RESEARCH ARTICLE

CYP2E1 rs2031920, *COMT* rs4680 Polymorphisms, Cigarette Smoking, Alcohol Use and Lung Cancer Risk in a Japanese Population

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Abstract

Background: Cytochrome P450 2E1 (*CYP2E1*) and catechol-O-methyltransferase (*COMT*) genes may contribute to susceptibility to lung cancer because of their critical involvement in mechanisms of carcinogenesis. Materials and Methods: We evaluated the role of *CYP2E1* rs2031920 and *COMT* rs4680 in a case-control study involving 462 lung cancer cases and 379 controls in Japanese. Logistic regression was used to assess adjusted odds ratios (OR) and 95% confidence intervals (CI). Multiplicative and additive interactions with cigarette smoking or alcohol use were also examined. Results: Neither *CYP2E1* rs2031920 nor *COMT* rs4680 was associated with lung cancer risk overall. However, smokers with the CC genotype of *CYP2E1* rs2031920 (OR = 3.57, 95% CI = 2.26 - 5.63) presented a higher risk of lung cancer than those with at least one T allele (OR = 2.91, 95% CI = 1.70 - 4.98) as compared to never-smokers with at least one T allele (reference). Subjects with excessive drinking and the CC genotype of *CYP2E1* rs2031920 had a significantly higher risk (OR = 2.22, 95% CI =1.39 - 3.56) than appropriate drinkers with at least one T allele. A similar tendency was observed between *COMT* rs4680 and either smoking or drinking habits. There were no multiplicative or additive interactions between the polymorphisms and either smoking or alcohol use. Conclusions: Our findings indicate that *CYP2E1* rs2031920 and *COMT* rs4680 are not major contributors to lung cancer risk in our Japanese population. Future studies on the genetics of lung cancer in Japanese and their environment interactions are required.

Keywords: Alcohol - *COMT* - *CYP2E1* - interaction - smoking

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Introduction

Lung cancer is a major cause of cancer-related death in developed countries, and the overall survival rate is still extremely poor. The World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) concluded in 1997 that alcohol use could possibly increase the risk of lung cancer (1997). Additionally, an increasing body of literature suggests that alcohol use may increase lung cancer risk after adjustment for cigarette smoking (Bandera et al., 2001). However, in 2007 the second expert report by the WCRF/AICR concluded that the evidence is so limited that no firm conclusion can be made (2007). Thus, the involvement of alcohol in the etiology of lung cancer is still under debate. A recent meta-analysis suggested a significant association between lung cancer risk and alcohol use (based on seven case-control studies combined, OR=1.33, 95% CI=1.07 - 1.66; based on 34 epidemiological studies combined, OR=1.15, 95% CI=1.02 - 1.30) (Bagnardi et al., 2015). Although tobacco smoking is an established risk factor for lung cancer, only approximately one in 10 smokers develops lung cancer in their lifetime (Doll and Peto, 1981), indicating interindividual variation in susceptibility to tobacco smoke. Lung cancer results from a complex interplay of genetic and environmental risk factors just like other common multifactorial diseases, such as cardiovascular disease, diabetes mellitus, and autoimmune disease.

Cytochrome P450 2E1 (CYP2E1) is an ethanol-inducible enzyme that metabolically activates various carcinogens, such as N-nitrosamines and benzene in tobacco smoke. Activation of N-nitrosamines may be associated with an increased risk of lung cancer. To date, a number of polymorphisms in the coding and non-coding regions of the *CYP2E1* gene have been reported. The most extensively evaluated *CYP2E1* polymorphisms are two point mutations in the 5'-flanking region [rs2031920 (-1019C>T) and rs3813867 (-1293G>C)], which are in complete linkage disequilibrium, and one point mutation in intron 6 [rs6413432 (7632T>A)]. In *CYP2E1*

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rs2031920, the minor allele (T allele) is associated with higher transcriptional activity and enzyme activity than the major allele (C allele) (Hayashi et al., 1991; Watanabe et al., 1994). Thus it is biologically plausible that this polymorphism in *CYP2E1* may be a risk factor for lung cancer. It is hypothesized that the T allele, which leads to higher *CYP2E1* activity, may be associated with an increased risk of lung cancer. Although many researchers have examined the association between these *CYP2E1* polymorphisms and lung cancer risk, the results remain inconclusive (Zhang et al., 2014; Ye et al., 2015; Zhai et al., 2015).

