Association Between Genetic Polymorphisms of the Cytochromes P-450 (1A1, 2D6, and 2E1) and the Susceptibility to Pancreatic Cancer

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Objectives : *Metabolic activation is a prerequisite for the carcinogenic effect of many carcinogens, and considerable interindividual variation exists in the metabolic capacity to activate the carcinogens. The cytochromes P-450 (CYPs) are responsible for the activation mechanism, and polymorphisms of the CYPs (CYP1A1, CYP2D6, and possibly CYP2E1) are known to be related to increased susceptibility to smoking- related Kreyberg type I lung cancer. The aim of this study is to clarify the relationship of genetic polymorphisms of the CYPs to susceptibility to pancreatic cancer, another smoking-related cancer.*

Methods : *We analyzed 45 samples from patients with pancreatic cancer and 53 samples from controls. DNA was isolated from blood samples and the CYP1A1, 2D6 and 2E1 genes were amplified by PCR. Analyzing the genotypes of the CYPs by allele-specific PCR or RFLP analysis, we assessed the allele frequencies for each mutation of the CYPs among the patients with pancreatic cancer and the controls.*

Results : *The allele frequencies for the mutation in the 3'-flanking region of the CYP1A1 among the cases and the controls were 0.31 and 0.36, respectively. The allele frequencies for the exon 7 mutation of the CYP1A1 were 0.16 and 0.23, respectively, but with no statistical significance. The frequencies for the mutant c2 allele of the CYP2E1 were 0.19 and 0.30, respectively, but with no statistical significance. Two persons homozygous for a gene deletion of the CYP2D6 were observed among control subjects; other mutations were not observed among either the patients or controls.*

Conclusion : *We could not find any evidence that polymorphisms of the CYPs are associated with increased susceptibility to pancreatic cancer.*

Key Words : *Pancreatic cancer, Cytochrome, Polymorphism*

INTRODUCTION

The etiology of pancreatic cancer is unknown, but cigarette smoking is a consistent risk factor in many epidemiologic studies. The risk increases with increased cigarette smoking[1-3].

The initial events in chemical carcinogenesis are often metabolic activation of the carcinogens to reactive intermediates and an interaction of these with DNA. Hence, interindividual variation in the ability to metabolize environmental procarcinogens and/or carcinogens may be responsible for the large variation in the susceptibility to chemical carcinogens.

The cytochrome P-450 (CYP) enzymes play important roles in the metabolism of various chemical carcinogens from the environment[4, 5]. Within a population, there exist genetic polymorphisms of

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cytochrome P-450 enzymes leading to interindividual variation in the metabolic capacity. Because cytochrome P-450 enzymes may either detoxify carcinogens or transform procarcinogens to ultimate carcinogens, genetic polymorphisms of the cytochromes P-450 may partly explain the interindividual variation in susceptibility to carcinogens. Three polymorphic enzymes, CYP1A1, CYP2D6, and CYP2E1, have been suggested as having some roles in human lung carcinogenesis[6-8].

The CYP1A1 is responsible for the metabolic activation of benzo[a]pyrene and numerous other polycyclic aromatic hydrocarbons in tobacco smoke. So far, two point mutations have been demonstrated. One is a T to C mutation at position 6235 in the 3'-flanking region (m1) which provides a restriction endonuclease cleavage site for Msp I , and the other is an A to G mutation at position 4889 within exon 7 (m2). Both mutant alleles have been reported to be associated with increased susceptibility to lung cancer[7, 9-12].

The CYP2D6 polymorphism is an autosomal recessive trait associated with impaired debrisoquine metabolism in 5-10% of the Caucasian population. The poor metabolizers have minimal or no capacity for metabolism of specific substrates such as debrisoquine. According to some epidemiological studies, poor metabolizers have a decreased risk of lung cancer[6, 13, 14].

The CYP2E1 is responsible for the activation of nitrosamine to ultimate carcinogens[8], and is induced by chronic ethanol consumption and cigarette smoking. The genetic polymorphism in the 5'-flanking region is known to be related with the inducibility of the enzyme[15]. Evidence of association between the CYP2E1 polymorphism and cancer risk is not strong.

