RESEARCH COMMUNICATION

Association between Polymorphisms in *UDP-glucuronosyltransferase 1A6* and *1A7* and Colorectal Cancer Risk

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Abstract

Genetic polymorphisms of uridine diphosphate-glucuronosyltransferases 1A6 (*UGT1A6*) and 1A7 (*UGT1A7*) may lead to genetic instability and colorectal cancer carcinogenesis. Our objective was to measure the interaction between polymorphisms of these repair genes and tobacco smoking in colorectal cancer (CRC). A total of 68 individuals with CRC and 112 non-cancer controls were divided into non-smoker and smoker groups according to pack-years of smoking. Genetic polymorphisms of *UGT1A6* and *UGT1A7* were examined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). We found a weak association of *UGT1A6* polymorphisms with CRC risk (crude odds ratio [OR], 1.65; 95% confidence interval [95%CI], 0.9-3.1, P=0.107; adjusted OR 1.95, 95%CI 1.0-3.8, P=0.051). The ORs for the *UGT1A7* polymorphisms were statistically significant (crude OR: 26.40, 95%CI: 3.5-198.4, P=0.001; adjusted OR: 21.52, 95%CI: 2.8-164.1, P=0.003). The joint effect of tobacco exposure and *UGT1A6* polymorphisms was significantly associated with colorectal cancer risk in non-smokers (crude OR, 2.11; 95%CI, 0.9-5.0, P=0.092; adjusted OR 2.63, 95%CI 1.0-6.7, P=0.042). In conclusion, our findings suggest that *UGT1A6* and *UGT1A7* gene polymorphisms are associated with CRC risk in the Japanese population. In particular, UGT1A6 polymorphisms may strongly increase CRC risk through the formation of carcinogens not associated with smoking.

Keywords: Gene polymorphisms - colorectal cancer (CRC) - UGT1A6 - UGT1A7

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer, and the leading cause of cancer death in the world (Siegel et al., 2012). Genetic susceptibility to this disease may result from inherited mutations in genes involved in carcinogen metabolism and DNA repair (Paz-Elizur et al., 2008; Joshi et al., 2009; Zhao et al., 2012). Uridine diphosphate-glucuronosyltransferases (UGTs) play an important role in glucuronidation of various endogenous and exogenous compounds such as bilirubin, steroid hormones, bile acids, anticancer drugs, tobacco-specific carcinogens, and dietary procarcinogens including heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) (Ciotti et al., 1997; van der Logt et al., 2004; Nagar et al., 2006). UGTs are classified into several families and subfamilies based on their structural and sequence homology (Jin et al., 1993). The locus codes of nine functional proteins of the UGT1A family differ only in their N-terminus as a result of alternate splicing of independent exon 1 regions to a shared C-terminus encoded by exons 2 to 5 (Nagar et al., 2006).UGT1A6 is expressed in several human tissues, and catalyses the glucuronidation of small planar phenols and primary aromatic amines (Nagar et al. 2006). Two functionally important SNPs arise in exon 1 of the *UGT1A6* gene, which result in T181A and R184S amino acid changes (*UGT1A6*8*), although alleles carrying only the T181A polymorphism (*UGT1A6*5*) or the R184S polymorphism (*UGT1A6*9*) have been described (http://www.pharmacogenomics.pha.ulaval.ca/files/content/sites/pharmacogenomics/files/Nomenclature/UGT1A/UGT1A6.htm). Metabolic rates of phenols by recombinant *UGT1A6*8* were lower than those of the most commonly occurring enzyme (Ciotti et al., 1997). UGT1A7 catalyses the conjunction and detoxification

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of several tobacco-related carcinogens (Nagar et al., 2006), and polymorphisms have been reported to result in the amino-acid changes N129K /R131K(*UGT1A7*2*), N129K/R131K/W208R (*UGT1A7*3*), and W208R (*UGT1A7*4*) (http://www.pharmacogenomics.pha.ulaval.ca/files/content/sites/pharmacogenomics/files/Nomenclature/UGT1A/UGT1A7.htm). UGT1A7 activity resulting from these polymorphisms were moderate to low compared with that of wild type *UGT1A7*1* (Guillemette et al., 2000; Zheng et al., 2001).

