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Polymorphism of *UDP-glucuronosyltransferase 1A7* gene: A possible new risk factor for lung cancer

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Abstract

UDP-glucuronosyltransferase (UGT) 1A7 detoxifies hydroxylated benzo-(α)-pyrenes and 2-hydroxyamino-1-methyl-6-phenylimidazo (4,5-β) pyridine. The purpose of this study was to evaluate whether *UGT1A7* polymorphisms are risk factors for lung cancer. A total of 113 Japanese patients with lung cancer and 178 healthy individuals were enrolled in this study. Genomic DNA was isolated from leukocytes. Exon 1 of *UGT1A7* was sequenced. Homozygous *UGT1A7*3/3* was observed in 17 (15%) of patients with lung cancer, and this incidence was significantly increased compared with the control group (4.5%, $P = 0.0036$). Multivariate logistic regression analysis demonstrated a significant association of lung cancer with Brinkmann index (odds ratio = 4.577, $P = 0.0004$) and homozygous *UGT1A7*3* (odds ratio = 4.020, $P = 0.0037$). The presence of *UGT1A7* polymorphisms was associated with lung cancer. Homozygous *UGT1A7*3* is a possible risk factor for lung cancer, at least in the Japanese population. Thus, determination of *UGT1A7* polymorphisms may provide an important clue to preventive measures against lung cancer.

Keywords: UDP-glucuronosyltransferase; Polymorphism; Lung cancer; Smoking; Detoxification; Risk factor

1. Introduction

Smoking is a well-established risk factor for lung cancer. The incidence of lung cancer has been shown to correlate proportionally with increases in cigarette sales [1]. Cigarette smoke contains several carcinogens, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanl (NNAL) or benzo-(α)-pyrene and hydroxylated benzo-(α)-pyrenes.

UDP-glucuronosyltransferases (UGTs) are a superfamily of enzymes that play fundamental roles in glucuronidation of several xenobiotics and endogenous compounds [2]. They detoxify a large variety of carcinogens. UGTs (UGT1 and UGT2) have been categorised into two families according to their sequence homology and substrate specificity. The UGT1 gene is located on chromosome 2q37, and it encodes nine functional proteins (UGT1, UGT1A3–1A10); the chromosomal locus also contains four pseudogenes (UGT1A2p, UGT1A11p–13p) [3]. The gene organisation is quite unique: exon 1, preceded by the respective promoter, is specific only for the UGT1A isoform; common exons 2–5 encode part of the UGT1A isoforms. Exon 1 and common exons 2–5 are responsible for mRNA expression of specific UGT1A isoforms, which determine their substrate specificity. *UGT1A7*, a member of the UGT1 family, is

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expressed in oral, laryngeal, oesophageal, gastric and small intestinal tissues [4–6]. mRNA and protein expression of *UGT1A7* mRNA in the lung is not clear; Guillamente and colleagues [7,8] showed evidence of mRNA expression in the lung, but another group reported absence of *UGT1A7* mRNA expression in the lung by Northern blot analysis.

UGT1A7 detoxifies hydroxylated benzo-(α)-pyrenes and 2-hydroxyamino-1-methyl-6-phenylimidazo (4,5-β) pyridine (*N*-hydroxy-PhIP), which is a carcinogen involved in digestive tract carcinogenesis [6,9,10]. *UGT1A7* may therefore constitute a cancer risk factor. Wild-type *UGT1A7* is termed *UGT1A7*1* (N129R131-W208). Genetic analyses have disclosed three different amino acids in the human *UGT1A7* gene differentiating three polymorphic *UGT1A7* alleles: *UGT1A7*2* (K129K131), *UGT1A7*3* (K129K131R208) and *UGT1A7*4* (R208) (Fig. 1). Other single nucleotide polymorphisms (*UGT1A7*5*–**9*) have been reported in Caucasians and African-Americans [11]. These polymorphisms are associated with reduced catalytic activity of the encoded protein [7,10,12]. *UGT1A7* allelic variations have been reported to be risk factors for orolaryngeal cancer [13], colorectal cancer [14], hepatocellular carcinoma [12,15] and pancreatic cancer [16]. The protein encoded by *UGT1A7*3* shows catalytic activity, and has been associated with the development of liver or colon cancer [10].