The aim of the present study is to clarify the association between polymorphisms of several CYPs (1A1, 2D6, and 2E1) known to be related to increased susceptibility to lung cancer and susceptibility to pancreatic cancer, another smoking-related cancer.

MATERIALS AND METHODS

1. Subjects

Forty-five Korean patients with pancreatic

cancer (28 males, 17 females; age range, from 34 to 95 years; mean$\pm$SD age, 60$\pm$11 years) who were diagnosed clinically, radiologically, or histologically, and fifty-three healthy control subjects (49 males, 4 females; age range, from 21 to 33 years; mean$\pm$SD age, 23$\pm$2 years) were included in this study. Among the 45 patients with pancreatic cancer, thirty-five patients had histologically confirmed adenocarcinoma. Twenty-five patients were smokers (24 males, 1 female), and among the smokers the consumption was 33.4$\pm$10.7 (mean$\pm$SD) packs-year.

2. Methods

Blood samples were collected in glass tubes that contained acid citrated dextrose solution B, and the buffy coat was separated and stored at -70℃ until use. Total genomic DNA of the white blood cells was extracted by the method of Blin et al[16].

The CYP1A1 genotypes ascribed to the mutation at position 6235 in the 3'-flanking region were determined according to the procedure of Kawajiri et al with some modifications[7]. Primers P79 (5'-AAGAGGTGTAGCCGCTGCACT-3') and P80 (5'-TAGGAGTCTTGTCTCATGCCT-3') were used for amplification of the 3'-flanking region of the CYP1A1 gene. Target DNA (about 500ng) was amplified in a 50-μL reaction mixture containing 10mM Tris (pH 8.3), 50mM KCl, 1mM MgCl$_2$, 100 μM of each dNTP, 1 unit Taq DNA polymerase, and 10 pmole of each primer P79 and P80. Thirty-four cycles of amplification were carried out under the following conditions : 30 sec at 94℃ for denaturation ; 1 min at 65℃ ; and 1 min at 72℃ for primer annealing and extension. The amplified products of 335 base pairs were digested with Msp I for 8 hours at 37℃, and the products were subjected to electrophoresis in a 2% agarose gel. Absence of the Msp I site in both alleles represents the homozygous wild-type genotype (wt1/wt1) and is characterized by single 335-bp fragment. Persons with mutations (m1) in homozygous state (m1/m1) show 206- and 129-bp fragments. Heterozygotes show 335-, 206-, and 129-bp fragments (Fig. 1).

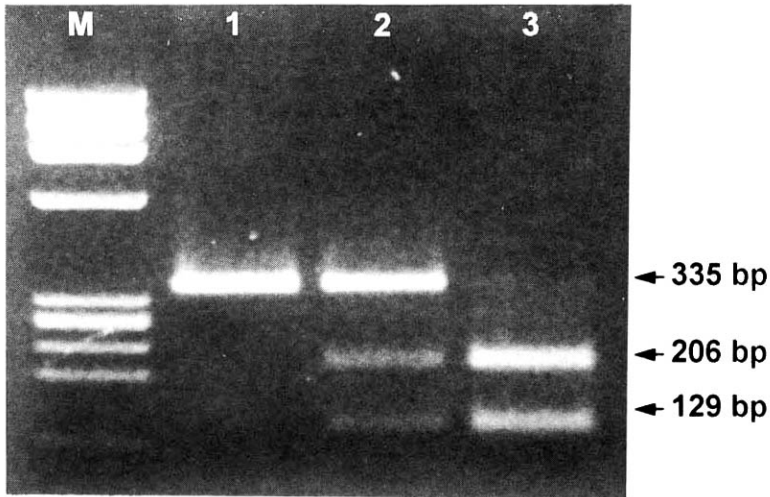The A to G transition at position 4889 within exon 7 of the CYP1A1 gene was determined by

Fig. 1. Polymorphisms of the CYP1A1 gene in the 3'-flanking region. The polymorphism was studied by PCR followed by *Msp* I digestion. The mutant allele has the *Msp* I site whereas the wild-type allele does not. Lane M, *Hae*III-digested ΦX174 DNA molecular weight markers; Lane 1, wild-type homozygote (wt1/wt1); Lane 2, heterozygote (wt1/m1); Lane 3, mutant homozygote (m1/m1).

