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Limitation of cigarette consumption by *CYP2A6*4*, **7* and **9* polymorphisms

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ABSTRACT: The whole gene deletion *CYP2A6*4*, the defect of the main nicotine oxidase, contributes to limiting lifelong and daily cigarette consumption. However, the effects on smoking habits of *CYP2A6*7* and **9*, two major functional polymorphisms common in Asian populations, have not been reported.

The present study examined the relationship between polymorphisms **4*, **7* and **9* with the smoking habits of 200 Japanese smokers who visited the Keio University Hospital (Tokyo, Japan).

The allele frequencies of **1* (wild type), **4*, **7* and **9* were 52, 17, 11 and 20%, respectively. When the three polymorphisms were considered simultaneously, the percentages of homozygous wild type, heterozygote, and homozygous mutants and compound heterozygotes were 26.0, 52.5 and 21.5%, respectively. Homozygous mutants and compound heterozygotes (n=43) smoked fewer cigarettes daily than heterozygotes (n=105) and homozygous wild-type individuals (n=52). Smokers with **7*/7*, **9*/**9* or **7*/**9* had lower daily cigarette consumption than smokers with **1*/**1*.**

In conclusion, polymorphisms **4*, **7* and **9* of *CYP2A6* were detected in approximately three out of four Japanese smokers, and their daily cigarette consumption was genetically modulated by these functional polymorphisms.

KEYWORDS: *CYP2A6*, genetic polymorphism, nicotine, smoking habit

Cigarette smoking is one of the main public health concerns. Recent research has provided evidence that smoking habits are influenced not only by environmental but also genetic factors. A meta-analysis of studies examining the smoking habits in twins found that genetic factors contributed significantly to both the onset and the persistence of smoking [1]. These observations suggest that a greater understanding of the genetic factors involved would help to solve smoking-related health issues. Nicotine is the chemical mainly responsible for smoking dependence. Falling concentrations of nicotine in the serum and cerebrospinal fluid are believed to promote the desire for further smoking. The metabolism of nicotine varies markedly among individuals or races [2–4], and genetic polymorphisms of *CYP2A6*, which belongs to the cytochrome P450 family and represents the main catalyst of nicotine transformation to cotinine, may explain these differences in activities [4, 5]. Several functional genetic polymorphisms of *CYP2A6* have been described. *CYP2A6*4*, the whole gene deletion common in Asian populations, evidently reduces the enzymatic activity [6]. The current authors have recently reported that carriers of *CYP2A6*4* are more likely to be light rather than heavy, lifelong daily smokers [7]. The other functional polymorphisms, *CYP2A6*7* and **9*, are also relatively common in Asians [5, 8, 9]. The **7* allele, a missense single nucleotide polymorphism in exon 9 (I471T) that decreases the enzymatic metabolism of nicotine to cotinine, has been detected in 8–16% of Japanese [5, 8, 10, 11] and 4% of Koreans [10]. The **9* allele, a single nucleotide polymorphism in the TATA box of the promoter region, which decreases the transcriptional activity [9] and *in vivo* metabolic activity [12] of *CYP2A6*, was detected in 16–22% of Asians [5, 9, 12]. Recent information indicates that **4*, **7* and **9* are three major functional polymorphisms of *CYP2A6* common in Asians, whereas other potentially functional polymorphisms, including *CYP2A6*2*, **3* and **5*, are rare in Japanese and Koreans [5, 11, 13]. However, a relationship between **7* or **9* and smoking habits has not been described. A previous report suggested that ~30% of Japanese have an impaired activity of CYP2A6 due to *CYP2A6*4* and that their smoking habits are influenced by this defective allele [7]. Therefore, it was hypothesised that the enzymatic activity of CYP2A6 may be modulated by these three polymorphisms in a large proportion of the Japanese population.

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The current study examined the association between the frequencies of *CYP2A6*4*, **7* and **9* and the daily and lifelong cigarette consumption (CC) and smoking history in a population of Japanese smokers.

