RESEARCH COMMUNICATION

CYP1A1 Polymorphisms and Risk of Lung Cancer in the Ethnic Kashmiri Population

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Abstract

The CYP1A1 category of enzymes plays a central role in the metabolic activation of major tobacco carcinogens. Several polymorphisms within the CYP1A1 locus have been identified and have been shown to be associated with lung cancer risk, particularly in Asian populations. Here we focused on the influence of three polymorphisms on lung cancer in ethnic Kashmiris, genotyping 109 lung cancer cases and 163 healthy controls by PCR-RFLP methods. While no polymorphic alleles in CYP1A1m4 (exon 7 thr to asn) site were detected in our population, the allele frequency of CYP1A1m1 (Msp1) and CYP1A1m2 (exon 7 ile to val) were 30.1 and 26.6 in controls and 44.5 and 38.9 in cases. The CYP1A1m1 and CYP1A1m2 variants were significantly associated with lung cancer susceptibility (ORs; 2.65, CI 95% = 1.562-4.49 and 2.24,CI 95%=1.35-3.73).This risk was prominent in case of SCC compared with AC or other types of lung cancer. Stratified analysis showed a multiplicative interaction between tobacco smoking and variant CYP1A1m1 genotype on the risk of SCC. The ORs of SCC for non-smokers were 2.08 and 3.15 for smokers. When stratified by pack years, effect was stronger in the heaviest smokers (ORs=6.00,95% CI=1.672-21.532).The interaction between tobacco smoking and variant CYP1A1m2 genotype followed similar pattern. Our findings thus support the conclusion that CYP1A1m1 and m2 polymorphisms are associated with the smoking related lung cancer risk in Kashmiri population.

Key Words: CYP1A1 polymorphisms - lung cancer - histology - tobacco smoking

Asian Pacific J Cancer Prev, **10**, 651-656

Introduction

Lung cancer mortality has increased rapidly during recent years in Asian countries. Cigarette smoking is the strongest established risk factor for lung cancer, but genetically determined variations in metabolism of tobacco derived carcinogens may affect individual susceptibility to lung cancer. However, although risk of lung cancer has been exclusively associated with tobacco smoking, fewer than 20% of smokers develop the disease (Carlsten et al., 2008), indicating that there may be important genetic components involved in the etiology; which makes identification of genetic susceptibility factors for lung cancer important in order to understand and prevent occurrence of the disease.

Several studies have suggested that genetic polymorphism in genes controlling carcinogen metabolism underlie individual variation in cancer susceptibility (Nebert et al., 1996; Gonzalez et al., 1997). Several metabolic enzymes have been investigated for their possible role in lung cancer susceptibility, including members of CYP450 super family. As phase 1 enzymes, these catalyze one of the first steps in the metabolism of carcinogens. This oxidising step often creates more reactive intermediates that are capable of binding with DNA and causing genetic mutations (Gonzalez et al.,1994; Bartsch et al., 2000). The Cyp super family of enzymes are the primary agents involved in oxidising carcinogens found in tobacco smoke, like polycyclic aromatic hydrocarbons (PAHs), nitrosoamines and arylamines (Spivack et al, 2001; Hukkanen et al., 2002). CYP1A1 polymorphisms were the first inthe CYP genes to be associated with lung cancer. Several important single nucleotide polymorphisms have been identified in the CYP1A1 locus. CYP1A1 genes contain 7 exons and have been localised to human chromosome no.15(15q22-qter). The CYP1A1m1 allele has a T-C mutation in 3'noncoding region, which has been associated with elevated enzyme activity (Petersen et al., 1991; Crofts et al.,1994; Kiyohara et al., 1994; Landi et al., 1994). An A-G transition in exon 7 creates the second allelic variant (m2),which leads to an amino acid substitution of val for ile in the heme binding region and results in an increase in microsomal enzyme activity (Cosma

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et al.,1993; Crofts et al.,1994; Kiyohara et al,1998).The variant m3 has a mutation in intron 7 (Crofts et al.,1993). Another polymorphism (m4), located two bases upstream of the m2 site, also causes an amino acid substitution of the Asn in heme binding region of the enzyme (Cascorbi et al., 1996).

