

Effects of *ADH1C*, *ALDH2*, and *CYP2A6* Polymorphisms on Individual Risk of Tobacco-Related Lung Cancer in Male Japanese Smokers^{*}

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Received June 25th, 2013; revised July 26th, 2013; accepted August 3rd, 2013

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ABSTRACT

Recent genome-wide association studies have identified several lung cancer susceptibility loci. We previously carried out a replication study in male Japanese smokers that focused on chromosome 5p15 (telomerase reverse transcriptase) and 3q28 (tumor protein p63) (Shimizu *et al.*, *Journal of Cancer Therapy*, Vol. 2, No. 5, 2011, pp. 690-696). The current study was performed to confirm the association of traditional susceptibility loci [*i.e.*, alcohol dehydrogenase 1C (*ADH1C*) and aldehyde dehydrogenase 2 (*ALDH2*)] in 1039 male Japanese smokers (573 lung cancer patients and 466 healthy control subjects) who were previously enrolled in a study to investigate the low odds ratio for lung cancer risk associated with functionally impaired and deletion polymorphisms in cytochrome P450 2A6 (*CYP2A6*). The minor allele frequency of rs671 in *ALDH2* (0.304) was significantly higher in lung cancer cases than in controls (0.226), with an odds ratio of 1.42 [95% confidence interval (CI) of 1.12 - 1.80, $p = 0.0033$]. No significant association of rs698 in *ADH1C* with lung cancer risk was found in this population of male Japanese smokers. For light smokers categorized according to the 50th percentile Brinkman index value among the control subjects (620 daily cigarettes \times years) and for the *CYP2A6**1 wild-type non-carrier sub-population, significantly high odds ratios of 1.98 and 1.68 (95% CI of 1.28 - 3.06, $p = 0.0022$, and 1.07 - 2.66, $p = 0.025$), respectively, were observed for rs671 in *ALDH2*. The present results support the association of *ALDH2* loci with lung cancers and suggest a specific effect of *ALDH2* loci resulting in a higher risk of lung cancer in light smokers. *CYP2A6* polymorphisms, including copy number polymorphisms, may lower the risk of heavy tobacco use-related lung cancer.

Keywords: Alcohol Dehydrogenase 1C; Aldehyde Dehydrogenase 2; Cytochrome P450 2A6; Lung Cancer; Tobacco

1. Introduction

Cigarette smoking and alcohol consumption affect susceptibility to etiologically complex diseases, including various tumors [1,2]. Polymorphisms of the alcohol-metabolizing enzymes alcohol dehydrogenase 1C (*ADH1C*) and aldehyde dehydrogenase 2 (*ALDH2*) influence the risks of various cancers such as those of the esophagus [3], breast [4], pancreas [5], stomach [6], mouth [7], lung [8], and liver [1]. The International Lung Cancer Consortium was established in 2004 and coordinates genotyping activities and ongoing genome-wide association studies

(GWAS) [9]. Recent association findings for smoking-related diseases implicate genetically derived individual differences [10-14]. Several reports from GWAS with respect to lung cancer risk in different ethnic populations have suggested a variety of susceptibility genes, such as telomerase reverse transcriptase (*TERT*, rs2736100) [15-17] and tumor protein p63 (*TP63*, rs4488809, rs9816619, or rs10937405) [17,18]. In one of our previous reports [19], we demonstrated that genetic polymorphisms in cytochrome P450 2A6 (*CYP2A6*) are determinants of smoking behavior and tobacco-related lung cancer risk, particularly for squamous cell and small cell carcinoma, which are known to be associated with cigarette smoking [13] in male Japanese heavy smokers. In addition, sig-

^{*}There are no competing financial interests in relation to this study.

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nificant associations of rs4488809 (*TP63*) and rs2736100 (*TERT*) with the risk of lung adenocarcinoma were identified [20]. Some of the single nucleotide polymorphisms (SNPs) found by GWAS have been investigated extensively; however, there have been few reports of the effects of SNPs in combination in a typical population.

