Original article

Role of *CYP1A1 Msp*I polymorphism in *CYP1A1* gene with susceptibility to lung cancer in Iranian patients

Majid Motovali-Bashi[1], Mostafa Biglari[2], Zohreh Hojati[1], Simin Hemati[3], Kian Khodadad[4]

[1] Assistant Professor, Department of Biology, School of Sciences, University of Isfahan, Isfahan, Iran. [2] Department of Biology, School of Sciences, University of Isfahan, Isfahan, Iran. [3] Assistant Professor, Department of Radiation Oncology, Isfahan University of Medical Sciences, Isfahan, Iran. [4] Assistant Professor, Chronic Respiratory Diseases Research Center, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

BACKGROUND: Lung cancer has remained the most prevalent malignancy worldwide. It is the fifth leading cause of cancer death in Iran. Nevertheless, during last few years a gradual permanent increase in its incidence has been reported. Although the crucial role of tobacco smoke in lung cancer initiation has long been established, it is tempting to hypothesize that genetic polymorphisms may contribute to lung cancer predisposition. *CYP1A1* gene encodes the main enzyme responsible for metabolic activation of several tobacco carcinogens. *CYP1A1 Msp*I (6235T→C) polymorphism is the most studied variation within the *CYP1A1*, impacts on the basal levels of metabolism and is believed to be associated with elevated lung cancer risk, mainly in Asian population. **METHODS**: We investigated the frequency of this genetic variation in Iranian lung cancer patients through a cross-sectional study. 65 lung cancer cases and 80 healthy controls were recruited. **RESULTS:** The present findings confirmed the low frequency of the variant *CYP1A1*2A* allele in the control group. A significant increased risk for lung cancer was observed among those who possessed heterozygous (**1/*2A*) genotype (Odds ratio = 2.79, 95% CI: 1.01-7.65). Adenocarcinoma was more frequent in non-smoker group (p = 0.00064); however, no significant increased risk was observed for squamous cell carcinoma and small cell carcinoma with respect to smoking. **CONCLUSIONS:** heterozygous (**1/*2A*) genotype may increase the risk of lung cancer.

KEYWORDS: Cytochrome P450, Lung Cancer, Polycyclic Aromatic Hydrocarbon, RFLP-PCR, Tobacco Smoke, Iran

BACKGROUND

Worldwide, lung cancer accounts for the most common cancer in men and women,[1] causes more than one million deaths each year.[2] However, in Iran it represents lower incidence than expected, since despite of high prevalence of smokers in Iranian male population, lung cancer remains fifth highest malignancy. It appears that its low incidence may be due to under reporting and difficult tissue diagnosis, although recent few studies have shown a gradual continuous increasing in lung cancer occurrence.[3,4]

It has been well established that chronic tobacco smoke inhalation is the most dominant risk factor for lung cancer.[5] Tobacco smoke is a mixture of several thousand chemicals, provides the main source of strong human carcinogens, including polycyclic aromatic hydrocarbons (PAHs), nitrosamines and aromaticamines.[6,7] However, not all the people exposed to tobacco smoke eventually develop the disease, indicating that in addition to various extent of smoking and different exposure to other environmental carcinogens, individual genetic differences in serious metabolizing genes might affect lung cancer carcinogenesis.[8-10]

The enzyme aryl hydrocarbon hydroxylase (AHH) encoded by *CYP1A1* gene, is a key member of more significant P450 enzymes, exhibits significant roles in the initial step of metabolic activation of vast numbers of environmental carcinogens.[11] AHH is predominantly expressed in extrahepatic tissues such as the lung, and appears to be more important in the metabolism of PAHs to their respective intermediates. These reactive electrophilic metabolites are high potent to bind to DNA, and lead to adducts formation, the chemical modifications to DNA that can precede mutation events.[12,13]

Several important polymorphisms have been described in the *CYP1A1* gene. The first detected variant allele is *CYP1A1*2A*, which consists of a T to C transition at nucleotide 3801, located in 3' non-coding region of the gene. This base substitution introduces a new restriction site for *Msp*I endonuclease and hence also known as *Msp*I.[14-16] Some studies that were conducted in Japanese showed a

Address for correspondence: Simin Hemati, Assistant Professor, Department of Radiation Oncology, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: hematti@med.mui.ac.ir
Received: 08.12.2011; **Revised:** 4.1.2012; **Accepted:** 08.02.2012

considerable high frequency for *Msp*I variant allele, whereas inconsistent with Japanese and Asian populations, this polymorphism was reported to be much less frequent in Caucasian populations.[15,17,18]

It has been demonstrated that following exposure to high inducible PAHs, individuals carrying *CYP1A1*2A* allele could represent enhanced regulation of transcription, or heightened enzymatic activity, although the precise mechanism underling this relation has not been determined.[19-21] However, a number of similar studies investigating the potential association between this genetic variation and lung cancer risk, have reported dissimilar consequences.[14,22,23]

In view of high prevalence of smoking in Iran, and the previously reported ethnic differences in association between *CYP1A1 Msp*I polymorphism and lung cancer risk, the present analysis has been designed to evaluate and compare the frequency of this genetic polymorphism in Iranian lung cancer patients, and to assess the possible association of this polymorphism to lung cancer predisposition.