STUDY POPULATION AND METHODS

In total, 200 Japanese smokers who visited the Keio University Hospital (Tokyo, Japan) were enrolled. The inclusion criteria were: 1) aged ⩾50 yrs; 2) lifelong CC ⩾10 pack-yrs; and 3) absence of respiratory disease, except chronic obstructive pulmonary disease (COPD), which was present in 70% of the study participants. A physician directly questioned the participants regarding their smoking history, including average daily CC, duration of smoking and cessation of smoking. The study protocol was approved by the Ethics Committee of the Keio University Hospital. Informed consent was obtained from each participant.

Genotyping

Allele-specific PCR [14] and restriction fragment length polymorphism [6] methods were applied for the detection of **4*. An allele-specific PCR method [11] was modified for the detection of **7* [forward primer (wild type): 5′-CTC CCA GTC ACC TAA GGA CGT-3′; (mutant): 5′-CTC CCA GTC ACC TAA GGA CGC-3′; reverse primer: 5′-AAA ATG GGC ATG AAC GCC C-3′]. A previously described specific PCR method was utilised for allele **9* [12].

Statistical analysis

Values are presented as mean±SD. The Mann-Whitney U-test was used to compare daily CC between homozygous wild type and other genotypes, as shown in figure 1. The Bonferroni/Dunn test was performed to compare mean values among three groups as shown in figure 2 and table 1. An unpaired t-test was used to compare mean values between two groups as shown in table 1. A p-value <0.05 was considered significant for the Mann-Whitney U-test and unpaired t-test. A p-value <0.0167 was considered significant for the Bonferroni/Dunn test.

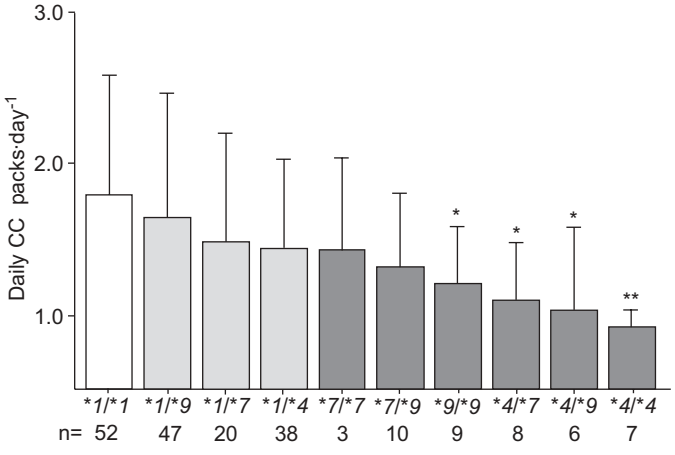

FIGURE 1. Daily cigarette consumption (CC) in smokers with genotypes *CYP2A6*1*, **4*, **7* and **9*. □: homozygous wild type; ▒: heterozygotes; ■: homozygous mutants or compound heterozygotes. *: $p<0.05$; **: $p<0.01$ *versus* **1/*1*.

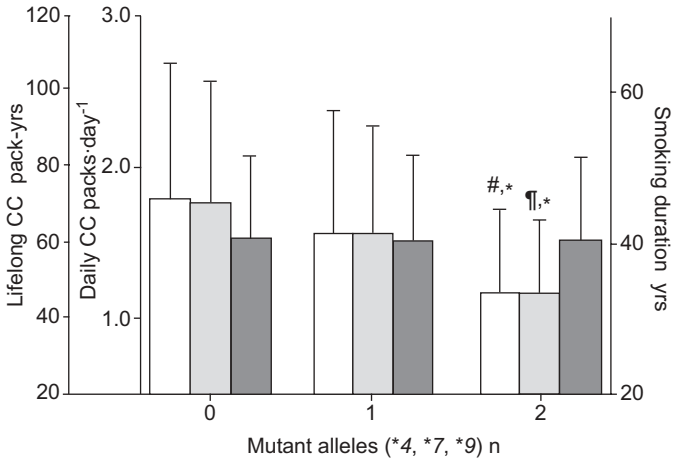

FIGURE 2. Comparison of smoking habits among smokers grouped according to the number of defective alleles (0: n=52; 1: n=105; 2: n=43); *CYP2A6*4*, **7* or **9*. CC: cigarette consumption. □: lifelong CC; ▒: daily CC; ■: smoking duration. #: $p=0.0001$; ¶: $p<0.0001$ *versus* the same variable in group 0. *: $p<0.01$ *versus* the same variable in group 1.