We performed further study of the traditional candidate genes for lung cancer risk (*i.e.*, *ADH1C* and *ALDH2*) in a population of male Japanese smokers. Herein, we confirm the increased susceptibility to lung cancers associated with *ALDH2* SNPs, especially for light smokers. Also, *CYP2A6* polymorphisms, including copy number polymorphisms that were not fully studied in GWAS (except for one computer-based special analytical study [18]), were found to be a major influence reducing the risk of heavy tobacco use-related lung cancer.

2. Materials and Methods

2.1. Subjects

This study was approved by the ethics committees of Hokkaido University and Showa Pharmaceutical University. The sample population comprised 1039 unrelated male Japanese smokers (573 case and 466 control subjects) selected from the 1705 participants of our previous study [19,20]. The other 666 subjects could not be included in this study because of genomic DNA sample limitations; there were apparently no differences in demographic factors such as ages and smoking status between case and control groups after exclusion of these subjects. The patient group consisted of 573 men who had received a pathological diagnosis of lung cancer [squamous cell or small cell carcinoma (n = 285) or adenocarcinoma (n = 288)] with a mean (\pm SD) age of 63.4 \pm 9.3 years (range 29 - 86 years). The control group consisted of 466 male smokers with a mean age of 52.2 \pm 12.2 years (range 20 - 92 years) without a history of cancer. The age of the lung cancer patients was defined at the time of pathological diagnosis of lung cancer.

Smokers included current and ex-smokers and were defined as individuals who had ever smoked cigarettes with a minimum smoking history of 10 cigarettes per day for at least 1 year. Light and heavy smokers were categorized by the 50th percentile Brinkman index value (daily cigarettes \times years) among control subjects and was found to be less than 620 (n = 233) and more than or equal to 620 (n = 233), respectively. Pathological classification of lung cancers was determined by at least three pathologists according to the criteria described in the literature [19].

2.2. Genotyping

Genomic DNA was prepared from peripheral lymphocytes [19]. Genotyping of *ADH1C* Ile350Val (rs698) [21] and *ALDH2* Glu504Lys (rs671) [3] polymorphisms was carried out according to previously described methods. Genotyping of *CYP2A6* was carried out by the methods described previously [19,20] with respect to rs5031016 for *CYP2A6**7 and rs28399433 for *CYP2A6**9 and the whole-gene deletion polymorphism of *CYP2A6* (*CYP2A6**4). *CYP2A6* variants were major alleles in control.

2.3. Statistical Analyses

The associations between the genotype distributions and patient status were assessed by odds ratios and 95% confidence intervals (CIs) that were calculated by unconditional logistic regression adjusting for age and cigarette smoking (Brinkman index value), unless otherwise mentioned. A *p* value less than 0.05 was considered to be statistically significant. Statistical computations were carried out using the statistical software SAS, version 5.1 (SAS Institute, Inc., Cary, NC) or PLINK 1.07 [22].

3. Results

Table 1 shows the association results of polymorphisms in three genes analyzed in terms of lung cancer risk in a

Table 1. Association of polymorphisms of *ADH1C*, *ALDH2*, and *CYP2A6* with lung cancer risk.

SNP	Allele (1/2)	Case			Control			Minor allele frequency		Odds ratio (95% CI) ^a	<i>p</i> value ^a	
		11	12	22	11	12	22	Case	Control			
<i>ADH1C</i>												
rs698	A/G ^b	503	62	2	413	51	1	0.058	0.057	1.00 (0.65 - 1.52)	0.989	
<i>ALDH2</i>												
rs671	G/A ^b	272	252	48	275	170	20	0.304	0.226	1.42 (1.12 - 1.80)	0.0033	
<i>CYP2A6</i> ^c												
Reduced activity or null allele		*1/*4,*7,*9	151	278	144	90	219	157	0.506	0.428	0.77 (0.63 - 0.94)	0.011

