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Different Effects of *TERT*, *TP*63, and *CYP*2*A*6 Polymorphism on Individual Risk of Tobacco-Related Lung Cancer in Male Japanese Smokers

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ABSTRACT

Recent genome-wide association studies have identified lung cancer susceptibility loci, *such as chromosome* 5*p*15 (*telomerase reverse transcriptase*, *TERT and cleft lip and palate transmembrane protein* 1-*like*, *CLPTM*1*L*), 15*q*25 (*nicotinic cholinergic receptor α*, *CHRNA*3-*CHRNA*5), *and* 3*q*28 (*tumor protein p*63, *TP*63). *Replication study was performed to confirm the association of the recently-identified susceptible loci* (*i.e.*, *TERT-CLPTM*1*L*, *CHRNA*3-*CHRNA*5, *and TP*63) *in a total of* 1460 *male Japanese smokers* (885 *lung cancer cases and* 575 *healthy control subjects*), *which were previously studied for a low odds ratio of impaired or deletion polymorphism in cytochrome P*450 2*A*6 (*CYP*2*A*6) *for lung cancer risk*. *The minor allele frequency* (0.442) *of rs*2736100 *on* 5*p*15 (*TERT*) *was significantly higher in lung cancer cases than that* (0.395) *of controls*, *with an odds ratio of* 1.27 (95% *CI of* 1.07 - 1.50, p = 0.00504). *A series of subgroup analyses revealed the significant associations of rs*4488809 (*TP*63, *odds ratio of* 1.21, p = 0.0422) *and rs*2736100 (*TERT, odds ratio of* 1.47, $p = 6.40 \times 10^{-5}$) *with the risk of lung adenocarcinoma*. *No significant association of CHRNA*3-*CHRNA*5 *and CLPTM*1*L was found in this population*. *The present results support replication of the association of TERT and TP*63 *loci with lung adenocarcinomas and suggest subtype-specific effects of these loci on higher risk of lung cancer in smokers*. *The CYP*2*A*6 *including copy number polymorphism*, *uninvestigated in large-scale genome-wide association studies*, *may influence lower risk to heavy tobacco use-related lung cancer*.

***Keywords*:** *TERT*, *TP*63, *Cytochrome P*450 2*A*6, *Lung Cancer*, *Tobacco*

1. Introduction

Recent association findings for nicotine dependence, smoking behavior, and smoking-related diseases implicate genetically derived individual differences [1-5]. Among several reports from genome-wide association studies (GWAS), nicotinic cholinergic receptor *α* (*CHRNA*3-*CHRNA*5, rs1051730 or rs667282) has been identified as a lung cancer susceptible locus [6-8]. Similarly, recent GWAS with respect to lung cancer risk in different ethnic populations have suggested a variety of susceptible genes, such as telomerase reverse transcriptase (*TERT*, rs2736100) [7,9,10], cleft lip and palate transmembrane protein 1-like (*CLPTM*1*L*, rs401681) [6,11], HLA-B associated transcript-3 (*BAT*3, rs3117582) [6], and mutS homolog 5 (*MSH*5, rs3131379) [6]. Tumor protein p63 (*TP*63, rs4488809, rs9816619, or rs10937405) was newly identified as a susceptible gene for lung adenocarcinomas in the Japanese and Korean from GWAS [10]. In our previous report [12], we demonstrated that genetic polymorphisms in cytochrome P450 2A6 (*CYP*2*A*6) are determinants of smoking behavior and tobacco-related lung cancer risk, particularly squamous cell and small cell carcinoma, which are known to be associated with cigarette smoking [4], in heavy smokers of male Japanese. Some of the single nucleotide polymorphisms (SNPs) found by GWAS have been investigated extensively, however, there have been few reports as to newly identified SNPs, including *TP*63 [10].

In the current study, we performed a replication study of the recently-identified candidate genes (*i.e.*, *CHRNA*3-

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*CHRNA*5, *TERT-CLPTM*1*L*, and *TP*63) in a population of male Japanese smokers. Herein, we report the replication of *TP*63 and *TERT* SNPs in one of the subgroups (lung adenocarcinoma). The polymorphisms of *CYP*2*A*6, including copy number polymorphism (CNP), which has not been studied yet in the platform of GWAS, may mostly influence susceptibility to heavy tobacco use-related lung cancer risk.