The oxidative stress level, which is an important mechanism in the development of cancer, may be modulated by catechol-O-methyltransferase (COMT). Several *COMT* polymorphisms have been reported, most notably rs4680 (Val158Met (472G>A) in exon 4) that converts a G (valine, high activity) to an A (methionine, low activity), resulting in a 3- to 4-fold lower *COMT* enzyme activity (Syvanen et al., 1997). It has been suggested that carriers of the A allele may exhibit lowered protective activity against oxidative stress, thereby promoting DNA damage and tumor progression (Zienolddiny et al., 2008). Furthermore, genetic polymorphisms involved in dopamine-related genes such as *COMT* have been associated with smoking and drinking behaviors (Uhl et al., 1998; Redden et al., 2005). Therefore, it is plausible that *COMT* rs4680 may be linked to lung cancer risk. Several studies examined the relationship between *COMT* rs4680 and lung cancer risk, with contradictory findings (Zhang et al., 2013; Tan and Chen, 2014; Peng et al., 2015). A recent meta-analysis found that no significant association between *COMT* rs4680 (AA + AG vs. GG) and lung cancer risk, with the summary OR of 1.14 (95% CI=0.90 - 1.44) (Peng et al., 2015). However, with a stratified analysis by the genotyping method, there was a significant association between lung cancer risk and rs4680 (OR=1.30, 95% CI=1.04 - 1.62) (Peng et al., 2015).

Studying gene-environment interactions in relation to lung cancer risk will be valuable because positive findings would clearly implicate disease-causing exposures, clarify lung cancer etiology, and point to environmental modifications for disease prevention. Smokers (established high risk population)/drinkers (suspected high risk population) with a genotype linked to smoking and/or drinking habits may be more susceptible to lung cancer than expected from the independent effects of the two (smoking/drinking and genetic polymorphism) separate factors. As smoking and/or drinking may interact with the *CYP2E1* and/or *COMT* enzyme, we conducted a case-control study of lung cancer in a Japanese population with special reference to the interaction between *CYP2E1* rs2031920 and *COMT* rs4680 and either cigarette smoking or alcohol use.

Materials and Methods

Study subjects and data collection

Subjects with histologically confirmed primary lung cancer were recruited from 1996 to 2008 at the Kyushu University Hospital (Research Institute for Diseases of the Chest, Kyushu University). Histological types were categorized into four major types according to the International Classification of Diseases for Oncology (ICD-O), second edition (Percy et al., 1990.): adenocarcinoma (8140, 8211, 8230–8231, 8250–8260, 8323, 8480–8490, 8550–8560, 8570–8572), squamous cell carcinoma (8050–8076), small cell carcinoma (8040–8045) and large cell carcinoma (8012–8031, 8310). Three hundred and seventy-nine potential controls with no prior history of cancer were recruited on a voluntary basis at the Fukuoka Prefectural Government and Kyushu University during the same period. Based on their family names, all subjects were possibly unrelated ethnic Japanese. The patients and controls self-reported information on the details of their smoking habits, alcohol use, years of education, and environmental tobacco smoke exposure from spouses, all of which may be independent or contributing risk factors for lung cancer.

The study protocol was approved by our institutional review board, and all participants provided written informed consent.

Genetic analysis

Genomic DNA was extracted from blood samples. Genotyping was conducted with blinding to case/control status. The *CYP2E1* rs2031920 and *COMT* rs4680 polymorphisms were determined by the methods described by Ulusoy et al. (Ulusoy et al., 2007) and Hirata et al. (Hirata et al., 2008), respectively. For quality control, both assays were repeated on a random 5% of all samples and the replicates were 100% concordant.

Statistical analysis

Comparisons of means, proportions and medians were based on the unpaired t test, chi-square test and Wilcoxon rank-sum test, respectively. The distribution of the *CYP2E1* rs2031920 and *COMT* rs4680 genotypes in controls was compared with that expected from Hardy-Weinberg equilibrium (HWE) by the chi-square (Pearson) test. Unconditional logistic regression was used to compute the odds ratios (ORs) and their 95% confidence intervals (CIs), with adjustments for several covariates. Subjects were considered current smokers if they smoked or stopped smoking less than one year before either the date of diagnosis of lung cancer or the date of completion of the questionnaires (controls). Never-smokers were defined as those who had never smoked in their lifetime. Former smokers were those who had stopped smoking one or more years before either the date of diagnosis of lung cancer or the date of completion of the questionnaires (controls). Based on "Healthy Japan 21" (National Health Promotion in the 21st Century), heavy drinkers were defined as those who drank more than 60g of alcohol per day . As "Healthy Japan 21" has emphasized drinking an appropriate volume of alcohol (20g of alcohol per day), appropriate drinkers were defined as those who did not exceed 20g of alcohol intake per day. The appropriate volume of alcohol use may have a protective effect on life expectancy and morbidity (Holman et al., 1996). Unlike cigarette smoke, ingested alcohol is eliminated

