SMOKING

Association of CYP2A6 deletion polymorphism with smoking habit and development of pulmonary emphysema

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METHODS

Participants

Genomic DNA was isolated from peripheral blood obtained from Japanese subjects with a significant smoking history who visited the outpatient clinic of Keio University Hospital between 1998 and 2002 for the diagnosis or treatment of COPD (smoker group, n=203, 189 men). The inclusion criteria for the smoker group were lifelong CC ≥10 pack years and age ≥50 years. Two hundred and fifteen patients from about 1500 outpatients with respiratory diseases thought to be COPD met the inclusion criteria. Twelve subjects were excluded because of accompanying giant bullae (n=2), pulmonary fibrosis (n=3), diffuse bronchiectasis (n=1), bronchial asthma (n=4), and lung cancer compromising pulmonary function (n=2), leaving 203 subjects who were judged eligible for further analysis. The subjects in this group were either current smokers (n=92) or ex-smokers (n=111), defined as those who had stopped smoking for at least 6 months. All subjects were directly interviewed and the number of cigarettes consumed per day, the duration of smoking, and the time elapsed since quitting smoking were recorded as accurately as possible. Close attention was paid to the change in the daily number of cigarettes and smoking. CC was calculated from mean number of cigarettes smoked. CC was calculated from mean smoking (years) and used for further analysis.

Background: Nicotine is responsible for smoking dependence and is mainly metabolised by CYP2A6. Several types of genetic polymorphism of CYP2A6 have been reported, but their relation to smoking habit and chronic obstructive pulmonary disease (COPD) phenotypes has not been fully clarified.

Methods: 203 current or ex-smokers (lifelong cigarette consumption (CC) ≥10 pack years) with subclinical and established COPD phenotypes were clinically evaluated and pulmonary function tests and a chest CT scan were performed (smoker group). The non-smoker group consisted of 123 healthy volunteers. CYP2A6 genotypes were determined in both groups.

Results: The percentage of subjects with a CYP2A6del allele (genotype D) was lower in heavy smokers (20.5%, n=88, CC ≥60 pack years) than in light smokers (37.4%, n=115, CC 10–59 pack years, χ²=6.8, p=0.01) or non-smokers (36.1%, n=122, χ²=6.0, p=0.01), lower in ex-smokers (20.7%, n=111) than in current smokers (41.3%, n=92, χ²=10.1, p<0.01); and lower in smokers with a high LAA (low attenuation area) score on the chest CT scan (18.4%, n=76, LAA >8.0) than in those with a low LAA score (37.0%, n=127, LAA <8.0, χ²=7.8, p<0.01).

Conclusions: Subjects with the CYP2A6del allele tend not to be heavy habitual smokers but can be light habitual smokers. The CYP2A6del polymorphism may inhibit smokers from giving up smoking, but appears to function as a protective factor against the development of pulmonary emphysema independent of smoking habit.

METHODS

Participants

Genomic DNA was isolated from peripheral blood obtained from Japanese subjects with a significant smoking history who visited the outpatient clinic of Keio University Hospital between 1998 and 2002 for the diagnosis or treatment of COPD (smoker group, n=203, 189 men). The inclusion criteria for the smoker group were lifelong CC ≥10 pack years and age ≥50 years. Two hundred and fifteen patients from about 1500 outpatients with respiratory diseases thought to be COPD met the inclusion criteria. Twelve subjects were excluded because of accompanying giant bullae (n=2), pulmonary fibrosis (n=3), diffuse bronchiectasis (n=1), bronchial asthma (n=4), and lung cancer compromising pulmonary function (n=2), leaving 203 subjects who were judged eligible for further analysis. The subjects in this group were either current smokers (n=92) or ex-smokers (n=111), defined as those who had stopped smoking for at least 6 months. All subjects were directly interviewed and the number of cigarettes consumed per day, the duration of smoking, and the time elapsed since quitting smoking were recorded as accurately as possible. Close attention was paid to the change in the daily number of cigarettes and smoking. CC was calculated from mean number of cigarettes smoked. CC was calculated from mean smoking (years) and used for further analysis.