The relationship between CYP1A1 polymorphism and lung cancer risk in various ethnic populations have been investigated in several studies (Bartsh et al., 2000). In Asian populations CYP1A1 m1 and m2 polymorphisms have generally been associated with moderate to increased risk of lung cancer compared to Caucasians and African-American, where these polymorphisms are much less common (Cosma et al., 1993; Garte et al.,1998). In Japanese and Chinese, CYP1A1 polymorphisms have been associated with increased lung cancer risk, especially in relation to tobacco smoking (Hong et al., 1998; Sugimura et al.,1998; Bartsch et al., 2000; Song et al., 2001). the studies on North Indian populations (Sobti et al., 2003; 2004) have reported that CYP1A1*2A and CYP1A1*2C polymorphism is not significantly associated with lung cancer risk, though the risk was found to increase in heavy smokers. Similarly a study on South Indian population (Sreeja et al., 2005) reported significant association of CYP1A1*2A polymorphism with lung cancer risk which however decreased with cigarette smoking. Another study (Parag et al, 2008) showed that CYP1A1 polymorphism is an important modifying factor in determining susceptibility to lung cancer. Significant increase in the risk in the individuals carrying variant genotypes of CYP1A1 and GSTM1 have further provided evidence that gene–gene interaction may play an important role in the development of lung cancer. Likewise significant interactions of CYP1A1 genotypes with tobacco, both in the form of tobacco smoking or tobacco chewing and alcohol have demonstrated the important of gene environment interactions in modifying the susceptibility to lung cancer.

In view of the prevalence of tobacco smoking and lung cancer in Kashmir valley we examined three polymorphisms m1, m2 and m4 in CYP1A1 to investigate its possible involvement in lung cancer of Kashmiri population .

Materials and Methods

Study subjects

This study consists of 109 lung cancer cases and 163 cancer free controls. The cases with histologically confirmed primary lung cancer were recruited from June 2006 to April 2008 in Sheri-Kashmir Institute of Medical Sciences, Soura, Kashmir J&K. Control samples were obtained from the OPD of same hospital. Each participant was personally interviewed to obtain detailed information on demographic characteristics and lifetime history of tobacco use. This protocol was approved by the local Institutional review board and ethical clearance committee. Informed consent was obtained from all subjects prior to initiation of interview and biospecimen collection.

Genotyping

Genomic DNA was isolated using standard proteinase k digestion, phenol/chloroform extraction and ethanol precipitation method from whole blood samples of both cases and controls. CYP1A1 genotypes at m1,m2 and m4 sites were analyzed by PCR-RFLP methods as previously described (Cascorbi et al,1996; Nakachi et al,1991) .The primers used for m1,m2 and m4 sites are shown below;

M1F 5' cag tga aga ggt gta gcc gct 3 ' and M1R 5 ' tag gag tct tgt ctc atg cct 3 'M2F & M4F 5 ' ttc cac ccg ttg cag cag gat agc c 3 ' and M2R & M4R 5 ' ctg tct ccc tct ggt tac agg aag 3 '

The M1F &M1R primers generated a product of 340bp, while M2F & M2R and M4F & M4R generated a 204bp product. Each PCR reaction mixture(25µl) contained 100ng template DNA, 0.2µM each primer, 0.2mM each dNTP, 1.0U Taq polymerase (Fermentas). To amplify the fragments containing site m1,m2 & m4 the reaction involved an initial denaturation step of 7 min at 95°C ,followed by 35 cycles of 30 sec at 95°C ,1 min at 61°C for m1 (63°C for m2 & m4) & 35 sec at 72°c and a final elongation step of 7 min at 72°C.

The restriction enzyme Msp1 (fermentas) was used to distinguish the m1 polymorphism; gain of a Msp1 restriction site occurs in the polymorphic allele, the wildtype allele shows a single band representing the entire 340 bp fragment and variant allele results in two fragments of 200 bp and 140 bp. The restriction enzymes BsrD1 & Bsa1(fermentas) were used to distinguish the m2 & m4 polymorphisms, respectively from the same 204 bp product in two different reactions. Both cleavage sites were lost in the case of the mutations and resulted in a single band, whereas, the wild type alleles generated 149 and 55bp (for m2

Table 1. Demographic Characteristics of the Study Subjects

Characteristic	Cases	Controls	OR (95%CI)	P value
Gender				
Female	22 (20.2)	68 (41.7)	1.00	0.0002
Male	87 (79.8)	95 (58.3)	2.83 (1.60-4.96)	
Age (years)				
<50	35 (32.1)	85 (52.1)	1.00	0.0011
≥50	74 (67.9)	78 (47.9)	2.30 (1.37-3.82)	
Mean (Range)	52.7 (26-80)	53.2 (30-75)		
Smoking				
No	25 (23.0)	65 (39.8)	1.00	0.0036
Yes	84 (77.0)	98 (60.2)	2.22(1.286-3.831)	
Pack Years				0.0411
<25	51(69.8)	59 (76.1)	2.24 (1.24-4.06)	
				0.0384
≥25	33 (30.2)	39 (23.9)	2.20 (1.14-4.20)	
Histological type				
SCC	69 (63.3)			
AC	18 (16.5)			
Others[a]	22 (20.2)			

