CT scanning-based phenotypes vary with ADRB2 polymorphisms in chronic obstructive pulmonary disease

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Summary

Background: Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease that is characterized by varying degrees of involvement of airway and lung parenchyma. Although cigarette smoke is the major risk factor for COPD, the principal determining factors of involvement of the airway or lung parenchyma have not been clearly defined. Genetic variability in COPD patients might influence the varying degrees of involvement of airway and parenchyma. We therefore studied whether airway and parenchyma involvement might be associated with the ADRB2 genotype, which has been reported to be associated with COPD susceptibility and the bronchodilator response.

Methods: One hundred and eleven COPD subjects, whose post-bronchodilator FEV1/FVC values were less than 0.7, and who had histories of smoking exceeding 10 pack-years, were prospectively recruited from pulmonology clinics of 11 hospitals in Seoul, Korea. The degrees of involvement of airway and parenchyma were evaluated by volumetric computed tomography (CT) scans. In-house software automatically calculated luminal areas, airway wall areas, percentages of wall areas in segmental bronchi, emphysema indices, and mean lung densities in the whole lung parenchyma. The ADRB2 genotypes at codon 16 were determined for all patients.

Results: Gly16 was associated with lumen diameter, luminal area, and percentage of wall area in patients with COPD (p = 0.02), whereas neither wall area nor wall thickness differed with ADRB2 genotype. Neither emphysema index nor mean lung density was associated with ADRB2 genotype.

Conclusion: Gly16 variant in ADRB2 gene was associated with airway wall phenotypes measured using CT scanning in COPD patients.

Introduction

Chronic obstructive pulmonary disease (COPD) is defined as airflow limitation measured by spirometry, and is a heterogeneous disease characterized by varying degrees of involvement of airway and lung parenchyma. Chronic inflammation and structural changes are the major pathologic features of COPD. Although some subjects show emphysema as the predominant feature, remodeling in airways is seen to various extents in COPD patients. Although cigarette smoking is the most important risk factor, the mechanisms of persistent inflammation, the causes of structural damage, and the factors that modify the involvement of airway or parenchyma, have not been clearly elucidated.

Computed tomography (CT) imaging is a useful tool in the evaluation of morphological changes in COPD patients and can also be helpful in evaluating heterogeneous COPD phenotypes. CT phenotypes including airway wall thickening, and emphysema severity, have been related to different clinical characteristics and varying treatment responsiveness in COPD, and such phenotypes may be useful research biomarkers. Genetic variability in COPD patients might explain persistent inflammation and the varying degrees of involvement of airway and parenchyma. To date, there have been several reports regarding the association of CT phenotypes and particular genotypes. The phenotypes associated with particular genes are mainly the severity of emphysema and emphysema distribution. Recent technology has made it possible to evaluate airway thickness. Airway phenotypes can therefore be evaluated to examine whether there might be an association between such phenotypes and particular genotypes. A previous report suggested that gender may influence airway lumen and airway thickness. However, little research on the genetic basis of airway thickness has been conducted.

ADRB2 is one of the most studied genes for COPD susceptibility and work on this gene has yielded contradictory results. Some data suggest that ADRB2 genotype may be associated with bronchodilator responsiveness to both short-term and long-term use of β2-agonist in asthma treatment. Recently, we developed volumetric CT analysis tools for evaluation of emphysema and airway characteristics in COPD patients. Quantified phenotypes of emphysema and airway using volumetric CT might be helpful in searching for more genetically homogeneous subgroups displaying particular genetic influences.

We therefore investigated whether there might be an association between emphysema and quantitative CT...
phenotypes of the airway, and ADRB2 genotypes on the other, which could be associated with the pathogenesis of airway disease in COPD patients.