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 1460 unrelated male Japanese smokers (885 case and 575 control subjects) out of the 1705 participants of our previous study [12]. The remaining 245 subjects could not participate in this study because of sample limitations; there was apparently no difference after removal of these missing samples. The patient group consisted of 885 men who had received a pathological diagnosis of lung cancer (squamous cell or small cell carcinoma, n = 409 or adenocarcinoma, n = 476) with a mean (± SD) age of 62.6 ± 9.3 years (in the range 22 - 86 years). The control group consisted of 575 male smokers with a mean age of 52.9 ± 11.3 years (range 20 - 92 years) without a history of cancer. The age of the lung cancer patients was defined at the time when pathological diagnosis of lung cancer was first confirmed. 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 × years) among control subjects and were defined as less than 690 (n = 286) and more than or equal to 690 (n = 289), respectively. Pathological classification of lung cancers was determined by more than three pathologists according to the criteria described in the literature [12].

2.2. Genotyping

Genomic DNA was prepared from peripheral lymphocytes [12]. SNPs were selected from previous GWAS reports [6,8-11]; rs4488809, rs9816619, rs10937405, and rs4600802 on 3q28; rs2736100, rs401681, rs4634969, rs402710, and rs31489 on 5p15; rs3117582 and rs3131379 on 6p21.33 (containing *BAT*3-*MSH*5); and rs1051730, rs667282, rs8034191, rs206534, rs16969968, rs12910984, rs12914385, and rs6495309 on 15q25. By consideration of linkage disequilibrium (LD), SNPs in high LD to another SNP ($r^2 > 0.8$ in HapMap—Japanese in Tokyo (JPT)) were excluded and the most significantly-associated SNPs in each locus were analyzed [13]. The SNPs on 6p21.33 were excluded because this SNP is not polymorphic in HapMap JPT [13]. A total of 7 SNPs were genotyped using commercially available TaqMan predesigned probes and primers (Applied Biosystems, Foster City, CA) on ABI PRISM 7500 (Applied Biosystems). Genotyping for the *CYP*2*A*6 was carried out by the methods described previously [12].

2.3. Statistical Analysis

The associations between the genotype distributions and the patients' 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 by using the statistical software SAS, version 5.1 (SAS Institute, Inc., Cary, NC) or PLINK 1.07 [14].

3. Results

Table 1 shows the association results of eight genetic polymorphisms analyzed in terms of lung cancer risk in a population of 1460 male Japanese smokers. The minor allele frequencies of three SNPs on 3q28 (*TP*63) were not significantly different between case and control groups ($p \geq 0.186$). The SNP rs2736100 on 5p15 (*TERT*), but not rs401681 (*CLPTM*1*L*), was significantly associated with lung cancer risk (odds ratio of 1.27, 95% CI of 1.07 - 1.50, $p = 0.00504$). In two SNPs on 15q25 (*CHARN*3-*CHRAN*5), there was no significant association. The odds ratio for subjects carrying *CYP*2*A*6 variants was calculated to be 0.73 (95% CI of 0.56 - 0.90, $p = 0.000230$), suggesting a decreased risk of tobacco-related lung cancer in subjects with variants of *CYP*2*A*6, which had been previously seen in expected phenotype groups estimated from *CYP*2*A*6 gene analyses [12].

The subgroup analyses according to histological types of cancer were investigated by classifying the lung cancer cases into two groups, adenocarcinoma cases and squamous cell or small cell carcinoma cases (**Table 2**). A significantly higher odds ratio of 1.47 was observed for rs2736100 G allele in *TERT* (95% CI of 1.22 - 1.77, $p = 6.40 \times 10^{-5}$) in the adenocarcinoma subgroup; however, no significant association was observed in the squamous cell or small cell carcinoma subgroup. The SNP rs4488809 in *TP*63 showed significant association in the adenocarcinoma cases (odds ratio of 1.21, 95% CI of 1.01 - 1.46, $p = 0.0422$), suggesting roles of *TP*63 and *TERT* as possibly susceptible genes for lung adenocarcinoma. In contrast, significantly lower odds ratios of 0.74 ($p = 0.00203$) and 0.67 ($p = 0.000386$) were obtained for *CYP*2*A*6 both

Table 1. Association of polymorphisms of *TP*63, *TERT-CLPTM1L*, *CHRNA*3-*CHRNA*5, and *CYP*2*A*6 with lung cancer risk.