[a]Bronchio-alveolar carcinoma (11), mixed cell carcinoma (3), and non differentiated type (8)

Table 2. Cyp1A1 Genotype Frequencies in Cases and Controls

	Cyp1A1 m1 Genotype[a]				Cyp1A1 m2 Genotype[a]			
	W1/w1	W1/m1-m1/m1	OR (95%CI)[b]	P value[c]	W2 /w2	W2/m2-m2 /m2	ORs(95%CI)[b]	P value[c]
Controls (163)	78	85	1.00		84	79	1.00	
Lung Cancer (109)	28	81	2.65 (1.56-4.49)	0.0002	35	74	2.24 (1.35-3.73)	0.002
SCC (69)	15	54	3.30 (1.72-6.32)	0.0001	21	48	2.43 (1.33-4.41)	0.003
AC (18)	6	12	1.83 (0.66-5.11)	0.241	7	11	1.67 (0.62-4.52)	0.309
Others (22)[d]	7	15	1.96 (0.76-5.06)	0.157	7	15	2.27 (0.87-5.85)	0.083

[a]w refers to wild type genotype and m to mutant genotypes at the studied polymorphic site; [b]ORs and 95% CIs were calculated with Cyp1A1 wild-type genotypes (W1 /w1 & W2 /w2) as reference groups; [c]P value for chi square test for comparison with controls; [d]Bronchio-alveolar carcinoma (11), mixed cell carcinoma (3), and non-differentiated type (8)

Table 3. Genotype Frequencies and ORs of Cyp1A1 Polymorphisms for Different Smoking Categories

		Cases			Controls			Crude OR[a]
		n	W1/w1	W1/m1-m1/m1	n	W2 /w2	W2/m2-m2 /m2	
Cyp1A1m1	Total	109	28	81	163	78	85	2.65 (1.56-4.49)
	Non-smokers	25	7	18	65	28	37	1.94 (0.73-5.28)
	Smokers	84	21	63	98	50	48	3.12 (1.66-5.88)
	<20 pack years	51	11	40	59	26	33	2.81 (1.21-6.50)
	≥20 pack years	33	10	23	39	24	15	3.68 (1.38-9.84)
Cyp1A1m2	Total	109	35	74	163	84	79	2.24 (1.35-3.72)
	Non-smokers	25	10	15	65	29	36	1.20 (0.53-3.47)
	Smokers	84	25	59	98	53	43	2.78 (1.50-5.15)
	<20 pack years	51	16	35	59	32	27	2.59 (1.04-4.94)
	≥20 pack years	33	9	24	39	23	16	3.83 (1.41-10.4)

[a]ORs and 95% CIs were calculated with Cyp1A1 wild-type genotypes (W1 /w1 & W2 /w2) as reference groups

site) or 139 and 65 bp (for m4 site) bands, respectively. The restriction digested products were analysed by electrophoresis on 3% agarose gel containing ethidium bromide and visualised under uv illumination.

Statistical Analysis

Pearson's $\chi 2$ test was used to examine differences in distribution of genotypes studied between cases and controls. Odds ratios (ORs) with 95% confidence interval (CI) calculated were computed to estimate the association between certain genotypes or tobacco smoking and disease. Smokers were considered current smokers if they smoked up to one year before the date of diagnosis for cancer or upto the date of interview for controls. Information was collected on the number of cigarettes smoked per-day, the age at which the subject started smoking and the age at which the subject stopped smoking if the person was an ex-smoker. Pack-years smoked was calculated to indicate cumulative cigarette dose and lighter and heavier smokers were categorized by the approximate 50th percentile pack years value among controls i.e., <25 pack-years and ≥25 pack-years. All the statistical analysis were performed with the SPSS (v.11.6) .

