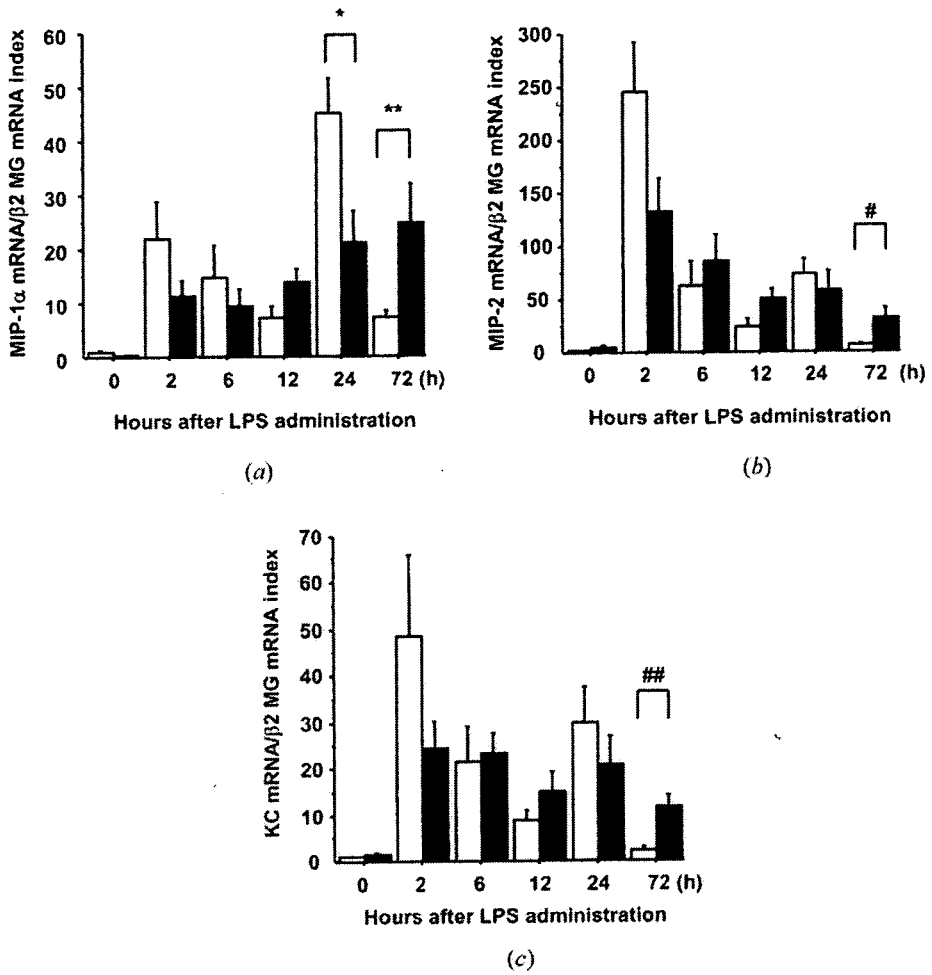

FIGURE 4 Whole lung expressions of MIP-1α, MIP-2, and KC in mice treated with intratracheal LPS. (*a*) MIP-1α expression was higher in 11-week-old mice than in 65-week-old mice at 24 hours (45.0 ± 6.6 vs. 20.9 ± 6.1, $^{*}P = 0.0146$), however, it was conversely higher in 65-week-old mice than in 11-week-old mice at 72 hours (24.7 ± 7.3 vs. 7.1 ± 1.3, $^{**}P = 0.0176$). (*b*) MIP-2 expression was higher in 65-week-old mice than in 11-week-old mice at 72 hours (30.6 ± 9.7 vs. 6.2 ± 2.3, $^{\#}P = 0.0155$). (*c*) KC expression was also higher in 65-week-old mice than in 11-week-old mice at 72 hours (11.2 ± 2.6 vs. 2.1 ± 0.7, $^{\#\#}P = 0.0015$). The number of animals is same as that of Fig. 2.

compared to their 11-week-old counterparts at 72 hours, but not in the early phases, after LPS administration.

Age-dependent differences in the levels of proinflammatory cytokines, including TNF-α and IL-1β, have remained controversial, although they have been investigated in various diseases in humans and animals. Some studies have shown higher plasma levels of proinflammatory cytokines, including TNF-α and/or IL-1β, in elderly individuals with endotoxiemia [11] as well as in aged animals [12–15] in various inflammatory diseases,

whereas lower levels of those cytokines have been, in contrast, reported by others [16, 17]. Antonini and colleagues demonstrated greater levels of TNF-α and IL-6 mRNA in the lungs of old rats at 7 days after pulmonary *Listeria monocytogenes* challenge [1], Saito and colleagues indicated enhanced induction of mRNA in lungs for IL-6 and IL-10 with aging at 6 hours after LPS intraperitonial challenge [18]. Recently, Gomez and colleagues showed that aged mice had an elevated pulmonary response characterized by higher neutrophilic inflammation, overexuberant production of neutrophilic chemoattractants, and elevated levels of IL-1β relative to young adult mice at 24 hours after intraperitoneal LPS administration [3]. The current study was consistent with their conclusion, and furthermore revealed an age-related kinetic diversity in proinflammatory cytokine mRNA expressions in the lungs, which might have been overlooked when examined at single time point.

Moreover, our finding that the expression of TNF-α and IL-1β exhibited high levels for over 24 hours, in addition to their immediate up-regulation following intratracheal LPS administration, may be in line with previous studies showing bimodal increases in serum TNF-α and/or IL-1β in sepsis models, although age-related differences have not been assessed [7, 19, 20]. One explanation for the bimodal-like increases in TNF-α and IL-1β mRNA expression in the lungs is that the early wave of cytokine mRNA expression is dependent upon cells resident in the injured lungs, such as epithelial cells and resident macrophages. The second phase may be produced by additional cell types, such as macrophages and neutrophils, recruited into the injured lungs from peripheral blood [12]. Although it is very important to determine the sources of cytokines in the lungs in each phase, in which the regulatory mechanism might differ by age, this issue needs to be investigated in future studies.

TNF-α and IL-1β reportedly play an important role in wound/injury repair in various mouse models, such as cutaneous injury [21] and acute colitis [22]. Furthermore, TNF-α and IL-1β stimulate phosphatidylcholine secretion in primary alveolar epithelial cells, thus suggesting a relationship with surfactant metabolism [23], whereas IL-1β promotes repair of injured alveolar epithelium [24, 25]. Considering that the up-regulation of TNF-α and IL-1β in later phases is followed by resolution of neutrophilic inflammation in the LPS-induced lung injury model, these cytokines may contribute to resolve inflammation. Taken together, these data suggest that dysregulation of TNF-α and IL-1β expression may be related to the delayed resolution of lung injury in 65-week-old mice.

Furthermore, we found that neutrophilic chemokines, such as MIP-1α, MIP-2, and KC, were significantly elevated in the lungs of 65-week-old mice when compared to their 11-week-old counterparts at 72 hours after LPS administration. From our results, the higher expression of MIP-1α,

MIP-2, and KC might partially explain the higher neutrophil accumulation in the lungs of 65-week-old mice at 72 hours.

There was the inconsistency of mortality in the current and previous study [4]. One likely explanation why no mice died in the present study is that, because 65-week-old mice in the current study was significantly larger (53.6 g on average) than the 65-week-old mice used in the previous study (41.6 g on average), the mice in the current study received less LPS dose per body weight (3.7 μg/g body weight on average) compared to the LPS dose used for the mice in the previous study (4.8 μg/g body weight on average).

A limitation of the current study is that the 65-week-old mice might not be old enough to be defined as "aged mice," given that the normal life span of male ICR mice is 77 to 83 weeks [26], and that 18- to 24-month-old mice have been usually used as aged mice in the other aging studies [3, 12, 14, 15, 18]. Further studies are required to determine whether the LPS-induced neutrophilic inflammation in the lungs is further enhanced in aged mice compared to 65-week-old mice, although we clearly demonstrated age-dependent differences between 11-week-old and 65-week-old mice in the current study.