Cigarette smoking is a primary risk factor for chronic obstructive pulmonary disease (COPD) and lung cancer, and cessation of smoking to reduce the risk of these diseases is a major medical concern. Since smoking dependence is significantly associated with the serum concentration of nicotine, it is important to understand nicotine metabolism in order to solve smoking related problems. Racial differences in serum levels of nicotine metabolites have recently been reported, suggesting the presence of genetic variance in enzymes related to nicotine metabolism. Nicotine is mainly metabolised by CYP2A6, a member of cytochrome P450. At least three types of genetic polymorphism of CYP2A6 have been reported: CYP2A6*2 T to A transition in exon 3, CYP2A6*3 gene conversions in exons 3, 6, and 8 between CYP2A6 and CYP2A7, and CYP2A6del, a whole gene deletion. The enzymatic activity of CYP2A6 has been found to be impaired in subjects with CYP2A6*2 or CYP2A6del allele, and a relationship between the genotypes and smoking habit has recently been proposed. These studies suggested the possibility that the presence of the mutant alleles could reduce the consumption of cigarettes. However, the detailed relationship between CYP2A6 polymorphism and smoking habit—including lifelong cigarette consumption (CC), daily CC, and smoking duration—has not been reliably established because of problems with the specificity of the genotyping method and the low frequency of defective alleles reported in these studies.

COPD develops primarily in heavy smokers, suggesting that smoking behaviour regulated by the serum nicotine level may be associated with the development of COPD. In addition, it is difficult to exclude the possibility that substances metabolised or activated by CYP2A6, including nicotine and procarcinogens, may be related to the pathogenesis of COPD. However, no authentic studies have shown an association between CYP2A6 genotypes and COPD manifestations. A study was undertaken to clarify the effects of the CYP2A6 genotype on smoking habit in a Japanese population in which the frequency of the CYP2A6del allele is expected to be high, and to elucidate the relationship between the CYP2A6 genotypes and COPD phenotypes.
Genomic DNA was also obtained from Japanese non-smoking healthy volunteers with few respiratory symptoms screened by a written questionnaire and few abnormalities on their chest radiograph (non-smoker group, n=123, 109 men). The inclusion criteria for the non-smoker group were lifelong CC <100 cigarettes and age ≥50 years. The smoking history of the subjects in this group was self-reported. Of 132 volunteers who met the inclusion criteria, nine were excluded because of respiratory symptoms (n=5) and chest radiographic abnormalities (n=4). This group included subjects who had no chance to smoke and who had tried smoking but did not continue. No subjects who smoked a pipe or cigars were included in either the smoker or non-smoker group.

Informed consent was obtained from each subject and the study protocol was approved by the ethical committee of Keio University Hospital.

Assessment of pulmonary function and emphysematous changes on chest CT scan
Vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV1.0) were measured in all subjects in the smoker group using an electronic spirometer (MFR-8200; Nihon Koden, Tokyo, Japan). Carbon monoxide transfer factor (T(CO)) was estimated by a 10-second breath holding in 170 subjects in the smoker group (Chestac-55V; Chest, Tokyo, Japan). The lung volume at the time of TLCO measurement (V(a)) was simultaneously determined and used for calculating T(CO)/V(a), the carbon monoxide transfer coefficient (KCO). All pulmonary function tests were performed at our pulmonary function laboratory by three expert technicians in a manner consistent with the criteria recommended by the American Thoracic Society.10 The functional impairment in smokers was assessed on the basis of either %KCO or %FEV1.0. The reference values of these pulmonary function parameters were obtained from data reported for healthy Japanese subjects.10

A chest CT scan was performed in all subjects in the smoker group (Proseed, GE Yokogawa Medical Systems, Tokyo, Japan) under the following conditions: 120 kVp, 200 mA, 1 second scan time, and 5 mm collimation. Low attenuation area (LAA) was visually assessed by the method of Goddard et al.11 Briefly, the whole lung was divided into six zones (left and right zones in the upper, middle, and lower lung fields). Low attenuation areas in each image section were scored on a scale from 0 to 4 (0=normal tissue, 1=25%, 2=25–50%, 3=51–75%, and 4=76–100%). The total (0–24) was defined as the LAA score. Evaluation of the LAA score was made by three pulmonologists in a blinded manner, and the mean score was used as a quantitative indicator of emphysematous change. The standard deviation (SD) in LAA scores for each subject determined by three examiners averaged 1.0 point, and we did not rectify the scatter in LAA scores between the examiners.