METHODS

Study population

We have investigated the frequency of this genetic variation in 65 Iranian patients with lung cancer compared to 80 healthy controls through a cross-sectional study (2009-2011).

The lung cancer patients included those who were first diagnosed by standard histopathological procedures at Chronic Respiratory Diseases Research Center, Masih Daneshvari Hospital, Tehran, Iran. To avoid any possible error in selection of primary lung cancer cases, blood collection was always performed under supervision of a medical oncologist.

The control group consisted of 80 cancer free volunteers, unrelated to patients, randomly selected among who refer to the clinics for regular health checks. Prior to blood sample collection a structured questionnaire was completed during a brief face to face interview to obtain demographic characteristics such as age, gender and smoking habits. For patients who were unable to participate in interview, data was collected from a person of subject's intimates or obtained from the medical file, registered by physicians at admission time. The Blood samples collected in tubes containing anticoagulant ethylenediaminetetraacetic acid (EDTA), immediately stored at - 20° C until DNA isolation.

Exclusion criteria were as follows: female gender, age, smoking habit, familial history of lung cancer and metastatic activity.

DNA Genotyping

Genomic DNA was extracted from peripheral white blood cells using a salting method. In order to analyze *CYP1A1 Msp*I polymorphism, a distinct region with 373 bp length including the variation site was amplified with PCR technique. Primers used for amplifying were (F: 5'-AGCAGTGAAGAGGTGTAGCCG-3') and (R: 5'-TAGAGAGGGCGTAAGTCAGCA-3'). Each 25 µl PCR reaction mixture consisted of 2 µl of 10 mM of each the forward and reverse primers, 2.5 µl of 10x solution buffer, 0.5 µl of a 10 µM of four mixed dNTPs, 0.75 µl of 50 mM of Mgcl2, o.25 µl of 5u/µl Taq DNA polymerase (Cinnagene, Co., Iran), 2 µl of genomic DNA (80ng/µl) and 15 µl of sterile H2o. The amplification reaction using thermal cycler was carried out as the following conditions: initial denaturation at 95° C in 3 minutes, followed by 35 cycles with melting at 95° C for 30 seconds, annealing at 62° C for 35 seconds, and primer extension at 72° C for 40 seconds. The PCR reaction was completed by a final extension step at 72° C for 10 minutes. The amplified products were then separated by electrophoresis through 1% gel agarose and visualized with ethidium bromide staining.

PCR amplified products were digested to determine genotypes. *Msp*I restriction enzyme (New England Biolabs) was used to distinguish the *CYP1A1 Msp*I polymorphism, at 37° C overnight. *Msp*I enzyme does not digest the wild type *CYP1A1*1* allele, and fragments included this allele remain intact (373 bp), while restriction digestion occurred at the presence of polymorphic *CYP1A1*2A* allele, generates two unequal 127 and 226 bp bands. The restricted products were analyzed by electrophoresis in 1% agarose gel.

Statistical Analysis

The chi-square test was used to compare the distribution of different genotypes and allele frequencies for the polymorphism among both patient and control categories. The same test was also used to determine possible significant association between lung cancer risk and *CYP1A1 Msp*I variant allele. The odd ratios (ORs) and their corresponding 95% confidence interval were calculated to estimate relative relationship between each *CYP1A1Msp*I variant allele and lung cancer. Independent t-test was used to compare numerical variables. Analysis was carried out with SPSS software version 16 (SPSS Inc., Chicago, IL, USA). A probability of P-value less than 0.05 was considered as statistically significant.

RESULTS

The general information of study population is illustrated in details in table 1. The mean age was calculated 53.3 years for the patients and 51.7 for the controls. There were no significant differences in the distribution of sex between cases and controls ($p > 0.05$). However, a significant difference in smoking status was obtained between two categories. Compared to healthy control group, more smokers were in lung cancer group ($p = 0.001$). The results of histology findings were as following (AC: Adenocarcinoma SCC: Squamous Cell Carcinoma SCLC: Small Cell Lund Carcinoma):

AC 22 (33.85)
SCC 9 (13.85)
SCLC 9 (13.85)
Other/unknown types 25 (38.45)

Table 2 depicts distribution of different lung cancer histological types. Compared to other types, adenocarcinoma was more frequent in patients, while cases with small cell lung carcinoma and squamous cell carcinoma were much more likely to have a positive smoking history, although no significant difference were observed ($p > 0.05$).

