GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Interaction between CYP 2C19*3 polymorphism and smoking in relation to laryngeal carcinoma in the Chinese Han population

J. Feng[1,4]*, L. Li[2]*, Y.-S. Zhao[3], S.-Q. Tang[4], H.-B. Yang[4] and S.-X. Liu[1]

[1]Department of Otolaryngology-Head and Neck Surgery,
West China Hospital of Sichuan University, Chengdu, P.R. China
[2]Department of Pathology, The Affiliated Hospital of North Sichuan Medical College, Nanchong, P.R. China
[3]Department of Cardiothoracia Surgery,
West China Hospital of Sichuan University, Chengdu, P.R. China
[4]Department of Otolaryngology, Head and Neck Surgery,
The Affiliated Hospital of North Sichuan Medical College, Nanchong, P.R. China

*These authors contributed equally to this study.
Corresponding author: S.-X. Liu
E-mail: liushixifj@163.com

Genet. Mol. Res. 10 (4): 3331-3337 (2011)
Received March 23, 2011
Accepted October 10, 2011
Published December 5, 2011
DOI http://dx.doi.org/10.4238/2011.December.5.9

ABSTRACT. Cytochrome P450 (CYP) 2C19 metabolizes arachidonic acid to biologically active epoxyeicosatrienoic acids, which strongly promote proliferation of cancer cells *in vitro* and *in vivo*. Knowing that smoking is the most important risk factor for laryngeal carcinoma, we examined the relationships between CYP2C19*3 polymorphism, smoking and laryngeal carcinoma in the Chinese Han population. In a Chinese Han case-control study of 300 laryngeal carcinoma patients and 300 healthy controls, we investigated polymorphism in the CYP2C19 gene by PCR-RFLP analysis. The CYP2C19*3 AG + AA genotype was significantly more prevalent in laryngeal carcinoma patients (6.67 *vs* 2.67%; P = 0.02). Multiple logistic regression analysis showed smoking (odds ratio (OR) = 6.353, 95% confidence interval (CI) = 4.413-9.144; P < 0.001) and alcohol consumption (OR =

2.607, 95%CI = 1.130-6.016; P = 0.025) as independent risk factors for laryngeal carcinoma; there was a significant interaction between CYP2C19*3 and smoking (OR = 17.842, 95%CI = 13.32-31.102; P = 0.009). We conclude that CYP2C19*3 polymorphism is significantly associated with laryngeal carcinoma in the Chinese Han population.

Key words: Laryngeal carcinoma; Cytochrome P450; Smoking; Epoxyeicosatrienoic acids

INTRODUCTION

Cytochrome P450 (CYP) epoxygenases metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs). Current research suggests that some CYP isoforms, such as CYP1A (Lundell and Wikvall, 2008), CYP2B (Fava et al., 2008), CYP2C (Node et al., 2001) and CYP2D (Fichtlscherer et al., 2004), participate in arachidonic acid metabolism. One human cytochrome P450 enzyme, CYP2C19, is abundantly expressed in endothelial and smooth muscle cells (Imig, 2000; Ercan et al., 2008) and is the key enzyme of EET synthesis. The addition of exogenous EETs markedly promotes the proliferation of cancer cells *in vitro* and *in vivo*. In neoplastic cell lines, added EET has been shown to increase the activation of MAPK and PI3K/Akt pathways and enhance phosphorylation of epidermal growth factor receptor (EGFR). EETs also inhibit carcinoma cell apoptosis through upregulation of the antiapoptotic proteins Bcl-2 and Bcl-xL and downregulation of the proapoptotic protein Bax (Jiang et al., 2005, 2007). These results suggest that the CYP2C19 plays a previously unrecognized role in the promotion of the neoplastic phenotype and in the pathogenesis of a variety of human cancers.