SNP	Allele (1/2)[a]	Case			Control			Minor allele frequency		Odds ratio (95% CI)[b]	*P* value[b]
		11	12	22	11	12	22	Case	Control		
3q28 (*TP*63)											
rs4488809	T/C	210	438	219	135	288	151	0.495	0.486	1.12 (0.95 - 1.33)	0.186
rs9816619	T/C	166	429	277	106	283	185	0.436	0.431	1.10 (0.93 - 1.31)	0.255
rs10937405	T/C	92	364	415	52	246	275	0.315	0.305	0.94 (0.79 - 1.13)	0.537
5p15 (*TERT-CLPTM1L*)											
rs2736100	G/T	178	419	279	94	265	214	0.442	0.395	1.27 (1.07 - 1.50)	0.00504
rs401681	A/G	95	389	386	61	255	257	0.333	0.329	1.01 (0.85 - 1.21)	0.901
15q25 (*CHRNA*3-*CHRNA*5)											
rs1051730	A/G	2	55	819	1	25	546	0.034	0.024	1.35 (0.82 - 2.23)	0.241
rs667282	T/C	224	424	225	133	277	152	0.499	0.483	1.08 (0.92 - 1.27)	0.362
19q13 (*CYP*2*A*6)[c]											
reduced or null allele	*1/*4,*7,*9	237	428	220	103	280	192	0.510	0.423	0.73 (0.56 - 0.90)	0.000230

[a]Minor allele in control was defined as allele 1. [b]Adjusted by age and Brinkman index (daily cigarettes × years). [c]Part of data from our previous report by Fujieda *et al.* [12].

Table 2. Association of polymorphisms of *TP*63, *TERT-CLPTM1L*, *CHRNA*3-*CHRNA*5, and *CYP*2*A*6 with lung cancer risk: subgroup analysis of histological types of lung cancer.

SNP	Allele	Adenocarcinoma (n = 476)				Squamous cell or small cell carcinoma (n = 409)			
		Minor allele frequency		Odds ratio (95% CI)[a]	*P* value[a]	Minor allele frequency		Odds ratio (95% CI)[a]	*P* value[a]
		Case	Control			Case	Control		
3q28 (*TP*63)									
rs4488809	T/C	0.518	0.486	1.21 (1.01 - 1.46)	0.0422	0.468	0.486	1.00 (0.80 - 1.25)	0.987
rs9816619	T/C	0.454	0.431	1.17 (0.97 - 1.41)	0.107	0.415	0.431	1.02 (0.81 - 1.27)	0.887
rs10937405	T/C	0.289	0.305	0.84 (0.69 - 1.03)	0.102	0.344	0.305	1.11 (0.88 - 1.39)	0.387
5p15 (*TERT-CLPTM1L*)									
rs2736100	G/T	0.475	0.395	1.47 (1.22 - 1.77)	6.40×10^{-5}	0.405	0.395	0.99 (0.80 - 1.23)	0.938
rs401681	A/G	0.337	0.329	1.03 (0.84 - 1.25)	0.809	0.328	0.329	1.00 (0.79 - 1.26)	0.984
15q25 (*CHRNA*3-*CHRNA*5)									
rs1051730	A/G	0.031	0.024	1.21 (0.70 - 2.10)	0.487	0.037	0.024	1.56 (0.83 - 2.91)	0.165
rs667282	T/C	0.501	0.483	1.08 (0.90 - 1.30)	0.404	0.498	0.483	1.12 (0.91 - 1.39)	0.296
19q13 (*CYP*2*A*6)[b]									
reduced or null allele	*1/*4,*7,*9	0.482	0.423	0.74 (0.55 - 0.93)	0.00203	0.542	0.423	0.67 (0.45 - 0.89)	0.000386

Lung cancer patients were classified into two groups, the adenocarcinoma group and the squamous cell or small cell carcinoma group. [a]Adjusted by age and Brinkman index (daily cigarettes × years). [b]Part of data from our previous report by Fujieda *et al.* [12].

in the adenocarcinoma group and squamous cell and small cell carcinoma group, respectively.

Genetic polymorphisms in the subgroups of smoking status were analyzed (**Table 3**). In this subgroup analysis, light smokers and heavy smokers were categorized according to the 50th percentile Brinkman index value (690 daily cigarettes × years) among the control subjects. In the light smokers, a significant higher odds ratio of 1.71 was observed for rs2736100 in *TERT* (95% CI of 1.29 - 2.26, $p = 0.000193$), whereas no significant association was observed (odds ratio of 1.06, $p = 0.558$) in the heavy smokers. On the other hand, a significantly lower odds ratio of 0.64 (95% CI of 0.42 - 0.86, $p = 6.02 \times 10^{-5}$) was obtained for *CYP*2*A*6 in heavy smokers.