Genotyping of CYP2A6
The CYP2A6(del) allele was detected by two different methods: (1) the two step polymerase chain reaction (PCR) method reported by Oscarson et al.8 and (2) the restriction fragment length polymorphism (RFLP) method described by Nunoya et al.9 (3) CYP2A6*2 and CYP2A6*3 were identified by the RFLP method reported by Chen et al.5 Oligonucleotide primer sequences and restriction enzymes used for these analyses were as follows: (1) 2A6ex7F; 5′-GGCCAAAC TGCCCTACATG-3′; 2A6ex8F; 5′-CATCTTCGTAAATGAC-3′; 2A6ex8F; 5′-CATTT CCTGGAATGAG-3′; 2A6R1: 5′-CAGCTTTAATTTGTGAGA CAT CAGACAA-3′; 2A6R2: 5′-AAAAATGCGATACGCC-3′; (2) 2A6-B6: 5′-CGATGGAAGGGCGACAAAGA GA-3′; 2A6-R5: 5′-CAGCGAGTGTACCTATGC-3′; 2A6-B7: 5′-CAGCGGAA GTTGTCCCTATGCTG-3′; (3) CYP2A6F03: 5′-CTTG ATCGATACA GGCGTGTTGA-3′; CYP2A6R06: 5′-CTGCTTGTGGT GTTTCTTTCC-3′. Values are presented as mean (SD). CC=lifelong cigarette consumption (pack years); duration=duration of smoking period (years); W=homozygous wild type; D=heterozygote or homozygous mutant for CYP2A6(del) allele.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>CYP2A6 genotype and smoking habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Genotype</td>
</tr>
<tr>
<td>All smokers</td>
<td>W</td>
</tr>
<tr>
<td></td>
<td>(n=203)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>W</td>
</tr>
<tr>
<td></td>
<td>(n=92)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>W</td>
</tr>
<tr>
<td></td>
<td>(n=111)</td>
</tr>
</tbody>
</table>

Values are mean (SD). CC=lifelong cigarette consumption (pack years); duration=duration of smoking period (years); W=homozygous wild type; D=heterozygote or homozygous mutant for CYP2A6(del) allele.

Data analysis
Values are presented as mean (SD). Genotype frequencies were compared between groups using the χ2 test. Mean values of age, CC, packs/day, smoking duration, LAA score, %KCO, and %FEV1.0 were compared between groups using the unpaired t test. Logistic regression analysis was performed to examine the effects of CYP2A6(del) genotype on CC, cessation of smoking, the severity of emphysematous changes, KCO, and airflow obstruction independent of other factors including age. A p value of <0.05 was considered significant.

RESULTS
CYP2A6*3 and CYP2A6(del) allele frequencies in non-smokers and smokers
Three CYP2A6 alleles (CYP2A6*1 (wild type allele), CYP2A6*3, and CYP2A6(del)) were identified in the Japanese subjects used in the study; no subject had CYP2A6*2. Based on these genetic results, smoking and non-smoking subjects were categorised into four subgroups, *1/*1 (n=220), *1/del (n=95), del/del (n=10), and *1/*3 (n=1). The allele frequencies of CYP2A6*1, CYP2A6*3, and CYP2A6(del) were 0.821, 0.002, and 0.176, respectively, which are qualitatively consistent with the values previously reported in Japanese or Chinese populations.8 10 12 Since only one non-smoking subject had the *1/*3 genotype, this subject was excluded from further analysis and *1/*1, *1/del, and del/del genotypes were designated as the wild type, heterozygote, and homozygous mutant, respectively; subjects with the *1/*1 genotype were defined as the W (wild type) group and those with the *1/del or del/del genotypes were defined as the D (deletion) group.