Recently, a common genetic variant, the CYP2C19*3 (CYP2C19 G636A) gene, was reported in some studies (Ercan et al., 2008). This polymorphism, 636 G→A substitution in the exon 4 region, changes the tryptophan codon to the termination codon, which leads to protein synthesis stopping earlier and the protein become functional defect for the deficiency connective zone of the hemochrome and the substrate. However, the role of this gene variant in laryngeal carcinoma has not been sufficiently investigated.

Smoking is the most important risk factor for laryngeal carcinoma (Cikojević et al., 2010). Death from laryngeal cancer is 20 times more likely for heavy smokers than for nonsmokers. The age-standardized risk of mortality from laryngeal cancer appears to have a linear relationship with increasing cigarette consumption. Furthermore, active smoking by patients with head and neck cancer is associated with significant increases in the annual rate of second primary tumor development compared to former smokers or nonsmokers.

Therefore, in the present study, we hypothesized that those with the CYP2C19*3 may have a higher risk for laryngeal cancer. We also speculated that there should be a synergistic interaction between the effect of smoking and the genetic variation for laryngeal cancer.

MATERIAL AND METHODS

Subjects

Study participants were recruited from patients admitted to the Department of Otolar-

yngology-Head and Neck Surgery, West China Hospital of Sichuan University from 2005 to 2010. Cases were consecutive primary untreated laryngeal carcinoma patients admitted to the Department of Otorhinolaryngology with histologically-confirmed squamous cell carcinoma of the larynx. Controls were selected from cancer-free patients admitted to the same hospital during the same time period with a broad range of diagnoses without matching cases by any covariate.

The study sample comprised 300 cases and 300 controls. Written informed consent was obtained from all study subjects, after which each subject provided a venous blood sample, which was collected in EDTA-coated tubes. This study was performed according to the Declaration of Helsinki and was approved by the ethics committee of the West China Hospital of Sichuan University.

Data collection

A standard questionnaire was administered to cases and controls by trained medical doctors to gather information on demographic variables, including cigarette smoking, drinking history, fruit and vegetable intake, and family history of cancer. The participants were asked to focus on the year prior to diagnosis (for controls the year prior to the interview date) when answering questions regarding lifestyle. Pack-years were calculated as years smoked multiplied by the current number (or previous number, for those who had quit) of cigarettes smoked/day divided by 20. For quantification of fruit and vegetable intake, an amount of 70 grams was considered an average portion of vegetables (both fresh and cooked vegetables included) and 100 grams were considered an average portion of fruit (Ashfield-Watt et al., 2004). Participants were classified as consuming ≥2 portions/day if they regularly consumed at least one portion of fruit and one portion of vegetables per day or if they consumed at least 2 portions of fruit or 2 portions of vegetables per day.

Genotyping of *CYP2C19**3 polymorphism

Genotyping was confirmed by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. The primer of *CYP2C19* was designed by the Primer Premier 5.0 software. The forward primer was 5'-CATCCTGGGCTGTGCT-3', and the reverse primer was 5'-AGGGCTTTGGAGTTTAGTG-3'; annealing temperature was 52°C. The PCR product (15 μL) was incubated with *Bam*HI (Fermentas Corporation) 5 U in a total volume of 25 μL overnight at 37°C, and the resulting fragments were separated on a 1.5% agarose gel. The presence of the G636A variant creates a *Bam*HI site producing two fragments of 263 and 133 bp. To verify the results, we used sequenced genomic DNAs as positive controls in our assays.

Statistical analysis

Data analysis was performed using the software Statistical Package for Social Sciences-SPSS for Windows (version 13.0, SPSS Institute, Chicago, IL). Hardy-Weinberg equilibrium was assessed by chi-square analysis. Differences in enumeration data between the cases and the controls were analyzed using the chi-square test. Differences in distributions of

genotypes and alleles between the cases and the controls were analyzed using the chi-square test. The distribution of allele frequencies was analyzed by 2 x 2 contingency tables using a 5% level of significance. Logistic regression analysis was used to estimate the odds ratio (OR) and its 95% confidence interval (CI) as a measure of the association between polymorphism of *CYP2C19* and risk of laryngeal carcinoma.