In summary, we investigated TNF-α, IL-1β, MIP-1α, MIP-2, and KC mRNA expression in the lungs at multiple time points during the course of LPS-induced lung injury. We demonstrated that TNF-α and IL-1β expression at 72 hours after injury was significantly higher in 65-week-old mice, and was accompanied by higher levels of expression of neutrophilic chemokines, when compared with 11-week-old mice. Although the role of TNF-α and IL-1β in the later phases of LPS-induced lung injury remains to be elucidated, these results will assist in understanding the age-related imbalance of inflammatory responses in the lungs.

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Characterisation of phenotypes based on severity of emphysema in chronic obstructive pulmonary disease

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Airflow limitation in chronic obstructive pulmonary disease (COPD) is caused by a mixture of small airway disease and emphysema, the relative contributions of which may vary among patients. Phenotypes of COPD classified purely based on severity of emphysema are not well defined and may be different from the classic phenotypes of "pink puffers" and "blue bloaters".

Methods: To characterise clinical phenotypes based on severity of emphysema, 274 subjects with COPD were recruited, excluding those with physician-diagnosed bronchial asthma. For all subjects a detailed interview of disease history and symptoms, quality of life (QOL) measurement, blood sampling, pulmonary function tests before and after inhalation of salbutamol (0.4 mg) and high-resolution CT scanning were performed.

Results: Severity of emphysema visually evaluated varied widely even among subjects with the same stage of disease. No significant differences were noted among three groups of subjects classified by severity of emphysema in age, smoking history, chronic bronchitis symptoms, blood eosinophil count, serum IgE level or bronchodilator response. However, subjects with severe emphysema had significantly lower body mass index (BMI) and poorer QOL scores, evaluated using St George's Respiratory Questionnaire (SGRQ), than those with no/mild emphysema (mean (SD) BMI 21.2 (0.5) vs 23.5 (0.3) kg/m^2, respectively; SGRQ total score 40 (3) vs 28 (2), respectively; $p<0.001$ for both). These characteristics held true even if subjects with the same degree of airflow limitation were chosen.

Conclusions: The severity of emphysema varies widely even in patients with the same stage of COPD, and chronic bronchitis symptoms are equally distributed irrespective of emphysema severity. Patients with the phenotype in which emphysema predominates have lower BMI and poorer health-related QOL.

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide,[1] and morbidity and mortality has been increasing in Japan[2] as in many other Western countries.[3] COPD is described as a disease state characterised by airflow limitation that is not fully reversible according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and the American Thoracic Society/European Respiratory Society consensus guidelines.[1 4 5] This airflow limitation is progressive and caused by a mixture of abnormal inflammatory responses in small airways and parenchymal destruction of the lungs, the relative contributions of which vary from person to person.[6]

Historically, typical phenotypes of COPD used to be known as "pink puffers" and "blue bloaters",[7 8] or A, B and X types.[9] This is because COPD had been recognised as a disease that is a mixture of chronic bronchitis and emphysema, with predominantly bronchitis and predominantly emphysema as the two extreme phenotypes.[10] However, a number of studies over the last three decades have revealed small airways as the most important site causing airflow limitation in COPD,[11–14] and parenchymal destruction (emphysema) is certainly a contributing factor to a variable extent through the loss of elastic recoil pressure.[1 4 5] Hogg *et al* recently re-demonstrated how important inflammatory changes in small airways are as a determinant of progression and severity in COPD.[15] The narrowing of small airways caused by inflammation and scarring and the blocking of small airway lumens with mucous secretions are thought to represent the primary pathology of airflow limitation.[6]

In this study we therefore examined the clinical phenotype based purely on the severity of emphysema quantitatively evaluated using high-resolution CT (HRCT) scanning. If the severity of emphysema varies despite the same degree of airflow limitation, subjects with COPD can be compared where the relative contributions of small airway disease and emphysema vary. For instance, subjects displaying little evidence of emphysema despite severe airflow limitation could be considered as showing a phenotype with predominantly small airway disease but not necessarily that of chronic bronchitis. This is the first report from the Hokkaido COPD cohort study, which was primarily designed to evaluate the natural history and prognosis of COPD as classified by severity of emphysema.

METHODS

Subjects

A total of 307 subjects with physician-diagnosed COPD were recruited at Hokkaido University Hospital and nine affiliated hospitals from May 2003 to the end of April 2005. All study protocols were approved by the ethics committees of all hospitals and all subjects provided written informed consent. All were either current or former smokers with a smoking history of at least 10 pack-years. Subjects diagnosed by respiratory physicians as having bronchial asthma or

Abbreviations: BDR, bronchodilator response; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; HRCT, high-resolution computed tomography; LAA, low attenuation area; QOL, quality of life; SGRQ, St George's Respiratory Questionnaire; TLCO, carbon monoxide transfer factor; VA, alveolar volume

* See end of article for all contributors to the Hokkaido COPD Cohort Study

bronchiectasis at entry to the study were excluded. Subjects were also excluded if they had active tuberculosis, any history of lung cancer, any history of lung resection, and any history of cystic fibrosis, allergic alveolitis or pulmonary fibrosis. Those who would compromise 5-year follow-up or accurate assessment of pulmonary function or who were receiving long-term supplemental oxygen therapy for >12 h/day were also excluded. To avoid interference with bronchodilator reversibility testing, those who had been taking non-selective β blockers for treatment of hypertension were excluded. On the first visit, diagnoses were reconfirmed by pulmonologists and established based on the spirometric criteria of the GOLD guidelines.[1] While 33 subjects were excluded from this study because the post-bronchodilator ratio of forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) was ≥0.7, these subjects were encouraged to join the subsequent follow-up study.

Chronic bronchitis symptoms

Well trained clinical research coordinators elicited disease history, smoking history and other information about all treatments. Chronic cough and sputum expectoration were considered to be present when they occurred on most days for ≥3 months/year and for ≥2 consecutive years.[16 17] To avoid a bias by patient reports about the presence of chronic sputum symptoms, the amount of sputum should be >10 ml/day for the definition described above and this was confirmed by clinical research coordinators for all subjects.

Pulmonary function tests

Rolling seal spirometers (Chestac; Chest MI Inc, Tokyo) or Fudac (Fukuda Denshi Co Ltd, Tokyo) were used for the spirometric measurements and carbon monoxide transfer factor (TLCO) at all hospitals. Further details of the procedures are provided in the online supplement available at http://thorax.bmj.com/supplemental. Predicted values of spirometric measurements were derived from the guidelines for pulmonary function tests issued by the Japanese Respiratory Society.[18] TLCO was measured by the single breath method. Results were corrected by alveolar volume (VA) and haemoglobin concentration. TLCO/VA values were compared with the predicted normal values.[18]

The reversibility of airflow limitation was evaluated by measuring spirometry before and 30 min after inhalation of salbutamol (0.4 mg). The bronchodilator response (BDR) was expressed in three ways: (1) as an absolute change in FEV_1, (2) as a percentage change from baseline FEV_1, and (3) as a percentage change from predicted FEV_1. Reversibility of airflow limitation was considered to be significant if an increase in FEV_1 was both >200 ml and 12% above pre-bronchodilator FEV_1 according to GOLD guidelines.[1]

HRCT scanning

Chest HRCT scans were performed in the supine position with the breath held at full inspiration. CT scanners used in nine hospitals are described in the online supplement available at http://thorax.bmj.com/supplemental. Technical parameters were as follows: 1 mm collimation, 120–140 kV, 75–350 mA, 0.75–1 s scanning time and 1–2 mm thickness. HRCT images were selected at three levels including the aortic arch, carina and 1–2 cm above the highest hemidiaphragm. Image interpretations were performed under −600 to −900 Hounsfield units (HU) window levels and 800–1500 HU window widths based on the best condition for detecting emphysema at each hospital.