Methods

Subjects and informed consent

The subjects for this study were extracted from a patient cohort entitled "The Korean Obstructive Lung Disease (KOLD) Cohort", which consists of patients with chronic obstructive pulmonary disease (COPD) or asthma. The KOLD Cohort was designed primarily to develop a systematic diagnostic model and an integrative prognostic factor of obstructive lung diseases. For the KOLD Cohort, the patients with chronic respiratory symptoms as well as airflow limitation or bronchial hyperresponsiveness have been and will be recruited in the pulmonary clinics of 11 hospitals in South Korea from June 2005 to October 2012. For this study, a kind of interim analysis of the KOLD Cohort study, a total of 145 patients were recruited from June 2005 to December 2006, and complete CT scanning data and other clinical information, such as blood analysis and data on pulmonary function were obtained.

In this study, the 118 COPD subjects extracted from the KOLD Cohort of 145 patients met all of the following criteria. They had post-bronchodilator FEV1/FVC values of <0.7 and had more than 10 pack-years of smoking history as well as no or minimal abnormalities on chest radiographs. Among 118 COPD subjects, only 111 patients were analyzed for this study after the exclusion of patients whose CT scans were not performed adequately.

Our Institutional Review Board approved the analyses of the clinical and imaging data. Individual informed written consent was obtained from all patients.

Measurements of airway and lung parenchyma using CT scans

Volumetric CT scans were performed on all patients using 16-channel, multidetector, CT machines of three manufacturers. These included the Somatom Sensation 16 (Siemens Medical Solutions, Forchheim, Germany), the GE Lightspeed Ultra (General Electric Healthcare, Milwaukee, WI), and the Philips Brilliance 16 (Philips Medical Systems, Best, Netherlands). Patients were scanned during suspended full inspiration and expiration in the supine position. CT parameters used in the different CT scanners were as follows: 16 × 0.75 mm collimation, 100 eff. mAs, 140 kVp (Somatom Sensation 16); 16 × 0.625 mm, 300 mAs, 140 kVp, Pitch 0.938, 0.5 s/rot (GE Lightspeed); and 16 × 0.75 mm, 133 mAs, 140 kVp, pitch 1, 0.75 s/rot (Philips 16). The acquired data were reconstructed using a standard algorithm with 0.625–0.8 mm thickness and 0.625–0.8 mm increment. The CT machines were calibrated every week with an AAPM standard phantom. The image data were stored in the Digital Imaging and Communications in Medicine (DICOM) format; this is the international standard for interconnecting medical imaging devices on standard networks.

Using in-house software, images of the whole lung were extracted automatically, and the attenuation coefficient of each pixel was measured and calculated. The cutoff level between normal lung density and a low-attenuation area was defined as −950 HU. From the CT data, the volume fraction of the lung below −950 HU (V950) and the mean lung density (MLD) were calculated automatically.

Measurements of the airway dimensions was performed near the origins of segmental bronchi (RB1, LB1 + 2) selected by a radiologist who was blind to clinical results, using in-house software. The software automatically detects the airway lumen, and the inner and outer boundaries of the airway wall, using a full-width-half-maximum (FWHM) method. The FWHM method is typical of objective, quantitative approaches to automatic airway measurement methods.19–21 The software was validated using polycr lively in-house software. The software automatically detects the airway lumen, and the inner and outer boundaries of the airway wall, using a full-width-half-maximum (FWHM) method. The FWHM method is typical of objective, quantitative approaches to automatic airway measurement methods.19–21 The software was validated using polycraryl tubes with variable inner diameters and wall thicknesses.22,23 In each segmental bronchus, the airway dimensions, including the wall area (WA), lumen area (LA), and wall area percentage (WA %), were measured. The wall area percentage was defined as WA % = WA/(WA + LA) × 100. The mean value of each segmental bronchus was used for statistical analysis. All of these analyses were performed by one of the authors (YL) who was blind to subjects’ background data.

Genotyping

ADRB2 genotyping was performed on all patients. Genomic DNA was prepared from blood for genotype analysis. Genotypes of codon 16 (rs1042713) were determined using polymerase chain reaction and restriction fragment length polymorphism (RFLP) techniques as previously described.16 Restriction digests were electrophoresed on 4% (w/v) agarose gels and visualized using ethidium bromide.