Table 3. Association of polymorphisms of *TP*63, *TERT-CLPTM*1*L*, *CHRNA*3-*CHRNA*5, and *CYP*2*A*6 with lung cancer risk: subgroup analysis of smoking status.

SNP	Allele	Minor allele frequency		Odds ratio (95% CI)[a]	*P* value[a]
		Case	Control		
Light smokers		(n = 220)	(n = 286)		
3q28 (*TP*63)					
rs4488809	T/C	0.525	0.489	1.20 (0.90 - 1.59)	0.213
rs9816619	T/C	0.447	0.435	1.09 (0.83 - 1.45)	0.529
rs10937405	T/C	0.308	0.298	0.98 (0.73 - 1.33)	0.917
5p15 (*TERT-CLPTM*1*L*)					
rs2736100	G/T	0.473	0.375	1.71 (1.29 - 2.26)	0.000193
rs401681	A/G	0.330	0.325	1.02 (0.76 - 1.37)	0.888
15q25 (*CHRNA*3-*CHRNA*5)					
rs1051730	A/G	0.041	0.026	1.46 (0.70 - 3.04)	0.309
rs667282	T/C	0.507	0.484	1.12 (0.85 - 1.46)	0.419
19q13 (*CYP*2*A*6)					
reduced or null allele	*1/*4,*7,*9	0.389	0.390	0.91 (0.64 - 1.17)	0.472
Heavy smokers		(n = 665)	(n = 289)		
3q28 (*TP*63)					
rs4488809	T/C	0.485	0.483	1.08 (0.88 - 1.34)	0.462
rs9816619	T/C	0.433	0.427	1.11 (0.89 - 1.37)	0.357
rs10937405	T/C	0.317	0.313	0.93 (0.74 - 1.16)	0.528
5p15 (*TERT-CLPTM*1*L*)					
rs2736100	G/T	0.432	0.415	1.06 (0.86 - 1.31)	0.558
rs401681	A/G	0.334	0.333	1.01 (0.81 - 1.27)	0.908
15q25 (*CHRNA*3-*CHRNA*5)					
rs1051730	A/G	0.031	0.021	1.27 (0.64 - 2.53)	0.488
rs667282	T/C	0.497	0.482	1.06 (0.86 - 1.31)	0.579
19q13 (*CYP*2*A*6)[b]					
reduced or null allele	*1/*4,*7,*9	0.550	0.455	0.64 (0.42 - 0.86)	6.02×10^{-5}

Light smokers and heavy smokers were categorized according to the 50th percentile Brinkman index value (690 daily cigarettes × years) among control subjects. [a]Adjusted by age and Brinkman index. [b]Part of data from our previous report by Fujieda *et al.* [12].

Consequently, together with the above-mentioned results, the further subgroup analysis was performed (**Table 4**) to understand different contributions of *TERT*, *TP*63, and *CYP*2*A*6 polymorphisms to odds ratios in tobacco use-related lung cancer risk. In patients with lung adenocarcinoma, the highest odds ratio of 1.75 (95% CI of 1.29 - 2.38, $p = 0.000345$) for rs2736100 in *TERT* was observed in light smokers. The lowest odds ratio of 0.60 (95% CI of 0.34 - 0.86, $p = 0.000148$) was obtained for *CYP*2*A*6 for squamous cell and small cell carcinoma in heavy smokers.

Table 4. Association of polymorphisms of *TP*63, *TERT-CLPTM1L*, *CHRNA*3-*CHRNA*5, and *CYP*2*A*6 with lung cancer risk: subgroup analysis of both histological types of lung cancer and smoking status.