The severity of emphysema was visually assessed by three independent pulmonologists according to the modified Goddard scoring system.[19] Six images were analysed in three slices in the lungs and an average score of all images was considered as a representative value of the severity of emphysema in each person. Each image was classified as normal (score 0), ≤5% affected (score 0.5), ≤25% affected (score 1), ≤50% affected (score 2), ≤75% affected (score 3) and >75% affected (score 4), giving a minimum score of 0 and maximum of 4. When the three independent pulmonologists disagreed in their evaluation, only the score assessed by the majority was taken.

Three-dimensional CT analyses were performed only in Hokkaido University Hospital (n = 137) to confirm the accuracy and reliability of visual assessment. The method of computerised assessment of emphysema for the whole lung is described in detail in the online supplement available at http://thorax.bmj.com/supplemental.

St George's Respiratory Questionnaire (SGRQ)

The SGRQ was used to assess health-related QOL. This is a supervised self-administered measure designed specifically for use in respiratory disease and contains three domains: symptoms (relating to cough, sputum, wheeze and shortness of breath); activity (relating to physical activity limited by breathlessness); and impact (relating to control, panic, medication and expectations).[20] A total score was calculated from all three domains.

Blood samples

Blood was taken from all subjects for the measurement of α_1-antitrypsin, leucocyte count, eosinophil count and immunoglobulin (Ig)E levels.

Statistical analysis

Data are shown as mean (SE) values unless otherwise specified. Skewed data were either transformed to logarithmic data or expressed as median values with interquartile range (IQR). Univariate analysis used χ^2 tests for categorical variables and one-way analysis of variance for quantitative variables with Scheffe's test as a post-hoc test for multiple comparisons. Relationships between two variables of quantitative data were examined using Spearman tests. For BMI and SGRQ scores, the

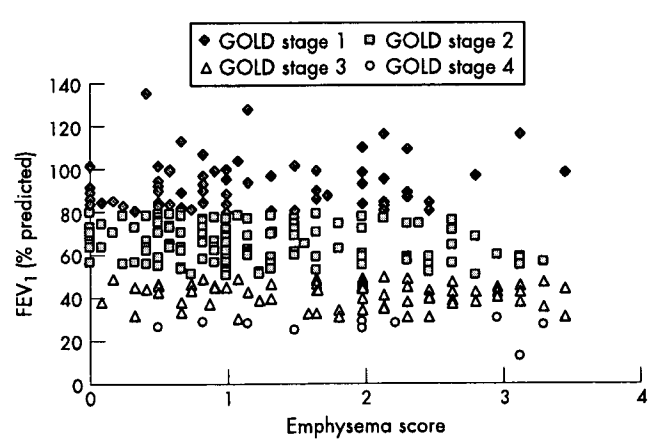

Figure 1 Relationship between emphysema score and forced expiratory volume in 1 s (FEV_1) % predicted in 274 subjects with chronic obstructive pulmonary disease (COPD). They were classified into four stages according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (stage 1, n = 64; stage 2, n = 127; stage 3, n = 71; and stage 4, n = 12). Severity of emphysema was highly variable despite the same stage of COPD, although the relationship between emphysema score and post-bronchodilator FEV_1 % predicted was significant overall ($r = -0.302$, $p<0.001$).

Table 1 Clinical characteristics of phenotypes based on severity of emphysema*

Characteristic	No/mild (n = 105)	Moderate (n = 124)	Severe (n = 45)	Total (n = 274)
Age (years)	70 (64–76)	72 (64–75)	71 (68–77)	71 (64–76)
Sex (male/female)	96/9	119/5	43/2	258/16
BMI (kg/m^2)	23.5 (0.3)	21.7 (0.3)†	21.2 (0.5)†	22.3 (0.2)
Smoking (pack-years)	58 (3)	65 (3)	66 (4)	63 (2)
Current smoker (%)	39/105 (37)	26/124 (21)	10/45 (22)	75/274 (27)
Clinical symptoms (%)				
Chronic cough	11/105 (11)	18/124 (15)	5/45 (11)	34/274 (12)
Chronic sputum	19/105 (18)	23/124 (19)	7/45 (16)	49/274 (18)
Chronic cough and sputum	8/105 (8)	16/124 (13)	5/45 (11)	29/274 (11)
Pulmonary function tests				
Pre-bronchodilator				
FVC (% predicted)	91.4 (2.0)	94.7 (2.0)	88.3 (3.1)	92.4 (1.3)
FEV_1 (% predicted)	63.0 (2.0)	56.9 (2.1)†	46.5 (3.0)†‡	57.5 (1.4)
Post-bronchodilator				
FVC (% predicted)	98.9 (1.8)	102.6 (1.8)	98.4 (2.9)	100.5 (1.2)
FEV_1 (% predicted)	69.5 (1.9)	62.7 (2.0)	52.0 (3.0)†‡	63.5 (1.3)
FEV_1/FVC	0.56 (0.01)	0.48 (0.01)†	0.42 (0.02)†‡	0.50 (0.01)
Transfer factor				
TLCO/VA (% predicted)	78.1 (2.2)	58.4 (1.8)†	41.6 (2.5)†‡	63.3 (1.5)
Blood analysis				
Eosinophils (log cells/μl)	2.26 (0.03)	2.16 (0.03)	2.19 (0.05)	2.20 (0.02)
Serum total IgE (log IU/ml)	1.87 (0.07)	1.72 (0.06)	1.74 (0.09)	1.78 (0.04)
α_1-antitrypsin (mg/dl)	128 (2)	129 (2)	135 (3)	129 (1)
Medications, no. of patients/ total (%)				
Anticholinergic agents	41/105 (39)	64/124 (52)	32/45 (71)†‡	137/274 (50)
β_2 agonists	29/105 (28)	43/124 (35)	18/45 (40)	90/274 (33)
Theophyllines	36/105 (34)	59/124 (48)†	27/45 (60)†	122/274 (45)
ICS	12/105 (11)	10/124 (8)	9/45 (20)	31/274 (11)

BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; TLCO, carbon monoxide transfer factor; VA, alveolar volume; ICS, inhaled corticosteroids; no/mild, no or mild emphysema (emphysema score <1); moderate, moderate emphysema (emphysema score ≥1–<2.5); severe, severe emphysema (emphysema score ≤2.5).
*Data shown as mean (SE) values except for skewed data which are expressed as median (interquartile range). Univariate analysis used χ^2 tests for categorical variables and one-way analysis of variance for quantitative variables with Scheffe's test as a post-hoc test for multiple comparisons.
†$p<0.05$ vs no/mild emphysema; ‡$p<0.05$ vs moderate emphysema.

Jonckheere-Terpstra test was used to examine trends for three groups of subjects. All statistical tests were two-sided and values of $p<0.05$ were considered statistically significant. Data were analysed using SPSS for Windows Version 12 (SPSS Japan, Tokyo).

RESULTS

There was a high degree of correlation between the subjective visual score of severity of emphysema for three CT images and an objective computerised quantification for the whole lung ($n = 137$, $r = 0.835$, $p<0.0001$) which was performed only for the subjects in Hokkaido University Hospital. This therefore justifies the use of visual assessment of emphysema for this multi-site study.

Figure 1 shows the relationship between the emphysema score and post-bronchodilator value of FEV_1 % predicted in all subjects with COPD. A weak but significant overall correlation was seen between the two parameters ($n = 274$, $r = -0.302$, $p<0.001$). However, a better correlation was seen between the emphysema score and TLCO/VA ($n = 273$, $r = -0.577$, $p<0.001$). An extremely wide variation in the severity of emphysema was seen with all stages of COPD. In other words, the severity of emphysema varied widely from none/mild to very severe even in subjects with the same level of airflow limitation. A similar finding was noted even if those subjects showing significant reversibility of airflow limitation ($n = 86$) as defined by the GOLD guidelines were excluded (data not shown).