Statistics

Analysis of variance (ANOVA) was used to analyze the baseline characteristics to determine any differences among genotypes. Statistical analyses of CT parameters were used to determine any differences between genotypes. Hardy–Weinberg equilibrium was tested by the chi-square method. Phenotypes tested included inspiratory and expiratory emphysema indices, inspiratory and expiratory mean lung densities, lumen area, lumen diameter, wall thickness, wall area, and wall area percentage. Inspiratory emphysema indices and expiratory emphysema indices were log transformed to approximate normal distributions. The associations between CT parameters and ADRB2 genotype were examined under the additive model assuming that an addition of each risk allele increases as the same amount of risk. For example, the model assumes that having two Gly alleles doubles the risk in comparison to having one Gly allele. The analysis was adjusted for age, gender, smoking status, body mass index (BMI), and baseline FEV1. A random effect model was used where hospital-influenced characteristics with CT phenotypes were modeled as random errors24 using Proc Mixed procedure of PC-SAS for Windows version 9.2 (SAS Institute, Cary, NC).
Results

Characteristics of subjects

One hundred and eleven patients (108 men and 3 women) were analyzed. Their mean (±SD) age, FEV1, and smoking history were 65.4 ± 7.4 years (range 47–81 years), 1.48 ± 0.57 L, and 45.9 ± 25.0 pack-years, respectively.

Amongst the 111 COPD patients, 40 patients were of the Arg/Arg genotype, 46 patients were Arg/Gly, and 25 patients were Gly/Gly. The prevalence of Arg16 polymorphisms did not significantly deviate from the Hardy–Weinberg equilibrium. The ages, FEV1 values, and smoking histories were not significantly different amongst the three groups of patients (Table 1).

Airway and lung parenchymal measurements using CT scanning according to genotype

Lumen area was significantly smaller in patients with Gly16 (p = 0.0225). Lumen diameter was significantly shorter (p = 0.0208) and wall area percentage was significantly higher in the presence of the Gly allele (p = 0.0205) (Table 2). Airway wall thickness and wall area were not associated with ADRB2 genotypes.

Inspiratory emphysema index, inspiratory mean lung density, expiratory emphysema index, and expiratory mean lung density did not vary with ADRB2 codon 16 genotype (Table 3).

Discussion

In this study, lumen area, lumen diameter and wall area percentage of airway were associated with the ADRB2 genotype whereas emphysema phenotypes were not. Our finding is the first report suggesting that genetic variability may influence airway phenotypes in COPD patients.

Our finding that COPD patients with the Gly16 allele in the ADRB2 showed smaller lumen areas and larger wall area percentages could be explained in several ways.

Firstly, the Gly16 allele in the ADRB2 might increase susceptibility to COPD. A report in Chinese claimed that Gly16 increased susceptibility to COPD.15 Although the claim was not supported by other reports of inconsistent findings,13,25 as race could have influenced association results, our finding suggesting smaller lumen areas in Gly16 alleles is compatible with the work showing the risk of COPD in Chinese subjects with Gly16 alleles.

Second, the Gly16 allele in ADRB2 might be related to a greater degree of airway obstruction. A previous report demonstrated that Gly16 was associated with better response to the long-term use of a short acting β2-agonist,17,26 leading others to hypothesize that a greater obstruction at baseline leads to better response to bronchodilator treatment in patients with the Gly16 genotype.27 Our findings also support the hypothesis that more obstruction is seen in patients with the Gly16 allele.

Lastly, Gly16 allele in the ADRB2 might be associated with the chronic bronchitis subtype. Previous report revealed that COPD patients with chronic bronchitis symptoms showed higher wall area percentage and thickness to diameter than COPD patients without chronic bronchitis symptoms.28 In our study, ADRB2 genotype was associated with wall area percentage but not with wall thickness nor with wall area. This result suggests an association with chronic bronchitis. However, we cannot rule out the possibility that this genotype is related to lumen dilation but not to airway wall phenotype. This warrants further research.