RESULTS

The frequencies of alleles **4*, **7* and **9* were 17, 11 and 20%, respectively. The homozygous wild type (**1/*1*) was detected in 52 out of the 200 subjects (26%), 105 were heterozygotes (52.5%), and 43 subjects were homozygous mutants and compound heterozygotes (21.5%). Heterozygotes for both **7* and **9* were expressed as **7/*9*, although haplotypes were not determined. No subject had more than or equal to three polymorphisms.

The number of subjects and daily CC corresponding to each genotype are shown in figure 1. Subjects with the **1/*1* genotype were the heaviest smokers followed, in order, by subjects with a single copy of **4*, **7* or **9*. The daily CC was lowest in the groups of homozygous mutants and compound heterozygotes, among whom those with **4/*4* were the lightest smokers. Among each genotype, the subjects with **4/*7*, **4/*9*, **9/*9* or **4/*4* smoked fewer cigarettes daily than those with **1/*1*, suggesting the inhibitory effects of **9* on daily CC besides **4*. The present results, that daily CC was fewer in the subjects with **4/*7* ($p<0.05$), but not in those with **1/*4* ($p=0.12$) compared with that in those with **1/*1*, implied the inhibitory effects of **7* on daily CC as well as **9*, as shown in figure 1.

The association between the number of variant alleles and smoking habits is summarised in figure 2. Smoking duration and age were similar among the groups (data not shown). When the relationship between the number of defective alleles (**4*, **7* and **9*) carried and smoking habits was evaluated, the lifelong and daily CC were significantly lower in subjects with homozygous mutants and compound heterozygotes than in those with a single or no polymorphism, as shown in figure 2. These results are consistent with synergistic inhibitory effects on lifelong and daily CC conferred by alleles **4*, **7* and **9*.

The isolated contribution of **7* and **9* to the smoking habits was further examined, independently of **4*. The results of this analysis are shown in table 1. Subjects with **7/*7*, **9/*9* or **7/*9*

TABLE 1 Association between the numbers of *7 or *9 allele and smoking habits

*7 or *9	n	Age yrs	Lifelong CC pack-yrs	Daily CC packs·day^{-1}	Duration yrs
Smokers without *4 allele					
0 (*1/*1)	52	65±9	71±36	1.76±0.80	41±11
1 (*1/*7 or *1/*9)	67	68±9	65±35	1.61±0.76	41±10
2 (*7/*7, *9/*9 or *7/*9)	22	68±9	54±21	1.28±0.47	43±10
p-value between 0 and 2		0.20	0.04	0.012#	0.38
Smokers with a *4 allele					
0 (*1/*4)	38	65±11	57±30	1.46±0.58	39±12
1 (*4/*7 or *4/*9)	14	66±9	37±16	1.11±0.66	37±12
p-value between 0 and 1		0.73	0.02*	0.06	0.51

Data are presented as mean±SD or n, unless otherwise indicated. CC: cigarette consumption. #: $p<0.0167$ *versus* group 0 (Bonferroni/Dunn test); *: $p<0.05$ *versus* group 0 (unpaired t-test).

had lower daily CC than the wild type (*1/*1). In addition, subjects with *4/*7 or *4/*9 had a significantly lower lifelong CC and tended to smoke fewer cigarettes daily than carriers of *1/*4.