Detailed analysis of the different *CYP1A1 Msp*I genotypes are presented in table 3. Since there were no individuals with **2A/*2A* genotype, who homozygous for *CYP1A1*2A* variant allele among both cases and controls, **2A/*2A* genotype was not shown.

The frequency of **1/*1* and **1/*2A* genotypes in the cases were estimated 81.5% and 18.4%, while these genotypes obtained in 95.5% and 7.5% of control subjects, respectively. Thus, the frequency of variant genotypes was low in two groups; however, a significant positive association was observed.

Table 1. General characteristics of the study population

Characteristics	Cases N (%)	Controls N (%)	P-value
Sex			
Men	37 (56.92)	47 (58.75)	0.855
Women	28 (43.08)	33 (41.25)	
Age			
Mean (SD)	53.39	51.71	-----
Range	34.67	34.81	
Smoking Status			
Non smokes	37 (56.92)	66 (82.50)	0.001
Smokers	28 (43.08)	14 (17.50)	
AC	22 (33.85)		
SCC	9 (13.85		
SCLC	9 (13.85		
Other/unknown types	25 (38.45)		

N: Number AC: Adenocarcinoma SCC: Squamous Cell Carcinoma SCLC: Small Cell Lund Carcinoma

Table 2. The relation between smoking and histological types of lung cancer

Histological types	Smokers	Non smokers	P-value
Adenocarcinoma	9 (40.90)	13 (59.10)	0.00064
Squamous carcinoma	6 (66.77)	3 (33.33)	0.193
Small cell carcinoma	7 (77.78)	2 (22.22)	0.062

Table 3. Distribution of *CYP1A1 MspI* genotypes

	CYP1A1 *Msp*	Cases N (%)	Controls N (%)	P-value	OR (CI 95%)
Genotype	*1/*1	53 (81.54)	74 (92.50)		
	1/*2A	12 (18.46)	6 (7.50)	0.047	2.79 (1.01-7.65)
Sum		56	80		

Table 4. Distribution of *CYP1A1 MspI* genotypes

	CYP1A1 *Msp*	Cases N (f)	Controls N (f)	P-value	OR (CI 95%)
Allele	CYP1A1*1	118 (0.91)	154 (0.963)		
	CYP1A1*2A	12 (0.09)	6 (0.037)	0.054	2.61 (0.98-6.91)
Sum		130	160		

between the **1/*2A* genotype and lung cancer risk, (p = 0.047, OR = 2.79 (1.01-7.65)). Nevertheless distribution of allele frequencies in cases and controls (Table 4) show that there were no significant positive association between the variant *CYP1A1*2A* allele and lung cancer risk (P = 0.054, OR= 2.61 (0.98-6.91)).

Comparison of *CYP1A1 Msp*I genotypes distribution in lung cancer histological types, as well as interaction of these genotypes and smoking was also assessed separately which in this case, no significant results were found (data not shown).

DISCUSSION

The current study was conducted to assess lung cancer susceptibility of Iranian patients for tobacco smoking and the possible role of the most characterized *CYP1A1 Msp*I polymorphism. As expected, the inevitable impact of tobacco smoke inhalation on predisposition to lung cancer was emphasized.

The association of tobacco smoke with different histological types of lung cancer showed a little further risk for small cell lung cancer, although no statistically significant difference was obtained. However, in contrast to our findings, nearly most previous studies suggest a higher risk for squamous cell cancer.

The present findings indicated no clear relationship between different lung cancer types and *CYP1A1 Msp*I polymorphism (data not shown). In this case, different investigations represented dissimilar results in Caucasian populations. However, several reports in Japanese and Chinese, where the mutant allele show higher frequencies, described a considerable relation between *CYP1A1* homozygous variant genotype and squamous cell carcinoma. For instance, Song et al.[21] reported a significant linkage between genetic *CYP1A1 Msp*I and lung squamous cell cancer among Chinese. A significant association between higher risk for the mentioned cancer type and the presence of at least one copy of mutant *CYP1A1*2A* allele in Asians was also reported.[12,17]

The pattern of genotype and allelic frequencies obtained in our investigation was almost confirmed the results of previous investigations in Caucasian population, since the variant allele was too low to found any *CYP1A1*2A* homozygous carriers. Nevertheless, a positive significant association was observed between heterozygous genotype and increased lung cancer risk (p = 0.047, OR = 2.79, 95% CI: 1.01-7.65). This finding differs from the most of reports in Caucasians, since in a few studies a marked relationship have been described. At the end, the limitations of present study could not be ignored. The small sample size might have had an effect on statistical analysis and further investigations on larger population are required to validate these findings. Therefore, in the near future, we will focus our efforts on more expanded population in Iranian lung cancer patients.