RESULTS

General characteristics of the participants

General characteristics of the study population are presented in Table 1. There were no significant differences between the laryngeal carcinoma patients and the control subjects in age and gender (both $P > 0.05$). However, there were significant differences in smoking, drinking, fruit and vegetable intake, and family history of cancer between the cases and the controls (all $P < 0.05$).

Table 1. Clinical characteristics of study participants.

	Laryngeal carcinoma (N = 300)	Control (N = 300)	P
Age (years; means ± SD)	63.4 ± 11.5	63.7 ± 11.4	0.844
Male gender (N, %)	265 (88.3)	267 (89.0)	0.764
Pack-years of smoking (N, %)			
0	62 (20.67)	186 (62.0)	<0.001
1-24	77 (25.67)	85 (28.33)	
>24	161 (53.67)	29 (9.67)	
Alcohol drinker (N, %)			
0 g/day	69 (23.0)	179 (59.67)	<0.001
1-30 g/day	132 (44.0)	99 (33.0)	
>31 g/day	99 (33.0)	22 (7.33)	
Fruit and vegetable intake (N, %)			
≥2 portions/day	124 (41.33)	208 (69.33)	<0.001
<2 portions/day	176 (58.67)	92 (30.67)	
Family history of cancer			
No	181 (60.33)	251 (83.67)	<0.001
Yes	119 (39.67)	49 (16.33)	

Distribution of the CYP2C19*3 genotype

The frequency of the *CYP2C19*3* A allele was significantly higher in laryngeal carcinoma patients than in controls (3.67 *vs* 1.5%; $P = 0.018$). The CYP2C19*3 AG + AA genotype was significantly more prevalent in laryngeal carcinoma patients (6.67 *vs* 2.67%; $P = 0.02$, Table 2).

Table 2. Genotype distribution of CYP2C19*3 polymorphism.

Group	N	Allele (%)		Genotype (N, %)		
		A	G	AA	AG	GG
Cases	300	3.67	96.33	2 (0.67)	18 (6.0)	280 (93.33)
Controls	300	1.50	98.50	1 (0.33)	7 (2.34)	292 (97.33)

Associations of traditional risk factors with laryngeal carcinoma

In participants who did not smoke, the *CYP2C19*3* A allele (AA or AG genotype) was associated with an increased risk of laryngeal carcinoma (OR = 1.51, 95%CI = 1.023-3.564) compared with GG genotype carriers. Among participants carrying *CYP2C19*3* GG genotypes, smoking was associated with a 5.88-fold higher risk (OR = 5.88, 95%CI = 3.232-10.435). However, smoking and carrying *CYP2C19*3* A allele (AA or AG genotype) were associated with a 25.94-fold increased risk for laryngeal carcinoma (OR = 25.94, 95%CI = 19.247-87.321) when compared to non-smoking *CYP2C19*3* GG genotypes (Table 3). These results indicate that there was a significant interaction between CYP2C19*3 and smoking in laryngeal carcinoma. Besides smoking, we found similar interactions between CYP2C19*3 and drinking, fruit and vegetable intake, and family history of cancer. However, by binary logistic regression analysis, we only found the interaction between CYP2C19*3 and smoking to remain significant (OR = 17.842 95%CI = 13.32-31.102; interaction $P < 0.001$; Table 4).

Table 3. Association of traditional risk factors with the CYP2C19*3 genotype in laryngeal carcinoma.