The relationship between *UGT1A7* polymorphism and lung cancer has not yet been evaluated. Whether *UGT1A7* is expressed in bronchial tissues is unknown. We hypothesised that functional alternations of *UGT1A7* would result in reduced detoxification of several carcinogens for lung cancer. To demonstrate this hypothesis, we carried out a preliminary study in a small group of subjects to evaluate whether *UGT1A7* polymorphism might be a risk factor for lung cancer.

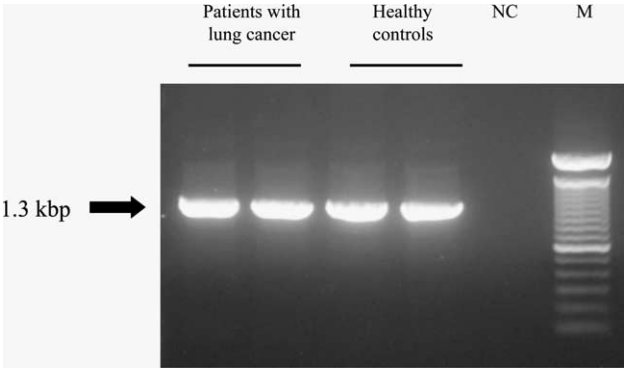

Fig. 1. PCR products of exon 1 of UGT1A7 from patients with lung cancer and from healthy individuals. After PCR, the primers were eliminated from the PCR product using Min Elute™ PCR purification kit (Quiagen, USA). The purified PCR products were separated on 1.2% agarose gel and visualised by ethidium bromide staining. The size of PCR product was 1294 bp. The amplified product contained the sequence of TATA box in the promoter region of *UGT1A7* bp, base pair; NC, negative control; M, molecular marker (100-bp DNA ladder).

2. Materials and method

2.1. Patients

A total of 113 (90 male and 23 female; median age 66 years) Japanese patients with lung cancer and 178 Japanese healthy individuals (115 male and 63 female; median age 63 years) were enrolled in this study. Our patients were incident cases; they were admitted to the Mie University Hospital or Mie Central Medical Center from May 2003 through January 2005. Lung tissue specimens were obtained from all patients with lung cancer by bronchoscopic or open lung biopsy. Histological classification of lung tumours was done according to the international staging system for lung cancer [17]. Detailed information on smoking habits was obtained from all patients and healthy subjects. The Brinkmann index (BI) was used as an indicator of smoking grade, and was calculated as follows: (average number of daily cigarettes consumption) × (years of smoking) [18]. Informed consent was obtained from all subjects, and the study was approved by the Ethical Committee of Mie University School of Medicine and Mie Central Medical Center.

2.2. Extraction of genomic DNA and amplification of UGT1A7

Genomic DNA was isolated from leukocytes using DNAQUICK (Dai-Nippon Phram. Co. Ltd, Osaka, Japan) according to the manufacturer's instructions. Exon 1 of *UGT1A7* was amplified by polymerase chain reaction (PCR) using specific primers. The primers used for amplification of TATA box and exon 1 were 5′-CGTCAAGGCCAAAAGCAT-5′ (Gene bank accession No. AF297093; nucleotides 98246–98263) and 5′-GTACAAAAGAATTCCTTAAACCAGAAA-3′ (AF297093; nucleotides 99540–99514), respectively. PCR mixtures were incubated at 94 °C for 2 min, and then PCR was performed at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min, during 30 cycles. A final extension at 72 °C for 8 min was performed to ensure complete extension of the PCR products.

2.3. Sequence of PCR product

The PCR products were directly sequenced by a dye terminating method using the ABI PRISM genetic analyser (PE Applied Biosystems, Foster city, California, United States of America (USA)). The sequence of primers used for the determination of TATA box and

exon 1 were 5′-CAGCACAGGGCATGATCT-5′ (AF297093; nucleotides 98296–98313) and 5′-CAGGGAAGCTGCTGGTAGT (AF297093; nucleotides 98626–98643), respectively.