^aAdjusted by age and Brinkman index (daily cigarettes \times years); ^bMajor allele in control was defined as allele 1; ^cPart of data from our previous reports by Fujieda *et al.* [19] and Shimizu *et al.* [20].

population of 1039 male Japanese smokers. The minor allele frequencies of *ADH1C* SNPs were not significantly different between case and control groups ($p \geq 0.05$). The SNP rs671 in *ALDH2* was significantly associated with lung cancer risk (odds ratio of 1.42, 95%CI of 1.12 - 1.80, $p = 0.0033$). The odds ratio for subjects carrying *CYP2A6* variants was calculated to be 0.77 (95%CI of 0.63 - 0.94, $p = 0.011$), suggesting a decreased risk of tobacco-related lung cancer in subjects with variants of *CYP2A6*, a finding previously seen in expected phenotype groups estimated from *CYP2A6* gene analyses [19,20].

Subgroup analysis according to histological cancer type classified the lung cancer cases into two groups: adenocarcinoma cases and squamous cell or small cell carcinoma cases (Table 2). High odds ratios of 1.40 and 1.41 (95%CI of 1.07 - 1.83, $p = 0.014$; 95%CI of 1.05 - 1.88, $p = 0.021$) were observed for the rs671 A allele in *ALDH2* in the adenocarcinoma subgroup and the squamous cell or small cell carcinoma subgroup, respectively. In contrast, a lower odds ratio of 0.72 (95% CI of 0.56 - 0.92, $p = 0.0099$) was obtained for *CYP2A6* variants in the squamous cell or small cell carcinoma subgroup than that of 0.79 (95% CI of 0.63 - 0.99, $p = 0.040$) in the adenocarcinoma group.

The effects of genetic polymorphisms were analyzed in terms of smoking status subgroups (Table 3). Light smokers and heavy smokers were categorized according to the 50th percentile Brinkman index value among the control subjects (620 daily cigarettes \times years). For light smokers, a significantly high odds ratio of 1.98 (95%CI of 1.28 - 3.06, $p = 0.0022$) was observed for rs671 in *ALDH2*, whereas no significant association was observed for heavy smokers (odds ratio of 1.24, $p = 0.130$). On the other hand, a significantly lower odds ratio of 0.74 (95%CI of 0.58 - 0.94, $p = 0.014$) was obtained for

CYP2A6 variants in heavy smokers.

Consequently, a further subgroup analysis was performed (Table 4) to understand the different contributions of polymorphisms of *ADH1C*, *ALDH2*, and *CYP2A6* to odds ratios in tobacco use-related lung cancer risk. In patients carrying no *CYP2A6**1 wild-type alleles, the highest odds ratio of 1.68 (95%CI of 1.07 - 2.66, $p = 0.025$) was observed for rs671 in *ALDH2*. The low but significant odds ratio of 1.32 (95%CI of 1.01 - 1.74, $p = 0.049$) was obtained in subjects harboring the *CYP2A6**1 wild-type allele.

4. Discussion

In the analysis of all subjects included in this study, rs671 in *ALDH2* was significantly associated with lung cancer risk ($p = 0.0033$), as shown in Table 1. A similar influence of *ALDH2* genetic polymorphism on the risk of lung cancer has been reported in Japanese [23] and Korean [24] populations. In the cancer type subgroup analysis shown in Table 2, the association of this SNP was detected in both the adenocarcinoma group and the squamous cell and small cell carcinoma group, with similar odds ratios of 1.40 ($p = 0.014$) and 1.41 ($p = 0.021$), respectively. These results suggest that *ALDH2* polymorphisms are associated with lung cancer risk, irrespective of the histological cancer type. Furthermore, in the subgroup analyses shown in Tables 3 and 4, rs671 in *ALDH2* was associated with lung cancer risk for light smokers (but not for heavy smokers) (Table 3) and for non-wild-type *CYP2A6* genotypes (associated with a low cigarette consumption phenotype) [19] (Table 4) with similar odds ratios of 1.98 ($p = 0.0022$) and 1.68 ($p = 0.025$), respectively.