ACKNOWLEDGEMENTS

This study was done at the University of Isfahan and supported by departments of Research/Technology and Graduate Studies. The authors are grateful to departments for their financial support. We also thank physicians and staffs from Masih Daneshvari Hospital for providing the samples and questionnaire data.

REFERENCES

1. Honma HN, De Capitani EM, Barbeiro AS, Costa DB, Morcillo A, Zambon L. Polymorphism of the CYP1A1*2A gene and susceptibility to lung cancer in a Brazilian population. J Bras Pneumol 2009; 35(8): 767-72.
2. Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002; 3(8): 461-9.
3. Hosseini M, Naghan PA, Karimi S, SeyedAlinaghi S, Bahadori M, Khodadad K, et al. Environmental risk factors for lung cancer in Iran: a case-control study. Int J Epidemiol 2009; 38(4): 989-96.
4. Hung RJ, Boffetta P, Brockmoller J, Butkiewicz D, Cascorbi I, Clapper ML, et al. CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis. Carcinogenesis 2003; 24(5): 875-82.
5. Kiyohara C, Shirakawa T, Hopkin JM. Genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of lung cancer. Environ Health Prev Med 2002; 7(2): 47-59.
6. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 2002; 21(48): 7435-51.
7. Hung RJ, Boffetta P, Brockmoller J, Butkiewicz D, Cascorbi I, Clapper ML, et al. CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a

pooled analysis. Carcinogenesis 2003; 24(5): 875-82.
8. Wu X, Zhao H, Suk R, Christiani DC. Genetic susceptibility to tobacco-related cancer. Oncogene 2004; 23(38): 6500-23.
9. Shields PG. Molecular epidemiology of smoking and lung cancer. Oncogene 2002; 21(45): 6870-6.
10. Hatagima A. Genetic polymorphisms and metabolism of endocrine disruptors in cancer susceptibility. Cad Saude Publica 2002; 18(2): 357-77.
11. Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer 2009; 9: 187.
12. Mota P, Moura DS, Vale MG, Coimbra H, Carvalho L, Regateiro F. CYP1A1 m1 and m2 polymorphisms: genetic susceptibility to lung cancer. Rev Port Pneumol 2010; 16(1): 89-98.
13. San JC, Cabanillas A, Benitez J, Carrillo JA, Jimenez M, Gervasini G. CYP1A1 gene polymorphisms increase lung cancer risk in a high-incidence region of Spain: a case control study. BMC Cancer 2010; 10: 463.
14. Wenzlaff AS, Cote ML, Bock CH, Land SJ, Santer SK, Schwartz DR, et al. CYP1A1 and CYP1B1 polymorphisms and risk of lung cancer among never smokers: a population-based study. Carcinogenesis 2005; 26(12): 2207-12.
15. Masson LF, Sharp L, Cotton SC, Little J. Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. Am J Epidemiol 2005; 161(10): 901-15.
16. Ishibe N, Wiencke JK, Zuo ZF, McMillan A, Spitz M, Kelsey KT. Susceptibility to lung cancer in light smokers associated with CYP1A1 polymorphisms in Mexican- and African-Americans. Cancer Epidemiol Biomarkers Prev 1997; 6(12): 1075-80.
17. Xu X, Kelsey KT, Wiencke JK, Wain JC, Christiani DC. Cytochrome P450 CYP1A1 MspI polymorphism and lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1996; 5(9): 687-92.
18. Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. Curr Drug Metab 2008; 9(2): 129-43.
19. Georgiadis P, Topinka J, Vlachodimitropoulos D, Stoikidou M, Gioka M, Stephanou G, et al. Interactions between CYP1A1 polymorphisms and exposure to environmental tobacco smoke in the modulation of lymphocyte bulky DNA adducts and chromosomal aberrations. Carcinogenesis 2005; 26(1): 93-101.
20. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. J Biol Chem 2004; 279(23): 23847-50.
21. Song N, Tan W, Xing D, Lin D. CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China. Carcinogenesis 2001; 22(1): 11-6.
22. Agundez JA. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab 2004; 5(3): 211-24.

How to cite this article: Motovali-Bashi M, Biglari M, Hojati Z, Hemati S, Khodadad K. Role of *CYP1A1 Msp*I polymorphism in *CYP1A1* gene with susceptibility to lung cancer in Iranian patients. J Res Med Sci 2012; 17(Spec 2): S242-S246.

Source of Support: All authors contributed to the designing and performing the study, **Conflict of Interest:** None declared.