2.4. Statistical analysis

Age and BI were expressed as median and range. The incidence of lung cancer in relation to age, gender, BI and *UGT1A7* polymorphism was first evaluated by univariate analysis. The distribution of gender, smoking habits and allelic frequency of *UGT1A7* polymorphism was analysed by χ^2 or Fisher exact test. Mann–Whitney *U* test was used to compare the mean of two variables. Multivariable logistic regression analysis and calculation of odd ratios were performed using the StatView software package for the Macintosh. The 95% confidence intervals (CI) of the OR were given. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical and histological data

Clinical characteristics of healthy individuals (controls) and lung cancer patients are described in Tables 1 and 2. The proportion of male and female was different between controls and patients; the number of males was significantly higher in lung cancer patients than in controls ($P = 0.0091$).

3.2. Association of UGT1A7 polymorphism with lung cancer

TATA box and exon 1 of *UGT1A7* were amplified from genomic DNA by polymerase chain reaction (PCR) using specific primers (Fig. 1). There was no mutation in the TATA box of *UGT1A7* in lung cancer and healthy control groups. The PCR product was sequenced and *UGT1A7* alleles were categorised into *UGT1A7*1* (wild-type), *UGT1A7*2*, *UGT1A7*3* and *UGT1A7*4* groups (Fig. 2, Table 3). *UGT1A7*5–*8* were not identified in any group. The genotype frequency for the three polymorphisms was found to be in Hardy–Weinberg equilibrium ($P = 0.32$ to K129,

Table 1
Univalidate analysis of clinical characteristics of healthy individuals and lung cancer patients

No. of subjects	Healthy individuals ($n = 178$)	Lung cancer ($n = 113$)	*P*-value
Gender (male/female)	115/63	90/23	$P = 0.0091$[a]
Age (years)	63 (29–99)	66 (37–88)	$P = 0.1202$[b]
Smoking (yes/ever/never)	91/24/63	79/7/27	$P = 0.005$[a]
Duration of smoking (years)	30 (0–60)	40 (0–65)	$P = 0.0009$[b]
BI	510 (0–2500)	800 (0–3000)	$P = 0.0001$[b]

BI, Brinkmann index.
[a] Data were analysed using χ^2 test.
[b] Mann–Whitney *U* test.

Table 2
Brinkmann index (BI) in healthy individuals and lung cancer patients

Smoking	Yes		No
	BI = or >400	BI < 400	
Lung carcinoma ($n = 113$)	84	3	26
Squamous cell carcinoma ($n = 38$)	34	1	3
Adenocarcinoma ($n = 47$)	23	2	22
Small cell carcinoma ($n = 23$)	22	0	1
Unclassified carcinoma ($n = 5$)	5	0	0
Healthy control ($n = 178$)	91	24	63

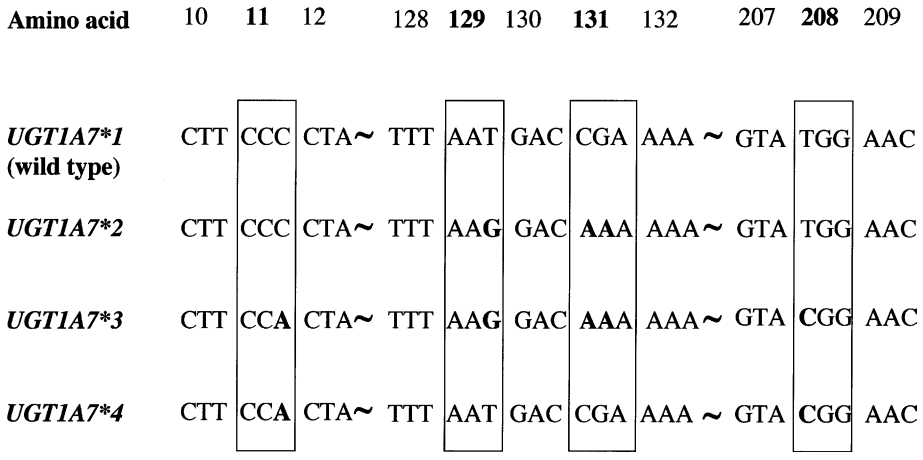

Fig. 2. Sequence of *UGT1A7*1*, *UGT1A7*2*, *UGT1A7*3* and *UGT1A7*4*. *UGT1A7* alleles: *UGT1A7*1* (N129R131W208), *UGT1A7*2* (K129K131W208), *UGT1A7*3* (K129K131R208) and *UGT1A7*4* (N129R131R208). Mutation CCC to CCA at position 11th amino acid is silent (P11–P11).

