genotypes and GSTM1 null genotype. This indicated that the interaction between CYP1A1 MspI genotypes and GSTM1 non-null genotype had no significant effect on familial aggregation of EC (Table 3).

Table 3. Interactions between CYP1A1 and GSTM1 gene polymorphisms in familial aggregation of EC.

Genotype	EC family	Non-EC family	OR (95%CI)	P value
MspI				
A, GSTM1 non-null genotype	3	26	0.153 (0.021-1.092)	
B+C, GSTM1 non-null genotype	10	11	1.741 (1.061-2.857)	0.35
A, GSTM1 null genotype	14	16	0.733 (0.382-1.407)	
B+C, GSTM1 null genotype	60	28	3.571 (1.738-3.346)	0.027

Risk factor analysis for familial aggregation of EC

Using logistic regression analysis, we found that the source of drinking water, intake of fresh vegetables and fruits, and the CYP1A1 MspI polymorphism were three statistically significant risk factors. Frequent intake of fresh vegetables and fruits was the protective factor (OR = 0.278, 95%CI = 0.137-0.551), while river water intake (OR = 3.468, 95%CI = 1.562-6.551) and the CYP1A1 MspI mutant genotype (OR = 2.732, 95%CI = 1.741-3.886) were the risk factors.

Table 4. Logistic regression analysis on GSTM1 gene polymorphisms and environmental factors.

	B	S.E.	Wald	Sig.	Exp(B)	95%CI for Exp(B)	
						Lower	Upper
History of pasteurization	-0.582	0.657	0.783	0.376	0.559	0.154	2.027
Smoking	-0.764	1.354	0.319	0.572	0.466	0.033	6.612
Alcohol consumption	-1.048	1.276	0.675	0.411	0.351	0.029	4.272
Intake of spicy food	0.017	0.775	0.000	0.983	1.017	0.223	4.641
Intake of salted and smoked food	0.164	0.675	0.059	0.809	1.178	0.313	4.425
Drinking water	-2.649	0.941	7.921	0.005	0.071	0.011	0.447
Intake of fresh vegetables and fruits	-1.811	0.721	9.707	0.013	0.237	0.205	0.626
MspI genotypes	2.047	0.887	10.891	0.001	2.675	1.508	4.108
GSTM1 gene polymorphism	0.712	0.968	0.529	0.612	1.172	0.378	3.697

B: Berta coefficient; S.E.: Standard error; Wald: Chi square value; Sig.: significance; Exp(B): Odds ratio.

DISCUSSION

The CYP1A1 gene is localized on chromosome 15q22-24. This gene catalyzes the oxidative metabolism of carcinogenic polycyclic aromatic hydrocarbons and aromatic amines present in cigarettes and the environment, leading to carcinogenicity and mutagenicity. The T/C genotype of CYP1A1 gene involves the mutation 264 bp downstream of non-encoding poly A at the 3' end, which can be recognized by the restriction endonuclease MspI. This mutation will enhance the catalytic activity or upregulate the expression of the CYP1A1 gene (Landi et al., 1994). Many studies have been devoted to the correlation between CYP1A1 MspI polymorphism and tumors. In relevant literature reports concerning lung cancer, prostate cancer, and head and neck cancers (Ji et al., 2012; Ding et al., 2013; Liu et al., 2013), individuals carrying mutant genotypes (CC+TC) have a higher incidence of tumors. Moreover, the interaction between these mutant genotypes and a history of smoking has an impact on tumor occurrence. Others have found that CYP1A1 MspI mutant genotypes are not necessarily associated with increased tumor incidence (Malik et al., 2010; Guo and Guo, 2012; Yu et al., 2012). The correlation between MspI mutant genotypes and tumor incidence, especially among those with familial history of tumors, remains to be confirmed. We found that MspI mutant genotypes contributed to familial aggregation of EC. Among EC families of the Kazakh ethnic group, the number of individuals carrying at least one CYP1A1 MspI mutant

genotype (T/C or C/C), or genotype B or C, was 5.05 times greater than that among non-EC families. This indicates that CYP1A1 MspI mutant genotypes contribute to familial aggregation. However, Moraes et al. (2012) did not discover any obvious correlation between CYP1A1 MspI gene polymorphism and tumor occurrence among Brazilian families with very high tumor incidence. This discrepancy may be explained by ethnic differences.

Four alleles of the GSTM1 gene have been identified so far. The null allele exhibits no activity of the GSTM1 gene, and there is a decline in detoxification capacity of specific carcinogens. Some studies (Liu et al., 2010; Aghajany-Nasab et al., 2011; Jiang et al., 2011) indicate that GSTM1 null genotype is associated with tumor occurrence. We found that the EC families and non-EC families differed significantly in distribution frequencies of GSTM1 genotypes ($P = 0.002$, $OR = 4.831$, $95\%CI = 1.802-12.987$). The frequency of GSTM1 null genotype was increased among EC families of the Kazakh ethnic group, 4.831 times of that among non-EC families. As reported by Zhang et al. (2011), the risk of EC in individuals carrying GSTM1 null genotype was 1.33 times greater than that in individuals carrying wild-type genotype among the Asian population. These results were consistent with ours.

The CYP1A1 and GSTM1 genes, which are respectively associated with metabolism and detoxification, may act in synergy. Their interaction can become an important risk factor. Chinese scholars reported that GSTM1 null genotype increased the risk of lung cancer, especially in those also carrying CYP1A1 gene polymorphism (Chen et al., 2001). Sam et al. (2010) also drew similar conclusions after investigation respiratory and digestive canal cancers among the Indian population. We found that the interaction between CYP1A1 MspI mutant genotypes and GSTM1 null genotype played an important role in familial aggregation of EC. This may be one underlying reason for the high incidence of EC among the Kazakh ethnic group in Xinjiang.