the use of allele-specific PCR[10]. Genomic DNA was first amplified for 35 cycles with the upstream primer P71 (5'-ATTAGGGTTAGTGGGAGGGACACG-3') and the downstream primer P72 (5'-GCTCAATGCAGGCTAGAATAGAAGG-3') to produce a 1472-bp fragment. Other conditions were identical to the scheme mentioned above. The amplified fragments were then reamplified for 14 cycles, alternatively with either the 5' wild-type specific primer P57 (5'-GAAGTGTATCGGTGAGACCA-3') or the 5' mutation-specific primer P58 (5'-GAAGTGTATCGGTGAGACCG-3'), and with the common 3' primer P72 under the following conditions : 30 sec at 94℃ for denaturation ; 1 min at 69℃ ; and 1 min at 72℃ for primer annealing and extension. A homozygous wild-type person (wt2/wt2) yields a 996-bp fragment with the wild-type specific primer pair P57/P72. A mutant homozygote at position 4889 (m2/m2) yields a 996-bp fragment with the mutation-specific primer pair P58/P72. A heterozygote yields 996-bp fragments in both reactions (Fig. 2).

Mutant CYP2D6 alleles were identified in two separate PCR reactions by use of primer pairs C (exon 3, sequence: 5'-GCCTTCGCCAACCACTCC

G-3') and D (intron 4 : 5'-AAATCCTGCTCTTCCGAGGC-3'); and E (exon 5: 5'-GATGAGCTGCTAACTGAGCCC-3') and F (intron 5: 5'-CCGAGAGCATACTCGGGAC-3') under the following conditions: 30 sec at 94℃ for denaturation; 30 sec at 59℃; and 30 sec at 72℃ for primer annealing and extension. Amplification with C and D for 30 cycles generates a 334-bp fragment with a G-to-A transition at the junction of intron 3/exon 4 which in affected individuals is resistant to digestion with the restriction enzyme *Bst*NI. The primer E contains a 1-bp mismatch to the CYP2D6 sequence, which makes the 268-bp PCR fragment produced from individuals with the exon 5 mutation sensitive to digestion with the restriction enzyme *Hpa*II at the site of the base-pair deletion. No PCR product is obtained in individuals who are homozygous for a gene deletion, in both PCR reactions[17].

The *Rsa*I and *Pst*I polymorphisms of the CYP2E1 gene were determined according to the procedure of Hayashi et al[15], by amplifying in PCR reaction for 30 cycles with primers J8 (5'-TTCATTCTGTCTTCTAACTGG-3') and J9 (5'-CCAGTCGAGTCTACATTGTCA-3') under the
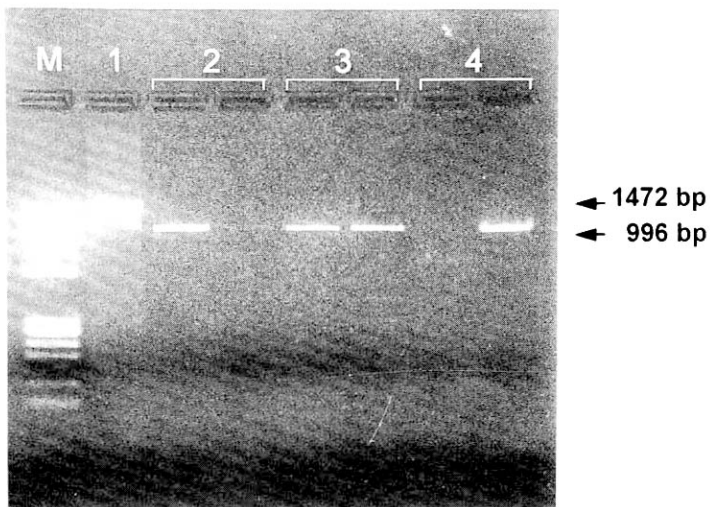
Fig. 2. Detection of the 4889 A to G transition in exon 7 by allele-specific
polymerase chain reaction. Genomic DNAs were amplified with primers P71
and P72. Then, allele-specific PCR was carried out with primers P57
(wild-type specific primer) and P72, and with primers P58 (mutation-specific
primer) and P72. Lane M, HaeIII-digested ΦX174 DNA molecular weight
markers; Lane 1, PCR product with primers P71 and P72; Lane 2, wild-type
homozygote (wt2/wt2); Lane 3, heterozygote (wt2/m2); Lane 4, mutant
homozygote (m2/m2).

