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ORIGINAL ARTICLE

Association between *UGT1A1**28*28 genotype and lung cancer in the Japanese population

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Abstract
Background Lung cancer is the leading cause of cancer death and is closely linked to tobacco smoking. Genetic polymorphisms in genes that encode enzymes involved in metabolizing tobacco carcinogens could affect an individual's risk for lung cancer. While polymorphism of UDP-glucuronosyltransferase1A1 (*UGT1A1*) is involved in detoxification of benzo(a)pyrene-7,8-dihydrodiol(–), a major tobacco carcinogen, the association between *UGT1A1* genotype and lung cancer has not been examined.
Methods We retrieved the clinical data of 5,285 patients who underwent systemic chemotherapy at Kyoto University Hospital. A total of 765 patients (194 lung cancer patients and 671 patients with other malignancies) with *UGT1A1* genotyping data were included in this analysis. We used logistic regression with recessive, dominant, and additive models to identify differences in genotype frequencies between lung cancer and other malignancies.
Results In the recessive model, *UGT1A1**28*28 genotype was significantly associated with lung cancer compared to other malignancies (odds ratio 5.3, $P = 0.0083$). Among lung cancer patients with a smoking history, squamous cell carcinoma was significantly predominant in patients with *UGT1A1**28*28 compared to those with other *UGT1A1* genotypes ($P = 0.024$).
Conclusion This is the first study to demonstrate a significant association between the homozygous *UGT1A1**28 genotype and lung cancer.

Keywords Lung cancer · Smoking · *UGT1A1*

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Introduction

Lung cancer is the leading cause of cancer death in the world [1]. Epidemiological studies have shown that lung cancer is definitely linked to tobacco smoking, especially in squamous cell carcinoma and small cell carcinoma [2]. Tobacco smoke contains dozens of carcinogenic agents that are harmful to humans [3, 4]. Genetic polymorphisms in genes that encode enzymes involved in metabolizing tobacco carcinogens may affect an individual's risk for lung cancer [5–7].

Among such genes, UDP-glucuronosyltransferase (*UGT1A1*) is involved in the detoxification of benzo(a)pyrene-7,8-dihydrodiol(–), a major tobacco carcinogen [8, 9]. Despite the strong association between tobacco smoking and lung cancer, the association between *UGT1A1* genotype and lung cancer has not been clarified. In this study, we examined the association between *UGT1A1* genotype and lung cancer prevalence.

Patients and methods

Patients

Using a prospective cohort database system (CyberOncology®; Cyber Laboratory Inc., Tokyo, Japan) and electronic medical records, we retrieved the clinical data of 5,285 patients who underwent systemic chemotherapy at Kyoto University Hospital (Kyoto, Japan) between January 2004 and June 2014. In this cohort, 1,276 lung cancer patients and 3,996 patients with other malignancies were registered. Among them, the patients who underwent *UGT1A1* genotyping were eligible for this study (Fig. 1). The patient characteristics retrieved included gender, age, smoking status (current/past/never), *UGT1A1* variant (*1, *6, or *28), and the primary site of malignancy. Pathology reports for the lung cancer patients were also reviewed in the electronic medical records.

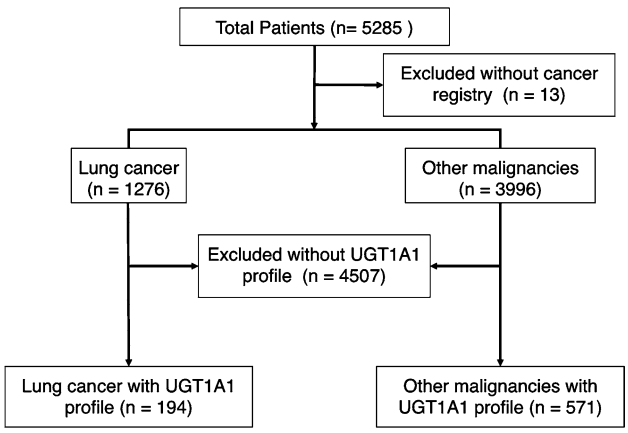

Fig. 1 Study flow

Genotyping

Genomic DNA was extracted from whole blood. Genotyping on *UGT1A1* variants was performed using the following methods.