from the body by various metabolic mechanisms and the alcohol elimination process begins almost immediately. Significant relationships between excessive drinking and lung cancer have been reported while appropriate drinking has not shown the same effects (Benedetti et al., 2009). In terms of alcohol consumption, the subjects were classified into the following three groups based on their intake for at least one year: those who drank more than 60g of alcohol per day (heavy drinkers), those who drank more than 20g of alcohol per day but not exceeding 60g per day (moderate drinkers) and those who drank less than 20g of alcohol per day (appropriate drinkers). Appropriate drinkers included infrequent and non-drinkers because the lung cancer risks were comparable among them (Kiyohara et al., 2010). Genotype impact was assessed by a score test for each genotype as follows: 0, homozygous for the major allele; 1, heterozygous; and 2, homozygous for the minor allele. The interaction between the genotypes and either smoking or alcohol use on the risk of lung cancer was statistically evaluated based on the likelihood ratio test, comparing the logistic models with and without (multiplicative scale) terms reflecting the product of the genotype and consumption status for interaction. In a logistic regression model, interaction is a departure from multiplicativity. Rothman has argued that interaction estimated as a departure from additivity better reflects biologic (additive) interaction (Rothman, 2002). Three measures for biologic interaction as departure from additivity, namely the relative excess risk due to interaction (RERI), attributable proportion (AP), and synergy index (SI), were calculated by the method described by Andersson et al. (Andersson et al., 2005). The RERI is the excess risk due to interaction relative to the risk without factor. AP refers to the attributable proportion of disease which is due to interaction in persons with both factors. SI is the excess risk from factor (to both factors) when there is interaction, relative to the excess risk from factor (to both factors) without interaction (Kalilani and Atashili, 2006). Additive interaction is absent if the RERI and AP are equal to zero and SI is equal to one.

All statistical analyses were performed using the computer program STATA Version 14.1 (STATA Corporation, College Station, TX). All P values were two-sided, with those less than 0.05 considered statistically significant. Because of the low power of the test for interaction, P values of less than 0.1 were used for statistical significance (Riegelman, 2004).

Results

The distributions of selected characteristics among subjects are summarized in Table 1. Our analysis included 462 lung cancer patients (242 with adenocarcinoma, 131 with squamous cell carcinoma, 69 with small cell carcinoma, and 20 with large cell carcinoma). As controls were not selected to match lung cancer patients on age and sex, there was a significant difference in age (P<0.001) and sex ratio (P<0.001) between lung cancer patients and controls. Similarly, there were significant differences between cases and controls in terms of years of education (P<0.001), smoking status (P<0.001), pack-years of smoking (P<0.001) and drinking status (P<0.001).

As shown in Table 2, the frequencies of CC (ancestral, Human SNP ancestral alleles are determined by comparison with primate DNA, so in general, they're based on chimpanzee sequence (NCBI)), CT, and TT genotypes of *CYP2E1* rs2031920 were 67.1 %, 29.7 %, and 3.25 % in cases and 63.1 %, 33.0 %, and 3.96 % in controls, respectively. The frequencies of GG (ancestral), GA, and AA genotypes of *COMT* rs4680 were 46.3 %, 45.0 %, and 8.66 % in cases and 49.3 %, 44.1 %, and 6.60 % in controls, respectively. Both genotypic distributions were consistent with HWE among controls. *CYP2E1* rs2031920 and *COMT* rs4680 genotypic distributions were not different between cases and controls. After adjustment for age, sex, education, smoking status, and drinking status, the CT and TT genotypes combined of *CYP2E1* rs2031920 gave OR=0.86 (95% CI=0.62-1.19). As compared with the GA and GG genotypes combined, adjusted OR for the AA genotype of *COMT* rs4680 was 1.48 (95% CI=0.81 - 2.71). After adjustment for age, sex, education, and drinking, current smoking (OR=4.42,