To emphasise the characterisation of phenotypes in COPD, all subjects were then classified into three groups based on severity of emphysema: (1) subjects with no/mild emphysema (emphysema score <1, percentage of low attenuation area (LAA) in the assessed lung <12.5% on average); (2) subjects with moderate emphysema (emphysema score ≥1–<2.5, percentage of LAA in the assessed lung <50% on average); and (3) subjects with severe emphysema (emphysema score ≥2.5). Table 1 shows the characteristics of the three groups. Although indices of airflow limitation and TLCO deteriorated as emphysema became more severe, no significant differences in age, sex, smoking history, blood eosinophil count or serum IgE levels were found. There was no α_1-antitrypsin deficiency in any subjects, and no significant difference was noted in the mean level of serum α_1-antitrypsin among the three groups. In terms of medication, anticholinergic agents or theophyllines were prescribed more often as emphysema became more severe, however β_2 agonists and inhaled corticosteroids were given to a similar extent in all three groups of subjects. The prevalence of chronic cough and/or sputum was remarkably similar among the three groups, indicating that the prevalence of chronic bronchitis is the same regardless of the severity of emphysema.

In terms of BDR to salbutamol, there was wide variation between subjects but a clear relationship was apparent between baseline FEV_1 and post-bronchodilator increase in FEV_1 when expressed as a percentage change from baseline FEV_1 (fig 2A). BDR was then compared among the three groups classified according to severity of emphysema. No significant differences were seen in absolute change in FEV_1 (no/mild emphysema, 173 (13) ml ($n = 105$); moderate emphysema, 163 (12) ml ($n = 123$); severe emphysema,150 (20) ml ($n = 45$)), percentage change from baseline FEV_1 (12.6 (1.2)%, 14.1 (1.3)% and 14.1 (2.0)%, respectively; fig 2B) or percentage change from predicted FEV_1 (6.5 (0.5)%, 6.1 (0.4)% and 5.6 (0.7)%,

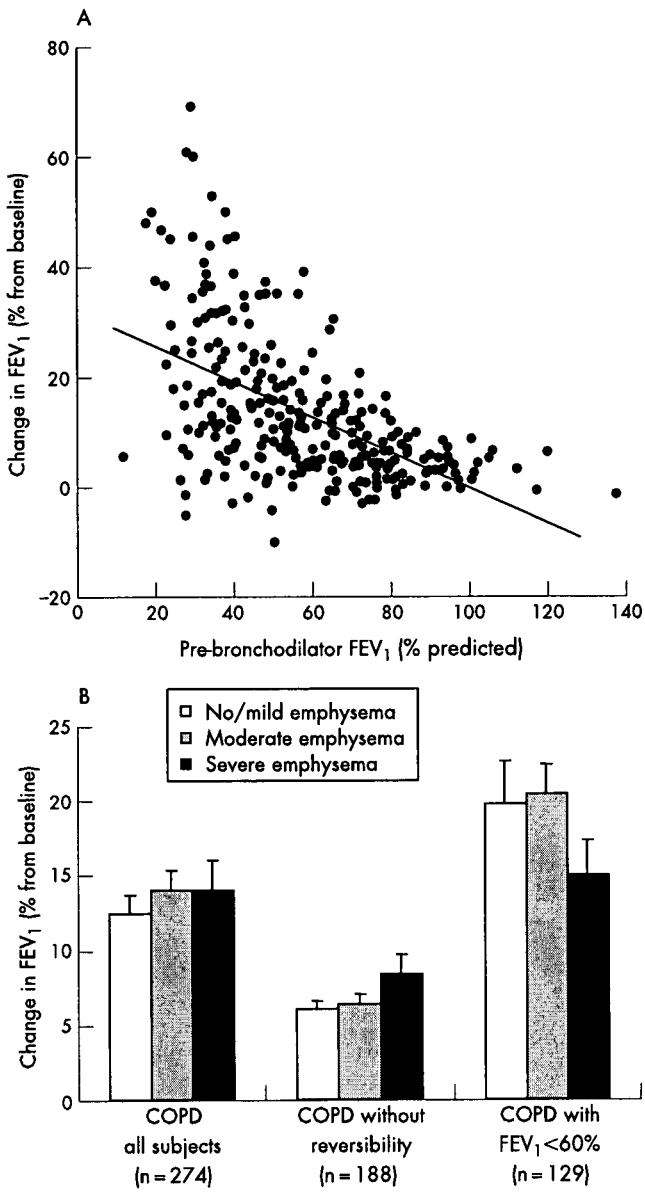

Figure 2 (A) Relationship between pre-bronchodilator forced expiratory volume in 1 s (FEV_1) and bronchodilator response (BDR) to β_2 agonist in all subjects with chronic obstructive pulmonary disease. (B) Comparison of BDR among three groups classified by severity of emphysema. Values are mean (SE).

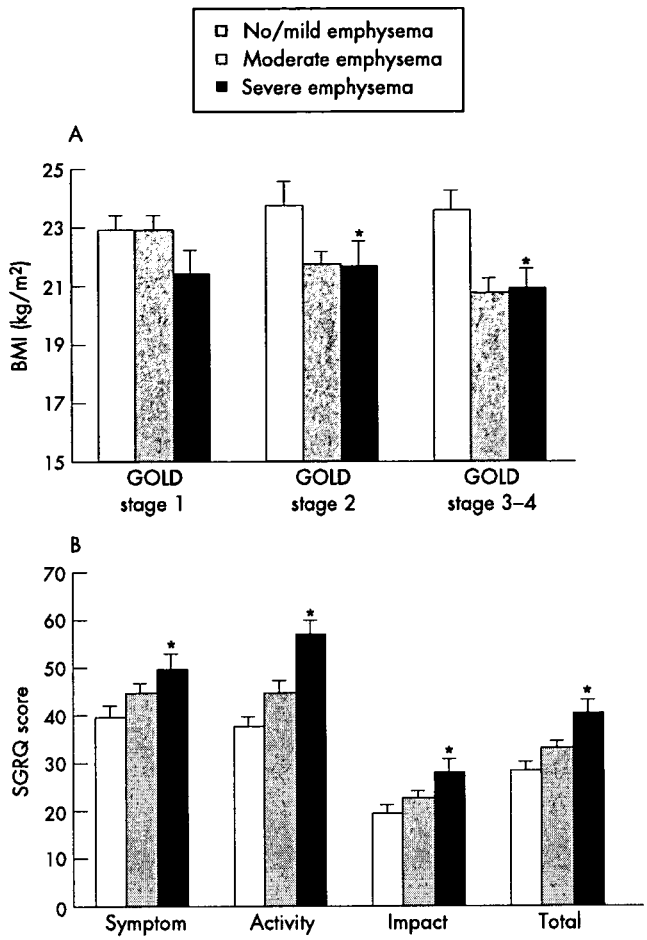

Figure 3 (A) Body mass index (BMI) and (B) St George's Respiratory Questionnaire (SGRQ) in the three groups classified by severity of emphysema. Values are mean (SE). Severity of emphysema is associated with lower BMI regardless of GOLD stage. See text for details of SGRQ data. The Jonckheere-Terpstra test was used to examine trends for the three groups of subjects (*$p<0.05$).

respectively). Significant differences between the groups were still absent even if we chose only subjects without reversibility of airflow limitation or those with FEV_1 <60% predicted, indicating that airflow limitation was perfectly comparable between the three groups (post-bronchodilator FEV_1 in no/mild emphysema, 46.9 (1.6)% predicted (n = 32); moderate emphysema; 44.8 (1.3)% predicted (n = 63); severe emphysema, 43.5 (1.7)% predicted (n = 35)).

Figure 3 shows data for BMI and health-related QOL. BMI was significantly lower as emphysema became more severe in all subjects (table 1). Of particular note is the fact that this held true even if subjects were compared separately based on the COPD stage (fig 3A) or only subjects with FEV_1 <60% predicted, indicating that airflow limitation was perfectly comparable between the three groups classified by the severity of emphysema (no/mild emphysema, 23.7 (0.6) kg/m^2, moderate emphysema, 21.1 (0.4) kg/m^2; severe emphysema, 21.1 (0.6) kg/m^2). All dimensions of SGRQ scores became significantly higher as emphysema became more severe (fig 3B). Statistical differences remained present in the activity and total scores of the SGRQ even for subjects with FEV_1 <60% predicted (activity score, 51 (4)%, 52 (3)% and 64 (3)%, respectively; total score, 37 (3)%, 40 (2)%, 45 (2)%, respectively). There was therefore a significant relationship between severity of emphysema and BMI overall ($r = -0.293$, $p<0.001$) and also with the SGRQ total score ($r = 0.231$, $p<0.001$).