Results

The demographic characteristics of study subjects are shown in (Table 1). There was no significant difference among cases and controls in terms of mean age. Although an effort was made to obtain a frequency match on smoking status between cases and controls, more smokers were present in case group compared to controls ($\chi 2$ =8.46, p value=0.004). Moreover, the cases had a higher value of pack-years smoked than controls; 30.2% of cases smoked ≥25 pack-years compared to 23.9% of controls ($\chi 2$ =7.92, p value=0.005). Genotyping shows that the allele frequencies for CYP1A1m1 and CYP1A1m2 were 44.5 and 38.9 in cases and 30.1 and 26.6 in controls. However, no polymorphic alleles were detectable in the m4 site of CYP1A1 locus . The distribution of CYP1A1 genotypes at m1 and m2 sites when compared among cases and controls, was found that 74.3% of cases carried CYP1A1 w1/m1 or m1/m1 genotype ,which was significantly higher than that of controls (52.1%) ($\chi 2$ test ,p value =0.0002). Similarly ,the CYP1A1m2 variant alleles were more prevalent among cases (67.8%) than controls (48.4%) ($\chi 2$ test ,p value =0.002). Those subjects who carried at least one CYP1A1m1 or m2 variant allele were at more than 2-fold higher overall risk for lung cancer. However, an elevated risk was observed only among SCC patients, no significant association between these polymorphisms and risk of AC and other types of lung cancer was observed (Table 2).

When lung cancer risk was examined with stratification of smoking status ,among smokers it was found those carrying CYP1A1 w1/m1 or m1/m1 genotype had an elevated risk compared to those carrying wild type genotype (OR=3.12 ,95% CI=1.656-5.876). Interestingly this risk was higher even in non-smokers who had a variant CYP1A1 m1 genotype compared to those with wild-type genotype.When stratified with pack years smoked, a joint effect of tobacco smoking and CYP1A1m1 allele was seen in individuals who consumed ≥25 pack-years. Similar pattern of gene smoking interaction was also seen among those who carried CYP1A1m2 variant allele (Table 3).

Table 4. Interaction of Cyp1A1 Genotypes and Tobacco Smoking on the Risk of Squamous Cell Carcinoma

		Cases			Controls			Crude OR[a]
		n	W1/w1	W1/m1-m1/m1	n	W2 /w2	W2/m2-m2 /m2	
Cyp1A1m1	Total	69	15	54	163	78	85	3.30 (1.72-6.32)
	Non-smokers	15	4	11	65	28	37	2.08 (0.60-7.23)
	Smokers	54	11	43	98	50	48	4.07 (1.88-8.80)
	<20 pack years	35	7	28	59	26	33	3.15 (1.88-8.35)
	≥20 pack years	19	4	15	39	24	15	6.00 (1.67-21.5)
Cyp1A1m2	Total	69	21	48	163	84	79	2.43 (1.34-4.42)
	Non-smokers	15	4	11	65	29	36	2.20 (0.63-7.64)
	Smokers	54	17	37	98	55	43	2.78 (1.38-5.59)
	<20 pack years	35	12	23	59	32	27	2.28 (0.96-5.42)
	≥20 pack years	19	5	14	39	23	16	4.00 (2.00-13.3)

[a]ORs and 95% CIs were calculated with Cyp1A1 wild-type genotypes (W1 /w1 & W2 /w2) as reference groups

Table 5. Interaction of Cyp1A1 Genotypes and Tobacco Smoking on the Risk of Adenocarcinoma

		Cases			Controls			Crude OR[a]
		n	W1/w1	W1/m1-m1/m1	n	W2 /w2	W2/m2-m2 /m2	
Cyp1A1m1	Total	18	6	12	163	78	85	1.83 (0.66-5.11)
	Non-smokers	7	2	5	65	28	37	2.42 (0.44-13.4)
	Smokers	11	4	7	98	50	48	1.82 (0.50-6.62)
	<20 pack years	8	3	5	59	26	33	0.99 (0.27-4.53)
	≥20 pack years	3	1	2	39	24	15	1.86 (0.16-22.3)
Cyp1A1m2	Total	18	6	12	163	84	79	2.12 (0.76-5.92)
	Non-smokers	7	1	6	65	29	36	4.83 (0.55-42.4)
	Smokers	11	5	6	98	55	43	1.53 (0.44-5.35)
	<20 pack years	8	3	5	59	32	27	1.97 (0.43-9.01)
	≥20 pack years	3	2	1	39	23	16	0.71 (0.06-8.51)

[a]ORs and 95% CIs were calculated with Cyp1A1 wild-type genotypes (W1 /w1 & W2 /w2) as reference groups

When the interaction of CYP1A1 polymorphisms and tobacco smoking was assessed separately for SCC and AC. It was found those carrying variant CYP1A1m1 genotype in case of SCC and were non-smokers, OR for variant m1 genotypes alone was 2.08(0.598-7.225) and OR for combined smoking and variant allele was 4.07(1.881-8.804), which was higher compared to those who had either only variant CYP1A1m1 genotype or were non-smokers only, indicating that the joint effect was multiplicative. Similar pattern was also observed for CYP1A1m2 variant genotype and tobacco smoking in SCC group although less pronounced (Table 4).