A recent meta-analysis has suggested that the *ADH1C*

Table 2. Association of polymorphisms of *ADH1C*, *ALDH2*, and *CYP2A6* with lung cancer risk: subgroup analysis of histological type of lung cancer.

SNP	Allele	Adenocarcinoma (n = 288)				Squamous cell or small cell carcinoma (n = 285)				
		Minor allele frequency		Odds ratio (95%CI) ^a	<i>p</i> value ^a	Minor allele frequency		Odds ratio (95%CI) ^a	<i>p</i> value ^a	
		Case	Control			Case	Control			
<i>ADH1C</i>										
rs698	A/G	0.046	0.057	0.75 (0.44 - 1.28)	0.297	0.071	0.057	1.30 (0.79 - 2.14)	0.305	
<i>ALDH2</i>										
rs671	G/A	0.289	0.226	1.40 (1.07 - 1.83)	0.014	0.319	0.226	1.41 (1.05 - 1.88)	0.021	
<i>CYP2A6</i> ^b										
Reduced activity or null allele	*1/*4, *7, *9	0.484	0.428	0.79 (0.63 - 0.99)	0.040	0.528	0.428	0.72 (0.56 - 0.92)	0.0099	

Lung cancer patients were classified into two groups, the adenocarcinoma group and the squamous cell or small cell carcinoma group. ^aAdjusted by age and Brinkman index (daily cigarettes \times years); ^bPart of data from our previous reports by Fujieda *et al.* [19] and Shimizu *et al.* [20].

Table 3. Association of polymorphisms of *ADH1C*, *ALDH2*, and *CYP2A6* with lung cancer risk: subgroup analysis of smoking status.

SNP	Allele	Minor allele frequency		Odds ratio (95%CI) ^a	<i>p</i> value ^a
		Case	Control		
Light smokers		(n = 117)	(n = 232)		
<i>ADH1C</i>					
rs698	A/G	0.043	0.052	0.75 (0.31 - 1.82)	0.524
<i>ALDH2</i>					
rs671	G/A	0.303	0.207	1.98 (1.28 - 3.06)	0.0022
<i>CYP2A6</i> ^b					
Reduced activity or null allele	*1/*4, *7, *9	0.389	0.390	0.88 (0.62 - 1.26)	0.500
Heavy smokers		(n = 456)	(n = 234)		
<i>ADH1C</i>					
rs698	A/G	0.062	0.062	1.07 (0.65 - 1.75)	0.791
<i>ALDH2</i>					
rs671	G/A	0.304	0.230	1.24 (0.94 - 1.65)	0.130
<i>CYP2A6</i> ^b					
Reduced activity or null allele	*1/*4, *7, *9	0.536	0.466	0.74 (0.58 - 0.94)	0.014

Light smokers and heavy smokers were categorized according to the 50th percentile Brinkman index value (620 daily cigarettes × years) among control subjects. ^aAdjusted by age and Brinkman index; ^bPart of data from our previous reports by Fujieda *et al.* [19] and Shimizu *et al.* [20].

Table 4. Association of polymorphisms of *ADH1C*, *ALDH2*, and *CYP2A6* with lung cancer risk: subgroup analysis of *CYP2A6* genotypes.

SNP	Allele	Minor allele frequency		Odds ratio (95%CI) ^a	<i>p</i> value ^a
		Case	Control		
<i>CYP2A6</i> *1 non-carrier		(n = 144)	(n = 156)		
<i>ADH1C</i>					
rs698	A/G	0.057	0.067	0.60 (0.28 - 1.30)	0.198
<i>ALDH2</i>					
rs671	G/A	0.306	0.205	1.68 (1.07 - 2.66)	0.025
<i>CYP2A6</i> *1 carrier		(n = 428)	(n = 309)		
<i>ADH1C</i>					
rs698	A/G	0.059	0.052	1.30 (0.77 - 2.19)	0.326
<i>ALDH2</i>					
rs671	G/A	0.304	0.236	1.32 (1.01 - 1.74)	0.049