Genotyping method 1 (January 2004–March 2011): polymerase chain reaction (PCR) was performed using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) with rTaq (Takara Bio, Shiga, Japan). The purified PCR products were then sequenced with a multicapillary DNA sequencer RISA384 System (Shimadzu, Kyoto, Japan). *UGT1A1**6 and *28 were genotyped [10].

Genotyping method 2 (April 2011–June 2014): genotyping analyses for *UGT1A1**6 and *28 were performed using Invader® *UGT1A1* assay kit (Sekisui Medical Co., Ltd., Tokyo, Japan).

Statistical analysis

Logistic regression analysis using recessive, dominant and additive models without covariates was performed to identify differences in genotype frequencies between patients with lung cancer and those with other malignancies. Fisher's exact test was used to test the association between *UGT1A1* genotype and pathological classification (squamous cell carcinoma vs other pathological types, small cell carcinoma vs other pathological types) in the lung cancer patients with a smoking history. We corrected for multiple testing using the Šidák correction [11] to control the family-wise error rate at 0.05. For logistic regression analysis, we corrected for six independent tests and the corrected significant threshold was 0.0085. For Fisher's exact test, the corrected significant threshold was 0.025 for two independent tests. Hardy–Weinberg equilibrium was also tested using the genetics package in R (https://cran.r-project.org/web/packages/genetics/index.html). Two-tailed P values <0.05 were considered significant. All statistical analyses were performed using R version 3.11 (http://www.r-project.org).

Ethics

This study was approved by the Ethics Committee of the Kyoto University Graduate School of Medicine (E2200). All patients provided written informed consent for *UGT1A1* genotyping and the use of the results for research purposes.

Results

A total of 765 patients with *UGT1A1* genotyping were included in this study. Among them, 194 patients had lung

Table 1 Patient characteristics

	Lung cancer	Malignancy excluding lung cancer
Characteristic	($n = 194$)	($n = 571$)
Age (years, range)	64 (36–84)	62 (13–82)
Sex (male/female)	143/51	349/222
Smoking history (current/past/never/unknown)	70/92/32/0	96/176/285/14
*UGT1A1**28 (%)		
−/−	147 (75.8)	447 (78.2)
−/+	40 (20.6)	120 (21.1)
+/+	7 (3.6)	4 (0.7)
*UGT1A1**6 (%)		
−/−	136 (70.1)	383 (67.1)
−/+	51 (26.3)	165 (28.9)
+/+	7 (3.6)	23 (4.0)

Table 2 Recessive, dominant, and additive model results

	Recessive		Dominant		Additive	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
*UGT1A1**28	5.3 (1.6–20.4)	0.0083[§]	1.2 (0.8–1.7)	0.47	1.3 (0.9–1.8)	0.16
*UGT1A1**6	0.9 (0.4–2.0)	0.79	0.9 (0.6–1.2)	0.44	0.9 (0.7–1.2)	0.46

OR odds ratio; *95% CI* 95% confidence interval

[§] Significant with multiple testing correction

cancer and 671 patients had other malignancies. Patient characteristics are summarized in Table 1. The allele frequencies of *UGT1A1**28 and *UGT1A1**6 were in Hardy–Weinberg equilibrium ($P = 0.18$ and $P = 0.33$, respectively) in patients with other malignancies.

In the recessive model, *UGT1A1**28*28 genotype was significantly associated with lung cancer (OR 5.3, $P = 0.0083$), whereas the *UGT1A1**6*6 genotype was not (OR 0.9, $P = 0.79$). Neither the dominant model nor the additive model showed any significant association with lung cancer (Table 2).