Table 1. Selected Characteristics of Lung Cancer Cases and Controls

Characteristics	Cases (n = 462)	Controls (n = 379)	*P**
Age (year), median (IQR)	68 (62 – 73)	58 (48 – 65)	<0.001
Male, n (%)	287 (62.1)	283 (74.7)	<0.001
Smoking status, n (%)			<0.001
Current smoker	198 (42.9)	129 (34.0)	
Former smoker	111 (24.0)	41 (10.8)	
Never smoker	153 (33.1)	209 (55.2)	
Pack-years, median (IQR)	38 (0 – 58)	0 (0 – 34)	<0.001
Alcohol use, n (%)			
Heavy drinkers	154 (33.3)	204 (53.8)	<0.001
Moderate drinkers	130 (28.1)	84 (22.2)	
Appropriate drinkers**	178 (38.5)	91 (24.0)	
Education, median (IQR)	12 (12 – 16)	16 (12 – 16)	<0.001
Histology, n (%)			
Adenocarcinoma	242 (52.4)		
Squamous cell carcinoma	131 (28.4)		
Small cell carcinoma	69 (14.9)		
Large cell carcinoma	20 (4.3)		

IQR, interquartile range; *P for χ^2 test; ** Appropriate drinkers include infrequent drinkers and non-drinkers

Table 2. Relation the *CYP2E1* rs2031920, and *COMT* rs4680 Polymorphisms, and the Risk of Lung Cancer

	Number (%) of		Adjusted*	P
	Cases	Controls		
CYP2E1 rs2031920				
CC (ancestral**)	310 (67.1)	239 (63.1)	1.0 (reference)	
CT	137 (29.7)	125 (33.0)	0.82 (0.59 – 1.16)	0.262
TT	15 (3.25)	15 (3.96)	1.32 (0.54 – 3.23)	0.549
	P†= 0.46	P‡ = 0.79	P-trend = 0.586	
CT + TT vs. CC			0.86 (0.62 – 1.19)	0.368
Prevalence of C allele	0.819	0.796		
COMT rs4680			1.0 (reference)	
GG (ancestral**)	214 (46.3)	187 (49.3)		
GA	208 (45.0)	167 (44.1)	0.98 (0.71 – 1.36)	0.895
AA	40 (8.66)	25 (6.60)	1.46 (0.78 – 2.73)	0.234
	P†= 0.45	P‡ = 0.13	P-trend = 0.474	
AA vs. GA + GG			1.48 (0.81 – 2.71)	0.207
Prevalence of G allele	0.688	0.714		

Adjusted for age, sex, education, smoking status and drinking status; **Defined by National Center for Biotechnology Information SNP database; †P for χ^2 test; ‡P for Hardy-Weinberg equilibrium test among controls

Table 3. Interaction of the *CYP2E1* rs2031920 and *COMT* rs4680 Polymorphisms in Relation to Lung Cancer

CYP2E1 rs2031920 genotype	COMT rs4680 genotype					
	GG +GA		P	AA		P
	No. cases/control	Adjusted OR (95% CI)*		No. cases/control	Adjusted OR (95% CI)*	
CT + TT	134/130	1.00 (reference)		15/10	1.54 (0.61 – 3.86)	0.357
CC	288/224	1.18 (0.84 – 1.67)	0.342	22/15	1.73 (0.76 – 3.96)	0.192

Multiplicative interaction measure*=0.95 (95% CI = 0.28 – 3.23, P =0.938); Additive interaction measure*Relative excess risk due to interaction (RERI) =0.23 (95% CI = -1.51 – 1.96, P = 0.799; Attributable proportion due to interaction (AP) =0.13 (95% CI = -0.80 – 1.06, P= 0.784); Synergy index (SI) =1.44 (95% CI = 0.08 – 25.8, P = 0.803); OR, odds ratio; CI, confidence interval; * Adjusted for age, sex, education, smoking and drinking

Table 4. Interaction of the *CYP2E1* rs2031920 Polymorphism and Either Cigarette Smoking or Alcohol Drinking in Relation to Lung Cancer

Genotype	Smoking*					
	Never		P	Ever		P
	No. cases/control	Adjusted OR (95% CI)**		No. cases/control	Adjusted OR (95% CI)**	
CT + TT	61/81	1.00 (reference)		91/59	2.91 (1.70 – 4.98)	<0.001
CC	92/128	1.10 (0.68 – 1.77)	0.698	218/111	3.57 (2.26 – 5.63)	<0.001