Table 3
Allelic frequency of *UGT1A7*

Group	Alleles	*n*	Allelic frequency
Control	*UGT1A7*1*	232	0.65
	*UGT1A7*2*	34	0.10
	*UGT1A7*3*	78	0.22*
	*UGT1A7*4*	12	0.03
Lung carcinoma	*UGT1A7*1*	131	0.58
	*UGT1A7*2*	20	0.09
	*UGT1A7*3*	71	0.31*
	*UGT1A7*4*	4	0.02

χ^2 test was used for data analysis.
* $P = 0.0138$.

$P = 0.32$ to K131 and $P = 0.25$ to R208, respectively). The allelic frequency of *UGT1A7*1*, *UGT1A7*2*, *UGT1A7*3* and *UGT1A7*4* was 0.65, 0.10, 0.22 and 0.03 in healthy controls, and 0.58, 0.09, 0.31 and 0.02 in lung cancer patients, respectively. The *UGT1A7*1* allele was distributed similarly in the two groups, but the allelic frequency of *UGT1A7*3* was significantly associated with lung cancer patients ($P = 0.0138$) (Table 3). The genotype frequency of *UGT1A7* in controls and lung cancer patients was summarised in Table 4. Eighty out of 178 (44.9%) healthy individuals had homozygous *UGT1A7*1* (wild-type). In the lung cancer group, homozygous *UGT1A7*1/1* was found in 39 out of 113 (34.5%) patients. Homozygous *UGT1A7*3/3* was observed in 17 (15.0%) patients with lung cancer, and it was more significantly prevalent in lung cancer than in the control group (4.5%, $P = 0.0036$) (Table 4).

Multivariate logistic regression analysis demonstrated that the occurrence of lung cancer is associated with BI (odds ratio = 4.577, $P = 0.0004$) and with the presence of homozygous *UGT1A7*3* polymorphism (odds ratio = 4.020, $P = 0.0037$). However, the presence of heterozygous *UGT1A7*3* polymorphism was not associated with lung cancer ($P = 0.6320$) (Table 5).

Table 5
Multivariate analysis of factors associated with the occurrence of lung cancer

	Odds ratio (95% CI)	*P*-value
Gender	1.549 (0.635–3.775)	0.3361
Brinkmann index = or >400	4.577 (1.972–10.625)	0.0004
Homozygous *UGT1A7*3*	4.020 (1.569–10.299)	0.0037
Heterozygous	1.141 (0.666–1.954)	0.6320

Data were analysed using multivariable logistic regression analysis.

4. Discussion

The association of cigarette smoking with the occurrence of lung cancer is well-established [19–21]. Smoking has also been associated with the occurrence of laryngeal, pharyngeal, bladder, kidney and pancreatic cancer [22]. Detection of risk factors is a fundamental step for the prevention of cancer.

UGT1A7 is expressed in the oral mucosa as well as in the epithelial layer of the digestive tract. Direct contact with environmental carcinogens including cigarette smoke has been reported to induce cancer in the organs of the digestive tract. In the present study, we found for the first time that *UGT1A7* polymorphism might also be a risk factor for lung cancer. However, whether the lungs express *UGT1A7* mRNA remains controversial.

The allelic frequency of *UGT1A7*1* is 0.36 23 or 0.44 [13] in normal Caucasians. Ando and colleagues [24] reported that the allelic frequency of *UGT1A7*1* is 0.59 in the Japanese population, and Wang and colleagues recently reported that the allelic frequency of *UGT1A7*1* is 0.69 in 60 healthy Japanese subjects [15]. In the present study, we found an allelic frequency of 0.65 in 178 healthy Japanese individuals. Overall, these observations suggest that wild-type *UGT1A7*1* allele is more frequent in the Japanese population.