All of the seven lung cancer patients with *UGT1A1**28*28 had a smoking history—three patients had squamous cell carcinoma, three had small cell carcinoma, and one had adenocarcinoma (classified to other types of carcinoma in this research). Among the rest of the lung cancer patients with a smoking history, 14 had squamous cell carcinoma, 74 had small cell carcinoma, and 67 had other types of carcinoma. The ratio of squamous cell carcinoma to other histological types in lung cancer was 3:1 in patients with *UGT1A1**28*28 and 14:67 in patients with other *UGT1A1* genotypes. Thus, squamous cell carcinoma was significantly predominant in lung cancer patients with *UGT1A1**28*28 ($P = 0.024$). In contrast, the ratio of small cell carcinoma to other histological types did not differ according to genotype ($P = 0.62$).

Discussion

To our knowledge, this is the first report of a significant association between *UGT1A1**28*28 genotype and lung cancer. In our study, *UGT1A1**28 genotype was associated with lung cancer only in the recessive model, whereas no significant association was identified in the dominant model or in the additive model. This finding is consistent with a previous study which reported that the level of benzo(a) pyrene-7,8-dihydrodiol(–) glucuronide formation in liver microsomes was lower in people with *UGT1A1**28*28 genotype compared with those with either *UGT1A1**1*28 or *1*1 genotype [8]. The allele frequency of *UGT1A1**28 in our study was 0.12, which is consistent with the allele frequency in Japanese individuals (0.09–0.15 [12–14]). Interestingly, the genotype frequency of *UGT1A1**28*28 was higher in our lung cancer patients (3.6 vs 1.3% [14]), while this difference did not reach statistical significance possibly because of the limited sample size.

By contrast, no significant association was observed between *UGT1A1**6 polymorphism and lung cancer in any of the models used in our analysis. Although *UGT1A1**28 is a TATAA box polymorphism in the promoter region that results in 70% reduction in expression levels [15–17], *UGT1A1**6 is a single-nucleotide polymorphism in the exon 1 region, which causes reduced bioactivities by 32%

among homogenous individuals [18]. Our results may suggest that the reduced expression levels of enzyme are a more important factor for the risk of lung cancer than reduced bioactivities.

In this study, squamous cell carcinoma was significantly predominant in the lung cancer patients with *UGT1A1**28*28 genotype and a smoking history. This finding is consistent with the results of a meta-analysis which showed that squamous cell carcinoma is more closely linked to tobacco carcinogens than is large cell carcinoma or adenocarcinoma [2]. These data indicated that squamous cell carcinoma may be more susceptible to reduced UGT1A1 enzyme activities toward tobacco carcinogens, such as benzo(a)pyrene-7,8-dihydrodiol(–).

Previous genome-wide association studies (GWAS) of lung cancer identified several susceptible genetic loci [19, 20]. However, *UGT1A1**28 has not been identified in these previous report by GWAS, because the commonly used GWAS panels did not include the repeat variants such as *UGT1A1**28. In this study, by targeting *UGT1A1* polymorphisms with the use of a hospital database, we revealed the association between *UGT1A1**28 polymorphism and lung cancer.

Our study has the following limitations. First, our results are obtained among from cancer patients without healthy controls, although the allele frequency of *UGT1A1**28 was compatible with previous reports as discussed above [12–14]. Second, because our study was carried out at a single institution in Japan, the results should be validated in another cohort or other ethnic groups.

In summary, our study revealed the risk of lung cancer by *UGT1A1**28 genotype and tobacco smoking.

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Compliance with ethical standards

Conflict of interest Dr. Narahara received research funding from Toyobo Biotechnology Foundation. Dr. Muto received research funding from Taiho Pharmaceutical and Mitsui Knowledge Industry. No other potential conflict of interest relevant to this article was reported.

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