SNP	Allele	Adenocarcinoma				Squamous cell or small cell carcinoma			
		Minor allele frequency		Odds ratio (95% CI)[a]	*P* value[a]	Minor allele frequency		Odds ratio (95% CI)[a]	*P* value[a]
		Case	Control			Case	Control		
Light smokers		(n = 160)	(n = 286)			(n = 60)	(n = 286)		
3q28 (*TP*63)									
rs4488809	T/C	0.525	0.489	1.23 (0.90 - 1.67)	0.194	0.525	0.489	1.07 (0.68 - 1.69)	0.766
rs9816619	T/C	0.447	0.435	1.11 (0.82 - 1.49)	0.516	0.450	0.435	1.03 (0.65 - 1.63)	0.901
rs10937405	T/C	0.292	0.298	0.90 (0.65 - 1.25)	0.534	0.350	0.298	1.33 (0.83 - 2.12)	0.234
5p15 (*TERT-CLPTM*1*L*)									
rs2736100	G/T	0.475	0.375	1.75 (1.29 - 2.38)	0.000345	0.467	0.375	1.67 (1.08 - 2.58)	0.0223
rs401681	A/G	0.339	0.325	1.11 (0.80 - 1.52)	0.540	0.308	0.325	0.88 (0.55 - 1.40)	0.580
15q25 (*CHRNA*3-*CHRNA*5)									
rs1051730	A/G	0.035	0.026	1.32 (0.59 - 2.96)	0.504	0.059	0.026	1.89 (0.69 - 5.15)	0.214
rs667282	T/C	0.484	0.484	1.05 (0.78 - 1.41)	0.770	0.567	0.484	1.37 (0.88 - 2.13)	0.160
19q13 (*CYP*2*A*6)[b]									
reduced or null allele	*1/*4,*7,*9	0.384	0.390	0.89 (0.60 - 1.18)	0.438	0.400	0.390	0.97 (0.55 - 1.39)	0.876
Heavy smokers		(n = 316)	(n = 289)			(n = 349)	(n = 289)		
3q28 (*TP*63)									
rs4488809	T/C	0.515	0.483	1.20 (0.95 - 1.52)	0.126	0.458	0.483	0.99 (0.77 - 1.27)	0.921
rs9816619	T/C	0.458	0.427	1.20 (0.94 - 1.52)	0.140	0.409	0.427	1.02 (0.79 - 1.31)	0.898
rs10937405	T/C	0.288	0.313	0.81 (0.63 - 1.06)	0.122	0.343	0.313	1.05 (0.81 - 1.37)	0.693
5p15 (*TERT-CLPTM*1*L*)									
rs2736100	G/T	0.474	0.415	1.30 (1.03 - 1.66)	0.0291	0.394	0.415	0.84 (0.66 - 1.07)	0.163
rs401681	A/G	0.337	0.333	0.99 (0.77 - 1.27)	0.916	0.331	0.333	1.03 (0.79 - 1.35)	0.814
15q25 (*CHRNA*3-*CHRNA*5)									
rs1051730	A/G	0.029	0.021	1.13 (0.54 - 2.38)	0.751	0.033	0.021	1.43 (0.64 - 3.21)	0.381
rs667282	T/C	0.510	0.482	1.10 (0.87 - 1.40)	0.416	0.486	0.482	1.06 (0.83 - 1.35)	0.663
19q13 (*CYP*2*A*6)[b]									
reduced or null allele	*1/*4,*7,*9	0.532	0.455	0.66 (0.41 - 0.91)	0.000937	0.566	0.455	0.60 (0.34 - 0.86)	0.000148

[a]Adjusted by age and Brinkman index (daily cigarettes × years). [b]Part of data from our previous report by Fujieda *et al.* [12].

4. Discussion

Recent GWAS have identified lung cancer susceptibility genes such as *TERT* [7,9], *CLPTM*1*L*, *CHRNA*3-*CHRNA5,* and *TP*63 [10]. We performed a replication study of these associations in a population of male Japanese smokers (a total of 1,460 subjects), which were previously studied for lung cancer risk [12]. Miki *et al*. [10] have identified that *TP*63 is a significant candidate gene determining individual risk of lung adenocarcinoma in the Japanese and Korean populations [10]. In that report [10], rs4488809 in *TP*63 showed the strongest association with lung adenocarcinoma risk. In this context, the present study replicated this association of rs4488809 with p = 0.0422 in *TP*63 in lung adenocarcinoma cases (**Table 2**). The SNP rs4488809 was associated with only adenocarcinoma risk, but not squamous cell and small cell carcinoma in the present study. Recent report for association results of *TP*63 in Caucasian populations showed significant association of SNPs in *TP*63 with squamous cell carcinoma susceptibility [15]. None of three SNPs tested in this study showed significant association for squamous cell carcinoma ($p \geq 0.765$, n = 278; data not shown), although the sample size was not large. Further analysis will be interested to clarify the cancer type-specific effects of *TP*63 polymorphism.