Multiplicative interaction measure**=1.12 (95% CI = 0.58 – 2.16, P =0.746)
Additive interaction measure**
Relative excess risk due to interaction (RERI) =0.56 (95% CI = -0.92 – 2.03, P = 0.457)
Attributable proportion due to interaction (AP) =0.16 (95% CI = -0.24 – 0.56, P= 0.433)
Synergy index (SI) =1.28 (95% CI = 0.63 – 2.59, P = 0.494)

Genotype	Drinking†					
	Appropriate		P	Excessive		P
	No. cases/control	Adjusted OR (95% CI)‡		No. cases/control	Adjusted OR (95% CI)‡	
CT + TT	55/84	1.00 (reference)		97/56	2.42 (1.41 – 4.16)	0.001
CC	123/120	1.52 (0.94 – 2.46)	0.088	187/119	2.22 (1.39 – 3.56)	0.001

OR, odds ratio; CI, confidence interval; * Current and former smokers were combined (ever-smokers); ** Adjusted for age, sex, education and drinking.; † Subjects who drink more than 20g of alcohol per day (excessive drinkers) and subjects who drink less than 20g of alcohol per day (appropriate drinkers); ‡Adjusted for age, sex, education and smoking

95% CI=2.78 - 7.04) and former smoking (OR=2.73; 95% CI=1.92 - 3.89) were associated with an increased risk of lung cancer (data not shown). As compared with appropriate drinking, heavy drinking (OR=1.68, 95% CI=1.12 - 2.50) and moderate drinking (OR=1.82, 95% CI =1.25 - 2.67) were associated with an increased risk of lung cancer (data not shown). To achieve adequate statistical power, current and former smokers were combined (ever smokers). Similarly, heavy and moderate drinkers were combined (excessive drinkers). Ever smoking (OR=3.17; 95% CI=2.28 - 4.39) and excessive drinking (OR=1.76; 95% CI=1.27 - 2.43) were significantly associated with an increased risk of lung cancer (data not shown). Based on these results, we designated the genotype (CC genotype of *CYP2E1* rs2031920 and AA genotype of *COMT* rs4680) that is presumed to increase the risk of lung cancer as the "at-risk" genotype. Using the GA and AA genotypes combined as the reference, adjusted OR for the CC genotype of *CYP2E1* rs2031920 was 1.16 (95% CI=0.84 - 1.62). Subjects without the "at-risk" genotype were bundled in one group for subsequent analysis. Although we examined associations between the polymorphisms

Table 5. Interaction of the *COMT* rs4680 Polymorphism and Either Cigarette Smoking or Alcohol Drinking in Relation to Lung Cancer

Genotype	Smoking*					
	Never		P	Ever		P
	No. cases/control	Adjusted OR (95% CI)**		No. cases/control	Adjusted OR (95% CI)**	
GG + GA	139/194	1.00 (reference)		283/160	3.21 (2.29 – 4.52)	<0.001
AA	14/15	1.57 (0.67 – 3.71)	0.302	26/10	4.47 (1.89 – 10.5)	0.001
Multiplicative interaction measure**=0.88 (95% CI = 0.26 – 2.95, P =0.840)						
Additive interaction measure**						
Relative excess risk due to interaction (RERI) = 0.68 (95% CI = -3.26 – 4.61, P = 0.735)						
Attributable proportion due to interaction (AP) = 0.15 (95% CI = -0.62 – 0.92, P= 0.698)						
Synergy index (SI) = 1.24 (95% CI = 0.38 – 4.05, P = 0.718)						

Genotype	Drinking†					
	Appropriate		P	Excessive		P
	No. cases/control	Adjusted OR (95% CI) ‡		No. cases/control	Adjusted OR (95% CI)**	
GG + GA	162/190	1.00 (reference)		260/164	1.74 (1.24 – 2.43)	0.001
AA	16/14	1.33 (0.56 – 3.12)	0.517	24/11	2.85 (1.19 – 6.86)	0.019
Multiplicative interaction measure‡ = 1.24 (95% CI = 0.37 – 4.19, P = 0.730)						
Additive interaction measure‡						
Relative excess risk due to interaction (RERI) = 0.79 (95% CI = -1.87 – 3.45, P = 0.560)						
Attributable proportion due to interaction (AP) = 0.28 (95% CI = -0.46 – 1.01, P = 0.459)						
Synergy index (SI) = 1.75 (95% CI = 0.31– 9.72, P = 0.538)						

OR, odds ratio; CI, confidence interval; * Current and former smokers were combined (ever-smokers); ** Adjusted for age, sex, education and drinking; †Subjects who drink more than 20g of alcohol per day (excessive drinkers) and subjects who drink less than 20g of alcohol per day (appropriate drinkers); ‡Adjusted for age, sex, education and smoking

and histological types, associations were similar across histological types (data not shown).