CYP2A6 genotype and smoking habit
Differences in smoking habit between subjects with the CYP2A6(del) genotypes are shown in Table 1. CC and number of cigarettes/day (packs/day) in the D group were significantly lower than in the W group in all smokers and in current smokers. Although the same trend was also observed for ex-smokers, the difference did not reach statistical significance. Duration of smoking did not differ between the genotypes in the groups.
for these parameters, the analysis was made by setting three
around the mean values obtained from all smokers studied.
we selected thresholds for these smoking related parameters
frequency between the subjects with different smoking habits,
table 2. To elucidate the difference in
day, duration of smoking and
relationship between lifelong CC, number of cigarettes/
different smoking habits
CYP2A6del
The relationship between lifelong CC, number of cigarettes/
CYP2A6del genotype frequency in subjects with
different smoking habits
The D group consisted of 53 subjects with the heterozygote
eight with the homozygous mutant in all smokers. When
the W group and the heterozygote and homozygous mutant
groups in all smokers were compared, CC was lower in the
homozygous mutant group (40 (10) pack years, p<0.01). The homozygous mutant group also
smoked fewer packs/day (0.96 (0.11)) than the W (1.61
pack years, p<0.05). The homozygous mutant group (40 (10) pack years) than in the W
group (65 (34) pack years, p<0.01, Kruskal-Wallis rank test
and Games-Howell test) or the heterozygote group (54 (31)
groups in all smokers were compared, CC was lower in the
W group and the heterozygote and homozygous mutant
subjects with different smoking habits
The relationship between lifelong CC, number of cigarettes/day,
duration of smoking and CYP2A6del genotypes is shown in
to elucidate the difference in CYP2A6del genotype frequency
between the subjects with different smoking habits,
we selected thresholds for these smoking related parameters
around the mean values obtained from all smokers studied.
Since there are no authentic criteria to define the thresholds
for these parameters, the analysis was made by setting three
cut off points for each parameter (CC: 40, 60, 80 pack years;
packs/day: 1.25, 1.5, 1.75; duration: 35, 40, 45 years). There
was no difference in the percentage of subjects with genotype D
(%) between non-smokers and all smokers. %D in heavy
smokers with CC >60 or >80 pack years (17.3%) was significantly
lower than in light smokers with CC 10–59 or 10–79
pack years (34.3%, p<0.05), respectively. However, the difference
was not statistically significant when the threshold for
CC was set at 40 pack years, although the tendency was similar
to that for a cut off point of 60 or 80 pack years. When the
smokers were divided into current and ex-smokers, %D was much lower in current smokers with a relatively heavy smoking
history of >80 pack years (16.7%) than in current smokers
with a light smoking history (CC 10–79 pack years, 50.0%,
p<0.01). In ex-smokers %D was lower in subjects with a relatively
heavy smoking history (CC >40 pack years, 16.0%) than in light smokers (CC 10–39 pack years, 33.3%, p<0.05).
When all smokers were included in the analysis, %D in the smoker group with a daily CC of >1.5 packs/day was lower
than in those with a lower daily CC (0.5–1.49 packs/day) and
than the non-smoker group. This tendency was seen more
clearly in current smokers than in ex-smokers. In current

### Table 2 Smoking habit and CYP2A6 genotype frequencies

<table>
<thead>
<tr>
<th></th>
<th>All smokers</th>
<th>Current smokers</th>
<th>Ex-smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Age %D</td>
<td>n Age %D</td>
<td>n Age %D</td>
<td>n Age %D</td>
</tr>
<tr>
<td>Total CC (pack years)</td>
<td>203 66 (9) 30.0</td>
<td>92 64 (8) 41.3</td>
<td>111 69 (10) 20.7††</td>
<td>122 56 (3) 36.1</td>
</tr>
<tr>
<td>10–59 packs/day</td>
<td>115 65 (10) 37.4</td>
<td>52 63 (9) 50.0</td>
<td>63 67 (11) 27.0</td>
<td></td>
</tr>
<tr>
<td>60+ packs/day</td>
<td>88 68 (9) 20.5**††</td>
<td>40 65 (8) 30.0</td>
<td>48 70 (8) 12.5††</td>
<td></td>
</tr>
<tr>
<td>0.5–1.49</td>
<td>99 68 (9) 38.4</td>
<td>44 65 (8) 56.8†</td>
<td>55 70 (10) 23.6</td>
<td></td>
</tr>
<tr>
<td>1.5+</td>
<td>104 65 (9) 22.1††</td>
<td>48 62 (8) 27.1**</td>
<td>56 67 (10) 17.9†</td>
<td></td>
</tr>
<tr>
<td>Duration (years) 10–39</td>
<td>80 59 (8) 33.8</td>
<td>39 57 (6) 38.5</td>
<td>41 61 (10) 29.3</td>
<td></td>
</tr>
<tr>
<td>40+</td>
<td>123 71 (10)** 27.6</td>
<td>53 69 (6)** 43.4</td>
<td>70 73 (7)** 15.7††</td>
<td></td>
</tr>
</tbody>
</table>