following conditions: 30 sec at 94°C for denatu-
ration; 1 min at 55°C; and 1 min at 72°C for
primer annealing and extension. The amplified
products were digested with Rsal or Psfl. From
the lengths of the restricted DNA fragments
produced by the two endonucleases, the geno-
types of CYP2E1 were determined. The amplified
products from individuals homozygous for the c1
allele are not digested with Psfl, but with Rsal,
whereas those from individuals homozygous for
the c2 allele are not digested with Rsa I , but with
Pst I (Fig. 3).

For group comparisons, the χ^2 test or Fisher's
exact test was used, and p<0.05 was considered
statistically significant.

RESULTS

Table 1 demonstrates the distribution of m1
allele conformations at position 6235 of the

CYP1A1 gene among pancreatic cancer patients
and control subjects. The allele frequencies for
wt1 and m1 were 0.69 and 0.31, respectively,
among the patients with pancreatic cancer, and
0.64 and 0.36 among the control subjects, with no
statistical difference. Also there was no significant
difference in the genotype distribution among the
cases and the controls. Table 2 shows the m1
allele frequencies among smokers and non-
smokers in patients with pancreatic cancer. Again,
no significant difference in the m1 allele frequency
between smokers and non-smokers was observed
in patients with pancreatic cancer.

Table 3 shows the distribution of three geno-
types and m2 allele frequencies at position 4889
of the CYP1A1 gene among the cases and the
controls. The allele frequencies for wt2 and m2
were 0.84 and 0.16, respectively, among the
patients with pancreatic cancer, and 0.765 and
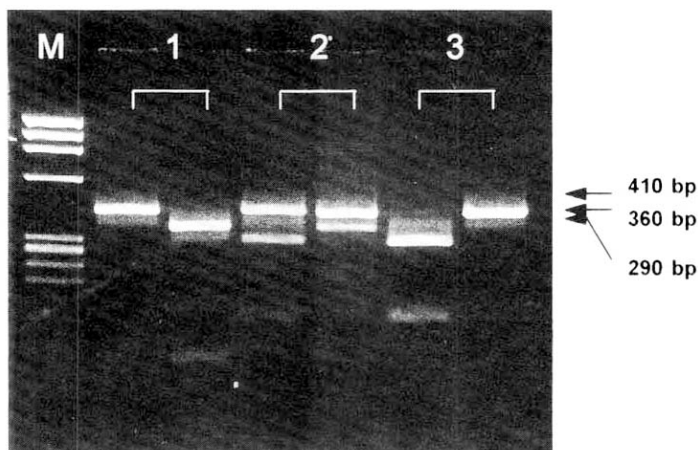0.235 among the control subjects, with no

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Fig. 3. Polymorphisms of the CYP2E1 gene in the 5'-flanking region. The polymorphism was studied by PCR followed by *Pst*I and *Rsa*I digestion. The c1 allele has the *Rsa*I site whereas the c2 allele has the *Pst*I site. Lane M, *Hae*III-digested ΦX174 DNA molecular weight markers; Lane 1, c1 homozygote (c1/c1); Lane 2, heterozygote (c1/c2); Lane 3, c2 homozygote (c2/c2).

Table 1. Genotype and Allele Frequencies According to the Polymorphisms at Nucleotide 6235 in the 3'-flanking Region of CYP1A1

Group	CYP1A1 genotype			Allele frequency	
	wt1/wt1	wt1/m1	m1/m1	wt1	m1
Healthy controls (n=53)	21 (39.6%)	26 (49.1%)	6 (11.3%)	0.64	0.36
Pancreatic cancer patients (n=45)	21 (46.7%)	20 (44.4%)	4 (8.9%)	0.69	0.31

CYP1A1, cytochrome P-4501A1; wt1, wild type (6235 T); m1, mutant type (6235 C)

statistically significant difference (χ^2=1.97). The genotype frequencies of m2 homozygotes among the cases and the controls showed no statistical difference (p=0.81, Fisher's exact test). Also, the division of the patients by smoking status led to no significant result (Table 2).