We have already reported that genetic polymorphisms of *NAT2* and *CYP1A2* in metabolic processes, and of *MUTYH* and *APEX1* DNA repair genes, contribute to CRC susceptibility in relation to smoking status in Japanese populations (Yoshida et al., 2007; Kasahara et al., 2008). We conducted a case-control study to evaluate the relevance to CRC risk by means of examining *UGT1A6* and *UGT1A7* gene polymorphisms, and assessed the effect of these genetic polymorphisms on CRC susceptibility in relation to smoking status.

Materials and Methods

Study Subjects

A total of 67 CRC patients (38 with colon cancer, 24 with rectal cancer and 5 with other carcinomas) were recruited between October 2003 and March 2005 at the Kobe Medical Center and Kobe Rosai Hospital in Kobe, Japan. The populations were included in previous studies that investigated genetic polymorphisms of metabolic or DNA repair enzymes (Yoshida et al., 2007; Kasahara et al., 2008). The controls consisted of 112 individuals who had not been currently or previously diagnosed with cancer, and were recruited between November 2002 and March 2003. Informed consent was obtained from all patients and controls, and all samples were coded after collection of blood and data. The amount of smoke exposure was calculated as pack-years, the product of number of years an individual had smoked and the average number of cigarettes smoked per day, converting to a standard pack of 20 cigarettes. The study design was approved by the Ethics Review Committee on Genetic and Genomic Research, Kobe University Graduate School of Medicine.

Genotyping

Genomic DNA was isolated from leukocytes in the previous studies (Yoshida et al., 2007; Kasahara et al., 2008). The genotypes of *UGT1A6* and *UGT1A7* were determined by PCR-RFLP analysis, as previously described (Logt et al., 2004; Chen et al., 2006).

Statistical Analysis

Logistic regression analysis was performed to assess the association between each genotype and CRC. Associations were expressed as odd ratios (OR) with 95% confidence intervals (95%CI) and P values of <0.05 were considered statistically significant. The ORs were computed to estimate the association between certain genotypes and CRC, and were adjusted for age, gender, and smoking habits. The subjects were divided into two groups according to pack-years of smoking: never-smoked (pack-years = 0), and smokers (pack-years >0). The gene-smoking interaction was also computed, and was adjusted for age and gender. Hardy-Weinberg equilibrium was tested using the goodness-of-fit Chi-square test to compare the observed genotype frequencies with the expected genotype frequencies among the control subjects. The statistical analysis was performed with PASW for Windows version 17.0 (SPSS Japan Inc., Tokyo, Japan).

Results and Discussion

We investigated the potential impact of genetic polymorphisms located in *UGT1A6* and *UGT1A7* on CRC risk. UGTs are thought to play a critical role in CRC because they are key enzymes in the metabolism of endogenous and exogenous compounds, including steroid hormones, xenobiotics and drugs (Nager et al., 2006). Many genetic polymorphisms in UGTs have been described, and some have been associated with increased CRC susceptibility (Fang & Lazarus, 2004; Tang et al., 2005). The present study is the first time that the association between *UGT1A6* and *UGT1A7* polymorphisms and CRC has been studied in a Japanese population.

Table 1 summarizes the distribution of characteristics of CRC patients and controls. There was no difference in the gender distribution (P=0.518) between males (patients, 53.7%; controls, 63.4%) and females (patients, 38.3%; controls, 36.6%). The mean age was 67.3 ± 11.0 years for patients and 67.6 ± 6.8 years for controls, respectively (P=0.859). Never-smokers comprised 55.2% of patients and 45.5% of controls, and smokers comprised 35.8% of patients and 51.8% of controls. Subtypes of the cases were as follows: colon, 56.7%; rectal, 35.8% and others, 7.5%.