DISCUSSION

This study has shown that the severity of emphysema varies widely despite the same disease stage in COPD. For instance, even in patients with moderate or severe COPD, some show very little evidence of emphysema while others have marked emphysema. Furthermore, some patients retain relatively normal pulmonary function despite the presence of severe emphysema. These observations support the findings of several past studies which argued against emphysema as the major cause of airflow limitation in COPD.[1 21–23]

All subjects were then classified into three groups based on the severity of emphysema. Although the small airways were not directly evaluated in this study, the extremely wide variation in severity of emphysema observed among subjects

with the same degree of airflow limitation appeared to indicate that the three groups might well represent differences in relative contributions of small airway disease and emphysema to airflow limitation. The most important findings in this study were that patients with severe emphysema had significantly lower BMI and worse QOL than patients with no/mild emphysema (predominantly small airway disease), despite similarities in age, smoking history, blood eosinophil count and IgE levels. Activity score, which includes dyspnoea on exertion, was particularly significant. These differences remained significant even if subjects with the same degree of airflow limitation were compared. Celli *et al*[24] recently proposed the BODE index, a simple multidimensional grading system, for predicting the risk of death in subjects with COPD. They demonstrated the importance of BMI, dyspnoea and exercise capacity index for assessment of subjects with COPD in addition to airflow limitation index. Several other reports support the notion that BMI and QOL, including dyspnoea, represent independent factors for the prognosis of COPD.[25–28]

Phenotyping of COPD described in the present study may thus have some clinical relevance in the management of patients with COPD. Another important consideration is that more attention should be paid to these phenotypes when studying the epidemiology, genetic background and pathogenesis of COPD.[29 30] In fact, an interesting study from Japan recently showed that body weight loss in COPD is associated with a novel polymorphism in secretory A_2-IID, an enzyme responsible for mobilisation of fatty acids including arachidonic acid from phospholipids, thereby potentially influencing systemic inflammation in COPD.[31] Possible reasons why severity of emphysema rather than airflow limitation itself is associated with lower BMI may be exaggerated systemic inflammatory response or increased work load of breathing in the emphysematous type of COPD; however, these speculations are beyond the scope of this study and need further investigation.

Historically, patients with COPD used to be classified as "pink puffers" or "blue bloaters",[7 8] or A, B and X types.[9] The phenotypic classification of COPD we propose here differs from classic phenotypes as the prevalence of chronic bronchitis symptoms was almost equal in the three groups studied. Indeed, we used to see far more subjects with COPD who were suffering from chronic bronchitis in the past than currently. However, in Japan at least, we have seen a dramatic decrease in the number of subjects diagnosed with COPD and chronic bronchitis over the last three decades.[32] Our data indicate that the decreasing frequency of chronic bronchitis in COPD over recent years does not reflect an increase of subjects with severe emphysema, but rather the manifestation of subjects with predominantly small airways disease which is not necessarily associated with bronchitis symptoms.

The reversibility of airflow limitation in COPD has long been a subject of debate.[33–35] In this study we found a wide variation between subjects in BDR to salbutamol, but no statistical differences according to phenotype based on severity of emphysema. These data led to two important speculations. First, conclusions of the present study were unlikely to be biased by the inadvertent inclusion of patients with bronchial asthma, particularly in the group with no/mild emphysema. Second, what is occurring in large or proximal airways (chronic bronchitis symptoms and reversibility of airflow limitation) may be independent of what is occurring in peripheral sites in the lungs (small airways disease and emphysema).

There are two limitations to this study. First, we used subjective visual scoring for assessment of emphysema severity rather than objective quantification. This is because we had to use various kinds of CT scanners for this study and could not obtain the Digital Imaging and Communications in Medicine (DICOM) images from all affiliated hospitals. However, all HRCT images were thin-slice (<2 mm) and we carefully optimised the data acquisition parameters as well as the parameters for data analysis at each hospital to obtain ideal images for assessment of emphysema. In addition, we showed that a visual emphysema score for three CT images was highly correlated with objective volume-based computerised assessment for the whole lung in almost half the subjects, and also found a significant correlation between the visual emphysema score and TLCO/VA as described previously.[36] Second, this study did not directly evaluate small airways disease, so we could not measure the real contribution of small airways disease to airflow limitation in any subjects. In parallel with this study, we attempted to develop new computer software using curved multiplanar reconstruction to obtain longitudinal images and to analyse accurately short-axis images of airways with inner diameter ≥2 mm located anywhere in the lungs. We recently published a paper which showed that airflow limitation in COPD is more closely related to the dimensions of the distal airways (sixth generation) than the proximal airways (third generation) in both upper and lower lobes.[37] The observed high correlation coefficients between FEV_1 percentage predicted and the dimensions of such distal airways are in sharp contrast to the weak but significant relationship between FEV_1 percentage predicted and severity of emphysema observed in this study. These data suggest that the site of small airways contributes more significantly to airflow limitation than emphysema in COPD. In other words, the contribution of the small airways may be vitally important in COPD regardless of the phenotype based on severity of emphysema.

In conclusion, this study has shown that the severity of emphysema is highly variable, even among subjects with the same stage of COPD, and that COPD phenotypes based on severity of emphysema clearly differ from the classic phenotypes of "pink puffers" and "blue bloaters". The prevalence of bronchitis symptoms and average bronchodilator responses to inhaled β_2 agonist were similar among the three groups classified according to severity of emphysema. However, the BMI was significantly lower and SGRQ scores were significantly worse in the phenotype with severe emphysema than in those with no/mild emphysema. Accordingly, classification of COPD based on CT scanning may provide distinct phenotypes and display great clinical relevance in the management of COPD. Further studies are ongoing in an attempt to examine possible differences in the natural history of the disease according to phenotypes based on the severity of emphysema.

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Further details are given in the online supplement available at http://thorax.bmj.com/supplemental.

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AMERICAN COLLEGE OF CHEST PHYSICIANS®

β_2-Adrenergic Receptor Genetic Polymorphisms and Short-term Bronchodilator Responses in Patients With COPD*

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Background: **COPD is characterized by a persistent airflow limitation that is not fully reversible; thus, the reversibility of airflow limitations in response to a bronchodilator is an important component of COPD. Several studies have established that two common nonsynonymous polymorphisms in the β_2-adrenergic receptor gene (*ADRB2*), Arg16Gly and Gln27Glu, have important effects in modulating responses to β_2-agonists; however, the effects of these polymorphisms on responses to β_2-agonists in patients with COPD is unknown.**

Objective: **To examine whether different genotypes at these two polymorphisms are related to differential responses to inhaled β_2-agonists in patients with COPD.**

Design and Participants: **A total of 246 patients with COPD who were participants in a longitudinal study of COPD (*ie*, the Hokkaido COPD cohort study) were studied. We compared short-term bronchodilator responses (BDRs) to salbutamol according to *ADRB2* genotypes at codons 16 and 27.**

Results: **The presence of the Arg16 allele was associated with lower BDRs to β_2-agonist inhalation. The mean (±SD) log (postbronchodilator FEV_1 − prebronchodilator FEV_1) values of Gly16 homozygotes (n = 65), Arg16Gly16 heterozygotes (n = 106), and Arg16 homozygotes (n = 75) were 2.19 ± 0.43, 2.09 ± 0.42, and 2.01 ± 0.42, respectively ($p < 0.05$). The genetic effects of the Arg16Gly polymorphism were independent of the severity of airflow limitation, age, and smoking status. The most common Arg16-Gln27 haplotype was also significantly associated with decreased BDRs to salbutamol ($p < 0.01$).**

