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Predicting Treatable Traits for Long-Acting Bronchodilators in Patients with Stable COPD

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Predicting Treatable Traits for Long-Acting Bronchodilators in Patients with Stable COPD

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강지은의 의학석사학위 논문을 인준함

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Abstract

Rationale: There is currently no measure to predict a treatability to a long-acting beta-2 agonist (LABA) or long-acting muscarinic antagonist (LAMA) in patients with COPD. We aimed to build prediction models for the treatment response to these bronchodilators.

Methods: We performed a prospective crossover study, in which each long-acting bronchodilator was given in a random order to 65 patients with stable COPD for 4 weeks, with a washout period in between. We analyzed fourteen baseline clinical traits, expression profiles of 31,426 gene transcripts, and damaged-gene scores of 6,464 genes acquired from leukocytes. Linear regression analyses were performed to build prediction models after using factor and correlation analyses.

Results: By a prediction model for a LABA, traits found associated with the treatment response were post-bronchodilator FEV_1, bronchodilator reversibility (BDR) to salbutamol, expression of 3 genes (CLN8, PCSK5, and SKP2), and damage scores of 4 genes (EPG5, FNBP4, SCN10A, and SPTBN5) (R^2 = 0.512, $p < 0.001$). Traits associated with the treatment response to a LAMA were COPD assessment test score, BDR, expression of 4 genes (C1orf115, KIAA1618, PRKX, and RHOQ) and damage scores of 3 genes (FBN3, FDFT1, and ZBED6) (R^2 = 0.575, $p < 0.001$). The prediction models consisting only of clinical traits appeared too weak to predict the treatment response, with R^2 = 0.231 for the LABA model and R^2 = 0.121 for the LAMA model.

Conclusion: Adding expressions of genes and damaged-gene scores to clinical traits may improve the predictability of treatment response to long-acting bronchodilators.

Keywords: long-acting beta-2 agonist; long-acting muscarinic antagonist; prediction model.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide and results in a substantial socioeconomic burden.[1)] The treatment regimen for a given patient is determined based on the patient's symptoms and history of exacerbations,[2)] and long-acting inhaled bronchodilators are the most essential pharmacological agents. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines recommend the regular use of a long-acting bronchodilator, either a long-acting beta-2 agonist (LABA) or long-acting muscarinic antagonist (LAMA) for group B COPD patients, i.e., symptomatic patients without frequent exacerbations, but there is no clear evidence to recommend one class of long-acting bronchodilators over another.[2)]

The therapeutic efficacy of long-acting bronchodilators in the treatment of stable COPD has been well established.[3)] They increase forced expiratory volume in 1 second (FEV_1) values and improve patient-reported outcomes, such as dyspnea, health-related quality of life, and exercise tolerance in COPD patients.[4-9)] They also reduce the risks of exacerbations and hospitalizations, compared with placebo.[10, 11)] However, evidence has shown clearly that not all patients respond equally to both.[12, 13)] A considerable number of patients show no response to bronchodilator treatment, and experience adverse effects, such as palpitations and dry mouth, leading to decreased adherence to treatment; thus, identifying potential responders and non-responders before a bronchodilator treatment is initiated is important.

Previous studies have reported several traits to be related to the variability in response to short-acting beta-2 agonists, including age,[14)] smoking,[15)] and baseline lung function.[16)] These findings suggest that individual clinical characteristics are possibly related to the variable responses to bronchodilator treatment. However, contrary to short-acting beta-2 agonist treatment, for long-acting bronchodilator treatment, no specific clinical characteristics have been found to be significant predictors of response. Previous reports on the genetic variation between beta-2 receptors and muscarinic receptors have suggested that targeted treatment would be beneficial in improving the therapeutic effect,[17-19)] but single-gene polymorphism was not enough to explain the variation in treatment response in COPD

patients.[20)]

If we could predict the treatment response to a particular class of bronchodilator based on patient traits before treatment, we would be better positioned to choose the most responsive medication for individual patients. More importantly, knowing traits associated with treatment response to a LABA or LAMA may expand our understanding of the pathophysiology of COPD and help to identify potential treatment targets. With the idea that genetic traits have a role in treatment response, we aimed to build a prediction model for long-acting bronchodilator treatment response, using patient clinical characteristics, as well as genetic traits, including gene expression and damaged-gene scores.

Materials and methods

Study design

This was a multicenter, prospective, open-label crossover study aimed at building a prediction model for long-acting bronchodilator treatment response in patients with COPD. Enrolled patients were instructed to discontinue all long-acting bronchodilators for at least 4 weeks, if they were using any. To build the prediction model, the following baseline traits were recorded for each patient: age, body mass index, smoking history, modified Medical Research Council dyspnea scale score, visual analog scale of cough and sputum score, the Korean version of the COPD assessment test (CAT) score, and pre- and post-bronchodilator (200 μg of salbutamol) spirometry, diffusing capacity, and lung volume measurements. In addition, blood samples for the expression profiles and damaged-gene scores were obtained from each patient.