Genotype distributions of *UGT1A6* adjusted for gender, age, and smoking habit, and corresponding allele frequencies are shown in Table 2. The genotype distributions and allele frequencies in patients are based on PCR–RFLP. All genotype distributions tested fulfilled the Hardy-Weinberg criteria. The distribution of variant *UGT1A6* alleles demonstrated a borderline significant association with CRC risk overall when *UGT1A6*1*1*

Table 1. Characteristics of Colorectal Cancer Cases and Control Subjects

Item	Patients		Controls		P-value
	n	%	n	%	
Number	67		112		
Gender:males	36	53.7	71	63.4	0.518[a]
females	26	38.3	41	36.6	
unknown	5	7.5	0	0.0	
Age: Mean ±S.D.	67.3 ±11.0		67.6 ±6.8		0.859[b]
Smoking status (Pack-years)					
Never	37	55.2	51	45.5	0.109[a]
Ever	24	35.8	58	51.8	
unknown	6	9.0	3	2.7	
Histological type					
colon	38	56.7			
rectum	24	35.8			
unknown	5	7.5			

[a]χ^2 analysis, [b]Student's T-test

Table 2. Genotype Distribution in Colorectal Cancer and Allele Frequency

Genotype	patients (n)	controls (n)	crude OR (95%CI)	P-value	adjusted OR (95%CI)[a]	P-value		Allele frequency alleles	patients%	controls%
UGT1A6[b]							*1	$T^{181}R^{184}$	70.7	78.9
*1/*1	33	69	1.00		1.00		*5	$A^{181}R^{184}$	0.0	0.9
variants	34	43	1.65 (0.9-3.1)	0.107	1.95 (1.0-3.8)	0.051	*8	$A^{181}S^{184}$	27.8	19.7
*1/*5	0	2					*9	$T^{181}S^{184}$	1.5	0.4
*1/*8	28	36								
*1/*9	1	1								
*8/*8	4	4								
*8/*9	1	0								
UGT1A7[c]										
*1/*1	1	32	1.00		1.00		*1	$N^{129}R^{131}W^{208}$	44.8	62.2
variants	66	80	26.40 (3.5-198.4)	0.001	21.52 (2.8-164.1)	0.003	*2	$K^{129}K^{131}W^{208}$	20.9	16.7
*1/*2	28	37					*3	$K^{129}K^{131}R^{208}$	28.4	20.3
*1/*3	30	37					*4	$N^{129}R^{131}R^{208}$	6.0	0.9
*2/*3	0	2								
*3/*3	0	2								
*3/*4	8	2								

[a]OR adjusted for gender, age, smoking habit; [b]*1*1 genotype includes patients or controls with two *1 (wild type) alleles; variant genotype included patients or controls with one or two *UGT1A6*5*, *UGT1A6*8* or *UGT1A6*9* alleles, [c]*1*1 genotype includes patients or controls with two *1 (wild type) alleles; variant genotype included patients or controls with one or two *UGT1A7*2, UGT1A7*3* or *UGT1A7*4*alleles

Table 3. *UGT1A6* Genotype Distribution in Relation to Colorectal Cancer Subtypes

Genotype	Colon						Rectal					
	patients (n)	controls (n)	crude OR (95%CI)	P-value	adjusted OR (95%CI)[a]	P-value	patients (n)	controls (n)	crude OR (95%CI)	P value	adjusted OR (95%CI)[a]	P value
UGT1A6[b]												
*1/*1	18	69	1.0		1.0		11	69	1.0		1.0	
variants	20	43	1.78 (0.9-3.7)	0.127	1.84 (0.8-4.0)	0.130	13	43	1.90 (0.8-4.6)	0.158	2.08 (0.8-5.3)	0.123

[a]OR adjusted for gender, age, smoking habit; [b]*1*1 genotype includes patients or controls with two *1 (wild type) alleles; variant genotype included patients or controls with one or two UGT1A6*5, UGT1A6*8 or UGT1A6*9 alleles

Table 4. *UGT1A6* Genotype Distribution in Relation to Smoking Status and Colorectal Cancer

Genotype	Non-Smokers (Pack-years=0)						Smokers (Pack-years>0)					
	patients (n)	controls (n)	crude OR (95%CI)	P-value	adjusted OR (95%CI)[a]	P-value	patients (n)	controls (n)	crude OR (95%CI)	P-value	adjusted OR (95%CI)[a]	P-value
UGT1A6[b]												
*1/*1	18	34	1.00		1.00		11	33	1.00		1.00	
variants	19	17	2.11 (0.9-5.0)	0.092	2.63 (1.0-6.7)	0.042	13	25	1.56 (0.6-4.1)	0.362	1.62 (0.6-4.3)	0.331