DISCUSSION

The present study revealed that approximately three out of four Japanese were carriers of *CYP2A6*4*, *7 or *9 alleles and that their daily cigarette consumption was genetically limited. It can be legitimately hypothesised that, in subjects whose activity of CYP2A6 is decreased, the elevated serum concentrations of nicotine suppress their desire to smoke the next cigarette. Recent studies have shown a higher serum nicotine/cotinine ratio in subjects with *4/*4, *7/*7 or *9/*9 rather than those with *1/*1 and that the inhibitory effects of *CYP2A6*7* on nicotine metabolism were comparable with those by *9 [10, 12]. However, the influence of these polymorphisms on limiting cigarette consumption was not evaluated in these studies. Based on the present authors' simultaneous analysis of *4, *7 and *9 (fig. 1), *4 seemed to be the strongest factor limiting the daily CC. However, *7 and *9 also appeared to contribute to limiting the lifelong and daily CC, independently of *4, as shown in table 1. The current results indicate that besides *4, *7 and *9 should also be considered when analysing inter-individual differences in nicotine metabolism. In future smoking cessation programmes, adjustments in the dose or duration of nicotine replacement exposure should be considered, particularly in Asian smokers.

The genotype frequencies of *CYP2A6* are highly variable among races. The reported frequencies of alleles *4, *7 and *9 are 11, 4 and 22%, respectively, in Koreans and 7, 3 and 16% in Chinese [9–12]. This is in contrast to 1 and 0% for alleles *4 and *7, respectively, in Caucasians [11]. However, the frequency of *9 is higher in Swedish (5%) and Turkish (7%) populations [9], such that this polymorphism may also have significant effects on smoking habits in Caucasians. Further studies will be necessary to develop optimal smoking cessation protocols by adjusting the nicotine replacement among various ethnic groups, particularly in Asians.

SWAN *et al.* [15] have recently demonstrated the effects of *CYP2A6* polymorphisms, including *4, *7 and *9, on nicotine clearance by analysing twin pair correlations. They demonstrated the significant genetic influence (59%) on total clearance of nicotine; however, they also suggested that the contribution of *CYP2A6* genotype was relatively small (5.2%) within the entire influence by genetic factors. The current authors speculate that these results were partly attributable to the low frequencies of the polymorphisms of *CYP2A6* (*4: 2.5%; *7: 0.0%; and *9: 6.2%) in enrolled subjects that mainly consisted of Caucasians. In addition, the study population largely comprised nonsmokers and, therefore, smoking behaviours could not be evaluated, which may be important in clinical settings.

Study limitations

The current study population included a large percentage of patients with COPD [7], whose smoking habits may have been influenced by manifestations of chronic respiratory disorders or by smoking cessation recommendations. However, the frequencies of alleles *4, *7 and *9 in the present study population were similar to those reported previously [6, 8, 9]. It is believed that the current results reflect the importance of *CYP2A6*7* and *9, although the present population was not precisely representative of a general Japanese population and included relatively heavy smokers. It should be also noted that the number of subjects with *7 or *9 in the current study, *e.g.* *7/*7 (n=3) or *4/*9 (n=6), were not always large enough to conclude the independent contribution of these polymorphisms to limiting CC. Previous studies suggested that *7 and *9 were always located on distinct alleles [10, 12]; however, haplotype analyses were not performed to confirm this fact in the present study. One other limitation of the study was the absence of a strict correlation between number of cigarettes smoked and actual amount of nicotine intake. Objective measurements reflecting nicotine intake and metabolism, such as serum concentrations of nicotine, urinary cotinine or exhaled carbon monoxide, were not made. However, the authors believe that true differences in nicotine intake among *CYP2A6* genotypes may be underestimated by the number of cigarettes smoked, since poor metabolisers of nicotine may absorb less nicotine from a single cigarette than smokers with the wild-type genotype, as previously reported [16].

In conclusion, the daily cigarette consumption was genetically limited by alleles **4*, **7* and **9*, the three main polymorphisms of *CYP2A6*, in a population of Japanese smokers. These observations may be particularly important for physicians in Asia. Genotyping of these polymorphisms may be the first practical step for smoking cessation programmes to tailor the nicotine replacement according to the genotypes of this critical enzyme, on the basis of their frequencies and effects on smoking habits.

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