We examined whether the *CYP2E1* rs2031920 genotypes had differential effects depending on the *COMT* rs4680 genotypes in relation to lung cancer risk (Table 3). Although the highest risk of the CC genotype of *CYP2E1* rs2031920 and the AA genotype of *COMT* rs4680 combined ("at-risk" genotypes combined) was observed (OR=1.73, 95% CI=0.76 - 3.96), the figure was not statistically significant. There were no additive or multiplicative interactions between *CYP2E1* rs2031920 and *COMT* rs4680.

Table 4 shows the modifying effect of the *CYP2E1* rs2031920 genotypes on the association of either smoking or drinking with lung cancer risk. Generally, the reference category is the absence of exposure (risk factor). Ever smokers with the CC genotype of *CYP2E1* rs2031920 (OR=3.57, 95% CI=2.26 - 5.63) had a higher risk of lung cancer than those with at least one T allele (OR=2.91, 95% CI=1.70 - 4.98), relative to never-smokers with at least one T allele (reference). The multiplicative and additive (RERI, AP and SI) interactions between the *CYP2E1* rs2031920 genotypes and smoking were far from significant. Among excessive drinkers, those with the CC genotype (OR=2.22, 95% CI=1.39 - 3.56) presented a somewhat lower risk of lung cancer than those with at least one T allele (OR=2.42, 95% CI=1.41 - 4.16), relative to appropriate drinkers with at least one T allele (reference). Again, four interaction measures did not reach statistical significance.

Table 5 shows the modifying effect of the *COMT* rs4680 genotypes on the association of either smoking or drinking with lung cancer risk. As is the case in *CYP2E1* rs2031920, a similar tendency between the *COMT* rs4680 genotypes and either smoking or alcohol use was observed. A gene-environment interaction was not suggested, with the combination of the "at-risk" genotype (AA genotype) and either ever smoking (OR=4.47, 95% CI=1.89 - 10.5) or excessive drinking (OR=2.85, 95% CI=1.19 - 6.86) conferring significantly higher risk, compared with at least one G allele and either no history of smoking or appropriate drinking. All interaction measures (multiplicative interaction, RERI, AP, and SI) were far from statistically significant.

Discussion

We determined the main effect of *CYP2E1* rs2031920 and *COMT* rs4680, and the interaction between these polymorphisms and either smoking or alcohol use on lung cancer risk using 462 cases of lung cancer and 379 controls. The frequency of the C allele of *CYP2E1* rs2031920 was 79.6% in controls and the genotypic distribution was consistent with HWE (Table 2). According to the HapMap SNP database (NCBI), the C allele frequency is most common among Yorubas (a West African ethnic group, 100%) and Caucasians (93.8%), and least common among Han Chinese (70.9%), with Japanese (80.8 %) intermediate between these groups. The frequency of the C allele in our study was similar to that of the HapMap SNP database. Similarly, the frequency of the G allele of *COMT* rs4680 was 71.4% in controls and the genotypic distribution was consistent with HWE (Table 2). The frequency of the G allele of *COMT* rs4680 in our study was close to that seen in the HapMap SNP database (71.5%) (NCBI).

Carriers of the T allele might have an increased ability to activate endogenous or exogenous neurotoxins and may therefore have an increased risk of developing lung cancer. The minor T allele of *CYP2E1* rs2031920 showed a non-significant decreased risk of lung cancer in this study, however. A recent meta-analysis showed that there was a

significant association between *CYP2E1* rs2031920 (CT + TT vs. CC) and lung cancer risk, with the summary OR of 0.80 (95% CI=0.71 - 0.90) (Zhai et al., 2015). The mechanism for how the T allele of the polymorphism might reduce the risk of lung cancer was not clear. The T allele may have a decreased ability to activate carcinogenic compounds other than N-nitrosamines and benzene. No difference in lung cancer risk was observed when stratified by sex or histological type (data not shown).