Traditional risk factor	CYP2C19*3 genotype	Cases (n)	Controls (n)	OR	95%CI
Smoking					
No	GG	59	180	1*	
No	AA or AG	3	6	1.54	1.023-3.564
Yes	GG	221	112	5.88	3.232-10.435
Yes	AA or AG	17	2	25.94	19.247-87.321
Drinking					
No	GG	64	175	1*	
No	AA or AG	5	4	3.42	2.125-7.868
Yes	GG	216	117	5.05	3.879-9.765
Yes	AA or AG	15	4	10.25	8.339-28.497
Fruit and vegetable intake					
≥2 portions/day	GG	121	205	1*	
≥2 portions/day	AA or AG	3	3	1.69	1.022-3.436
<2 portions/day	GG	159	87	3.10	2.545-5.657
<2 portions/day	AA or AG	17	5	5.76	4.653-8.343
Family history of cancer					
No	GG	177	246	1*	
No	AA or AG	4	5	1.11	0.986-1.989
Yes	GG	103	46	3.11	2.121-4.323
Yes	AA or AG	16	3	7.41	4.323-9.876

Data are presented as number of patients. CI = confidence interval; GG = homozygous G allele of CYP2C19*3 gene; AG = heterozygous allele of CYP2C19*3 gene; AA = homozygous A allele of CYP2C19*3 gene; OR = odds ratio. *Reference values.

Table 4. Logistic regression analysis results.

Risk factor	β	SE	Wald χ^2	P	OR	95%CI
Drinking	0.958	0.427	5.045	0.025	2.607	1.130-6.016
Smoking	1.849	0.186	99.000	0.000	6.353	4.413-9.144
CYP2C19* Smoking[§]	0.733	0.433	7.211	0.009	17.842	13.32-31.102

[§]Interaction between smoking and CYP2C19*3 A allele.

Limitation of this study

The present study was limited by the relatively small sample size. This may have led to weak statistical power and wide CIs when estimating odds ratios. The other study limitation is that we did not survey all the previously reported gene variations that could affect the metabolism of arachidonic acid and, therefore, contribute to the regulation of vascular tone.

DISCUSSION

In this study, we found that Chinese Han patients with laryngeal carcinoma had a higher frequency of the *CYP2C19*3* A allele than did controls. There was also significant synergism between this polymorphism and smoking in laryngeal carcinoma in the Chinese Han population.

We found that patients with the *CYP2C19*3* polymorphism had a higher possibility of laryngeal carcinoma. The *CYP2C19**3 mutant A allele changes the tryptophan codon to the termination codon which results in the protein synthesis stopping earlier and the protein become functional defect for the deficiency connective zone of the hemochrome and the substrate. Cytochrome P450 epoxygenases metabolize arachidonic acid to EETs. Some of the EETs are reported to have potential antimigratory, antioxidant, and antiapoptotic effects (Sun et al., 2002; Gauthier et al., 2004), and these effects help to explain the association of CYP2C19 with the mechanism of laryngeal carcinoma.

To our knowledge, we are the first to report this gene-environment interaction between smoking and the *CYP2C19*3* polymorphism in Chinese Han population. Compared to the nonsmokers carrying the *CYP2C19*3* GG genotype, the GG genotype smoker was associated with a 5.88-fold higher risk for laryngeal carcinoma. Interestingly, our interaction analysis showed that smoking could cause a significant 25.94-fold higher risk of laryngeal carcinoma patients with the *CYP2C19*3* A allele (AG + AA), compared to nonsmoking patients with the *CYP2C19*3* GG genotype.

Compared to the nonsmokers carrying the *CYP2C19*3* GG genotype, the GG genotype drinker was associated with a 5.05-fold increased risk for laryngeal carcinoma. However, for the CYP2C19*3 A allele carriers, drinking, fruit and vegetable intake of less than 2 portions per day, and family history of cancer showed respectively a 10.25-, 5.76- and 7.41-fold increased risk for laryngeal carcinoma. However, by multiple logistic analysis, only the interaction between CYP2C19*3 and smoking remained significant.