Conclusion: **The genetic effects of *ADRB2* gene polymorphisms may explain some of the variability in response to therapeutic doses of a short-acting β_2-agonists in patients with COPD.** ***(CHEST 2007; 132:1485–1492)***

Key words: Arg16Gly; β_2-adrenergic receptor; bronchodilator response; COPD; genetic polymorphism

Abbreviations: *ADRB2* = β_2-adrenergic receptor gene; BDR = bronchodilator response; β2AR = β_2-adrenergic receptor; ΔFEV_1 = postbronchodilator FEV_1 − prebronchodilator FEV_1; COLD = Global Initiative for Chronic Obstructive Lung Disease; HWE = Hardy-Weinberg equilibrium; LABA = long-acting β_2-agonist; SABA = short-acting β_2-agonist; SNP = single-nucleotide polymorphism

β_2-Agonists are the most common bronchodilators used to treat airflow limitations associated with obstructive airway diseases, including asthma and COPD. In a recent study[1] of 274 patients with COPD, we showed that the bronchodilator response (BDR) to salbutamol therapy was associated with a continuous phenotype in patients, with a wide interindividual variation. In patients with COPD, a higher reversibility of airflow obstruction has been proposed to predict a slower decline in FEV_1 and better survival.[2–5] Therefore, bronchodilator reversibility may be a useful indicator not only for assessing the clinical effect of treatment for COPD but also for predicting clinical outcome and survival. Interindividual variation in therapeutic responses to β_2-agonists can be determined by

several factors, including the degree of baseline airflow limitation,[1,6] smoking status,[7] age,[8] and genetic factors.[9,10]

The β_2-adrenergic receptor (β2AR) gene (*ADRB2*) is a small intronless gene on chromosome 5q31-q32. Three single-nucleotide polymorphisms (SNPs) in the coding region have been identified that alter the encoded amino acid,[11] including Arg16Gly and Gln27Gly. The β2AR mediates the physiologic responses of the airways, including bronchodilation, antiinflammatory actions, mucociliary clearance, and vascular endothelial permeability.[12] Genetic variations of the *ADRB2* gene have a significant physiologic role in regulating responses to exogenous β_2-adrenergic agonists[13,14]; some studies have indicated that they affect β2AR-induced receptor down-regulation,[15] the loss of bronchoprotection,[16] or the activation of extracellular signal-regulated kinases in airway smooth muscle cells that contributes to mitogenesis and exaggerated inflammatory cytokine expression.[17] Alterations in the *ADRB2* gene may also affect the signaling and function of other receptors that control airway contractility such as cholinergic receptors.[18]

Since the initial description of polymorphic variations in the coding region of the *ADRB2* in 1993,[19] the potential clinical importance of two common polymorphisms at codons 16 and 27 has been the subject of extensive study. Several clinical studies[13,14] have shown the genetic contribution of the Arg16Gly polymorphism to short-term BDR to β_2-agonists in patients with asthma. Longer term clinical responses to short-acting β_2-agonists (SABAs) and long-acting β_2-agonists (LABAs) have also been modified by the Arg16Gly polymorphism.[20–22] No studies, however, have investigated the genetic impacts of the *ADRB2* gene polymorphisms on BDR in patients with COPD. In the current study, we conducted a genetic association study between the Arg16Gly and Gln27Glu polymorphisms and short-term BDR to salbutamol, using Japanese patients with COPD who participated in the Hokkaido COPD cohort study.[1]

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Materials and Methods

Study Subjects

The recruitment of patients with COPD has been described previously.[1] In brief, the population consisted of 274 unrelated Japanese patients with COPD who were recruited from an outpatient setting from Hokkaido University Hospital in Sapporo, Japan, and nine other affiliated hospitals in the surrounding area. The diagnosis of COPD was made by respiratory physicians. The inclusion criteria for patients were as follows: met the spirometric criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines[23]; age $\geq$ 40 years; and current smoker or ex-smoker with a smoking history of $>$ 10 pack-years. Study approval was obtained from the governing ethics committees for each study center, and all patients gave written informed consent.

Pulmonary Function Tests

The procedures for pulmonary function tests have been previously described in detail.[1] Briefly, spirometry (*ie*, FVC, FEV_1, and FEV_1 percent predicted) was performed before and 30 min after bronchodilator therapy (salbutamol, 400 μg) was given. BDR was expressed by the three commonly used indexes recommended by the American Thoracic Society[24] or the European Respiratory Society.[25] The absolute BDR index was calculated as (postbronchodilator FEV_1 − prebronchodilator FEV_1) [ΔFEV_1] in milliliters. The change in the percent predicted index[25] was calculated as (ΔFEV_1/predicted FEV_1) × 100, and the change in the percentage of the prebronchodilator index[24] as (ΔFEV_1/prebronchodilator FEV_1) × 100.

We devoted meticulous attention to the accuracy of the spirometry tests.[1] Volume calibration using a calibration syringe had been performed by technicians in each hospital before examination, and a quality-control protocol was developed based on the criteria used in the Lung Health Study[26] to increase accuracy and decrease intraindividual variability.

DNA Genotyping

Among 274 patients with COPD, genetic samples were available for 264 patients. We identified the Arg16Gly and the Gln27Glu polymorphisms at the *ADRB2* gene using an assay combining kinetic real-time quantitative polymerase chain reaction with allele-specific amplification, as described previously.[27]

Statistical Analysis

To investigate the determinants of short-term BDR in patients with smoking-related COPD, we used stepwise linear regression models. Initially, we excluded a group of patients (n = 18) who exhibited a worsening of FEV_1 after β_2-agonist administration because the physiologic significance of BDR and its potential therapeutic value are obscured by the fact that some COPD patients respond to β_2-agonists with paradoxical bronchoconstriction. The paradoxical bronchoconstriction is caused by several mechanisms, including an IgE-mediated reaction to excipients in

the metered-dose inhaler, irritation secondary to propellants, preservatives, or turbulence of airflow due to inappropriate inhaler technique.[28]

Accordingly, we tested two independent models using 246 and 161 patients with COPD. Model 1 included all patients who did not experience a worsening of FEV_1 after β_2-agonist administration. In model 2, we confined our analysis to 161 patients whose bronchial reversibility or ΔFEV_1/prebronchodilator FEV_1 (percent predicted) was < 15%, because airflow limitation tends to be fixed, by definition, in COPD rather than demonstrate significant reversibility. A possibility may remain that the increased BDR only indicates the presence of coexisting asthma, although patients with physician-diagnosed asthma were carefully excluded in this study.[1] Spirometry may be helpful for the diagnosis of coexisting asthma, where at least a 12% improvement in FEV_1 either spontaneously, after the inhalation of a bronchodilator, or in response to a short course of glucocorticosteroid therapy may favor a diagnosis of asthma.[29] In the current study, we used the threshold of 15% as representing bronchial reversibility, which was proposed for FEV_1 by Nickerson et al[30] based on the measured coefficient of variation of FEV_1. In fact, of the 4,194 patients with mild COPD in the Lung Health Study,[31] BDR of > 15% of the prebronchodilator value occurred only in 2.6% of patients. Therefore, although current GOLD guidelines do not have any recommendation for positive bronchodilator reversibility testing,[23] an acute change that exceeds 15% of the baseline measurement is unlikely to have arisen by chance.

We examined the following independent variables in both models: age; sex; smoking status (current or past); smoking index (pack-years); body mass index; prebronchodilator FEV_1 (percent predicted); peripheral blood eosinophil counts; total serum IgE levels; the diffusing capacity of the lung for carbon monoxide divided by the alveolar volume; the presence of the regular use of β_2-agonists; the presence of the regular use of inhaled corticosteroids; and Arg16Gly and Gln27Glu genotypes. The Kolmogorov-Smirnov test for normality was applied for quantitative indexes to evaluate the distribution, and three indexes of BDR, smoking index, eosinophil counts, and total serum IgE levels, were log-transformed to approximate normal distributions. All clinical and genotype data were managed with a statistical software package (SYSTAT for Windows, version 11; SYSTAT; San Jose, CA).