As for the CYP2D6 polymorphisms, we could find no person who had a mutation either at the junction of intron 3/exon 4 or within exon 5 of the CYP2D6 gene in this study. However, 2 persons homozygous for a complete gene deletion were found among the controls.

Table 4 shows the genotype and allele fre-

quencies of CYP2E1 among the cases and controls. The allele frequencies for c1 and c2 were 0.81 and 0.19, respectively, among the cases, and 0.70 and 0.30, respectively, among the control subjects (χ^2 =3.35, p=0.07).

DISCUSSION

The cytochrome P-450 enzymes are the most important enzymes in the oxidative metabolism of many endogenous substances such as steroids, fatty acids, prostaglandins, drugs, and other foreign compounds, including many chemical car-

cinogens from the environment. Within a population, there exist genetic variations of cytochrome P-450 enzymes leading to interindividually different metabolic capacity. Since the initial event in chemical carcinogenesis is often metabolic activation of procarcinogens to ultimate carcinogens[4, 5], large variation in the susceptibility to chemical carcinogens may be related to the genetic variations of cytochrome P-450 enzymes. Polymorphisms are recognized for three or more cytochrome P-450 species, and recent studies have suggested that genetic polymorphisms of CYP1A1,

CYP2D6, and possibly CYP2E1, may be related to different interindividual susceptibility to lung cancer[6-8].

The CYP1A1 metabolizes polycyclic aromatic hydrocarbons such as benzo[a]pyrene to activated carcinogens such as diolepoxide[9]. The CYP1A1 gene is localized on the long arm of chromosome 15 and consists of seven exons and six introns. To date, two polymorphic loci are known. One is a T to C mutation at position 6235 in the 3'-flanking region (m1), which provides a restriction endonuclease cleavage site for *MspI*, and is related with the increased inducibility of the enzyme[7]. Possibly, the m1 mutation in the noncoding 3'-flanking region is a marker for alterations in the regulatory regions of the CYP1A1 at the 5' side. Peterson et al demonstrated relatively higher degree of inducibility in cultured lymphocytes from persons with wt1/m1 than from persons with wt1/wt1[18]. The other is an A to G mutation at position 4889 within exon 7 (m2), which results in replacement of isoleucine with valine and almost 2-fold higher activity of the enzyme[10]. These two loci are known to be linked very closely. Crofts et

Table 2. Mutant Allele Frequencies of the CYP1A1 According to the Smoking Status in Patients with Pancreatic Cancer

Group	Mutant allele frequency	
	m1	m2
Smokers (n=25)	0.32	0.18
Non-smokers (n=20)	0.30	0.125

CYP1A1, cytochrome P-4501A1; m1, mutant type (6235 C); m2, mutant type (4889 G)

Table 3. Genotype and Allele Frequencies According to the Polymorphisms at Nucleotide 4889 within Exon 7 of CYP1A1

Group	CYP1A1 genotype			Allele frequency	
	wt2/wt2	wt2/m2	m2/m2	wt2	m2
Healthy controls (n=53)	32 (60.4%)	17 (32.1%)	4 (7.5%)	0.765	0.235
Pancreatic cancer patients (n=45)	31 (68.9%)	14 (31.1%)	0 (0%)	0.84	0.16

CYP1A1, cytochrome P-4501A1; wt2, wild type (4889 A); m2, mutant type (4889 G)

Table 4. Genotype and Allele Frequencies According to the Genetic Polymorphisms in the 5'-flanking Region of CYP2E1

Group	CYP2E1 genotype			Allele frequency	
	c1/c1	c1/c2	c2/c2	c1	c2
Healthy controls (n=53)	27 (50.9%)	20 (37.7%)	6 (11.4%)	0.70	0.30
Pancreatic cancer patients (n=45)	31 (68.9%)	11 (24.4%)	3 (6.7%)	0.81	0.19*