CC=lifelong cigarette consumption; duration=duration of smoking period; %D=percentage of subjects with genotype D.
†p<0.05, ††p<0.01, v non-smokers.
Non-smokers were younger than the subgroups of smokers except those in the 10–39 years duration group of current smokers.

### Table 3 Contribution of D genotype to smoking habit in logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>All smokers (n=203)</th>
<th>Current smokers (n=92)</th>
<th>Ex-smokers (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition: Factor</td>
<td>χ² p value OR 95% CI</td>
<td>χ² p value OR 95% CI</td>
<td>χ² p value OR 95% CI</td>
</tr>
<tr>
<td>CC &gt;60</td>
<td>Genotype D</td>
<td>6.21 0.01** 0.44 0.23 to 0.84</td>
<td>3.71 0.05 0.42 0.18 to 1.02</td>
</tr>
<tr>
<td>Age [10 years]</td>
<td>Genotype D</td>
<td>2.97 0.08 1.31 0.96 to 1.77</td>
<td>1.17 0.28 1.32 0.80 to 2.19</td>
</tr>
<tr>
<td>Packs/day &gt;1.5</td>
<td>Genotype D</td>
<td>7.16 &lt;0.01** 0.42 0.23 to 0.80</td>
<td>8.26 &lt;0.01** 0.27 0.11 to 0.66</td>
</tr>
<tr>
<td>Duration ≥40</td>
<td>Age [10 years]</td>
<td>5.45 0.02* 0.69 0.51 to 0.94</td>
<td>3.22 0.07 0.62 0.36 to 1.03</td>
</tr>
<tr>
<td>Age [10 years]</td>
<td>Genotype D</td>
<td>0.09 0.77 0.89 0.41 to 1.93</td>
<td>0.03 0.85 1.11 0.36 to 3.48</td>
</tr>
<tr>
<td>Duration ≥40</td>
<td>Age [10 years]</td>
<td>51.2 &lt;0.01** 6.64 4.04 to 11.61</td>
<td>23.2 &lt;0.01** 15.4 5.06 to 46.9</td>
</tr>
</tbody>
</table>

CC=lifelong cigarette consumption [pack years]; duration=duration of smoking period [years]; OR=odds ratio; CI=confidence interval.
†p<0.05, **p<0.01.
OR and 95% CI correspond to having genotype D or aging every 10 years.

### Table 4 Clinical profiles of current and ex-smokers

<table>
<thead>
<tr>
<th></th>
<th>Current smokers</th>
<th>Ex-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>n %D Age CC Packs/day Duration LAA %Kco† %FEV1.0</td>
<td>n %D Age CC Packs/day Duration LAA %Kco† %FEV1.0</td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>92 41.3 64 (8) 60 (27) 1.50 (0.64) 41 (10) 4.5 (4.5) 84 (29) 76 (24)</td>
<td></td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>111 20.7 69 (10) 62 (37) 1.56 (0.79) 40 (11) 7.3 (5.8) 76 (32) 66 (30)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.01## &lt;0.01** 0.62 0.39 &lt;0.01** 0.10 0.01*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD).
CC=lifelong cigarette consumption [pack years]; duration=duration of smoking period [years]; %D=percentages of subjects with genotype D; LAA=low attenuation area score on CT scan; %Kco=carbon monoxide transfer coefficient; FEV1.0=forced expiratory volume in 1 second.
†p<0.05, **p<0.01, by test.
smokers %D was significantly lower in subjects with a higher daily CC than in those with a lower daily CC for all three thresholds examined (1.25 packs/day: 32.7% v 54.1%, p<0.05; 1.75 packs/day: 27.3% v 49.2%, p<0.05). %D was much higher in current smokers with a light smoking history (0.5–1.49 packs/day) than in non-smokers.