In contrast to SCC, no significant joint effect between tobacco smoking and CYP1A1 polymorphisms on the risk of AC was seen, although an excess risk related to CYP1A1 genotype was seen among nonsmokers (Table 5).

Discussion

In Asian population CYP1A1 polmorphisms have generally been associated with increased risk of lung cancer, compared with Caucasians and African-Americans (Cosma et al., 1993; Garte et al., 1998). Although a number of studies have been carried out in various ethnic populations to examine the role of CYP1A1 polymorphisms in lung cancer (Bartsh et al., 2000), no such study has been conducted in Kashmiri population. Here we investigated the prevalence of CYP1A1 polymorphisms and their association with the risk of lung cancer in ethnic Kashmiri population. No polymorphic alleles were detectable in the m4 site of CYP1A1 locus in our study subjects. However, we observed a significant difference in the distribution of CYP1A1m1 and m2 genotype frequencies in controls and cases. Our data shows an association between these polymorphisms and elevated risk of lung cancer, especially SCC type in Kashmiri population. These findings are consistent with previous studies conducted in various ethnic groups. Studies on Indian populations have shown a similar association between CYP1A1 variant and risk of lung cancer (Sobti et al., 2003; 2004). Another study on South Indian population showed higher prevalence of the CYP1A1 homozygous variant genotype was recorded among lung cancer patients compared to controls (Sreeja et al, 2005). In Japanese population an association between CYP1A1 polymorphisms and lung cancer was stronger for SCC compared to AC (Nakachi et al.,1991;1993; Hayashi et al., 1992).Also our results are similar to the one study conducted in Chinese population (Song et al., 2001).while our data shows an association of CYP1A1 variant with higher risk of SCC, no such risk was observed in case of AC. However, an elevated risk for AC was observed in non-smokers, suggesting that carcinogenic substances involved in the development of AC in non-smokers might also be the substrates for CYP1A1. These findings are consistent with that reported for Chinese population in Taiwan (Lin et al., 2000), showing an elevated risk for SCC type of lung cancer but not with AC type was significantly associated with the polymorphisms in the CYP1A1 and microsomal epoxide hydrolase genes.

Our results are inconsistent with the results obtained from Caucasians most likely due absence or rarity of the CYP1A1 polymorphisms in their population (Hirvonen et al., 1992; Shields et al., 1993; Alexandrie et al., 1994; Bouchardy et al., 1997). However, a study (Marchand et al, 1998) including Caucasians, Japanese and Hawaiians, showed an increased risk for lung SCC with CYP1A1 m1 polymorphism, especially when combined with a GSTM1 deletion, whereas, lung AC was closely associated with CYP2E1 wild-type genotype. Tobacco smoke contains BP and other PAHs, which are proven to induce lung carcinogenesis particularly SCC subtype (Deutsch et al,1998). Furthermore, TP53 mutational studies in lung cancers have revealed that G:C and T:A transversions are more common in SCC than in AC because of the presence of BP and other PAHs in tobacco smoke (Takeshima et al.,1993; Greenblatt et al., 1994). These findings provide strong evidence that PAHs are involved in the development of lung SCC. So it can be expected that the CYP1A1 polymorphisms, which result in altered expression or catalytic activity of the enzyme are an important modifier of genetic susceptibility to lung cancer, particularly SCC subtype caused by tobacco smoking, as this enzyme plays a central role in the activation of PAHs.

Our data also shows an interaction between tobacco smoking and variant CYP1A1 genotype. The heaviest smokers who had the variant CYP1A1 m1 genotype were at a higher risk for SCC type of lung cancer. This effect was not observed in AC type of lung cancer. A study carried out in north Indian population (Sobti et al., 2004) also supports our findings. Also a strong association between CYP1A1 polymorphisms and lung cancer has been observed among smokers compared to non-smokers (Wu et al., 1997). Our findings are also consistent to the study (Munish et al., 2009), who have reported that a greater than multiplicative interaction on the risk of lung cancer between the cumulative cigarette dose and the c1/c1 genotype of CYP2E1, another polymorphic enzyme responsible for the activation of carcinogens in tobacco smoke.

In conclusion our results show a statistically significant association between the polymorphisms of CYP1A1 gene and elevated risk of lung cancer, especially SCC type, in Kashmiri population. Additionally our data supports the evidence that CYP1A1 is an important determinant in susceptibility to tobacco induced lung cancer. Our data also supports the hypothesis the susceptibility to certain cancers may depend on the ethnic specific gene polymorphisms.

Acknowledgements

The authors would like to thank Dr Iftikhar Hussain for assistance in the statistical analysis. None of the authors had any possible conflicts of interest in this research.

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