The Hardy-Weinberg equilibrium (HWE) program[32] was used to compare observed numbers of genotypes with the numbers of genotypes expected under HWE. In 121 white patients with asthma, the BDR to β_2-agonists has been reported to be significantly related to the *ADRB2* gene haplotype pair but not to individual SNPs,[11] and the association of SNPs with BDR may depend on *cis* interactions between the *ADRB2* polymorphisms at codons 16 and 27. Therefore, the association between *ADRB2* haplotypes and the BDR was also examined using the Haplo.Score program of Schaid et al.[33] This program has the advantage that adjustment for covariates and the computation of simulation p values for each haplotype can be performed. The number of simulations for empirical p values was set at 1,000.

Results

Patient characteristics are shown in Table 1. Among 246 patients with COPD, 79 patients (32.1%) regularly used β_2-stimulants (SABAs, 29 patients; LABAs, 50 patients) and 27 patients (11%) used inhaled corticosteroids regularly. The two patient groups, which were stratified according to the extent of BDR (*ie*, < 15% and ≥ 15%), differed in prebronchodilator FEV_1 and GOLD stage. The frequencies of patients who were receiving long-term treatment with β_2-stimulants were also significantly different between the two groups; a lower frequency was noted in patients whose reversibility was < 15%.

The prevalence of Arg16Gly and Glu27Gln polymorphisms did not significantly deviate from the HWE in either population examined. In model 1, using three indexes of BDR, the Arg16Gly genotype, prebronchodilator FEV_1 percent predicted, smoking status, and peripheral blood eosinophil counts were consistently entered into the model to determine BDR by stepwise linear regression analysis (Tables 2, 3). In model 2, the Arg16Gly genotype, prebronchodilator FEV_1 percent predicted, and smoking status were consistently entered into the model (Table 2). The other polymorphism (Gln27Glu) was not associated with BDR and was removed in both models.

The results suggest that the presence of the Arg16 allele was associated with lower BDR after β_2-agonist inhalation in patients with COPD. For example, the mean (± SD) log ΔFEV_1 values of Gly16 homozygotes (n = 65), Arg16Gly16 heterozygotes (n = 106), and Arg16 homozygotes (n = 75) were 2.19 ± 0.43, 2.09 ± 0.42, and 2.01 ± 0.42, respectively, in model 1; and 2.01 ± 0.42 (n = 38), 1.93 ± 0.42 (n = 72), and 1.79 ± 0.42 (n = 51), respectively, in model 2 (Table 3). In both models, the genetic effects of the Arg16Gly polymorphism were codominant with heterozygotes showing intermediate levels of response to salbutamol when compared with both Arg16 and Gly16 homozygote genotypes. Current smoking was associated with a lower BDR in both models (model 1: mean log ΔFEV_1 value of current smokers, 2.00 ± 0.43 [n = 64]; mean log ΔFEV_1 value of past smokers, 2.12 ± 0.42 [n = 182]).

The multiple regression models (Table 2) resulted in an adjusted R^2 of < 10% for the absolute response and change in percent predicted indexes, whereas the adjusted R^2 was 33% for the change in percentage predicted prebronchodilator FEV_1. The R^2 value for the prediction equation of the change in percent predicted prebronchodilator FEV_1 index was largest as a consequence of having the prebronchodilator FEV_1 value both as the index denominator and as one of the explanatory variables in the models.

When the influence of the *ADRB2* haplotype on the absolute response was examined, the Arg16-Gln27 haplotype demonstrated the lowest haplotype-specific score (model 1, −2.46; model 2, −2.57) [Table 4]; these results are compatible with the results of single-SNP analyses.

Discussion

This is the first study to demonstrate that the development of a BDR to a SABA in patients with COPD is

Table 1—*Patient Characteristics

Characteristics	Reversibility		p Value†
	< 15% (n = 161)	≥ 15% (n = 85)	
Age, yr	68.7 (8.5)	70.9 (7.5)	0.044
Sex			0.51
Male	150	81	
Female	11	4	
Smoking, pack-yr	62.7 (30.5)	64.3 (31.1)	0.7
Smoking status			0.34
Current	45	19	
Past	116	66	
BMI	22.2 (2.97)	22.6 (3.53)	0.31
Prebronchodilator FEV_1, % predicted	64.7 (21.2)	41.7 (13.3)	<0.00001
GOLD stage‡			
1	51	5	<0.00001
2	75	41	
3	30	34	
4	5	5	
DLCO/VA ratio, % predicted	63.3 (23.4)	65.5 (24.6)	0.51
Log (eosinophil counts)	2.19 (0.32)	2.27 (0.34)	0.068
Log (total IgE levels)	1.82 (0.72)	1.72 (0.68)	0.32
Atopy,§ %	24.5	22.2	0.69
Regular use of β_2-stimulants, %	27.3	41.2	0.027
Arg46Gly, No.			
Arg/Arg	51	24	0.39
Arg/Gly	72	34	
Gly/Gly	38	27	
Glu79Gln, No.			
Glu/Glu	135	65	0.23
Glu/Gln	25	17	
Gln/Gln	0	1	

*Values are given as the mean (SD), unless otherwise indicated. BMI = body mass index; DLCO = diffusing capacity of the lung for carbon monoxide; VA = alveolar volume.

†Comparisons were made by χ^2 test or two-sample t test, where appropriate.

‡GOLD stage I is defined as an FEV_1/FVC ratio of < 70% and FEV_1 > 80% predicted; GOLD stage II is defined as an FEV_1/FVC ratio of between < 70% and 50% predicted and an FEV_1 of ≤ 80% predicted; GOLD stage III is defined as an FEV_1/FVC ratio of between < 70% and 30% predicted and an FEV_1 of ≤ 50% predicted; and GOLD stage IV is defined as an FEV_1/FVC ratio of < 70% and an FEV_1 of ≤ 30% predicted or an FEV_1 of < 50% predicted plus chronic respiratory failure.

§Defined as positive specific IgE responses to at least one of 14 common inhaled allergens.

associated with *ADRB2* polymorphisms. A greater BDR developed in COPD patients with homozygous Gly16 than in those with homozygous Arg16; whereas a BDR developed in heterozygote patients that was between that of the two types of homozygote patients. The differences in BDR may contribute to differences in disease outcomes, including lung function, the severity of dyspnea, exercise capacity, and exacerbations.[3–5] An altered function of the receptor due to the Arg16Gly polymorphism may be one of several mechanisms that leads to the development of particular disease outcomes in relation to BDR. Therefore, identification of the Arg16Gly polymorphism as a genetic factor influencing variability in responses to β_2-agonists could have important implications for COPD therapy and could define subgroups of COPD patients with differential responses.

The mechanism underlying our findings remains unclear, with conflicting results of a genetic influence of *ADRB2* gene polymorphisms on the BDR to β_2-agonists. Clinical studies[20–22] performed over the past few years using asthmatic patients have concentrated on the Arg16Gly polymorphism, showing that the Arg16 variant is associated with a poorer response to regular SABAs or LABAs; our findings in the current study are compatible with the results of those studies. In contrast, our findings are in apparent contradiction with a report by Martinez et al[14] showing that asthmatic children who were homozygotes for Gly16 had significantly less β_2-agonist responsiveness than those who were homozygotes for Arg16. Among Puerto Ricans with asthma, the Arg 16 allele was also associated with a greater BDR using both family-based and cross-sectional tests.[13] It is possible that the mechanisms by which polymorphisms in the *ADRB2* gene determine responses to β_2-agonists in patients with asthma may be different from the mechanism among patients with COPD. In

Table 2—*Linear Regression Analyses for Determinants of BDR in Patients With COPD