There was no difference in %D with duration of smoking when all smokers were included, or when current smokers were analysed separately. However, %D tended to be lower in ex-smokers whose duration of smoking was relatively longer (35 years: 15.2% v 34.3%, p<0.05). %D in ex-smokers with a relatively heavy smoking history for both lifelong and daily CC and duration of smoking was lower than in non-smokers.

The number of subjects with the wild type, heterozygote, and homozygous mutant was 78, 42, and 2, respectively, in the non-smoker group, and 142, 33, and 8, respectively, in the smoker group. The genotype frequency distributions in these groups did not deviate from the Hardy-Weinberg equilibrium.

The age adjusted effect of %D on smoking habit determined by logistic regression analysis is shown in table 3. Genotype D was confirmed to function as an independent factor, reducing daily and lifelong CC but not the duration of smoking in all smokers or in current smokers. This tendency was not evident in ex-smokers. Aging was found to be an important factor in determining the duration of smoking in both current and ex-smokers.

### Table 5: Contribution of D genotype to cessation of smoking in logistic regression analysis

<table>
<thead>
<tr>
<th>Independent factor</th>
<th>χ²</th>
<th>p value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype D</td>
<td>9.41</td>
<td>&lt;0.01**</td>
<td>0.36</td>
<td>0.19 to 0.69</td>
</tr>
<tr>
<td>Age [10 years]</td>
<td>11.46</td>
<td>&lt;0.01**</td>
<td>1.76</td>
<td>1.27 to 2.45</td>
</tr>
<tr>
<td>CC [10 pack years]</td>
<td>0.48</td>
<td>0.49</td>
<td>0.97</td>
<td>0.88 to 1.06</td>
</tr>
</tbody>
</table>

### Table 6: Association of CYP2A6 genotype with LAA score, %KCO, and %FEV₁₀

<table>
<thead>
<tr>
<th>LAA score</th>
<th>n</th>
<th>Age</th>
<th>CC</th>
<th>%D</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8.0</td>
<td>127</td>
<td>65 (9)</td>
<td>59 (32)</td>
<td>37.0</td>
</tr>
<tr>
<td>≥8.0</td>
<td>76</td>
<td>69 (9)</td>
<td>66 (34)</td>
<td>18.4**</td>
</tr>
<tr>
<td>%KCO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td>115</td>
<td>65 (9)</td>
<td>59 (29)</td>
<td>34.8</td>
</tr>
<tr>
<td>&lt;65</td>
<td>55</td>
<td>71 (8)</td>
<td>70 (39)</td>
<td>18.2†</td>
</tr>
<tr>
<td>%FEV₁₀</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>146</td>
<td>65 (10)</td>
<td>59 (31)</td>
<td>32.2</td>
</tr>
<tr>
<td>&lt;50</td>
<td>57</td>
<td>71 (8)</td>
<td>69 (38)§</td>
<td>24.6</td>
</tr>
</tbody>
</table>

### Table 7: Contribution of D genotype to LAA score, %KCO, and FEV₁₀ independent of age and cigarette consumption in logistic regression analysis

<table>
<thead>
<tr>
<th>Definition</th>
<th>Independent factor</th>
<th>χ²</th>
<th>p value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAA ≥8 (76/203, 37%)</td>
<td>Genotype D</td>
<td>6.41</td>
<td>0.01*</td>
<td>0.40</td>
<td>0.20 to 0.81</td>
</tr>
<tr>
<td>CC (10 pack years)</td>
<td>Age [10 years]</td>
<td>7.48</td>
<td>0.01*</td>
<td>1.58</td>
<td>1.14 to 2.18</td>
</tr>
<tr>
<td>%KCO ≥65</td>
<td>Genotype D</td>
<td>4.57</td>
<td>0.03*</td>
<td>0.40</td>
<td>0.17 to 0.93</td>
</tr>
<tr>
<td>CC (10 pack years)</td>
<td>Age [10 years]</td>
<td>14.46</td>
<td>&lt;0.01**</td>
<td>2.19</td>
<td>1.46 to 3.29</td>
</tr>
<tr>
<td>%FEV₁₀ &lt;50</td>
<td>Genotype D</td>
<td>0.43</td>
<td>0.51</td>
<td>0.78</td>
<td>0.37 to 1.64</td>
</tr>
<tr>
<td>CC (10 pack years)</td>
<td>Age [10 years]</td>
<td>14.34</td>
<td>&lt;0.01**</td>
<td>2.04</td>
<td>1.41 to 2.95</td>
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**CYP2A6del genotype frequency and severity of emphysema, impairment of KCO, and airflow obstruction**