Our finding that genetic variability in the ADRB2 may influence airway phenotypes in COPD patients could raise an important issue in clinical practice. Since airway phenotypes are important in COPD, factors influencing these phenotypes can be a target for drug development.

There are some limitations in this study. First, according to a recent report results may differ with the use of various types of CT scanners, and with the radiation doses used.29 To minimize the variation of the result, we modified the imaging protocols with similar reconstruction methods, resolution, and radiation dose. However, usage of different machines from different vendors with different imaging protocols may increase the measurement errors, resulting in weakening the correlation with the CT phenotypes.

Table 1 Baseline characteristics of 111 subjects with COPD by ADRB2 genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Arg/Arg</th>
<th>Arg/Gly</th>
<th>Gly/Gly</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (male/female)</td>
<td>40 (39/1)</td>
<td>46 (45/1)</td>
<td>25 (24/1)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.3 ± 6.6</td>
<td>65.0 ± 8.5</td>
<td>66.3 ± 6.5</td>
<td>0.76</td>
</tr>
<tr>
<td>Baseline FEV1 (L)</td>
<td>1.41 ± 0.58</td>
<td>1.53 ± 0.53</td>
<td>1.45 ± 0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Smoking (pack-years)</td>
<td>46.4 ± 25.5</td>
<td>50.0 ± 27.2</td>
<td>38.3 ± 17.8</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Data are presented as mean (±SD), unless otherwise noted. Analysis of variance (ANOVA) was used to calculate p values.

Table 2 Airway wall indices using CT scanning according to ADRB2 genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Arg/Arg</th>
<th>Arg/Gly</th>
<th>Gly/Gly</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area (mm²)</td>
<td>11.1 ± 2.9</td>
<td>10.7 ± 2.6</td>
<td>9.5 ± 3.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Lumen diameter (mm)</td>
<td>3.63 ± 0.48</td>
<td>3.58 ± 0.46</td>
<td>3.34 ± 0.55</td>
<td>0.02</td>
</tr>
<tr>
<td>Airway thickness (mm)</td>
<td>1.23 ± 0.12</td>
<td>1.21 ± 0.12</td>
<td>1.24 ± 0.10</td>
<td>0.59</td>
</tr>
<tr>
<td>Wall area (mm²)</td>
<td>17.8 ± 5.4</td>
<td>17.7 ± 5.2</td>
<td>16.4 ± 6.0</td>
<td>0.22</td>
</tr>
<tr>
<td>Wall area (%)</td>
<td>63.6 ± 4.2</td>
<td>63.7 ± 4.2</td>
<td>66.4 ± 5.4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Mean values (±SEM) for data are shown. Associations of gly16 polymorphism and CT parameters were estimated assuming additive genetic model (adjusting age, sex, smoking, FEV1, BMI, and random effects of recruited hospitals).
Secondly, we could not measure small airways, but rather only large airways, so this study may only reflect large airways. According to one study, however, large airways rather than small airways.30

Thirdly, we analyzed only codon 16 in our ADRB2 genotyping. It is suggested that ADRB216 is of functional importance and this genotype has been extensively studied in both clinical trials, and to define functional aspects of COPD.17,27 Although there may be unsuspected between-race differences in the importance of this codon, and although exploration of other haplotypes might be informative,31 codon 16 seems, at present, to be the most informative.

Lastly, most of our patients were male, and there may be gender differences in CT phenotypes of COPD subjects.12 Our results may therefore not apply to female patients.

In conclusion, ADRB2 gene polymorphism was associated with airway wall phenotypes measured using CT scanning in COPD patients. CT phenotype differences may be related to different subtypes that may be explained by genetic factors. Future research might include replication in additional cohorts and identification of functional relevance.

Conflict of interest

None declared.

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References