[a]OR adjusted for gender, age, smoking habit; [b]*1*1 genotype includes patients or controls with two *1 (wild type) alleles; variant genotype included patients or controls with one or two UGT1A6*5, UGT1A6*8 or UGT1A6*9 alleles

was used as reference (crude OR, 1.65; 95%CI, 0.9-3.1, P=0.107; adjusted OR 1.95, 95%CI 1.0-3.8, P=0.051). Table 3 summarizes the genotype distribution for colon and rectal cancer, showing the OR adjusted for gender, age and smoking habits. The adjusted ORs for the variant *UGT1A6* alleles showed no statistically significant risk for either colon or rectal cancer (OR 1.84, 95%CI 0.8-4.0, P=0.130 for colon cancer; OR 2.08, 95%CI 0.8-5.3, P=0.123 for rectal cancer). The OR for the joint effect of tobacco exposure (pack-years) and the three polymorphisms, adjusted for gender and age, are shown in Table 4. The variant *UGT1A6* alleles showed a significant association with CRC risk in non-smokers (crude OR, 2.11; 95%CI, 0.9-5.0, P=0.092; adjusted OR 2.63, 95%CI 1.0-6.7, P=0.042), but did not in smokers (crude OR 1.56, 95%CI 0.6-4.1, P=0.362; adjusted OR 1.62, 95%CI 0.6-4.3, P=0.331). Logt et al. (2004) reported a significant association of CRC and the presence of variants in *UGT1A6*, although another study of colon cancer observed no association with genetic variants in *UGT1A6* (Chan et al., 2005). We also found a weak relationship between CRC risk and *UGT1A6* polymorphisms in Japanese individuals. In particular, we confirmed that the association of *UGT1A6* polymorphisms for CRC risk was strongly increased compared with that in non-smokers. The genetic variations in *UGT1A6* gene associated with the less frequent variants have a 30–50% lower enzyme activity (Ciotti et al., 1997). UGT1A6 metabolizes heterocyclic amines (HCAs), which are formed in protein-rich foods, such as meat, as a result of pyrolysis during cooking (Orzechowski et al., 1994). We also did not find any evidence of effects of modification for the polymorphisms of *CYP2A9* enzyme (data not shown), although polymorphisms related to *UGT1A6* associated with CRC-risk were reported (Hubner et al., 2006). Furthermore, the presence of these *UGT1A6* polymorphisms was not associated with lung

cancer in Japanese (data not shown). Thus, individuals bearing variant *UGT1A6* genotypes associated with reduced enzyme activity to detoxify food-derived HCAs sufficiently may therefore be at an increased risk for CRC without smoking.

Genotype distributions of *UGT1A7* adjusted for gender, age, and smoking habit, and corresponding allele frequencies are shown in Table 1. The distribution of variant *UGT1A7* alleles were significantly associated with CRC risk when *UGT1A7 *1*1* was used as reference are shown in Table 1 (crude OR: 26.40, 95%CI: 3.5-198.4, P=0.001; adjusted OR: 21.52, 95%CI: 2.8-164.1, P=0.003). Our study supports that the *UGT1A7* polymorphisms were associated with a significantly increased risk of CRC in Japanese patients, which is similar to observations made in Chinese and Caucasian populations (Strassburg et al., 2002; Logt et al., 2004; Chen et al., 2006). Moreover, the presence of *UGT1A7* polymorphisms was also associated with lung cancer in Japanese (Araki et al., 2005). UGT1A7 catalyses the glucuronidation of tobacco smoke-derived polycyclic aromatic hydrocarbons (PAHs), such as benzo-(a)pyrene metabolites, as well as dietary-derived HCAs, all of which are known carcinogens (Nager et al., 2006). *UGT1A7* polymorphisms shown to result in a significant reduction of enzyme activity are risk factors for CRC (Guillemette et al., 2000; Logt et al., 2004). However, we did not thoroughly investigate the association between CRC and *UGT1A7* polymorphisms with smoking status, because our overall population was relatively small and limited our ability to determine gene–environment interactions with respect to smoking status.

In conclusion, our findings suggest that *UGT1A6* and *UGT1A7* gene polymorphisms are associated with risk of CRC in Japanese populations. These data may contribute to altered *UGT1A6* and *UGT1A7* expression and altered carcinogen detoxification between individuals. In particular, *UGT1A6* polymorphisms are highly detrimental for CRC risk with respect to the formation of carcinogens not related to smoking. These variations need to be further verified as predictive biomarkers in a larger population.

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