As for *COMT* rs4680, the AA genotype was nonsignificantly associated with an increased risk of lung cancer in this study. It is consistent with the hypothesis that the A allele (low *COMT* activity) may be linked to decreased protective activity, thereby promoting DNA damage and tumor progression. Moreover, it has been shown that the *COMT* polymorphisms are associated with estrogen metabolism and possibly nicotine and alcohol dependence through the breaking down of catecholamines in the brain (Ball and Knuppen, 1980; Colilla et al., 2005). As *COMT* plays an important role in estrogen metabolism by converting catechol estrogens to stable conjugates, differential findings by sex can be expected. A meta-analysis based on only two studies (Cote et al., 2009; Lim et al., 2012) with non-significant results found a significant association between *COMT* rs4680 and lung cancer risk among women under the heterogeneous model (AG vs. GG, OR=1.190, 95% CI=1.001-1.422) (Tan and Chen, 2014). However, in the present study *COMT* rs4680 appeared to have no influence on the risk of lung cancer when stratified by sex or histological type (data not shown).

As both polymorphisms may be linked to smoking and drinking susceptibility and/or behavior, we simultaneously evaluated the potential interaction between these two genetic polymorphisms. There were no multiplicative or additive interactions between *CYP2E1* rs2031920 and *COMT* rs4680. The interaction between these two polymorphisms requires further study with more samples in different racial and ethnic populations. Findings from polymorphism-polymorphism interaction analyses must be interpreted with caution due to the reduced numbers of observations in the subgroups. Replication of these findings in different populations is very important before any causal inference can be drawn.

It is widely accepted that lung cancer development requires environmental factors acting on a genetically predisposed individual. We evaluated whether interactions existed between *CYP2E1* rs2031920 and *COMT* rs4680 and either smoking or alcohol use (Tables 4 and 5). The *CYP2E1* enzyme is involved in the metabolic activation of carcinogens found in cigarette smoke and the C allele of *CYP2E1* rs2031920 is linked to higher enzymatic activity (Watanabe et al., 1994; Hayashi et al., 1991). The C allele of *CYP2E1* rs2031920 is also associated with greater alcohol consumption (Hayashi et al., 1991; Iwahashi et al., 1994; Sun et al., 2002). Thus, the C allele may be an "at-risk" allele. Alcohol use and smoking are known to be highly correlated behaviors. Since a blunted dopaminergic neurotransmission in the reward system seems to play a critical role in the development of nicotine (smoking behavior (Munafo et al., 2011)) or alcohol dependence (higher dose of alcohol (Kauhanen et al., 2000)), it can be hypothesized that the A allele, which is related to lower *COMT* activity (Syvanen et al., 1997), of rs4680 may be associated with an increased risk of lung cancer. There were no multiplicative or additive interactions between the two polymorphisms and either smoking or alcohol use (Tables 4 and 5). The significantly high ORs were attributed largely to the effect of ever smoking (OR=3.17) or excessive drinking (OR=1.76). To the best of our knowledge, no lung cancer studies on additive interaction between the two polymorphisms and either smoking or drinking have been previously reported.

Our study design has several limitations. First, our study may have included a bias due to the self-reporting of smoking and drinking habits (misclassification bias). However, discrepancies between self-reported smoking habits and biochemical verification are minimal among the general population (Wong et al., 2012; van der Aalst and de Koning, 2016). Similarly, although the validity of self-reported alcohol intake has been debated and questioned in many studies, overall the validity of self-reports on alcohol consumption is relatively high (Alvik et al., 2005; Bountziouka et al., 2012; Sam et al., 2014). This indicates that self-reported measures are acceptable as well as cost-effective. Second, controls were younger than cases. However, after adjustment for age in logistic regression analysis the differences between the crude and adjusted ORs were small, indicating that age did not have a strong influence on the risk estimates of the polymorphisms. Third, the moderate sample size limited the statistical power of our study and large well-designed studies are warranted to confirm our findings, particularly the polymorphism-polymorphism and polymorphism-environment interactions.

In conclusion, this study suggests that *CYP2E1* rs2031920 and *COMT* rs4680 polymorphisms are not potential contributors to lung cancer risk in a Japanese population. We do not find evidence for interactions between smoking or alcohol use and the two polymorphisms affecting lung cancer. Future studies involving larger control and case populations and better exposure histories will undoubtedly lead to a more thorough understanding of the roles of *CYP2E1* and *COMT* in lung cancer development.

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