The relationship between the CYP2A6del genotype and severity of emphysematous changes examined by LAA scores on CT images, impairment of KCO, and airflow obstruction is shown in table 6. The morphological severity of emphysema was assessed by dividing the smokers into two groups with LAA scores of ≥8.0 or <8.0. Since an LAA score of 24 implies that most of the lung fields have emphysematous changes, an LAA score of 8 indicates that about one third of the total lung fields is occupied by areas of emphysema. %D in smokers with LAA scores of ≥8.0 was significantly lower than in those with LAA scores of <8.0. These two groups were matched for CC but not for age distribution.
The functional severity of emphysema was assessed from %Kco. The threshold for Kco was taken to be 65% of the reference value—that is, subjects with %Kco of <65% and those with %Kco of ≥65%, the difference being qualitatively similar to that obtained with LAA scores. In the analysis of %Kco, however, age distribution and CC were not matched between the groups. There was no significant difference in %Kco between the groups divided by %FEV1.0.

Logistic regression analysis was performed to examine whether the D genotype functioned as an independent factor to modify the development of emphysema and airflow obstruction apart from aging and the amount of cigarette smoked (table 7). The D genotype was found to reduce the risk of enhancement of the LAA score and impairment of %Kco, but it was not related to the extent of airflow obstruction. Unlike the D genotype, aging was found to be an important factor in promoting emphysematous changes (LAA score and %Kco) and deteriorating airflow obstruction (%FEV1).

**DISCUSSION**

**Contribution of CYP2A6del genotype to smoking habit**

Analysis of the results of lifelong CC and CYP2A6del genotype frequency showed that the presence of this deletion allele prevented subjects from becoming heavy smokers (CC ≥60 pack years, table 2). The association of CYP2A6del genotypes with lifelong CC, number of cigarettes per day, and duration of smoking suggested that the deletion allele limited the number of cigarettes/day rather than the duration of smoking, so that packs/day nearly paralleled lifelong CC, especially in current smokers (tables 1–3). It is reasonable to speculate that the number of cigarettes consumed per day is limited by the increased serum levels of nicotine in subjects with the deletion allele. These observations are consistent with previous findings that impaired function of CYP2A6 could reduce cigarette consumption. However, this is only true for heavy smokers with a lifelong CC of ≥60 pack years, and does not apply to relatively light smokers with a CC of 10–59 pack years. The frequency of the CYP2A6del genotype in these light smokers was comparable to that in non-smokers (table 2), suggesting that this deletion allele could not always protect subjects from becoming habitual smokers. It is interesting that the frequency of the D genotype was higher in current smokers with a CC of 10–59 pack years than in those with a high LAA score, although these groups were matched for lifelong CC (table 6). These observations were not applicable for %Kco of CC which is known to be closely correlated with the severity of pulmonary emphysema. Furthermore, the protective effects of the D genotype against emphysema were confirmed by logistic regression analysis adjusted for age and cigarette consumption (table 7). These findings suggest that the CYP2A6del mutation directly acts as an intrinsic factor against the development of emphysematous changes, independent of the function limiting the amount of CC. In the present analysis we have used lifelong CC (pack years) but not daily CC (packs/day) as an overall measure of exposure to smoking because, in terms of potential to promote emphysematous changes leading to impaired lung function, lifelong CC is better than packs/day as the latter does not take into account duration of smoking, although CC is significantly influenced by age. We have eliminated the effect of age contained implicitly in CC by applying logistic regression analysis in which the effect of age and that of CC can be assessed separately.

There are a number of other confounding factors related to the development of emphysema and airflow obstruction besides age and CC such as race, sex, air pollution, and social status. The contribution of the genotype D to the severity of emphysema and impaired Kco was not as much as that of age, indicating that further investigations are needed to clarify the relationship between this polymorphism and the development of pulmonary emphysema.