An important contribution of *TERT* on 5p15 variants to lung cancer has been suggested by multiple research groups [7,10,16]. The rs2736100 in *TERT* were significantly associated with lung cancer risk (p = 0.00504), as shown in **Table 1**. In subgroup analysis shown in **Table 4**, association of this SNP was detected in both adenocarcinoma group and squamous cell or small cell carcinoma group in light smokers, with similar odds ratios of 1.75 (p = 0.000345) and 1.67 (p = 0.0223), respectively. These results suggest that *TERT* polymorphisms would be associated with non-smoking-related lung cancer outside the context of histological types of cancer. Data reported by Wang *et al*. [16], demonstrating that *TERT*-*CLPTM*1*L* variants showed the strongest association for adenocarcinoma and weak but similar trend for squamous cell carcinoma in never-smokers, supported our preset results. On the other hand, *CHARN*3-*CHRAN*5 also located on 15q25 has been reported one of the most import factors identified in GWAS in Caucasian populations [7,9]; however, the association of these variants were not replicated in this study (**Table 1**). The cause of these differences was probably because of their extremely low frequency in the Asian population, as has been reported by other Japanese group [17]. Wu *et al.* [18] have reported that different SNPs on *CHARN*3-*CHRAN*5 locus, rs2036534C > T, rs667282C > T, rs12910984G > A, and rs6495309T > C, would be associated with increased lung cancer risk in Chinese populations. However, these associations were not replicated in this study ($p \geq 0.196$, data not shown). Contribution of SNPs on *CHARN*3-*CHRAN*5 to susceptibility of lung cancer may be concluded to be small in the Asian populations.

In an analysis of all the subjects tested, *CYP*2*A*6 variant was significantly associated with lung cancer risk, with the lowest p-value of 0.000230 among polymorphisms tested in this study (**Table 1**). As we previously reported [12], *CYP*2*A*6 was suggested to be associated with smoking-dependent lung cancer risk outside the context of histological types of cancer (**Table 4**). However, in recent reports on GWAS, *CYP*2*A*6 polymorphism was not identified as a risk factor of lung cancer. This is partly because the SNPs analyzed in this study (rs5031016 for *CYP*2*A*6*7 and rs28399433 for *CYP*2*A*6*9) were not included in the platforms of the previous GWAS. Most of GWAS did not detect CNP, although whole-gene deletion type polymorphism of the *CYP*2*A*6 (*CYP*2*A*6*4) was observed at relatively high frequency in the Asian populations [12]. The ethnic difference in the *CYP*2*A*6 allele frequency between Caucasians and Japanese should be also considered in terms of lower frequencies of *CYP*2*A*6*9 and rare *CYP*2*A*6*4 and never *CYP*2*A*6*7 detected in Caucasians than those highly found in the Asians (~15% - 20%) [12]. From these ethnic differences in polymorphic frequencies, the contribution of *CYP*2*A*6 polymorphisms in Caucasians might be smaller. Further analysis is needed to evaluate the contribution of *CYP*2*A*6 polymorphisms in the studies of large sample size.

Overall, the different roles of *TERT*, *TP*63, and *CYP*2*A*6 variants were shown in subgroups indicating smoking status and lung cancer types in male Japanese smokers. Taken together the candidate genes identified in the recent GWAS, lung cancer risk in smokers may be accounted for by individual smoking status and multiple gene variations such as *TERT*, *TP*63, and *CYP*2*A*6 polymorphism. In conclusion, we firstly clarified that the association of *TP*63 was replicated in the lung adenocarcinoma group of an independent Japanese population. Genetic variation in *CYP*2*A*6 may mostly influence susceptibility to heavy tobacco use-related lung cancer risk in male Japanese smokers.

5. Competing Interests

There are non-financial competing interests in this study.

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Abbreviations

CI: confidence interval; *CLPTM*1*L*: cleft lip and palate transmembrane protein 1-like; CNP: copy number polymorphism; *CYP*2*A*6: cytochrome P450 2A6; GWAS: genome-wide association studies; *CHRNA*: nicotinic cholinergic receptor α; LD: linkage disequilibrium; SNP: single nucleotide polymorphism; *TERT*: telomerase reverse transcriptase; and *TP*63: tumor protein p63.