Smoking is an established cause of cancer of several organ sites, including the lung, larynx, oral cavity (including pharynx), and esophagus. Smoking itself can alter some particular tumor progression via signaling transduction and thus modify the metabolism of EETs (Ye et al., 2004). Laryngeal carcinoma patients with both a smoking habit and *CYP2C19*3* genetic variation were expected to be more prone to oxidative stress damage. This effect derives not only from impaired EET functions, but also the possible influence from extract materials of the cigarettes, especially nicotine. A previous study showed that some arachidonic acid metabolites, including some EETs, could promote the proliferation of cancer cells *in vitro* and *in vivo* (Jian-Gang et al., 2007), which could also be activated by nicotine (Kurahashi et al., 2003). Our findings need further investigation to explain the mechanism of this gene-environment or other possible pharmacogenetic interactions.

CONCLUSIONS

We found a significant association between the *CYP2C19*3* polymorphism and laryngeal carcinoma in Chinese Han people. There is significant synergetic effect between *CYP2C19*3* polymorphism and smoking on the risk factor for laryngeal carcinoma.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#30872850) and Science Supporting Foundation of Sichuan (#2008sz0186).

REFERENCES

Ashfield-Watt PA, Welch AA, Day NE and Bingham SA (2004). Is 'five-a-day' an effective way of increasing fruit and vegetable intakes? *Publ. Health Nutr.* 7: 257-261.

Cikojević D, Gluncić I and Klancnik M (2010). Cigarette smoking and progression of laryngeal lesions. *Coll. Antropol.* 34 (Suppl 1): 45-48.

Ercan B, Ayaz L, Cicek D and Tamer L (2008). Role of CYP2C9 and CYP2C19 polymorphisms in patients with atherosclerosis. *Cell Biochem. Funct.* 26: 309-313.

Fava C, Montagnana M, Almgren P, Rosberg L, et al. (2008). The V433M variant of the CYP4F2 is associated with ischemic stroke in male Swedes beyond its effect on blood pressure. *Hypertension* 52: 373-380.

Fichtlscherer S, Dimmeler S, Breuer S, Busse R, et al. (2004). Inhibition of cytochrome P450 2C9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. *Circulation* 109: 178-183.

Gauthier KM, Falck JR, Reddy LM and Campbell WB (2004). 14,15-EET analogs: characterization of structural requirements for agonist and antagonist activity in bovine coronary arteries. *Pharmacol. Res.* 49: 515-524.

Imig JD (2000). Epoxygenase metabolites. Epithelial and vascular actions. *Mol. Biotechnol.* 16: 233-251.

Jiang JG, Chen CL, Card JW, Yang S, et al. (2005). Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. *Cancer Res.* 65: 4707-4715.

Jiang JG, Ning YG, Chen C, Ma D, et al. (2007). Cytochrome p450 epoxygenase promotes human cancer metastasis. *Cancer Res.* 67: 6665-6674.

Kurahashi K, Nishihashi T, Trandafir CC, Wang AM, et al. (2003). Diversity of endothelium-derived vasocontracting factors-arachidonic acid metabolites. *Acta Pharmacol. Sin.* 24: 1065-1069.

Lundell K and Wikvall K (2008). Species-specific and age-dependent bile acid composition: aspects on CYP8B and CYP4A subfamilies in bile acid biosynthesis. *Curr. Drug Metab.* 9: 323-331.

Node K, Ruan XL, Dai J, Yang SX, et al. (2001). Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J. Biol. Chem.* 276: 15983-15989.

Sun J, Sui X, Bradbury JA, Zeldin DC, et al. (2002). Inhibition of vascular smooth muscle cell migration by cytochrome p450 epoxygenase-derived eicosanoids. *Circ. Res.* 90: 1020-1027.

Ye YN, Liu ES, Shin VY, Wu WK, et al. (2004). Nicotine promoted colon cancer growth via epidermal growth factor receptor, c-Src, and 5-lipoxygenase-mediated signal pathway. *J. Pharmacol. Exp. Ther.* 308: 66-72.