Model	Coefficient	F Value	p Value
Model 1† (n = 246)			
ΔFEV_1 (in mL)			
Smoking status	0.11	2.87	0.092
Prebronchodilator FEV_1 (in % predicted)	−0.0035	7.59	0.0063
Log (eosinophil count)	0.18	4.38	0.037
Arg16Gly polymorphism	0.088	4.94	0.027
ΔFEV_1/predicted FEV_1‡			
Smoking status	0.12	4.37	0.038
Prebronchodilator FEV_1 (in % predicted)	−0.0032	7.71	0.006
Log (eosinophil counts)	0.13	2.75	0.099
Arg16Gly polymorphism	0.07	4.54	0.034
ΔFEV_1/prebronchodilator FEV_1§			
Smoking status	0.13	5.03	0.026
Prebronchodilator FEV_1 (in % predicted)	−0.011	94.1	<0.0001
Log (eosinophil counts)	0.11	2.24	0.14
Arg16Gly polymorphism	0.069	4.41	0.037
Model 2 (n = 161)			
ΔFEV_1 (in mL)‖			
Smoking status	0.15	2.04	0.043
Prebronchodilator FEV_1 (in % predicted)	0.0027	1.7	0.09
Arg16Gly polymorphism	0.11	2.52	0.013
ΔFEV1/predicted FEV1¶			
Age	0.0077	2.27	0.024
Smoking status	0.14	2.25	0.026
Prebronchodilator FEV_1 (in % predicted)	0.0033	2.46	0.015
Arg16Gly polymorphism	0.11	2.83	0.0053
ΔFEV_1/prebronchodilator FEV_1#			
Age	0.0063	1.89	0.06
Smoking status	0.16	2.53	0.012
Prebronchodilator FEV_1 (in % predicted)	−0.0041	−3.05	0.0027
Arg16Gly polymorphism	0.11	2.86	0.0048

*Variables that had an F value of ≥ 3.0 were entered into each model.
†Adjusted R^2 = 0.076 (SE, 0.43).
‡Adjusted R^2 = 0.074 (SE, 0.39).
§Adjusted R^2 = 0.32 (SE, 0.39).
‖Adjusted R^2 = 0.053 (SE, 0.42).
¶Adjusted R^2 = 0.095 (SE, 0.35).
#Adjusted R^2 = 0.15 (SE, 0.35).

addition, the differences in age (childhood or adult asthma) or ethnicity (Japanese, white, Hispanic, or Puerto Rican) may also underlie these inconsistent results in the literature. Potential interactions between alleles at different SNPs in and around the *ADRB2* gene may lead to inconsistent results because *ADRB2* haplotypes have been described to occur at different frequencies based on ethnicity.[11]

Screens of the *ADRB2* gene in Japanese subjects have revealed 15 SNPs within the 3-kb promoter and 1.2-kb structural regions,[34] which identified five common haplotypes (> 5%). Although the detection

Table 3—*Association Between the Arg16Gly Polymorphism and BDR

Model	GlyGly	ArgGly	ArgArg
Model 1 (n = 246)			
Log (ΔFEV_1)	2.19 (0.43)	2.09 (0.42)	2.01 (0.42)
Log (ΔFEV_1/prebronchodilator FEV_1)	0.76 (0.39)	0.67 (0.38)	0.6 (0.38)
Log (ΔFEV_1/FEV_1 % predicted)	1.05 (0.39)	0.95 (0.38)	0.89 (0.38)
Model 2 (n = 161)			
Log (ΔFEV_1)	2.01 (0.42)	1.93 (0.42)	1.79 (0.42)
Log (ΔFEV_1/prebronchodilator FEV_1)	0.82 (0.35)	0.73 (0.35)	0.61 (0.35)
Log (ΔFEV_1/FEV_1 % predicted)	0.6 (0.36)	0.51 (0.36)	0.38 (0.36)

*Values are given as adjusted means (SD).

Table 4—*Association Between ADRB2 Haplotypes and the Absolute BDR in Patients With COPD*

Haplotype	Position 16	Position 27	Haplotype Frequency	Haplotype-Specific Score*	Simulation p Value†
246 patients with COPD					
1	Arg	Gln	0.514	−2.46	0.006
2	Arg	Glu	0.003	−1.02	0.23
3	Gly	Glu	0.087	1.62	0.084
4	Gly	Gln	0.396	1.82	0.055
161 patients with COPD					
1	Arg	Gln	0.532	−2.57	0.006
2	Arg	Glu	0.005	−0.14	0.87
3	Gly	Glu	0.073	1.34	0.18
4	Gly	Gln	0.39	2.01	0.036

*Negative haplotype-specific scores were associated with a protective effect, and positive haplotype-specific scores were associated with increased risk.

†Simulation p values of haplotype associations were calculated by the Haplo. Score algorithm developed by Schaid et al,[33] while controlling for smoking status (current or past) and prebronchodilator FEV_1 percent predicted.

of Arg16Gly and Gln27Glu does not allow us to dissect the role of specific haplotypes on BDR in patients with COPD, the most important polymorphisms appear to be nonsynonymous polymorphisms at loci 16, 27, and 164, as well as a polymorphism involving amino acid 19 of the 5′ leader cistron of the β2AR, which is also known to influence levels of β2AR expression in cells. Of note, both the Glu27 and Cys19 alleles, which are in complete linkage equilibrium, are rare among Japanese people. In addition, the Ile164 allele has not been identified in the Japanese population. Thus, although genetic variations other than Arg16Gly and Gln27Glu may also be important,[35] we believe that the observed association between Arg16Gly polymorphism and BDR in the present study reflects the consequences of this functional polymorphism.

In addition to the Arg16Gly polymorphism, peripheral blood eosinophil counts, FEV_1 percent predicted, and current smoking showed some influence on indices of BDR to salbutamol in the current study, which is in line with previous findings.[6–8,36,37] Possible explanations for why current smoking was associated with decreased BDR may include more uneven ventilation and earlier small airway closure,[38] excess mucus lining in the airways,[39] reduced density of the β2ARs, and decreased ligand binding to β2ARs[40] in current smokers. Of note, exposure to cigarette smoke may influence the genetic effects of β_2-agonists, as it has been shown[41] that subjects who were homozygous for Arg16 and who smoked had an almost eightfold increased risk for the development of asthma compared with subjects who did not carry the Arg16 and who did not smoke. In addition, comprehensive gene expression profiling analysis on normal human airway epithelial cells has revealed that cigarette smoking induces altered expressions of a number of genes related to matrix degradation, tissue repair, immune response, and inflammation.[42] Therefore, it is possible that the genetic effects of the Arg16Gly are influenced by these altered gene expressions in current smokers.

An association between peripheral blood eosinophil counts and the absolute BDR was observed in a model 1, which included COPD patients with a BDR of ≥ 15%, but not in a model 2, which excluded COPD patients with a BDR of ≥ 15%. This finding may also support the contention that the presence of a nonfixed airflow limitation identifies patients with COPD who have some pathologic features of asthma with some therapeutic relevance on one hand,[36,37,43] whereas, on the other hand, airway irreversibility may develop in some patients with severe asthma. Although we carefully excluded physician-diagnosed bronchial asthma based on clinical features in this study, such exclusion criteria might not have been perfect.

BDR is a continuous phenotype that is determined by multiple environmental and genetic factors rather than by a single major factor. Previous analyses of 72 families ascertained through patients with severe, early-onset COPD[9] revealed that the heritability of BDR ranges from 10.1 to 26.3%. It is, therefore, not surprising that our regression models including smoking, lung function, and the Arg16Gly polymorphism explained only 5 to 9% (R^2) of the total variability in the absolute response. Furthermore, this weak association may reflect the inherent character of the bronchodilator phenotype, which has substantial day-to-day variation and poor reproducibility.[6,30,44] We also recognize that the small number of participants limits our study. Nevertheless, some of the interindividual differences in BDR might be explained by the *ADRB2* gene. A larger prospective study on groups of patients with COPD with known *ADRB2* polymorphisms will be required to confirm our findings.

In conclusion, we have found preliminary evidence that the Arg16Gly polymorphism in the *ADRB2* gene has a significant physiologic role in regulating responses to exogenous β2AR agonists in patients with COPD. Because these drugs are the most widely used agents in the treatment of asthma and COPD, our findings have important clinical implications for our understanding of the genetic factors determining short-term responsiveness to bronchodilator therapy in patients with chronic obstructive airway diseases.

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