Among the *UGT1A7* polymorphisms evaluated in our Japanese population, the incidence of *UGT1A7*3*

Table 4
Association of the UGTl A7 polymorphism with histology of lung carcinoma

UGT1A7 alleles	Control ($n = 178$)	Lung carcinoma				
		Total ($n = 113$)	scc ($n = 38$)	adeno ($n = 47$)	sclc ($n = 23$)	Unknown ($n = 5$)
*UGTlA7*1/1*	80	39	10	16	12	1
*UGTlA7*l/2*	15	16	4	10	1	1
*UGTlA7*l/3*	45	33[a]	12	13	7	1
*UGTlA7*l/4*	12	4	4	0	0	0
*UGTlA7**2/2	1	0	0	0	0	0
*UGTlA7*2/3*	17	4	3	0	0	1
*UGTlA7*3/3*	8	17[b]	5	8	3	1
*UGTlA7*3/4*	0	0	0	0	0	0
*UGTlA7*4/4*	0	0	0	0	0	0

Data were analysed using χ^2 test.
[a] $P = 0.5482$, compared with control.
[b] $P = 0.0036$, compared with control, scc, squamous cell carcinoma; sclc, small cell lung carcinoma; adeno, adenocarcinoma.

was significantly associated with the presence of lung cancer. Results of the multivariate regression analysis also suggest that *UGT1A7* polymorphism is an important risk factor for lung cancer. This association may differ among different ethnic groups. For example, allelic frequency of *UGT1A7*3* in normal Caucasians has been reported to be 0.17 14 or 0.30 23, and Köhle and colleagues [23] reported an allelic frequency of 0.30 and 0.38 in Caucasians and Egyptians, respectively. However, in the present study, we found an allelic frequency of 0.22 in healthy Japanese subjects. Thus, it appears that the allelic frequency of *UGT1A7*3* differs among distinct ethnic groups. This ethnic factor may explain the positive relationship between *UGT1A7*3* and lung cancer observed in our Japanese population.

UGT1A7 catalyses the glucuronidation of benzo-(α)-pyrene metabolites and heterocyclic amines including PhIP [6,9,10]. The enzymatic activity of *UGT1A7*2* (54%), *UGT1A7*4* (36%) and *UGT1A7*3* (17%) toward benzo [α] pyrene metabolites was reduced compared with *UGT1A7*1* (100%) [24]. Therefore, it is conceivable that the enzymatic activity of homozygous *UGT1A7*3* against xenobiotics contained in cigarette smoke is also reduced. Genetic alternation with reduced enzymatic activity of *UGT1A7* would affect the mucosal detoxification of airborne or dietary xenobiotics allowing their free passage to the systemic circulation. This may result in systemic accumulation of several carcinogens that may favour the development of lung cancer. These observations suggest that determination of *UGT1A7* polymorphism may be useful to identify individuals susceptible to lung cancer and to take measures to prevent development of the disease.

It has been reported recently that human *UGT1A9* and *UGT2B7* catalyse the glucuronidation of NNAL, another major metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Glucuronidation of NNAL is known to be an important detoxification pathway that may be involved in the prevention of lung cancer [25]. Whether polymorphisms of *UGT1A9* and/or *UGT2B7* are risk factors for lung cancer remain to be determined. *UGT1A9* is expressed in lung tissues [26]. Concurrent polymorphisms of *UGT1* (*UGT1A6*2* and *UGT1A7*3*) have frequently been reported in Caucasians and Egyptians [23]. Thus, it is possible that in addition to *UGT1A7*, polymorphisms of other alleles of *UGT1A*s may also be involved in the impaired detoxification mechanism of inhaled carcinogens. Furthermore, expression of xenobiotic-metabolising enzymes other than UGTs has also been reported in human bronchial mucosa and peripheral lung tissues [27]. Deficient function of these enzymes may also be associated with lung cancer. For example, blood glutathione level and glutathione-*S* transferase activity have been associated with the occurrence of non-small cell lung carcinoma [28]. Measurement of the activity of these enzymes together with that of UGT families may also useful to predict the risk for lung cancer.

In brief, the results of this preliminary study in a small number of lung cancer patients suggest that *UGT1A7* polymorphism might be associated with lung cancer. Homozygous *UGT1A7*3* was found to be a risk factor for lung cancer, at least in the Japanese population. Based on this observation, determination of *UGT1A7* polymorphisms may provide useful information about preventive measures against lung cancer. However, further study in a larger population should be carried out to confirm these findings.

Conflict of interest statement

None declared.

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