*CYP2A6**1 non-carriers and carriers were categorized according to the presence of *CYP2A6**1 wild-type allele. ^aAdjusted by age and Brinkman index; ^bPart of data from our previous reports by Fujieda *et al.* [19] and Shimizu *et al.* [20].

rs698 polymorphism may contribute to cancer risk among Africans and Asians [25]. In this study, however,

the association of this variant with lung cancer risk was not replicated (**Table 1**). No association of this SNP with

esophageal cancer risk in a Chinese population has also been reported [26]. The cause of these different findings is probably the extremely low frequency of this SNP in the Asian population, as has been reported in a European cohort [6].

Another meta-analysis (which included our previous study [19]) suggested that *CYP2A6**4 is associated with susceptibility to lung cancer in Asians [27]. The ethnic differences in *CYP2A6* allele frequencies between Caucasians and Japanese should be also considered: lower frequencies of *CYP2A6**9, *CYP2A6**4 (rare) and *CYP2A6**7 (never detected) are evident in Caucasians, whereas these alleles are relatively common in Asians (from about 15% to 20% for each allele) [19]. The whole-gene deletion of *CYP2A6* may decrease the risk of lung cancer in Asians [19,27]. In the analysis of all subjects in the current study, *CYP2A6* variants were significantly associated with a decrease in lung cancer risk (**Table 1**). As we previously reported [19,20], *CYP2A6* seems to be associated with smoking-dependent lung cancer risk (**Tables 3 and 4**). We recently reported the different effects of *TERT*, *TP63*, and *CYP2A6* polymorphisms [20] on associations of individual lung cancer risk in a population of male Japanese smokers who were previously studied for lung cancer risk [19].

Overall, the different roles of *ALDH2* and *CYP2A6* variants were highlighted in subgroups indicating smoking status in male Japanese smokers. Taken together with the candidate genes identified in GWAS, lung cancer risk in Asian smokers may be accounted for by individual smoking status and multiple gene variations such as those in *TERT*, *TP63*, and *CYP2A6*, as reported previously [20], and those in *ALDH2* and *CYP2A6* as demonstrated in this study. Individual lung cancer risk and the details of chemical metabolism or disposition will be difficult to elucidate [28], but the following mechanisms are most likely involved. Carcinogenic nicotine-derived N-nitrosamines are probably genotoxically activated by polymorphic *CYP2A6* [29], and reactive oxygen species in tobacco smoke may contribute to lung carcinogenesis. Detection of lipid peroxidation-induced DNA adducts have been reported in human autopsy tissues [30]. Severe systemic acetaldehydemia may cause multicentric field cancerization [3], because acetaldehyde has been also detected in mainstream cigarette smoke [31].

In conclusion, we clarified that the previously established association of *ALDH2* with lung cancer risk was replicated in the current case-control study of an unrelated population of male Japanese smokers for all participants and for all subgroup analyses, including a high odds ratio for the light-smoker subgroup. In addition, our findings suggest that genetic variation in *CYP2A6* may be a major influence on heavy tobacco use-related lung

cancer risk in male Japanese smokers.

5. Acknowledgements

This work was supported in part by the Ministry of Education, Culture, Sports, Science and Technology of Japan and by an SRF Grant for Biomedical Research in Japan. The authors thank Drs. Masaki Fujieda, Kazuma Kiyotani, Hirotooshi Dosaka-Akita, Yuichi Sawamura, Jun Yokota, Asaki Kase, Ren Sawaya, and Norie Murayama for their support.

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Abbreviations

ADH1C: alcohol dehydrogenase 1C;
ALDH2: aldehyde dehydrogenase 2;
CI: confidence interval;
CYP2A6: cytochrome P450 2A6;

GWAS: genome-wide association studies;
SNP: single nucleotide polymorphism;
TERT: telomerase reverse transcriptase;
TP63: tumor protein p63.