* χ^2 = 3.35, p = 0.07 vs. healthy controls. CYP2E1, cytochrome P-4502E1

al reported that the inducibility of the CYP1A1 is related with the m2 mutation in exon 7, but not with the m1 mutation in individuals who have only one type of mutation. These results suggest that the increased inducibility of the CYP1A1 in individuals with the m1 allele may be merely due to the linkage of the m1 allele to the m2 allele[19]. Anyway, individuals homozygous for the m1 or the m2 allele are known to have about threefold higher risk to lung cancer, especially smoking-related Kreyberg type I lung cancer, and the risk is much higher (7.31~fold) at low levels of cigarette consumption[7, 9-12].

In this study, we could not find any evidence that genetic polymorphisms of the CYP1A1 are associated with increased susceptibility to pancreatic cancer. It is in concordance with an other recent study by Okada et al[20]. These results may be due to the relatively high proportion of non-smokers in patients with pancreatic cancer in our study. In this study, the proportion of smokers in the patient group (male : 85.7%, female : 5.9%) was similar to that in age-matched Korean population (male : 77.6%, female : 13.6%)[21]. Different age and sex distributions between the patients and the controls in our study may also influence the results. Another possible explanation is a relatively lower risk of pancreatic cancer by smoking than that of lung cancer. Pulmonary tissue is directly exposed to high concentration of activated carcinogens because the CYP1A1 is expressed and induced by smoking in lung tissue[22]. On the other hand, pancreatic tissue is indirectly exposed to carcinogens which are removed by detoxification enzymes such as glutathione transferase M1 in the liver. It is also possible that increased risk of pancreatic cancer due to smoking is not associated with polycyclic aromatic hydrocarbons but with other carcinogens or risk factors linked to cigarette smoking.

The CYP2D6 metabolizes debrisoquine, metoprolol, codeine, dextromethorpan, sparteine, etc. The CYP2D6 polymorphism is an autosomal recessive trait associated with impaired debrisoquine metabolism in 5-10% of the Caucasian population[23]. This metabolic defect usually reflects absence of the CYP2D6 protein due to by mutations in the CYP2D6 gene in affected individuals,

which results in the poor metabolizer phenotype. A G-to-A transition at the junction of intron 3/exon 4, single base-pair deletion in exon 5, or a gene deletion is responsible for the absence of the enzyme activity in about 90% of poor metabolizers[24, 25]. Poor metabolizers are known to have reduced susceptibility to lung cancer[6, 13, 14]. However, it is not known which carcinogen is metabolized by the CYP2D6 enzyme. Our results showed that poor metabolizers are quite rare in the Korean population, so it is unlikely that polymorphisms of the CYP2D6 are associated with increased susceptibility to cancer in the Korean population.

The CYP2E1 metabolizes nitrosamine in foods or cigarette smoke to activated carcinogens[8], and is induced by chronic ethanol consumption and smoking[26, 27]. Recently, the presence of genetic polymorphisms of this enzyme was confirmed by Hayashi et al. The *Rsa*I and *Pst*I polymorphisms in the 5'-flanking region of the CYP2E1 gene are related to the transcriptional regulation of the enzyme. The transcriptional activity of the c2 gene was 10 times higher than that of the c1 gene in studies with the chloramphenicol acetyltransferase assay using Hep G2 cells[15]. However, it is uncertain whether CYP2E1 induction following chronic ethanol ingestion differs in vivo according to the genotypes. So far, it is uncertain whether polymorphisms of the CYP2E1 are associated with the increased susceptibility to cancer in human beings. In our study, the frequencies for the c2 allele among the cases and the controls are 0.18 and 0.30, which was not statistically significant at the 95% level, but significant at the 90% level. However, this difference may be due to the small sample size because the observed frequency for c2 allele among the patients with pancreatic cancer is quite similar to those among the control subjects, patients with alcoholic liver disease, and patients with hepatocellular carcinoma in the Korean population (unpublished data), and to those among healthy controls in the Japanese and the Chinese populations (0.25-0.35)[28-30].

In conclusion, we could find no evidence that polymorphisms of the cytochromes P-450 (1A1, 2D6, and 2E1) are associated with increased susceptibility to pancreatic cancer.

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