The protective effect of the genotype D against the development of emphysema was obscured when %FEV1 was used as an indicator to judge the severity of COPD (tables 6 and 7). The severity of airflow obstruction represented by %FEV1 is known to be determined by various factors including emphysematous changes, airway wall thickening, and airway hyperreactivity. The absence of a definite association between the CYP2A6 mutation and %FEV1 may not therefore be unreasonable. Our observations suggest that unspecified substances metabolised by CYP2A6 may be responsible for the difference in LAA score between smokers with different genotypes, and are related to (or modify) the inflammatory processes specific to emphysematous changes such as elastase-antielastase imbalance and interaction between oxidants and antioxidants. Miyamoto et al found that the CYP2A6del allele reduced the risk of lung cancer in Japanese
populations, which may be explained by inhibition of the activation of procarcinogens in subjects with the deletion allele. Smoking habit was not considered in that report, although CC is also important as a risk factor for lung cancer. Cantlay et al. suggested a possible association between a genetic polymorphism in CYP1A1, which is also known to activate procarcinogens, and the development of both lung cancer and pulmonary emphysema. However, the mechanisms causing the difference in emphysematous changes between the genotypes were not described.

Critique of methods
One criticism of studies of CC is that there is no reliable threshold for distinguishing between heavy and light smokers, which should be determined not only from lifelong and/or daily CC but also by the duration of smoking. We therefore evaluated the difference in CYP2A6del genotype frequency at different thresholds of lifelong CC, daily CC, and duration of smoking, and investigated whether the essential tendency would be changed qualitatively when the threshold was moved to other values. Since the frequency of the CYP2A6del genotype was consistently assessed at most of the different thresholds studied, we adopted thresholds for lifelong CC, daily CC, and duration of smoking of 60 pack years, 1.5 packs/day, and 40 years, respectively, and these were used in subsequent analyses (table 3).

Another criticism is that there are no authentic criteria for defining the severity of emphysema from the LAA score and Kco. We have previously shown that the 95% confidence limit of the relative area of LAA (%LAA) examined in non-smoking controls with no signs of pulmonary disease averaged 25%. Furthermore, Mishima et al. found that the %LAA never exceeded 30% in smokers with a small amount of airflow obstruction. These findings suggest that pathological emphysema can be judged to be present when 25% LAA is taken as the threshold, while the extent of morphological severity of emphysema can be approximately assessed when 30% is used as the threshold. We therefore assumed that an LAA threshold of 8 (corresponding to 33% LAA) would distinguish smokers with significant emphysema from those with less emphysema.

We assumed that Kco was significantly impaired when it was lower than 65% of the reference value. Based on the SD of Kco reported for healthy Japanese subjects,19 we found that the 95% confidence limit of this parameter corresponded to 68% of the reference value. We therefore took 65% of the reference value as the threshold for judging the impairment in Kco. Significant correlation was found between %Kco and the LAA score, expressed by the equation: LAA score = 16.05 – 0.120 x %Kco (r=0.70, p<0.0001). Based on this equation, the LAA score corresponding to 65% of the reference value of Kco was found to be 8.3, which is consistent with the threshold used for judging the morphological severity of emphysema on CT images.

The severity of airflow obstruction in smokers of CYP2A6del genotype was evaluated according to the criteria recommended by the GOLD guideline.23 Using a threshold value of 50% of predicted FEV1, smokers were divided into two groups (≥50% and <50% FEV1); %FEV1 was not closely correlated with LAA score (n=203, r=0.50, p<0.0001).

We conclude that (1) the CYP2A6del allele appears to restrict the amount of CC in heavy smokers but not in light smokers, (2) the CYP2A6del allele may inhibit smokers from quitting smoking, and (3) the protective role of the CYP2A6 deletion allele in pulmonary emphysema seems to be derived from impaired activity of this enzyme, independent of its effect on regulating CC. These findings suggest that determination of the genotype will be useful in efficiently withdrawing patients from nicotine dependence in smoking cessation protocols with nicotine containing materials, and will give a new insight into the pathogenesis of smoking induced pulmonary emphysema.

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