It is generally believed that EC is a result of the combined action of genetic and environmental factors. Among various studies, smoking, consumption of alcohol, intake of spicy and hot food, and fast eating speeds are commonly associated with EC since they cause damage to esophageal mucosa. Liu et al. (2008) reported that irregular eating patterns and preference for hot and hard food contributed to incidence of EC in the Huai'an region. Logistic regression analysis was carried out in this study to identify the risk factors of familial aggregation of EC. Three risk factors of statistical significance were determined including the source of drinking water, intake of fresh vegetables and fruits, and CYP1A1 MspI mutant genotypes. Among them, frequent intake of fresh vegetables and fruits was the protective factor, while river water intake and CYP1A1 MspI mutant genotypes were the risk factors. An earlier study found that the risk of EC was increased by 4.763 times among Kazakh people who drank river water (Wang et al., 2007).

We demonstrated that CYP1A1 and GSTM1 gene polymorphisms played a role in familial aggregation of EC among the Kazakh ethnic group in Xinjiang. River water intake and CYP1A1 MspI mutant genotypes were the risk factors of familial aggregation of EC, contributing to the high incidence in EC families. However, we did not prove the correlation of smoking and alcohol consumption to familial aggregation of EC owing to limited sample size and differences in major risk factors across the region. The sample size will be increased for stratified analysis in the future in order to identify additional causes for familial aggregation of EC among the Kazakh ethnic group in Xinjiang.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

- Aghajany-Nasab M, Panjehpour M, Samiee SM, Rahimi F, et al. (2011). Glutathione S-transferase mu gene variants and colorectal cancer development-use of sequence-specific probes for an Iranian population. *Asian Pac. J. Cancer Prev.* 12: 1511-1515.
- Chen S, Xue K, Xu L, Ma G, et al. (2001). Polymorphisms of the CYP1A1 and GSTM1 genes in relation to individual susceptibility to lung carcinoma in Chinese population. *Mutat. Res.* 458: 41-47.
- Ding G, Xu W, Liu H, Zhang M, et al. (2013). CYP1A1 MspI polymorphism is associated with prostate cancer susceptibility: evidence from a meta-analysis. *Mol. Biol. Rep.* 40: 3483-3491.
- Guo R and Guo X (2012). Quantitative assessment of the associations between CYP1A1 polymorphisms and gastric cancer risk. *Tumour Biol.* 33: 1125-1132.
- Ji YN, Wang Q, Lin XQ and Suo LJ (2012). CYP1A1 MspI polymorphisms and lung cancer risk: an updated meta-analysis involving 20,209 subjects. *Cytokine* 59: 324-334.
- Jiang Z, Li C and Wang X (2011). Glutathione S-transferase M1 polymorphism and bladder cancer risk: a meta-analysis involving 33 studies. *Exp. Biol. Med. (Maywood)* 236: 723-728.
- Landi MT, Bertazzi PA, Shields PG, Clark G, et al. (1994). Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics* 4: 242-246.
- Liu L, Wu G, Xue F, Li Y, et al. (2013). Functional CYP1A1 genetic variants, alone and in combination with smoking, contribute to development of head and neck cancers. *Eur. J. Cancer* 49: 2143-2151.
- Liu R, Yin LH, Pu YB, et al. (2008) Genetic and environmental risk factors and risk of esophageal cancer in Huaian. *Cancer dist. mut.* 20: 367-370.
- Liu R, Yin L, Pu Y, Li Y, et al. (2010). Functional alterations in the glutathione S-transferase family associated with enhanced occurrence of esophageal carcinoma in China. *J. Toxicol. Environ. Health A.* 73: 471-482.
- Malik MA, Upadhyay R, Mittal RD, Zargar SA, et al. (2010). Association of xenobiotic metabolizing enzymes genetic polymorphisms with esophageal cancer in Kashmir Valley and influence of environmental factors. *Nutr. Cancer* 62: 734-742.
- Moraes LN, Borges MF, Sousa PA, Venere PC, et al. (2012). Lack of association of CYP1A1-MspI SNP and GSTM1 null genotypes with cancer in a Brazilian family with unusually high cancer incidence. *Genet. Mol. Res.* 11: 1610-1617.
- Sam SS, Thomas V, Reddy KS, Surianarayanan G, et al. (2010). Gene-environment interactions associated with CYP1A1 MspI and GST polymorphisms and the risk of upper aerodigestive tract cancers in an Indian population. *J. Cancer Res. Clin. Oncol.* 136: 945-951.
- Wang XM, Jie ES, Ma YQ, Chen B, et al. (2007). Xinjiang kazakh esophageal cancer risk factors case-control study. *China Public Health* 23: 737-738.
- Yu L, Sun L, Jiang YF, Lu BL, et al. (2012). Interactions between CYP1A1 polymorphisms and cigarette smoking are associated with the risk of hepatocellular carcinoma: evidence from epidemiological studies. *Mol. Biol. Rep.* 39: 6641-6646.
- Zhang CX, Chai YR, Wang P, et al. (2011). GSTM1 gene polymorphism and risk of esophageal cancer meta analysis. *China Public Health* 27: 241-243.
- Zhao CH, Liu HX and Bu XM (2004). HMLH3 mismatch repair gene mutations in familial gastric cancer. *World Chin. J. Digest.* 12: 1030-1033.