Patients were randomly assigned to either a beta-2 agonist (150 μg of indacaterol single-dose dry powder inhaler [Onbrez Breezhaler; Norvatis, Basel, Switzerland]) or a muscarinic antagonist (18 μg of tiotropium [Spiriva Handihaler; Boehringer Ingelheim, Ingelheim, Germany]) for the first 4 weeks. Then, after a 4-week washout period in between, the other medication was administered for the final 4 weeks (Figure 1). Each medication was administered once daily by inhalation in the morning. All patients were given instructions on inhaler device use prior to treatment. They were asked to visit the clinic at weeks 4, 8, and 12 for spirometry testing, and to report respiratory symptoms and adverse events if they experienced any. Compliance with inhaler treatment was determined by patient self-report. We categorized the level of compliance into 4 categories: good compliance = 75–100% of the prescribed inhaler medication was reported used; borderline compliance = 50–75% reported used; poor compliance = 25–50% reported used; and minimal compliance = 0–25%.

If a patient experienced an acute COPD exacerbation that required a medication modification during the study period, the patient was removed from the study. All respiratory medications were permitted except for other inhaled long-acting bronchodilators, corticosteroids, and macrolide antibiotics. Salbutamol was allowed as a symptom reliever. The objective of the study was to build prediction models for the treatment response to a

LABA and LAMA. A significant treatment response was defined as an increase of 100 mL in FEV_1.[21)]

Patients

Patients were recruited at 5 institutions in South Korea. Criteria for inclusion were an age of 45 years or more, a smoking history of at least 10 pack-years, experience of exertional dyspnea for more than a year, and a diagnosis of moderate COPD according to the GOLD criteria (post-bronchodilator FEV_1 between 50% and 80% of predicted, in the presence of post-bronchodilator FEV_1/FVC ratio of <0.70).

Exclusion criteria were a COPD exacerbation or respiratory infection in the 4 weeks before screening, a history of asthma, and the presence of bronchiectasis or sequelae of tuberculosis. Patients with a comorbid condition that would affect the results of the study were also excluded. The study protocol was approved by the Asan Medical Center Institutional Review Board (IRB No. 2014-0298). The study was registered with the Clinical Research Information Service (CRIS) before patient enrollment began; CRIS is a not-for-profit online registration system for clinical researchers established by the Korean Centers for Disease Control and Prevention (registration No. KCT0001092). CRIS is part of the World Health Organization International Clinical Trials Registry Platform. All patients gave written informed consent.

RNA microarray analysis for gene expression

RNA microarray analysis was performed for 31,426 gene transcripts by a specialized company, Macrogen (Seoul, South Korea). The method for RNA preparation and processing is illustrated in the Supplementary file. In brief, the amplified and purified RNA was reverse-transcribed to cDNA, and labeled cRNA was hybridized to the human HT-12 expression version 4 bead array, according to the manufacturer's instructions (Illumina, Inc., San Diego, USA). The array signal was detected using Amersham Fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK). The arrays were scanned with an Illumina Bead Array Reader confocal scanner. Array data export processing and analysis were performed using Illumina BeadStudio, version 3.1.3 (Gene Expression Module, version

3.3.8). All data analysis and visualization of differentially expressed genes was conducted using R, version 2.4.1 (www.r-project.org).

DNA exome sequencing

The exome sequencing was also performed by Macrogen (Seoul, South Korea). DNA extracted from leukocytes were used for whole exome next-generation sequencing. The method for DNA preparation is illustrated in the Supplementary file. The qPCR Quantification Protocol Guide was used for quantifying the final purified product, and the exome sequencing was performed using the HiSeq™ 2500 platform (Illumina, San Diego, USA).

Damaged-gene score calculation methods

The damaged-gene score represents the overall impact of a nonsynonymous mutation in a gene, with lower scores indicating more damage in the molecular function of the gene. The damaged-gene score was calculated using the sorting intolerant from tolerant (SIFT) algorithm.[22] A filtering strategy based on rare (minor allele frequencies in 1,000 genome < 0.01) and damaged (SIFT threshold < 0.05) variants identified 6,464 genes, and the damaged-gene score was individually computed using the geometric mean of the SIFT score for nonsynonymous variants of the genes. The 1,000 Genome Data is a single-variation catalogue with allele frequencies in 2,504 healthy individuals, and the data have been used as a basis for filtering rare variants in several studies.[23] The SIFT score, which indicates the degree of damage of the individual variant, has already been shown in many studies to be indicative of the degree of damage to the gene.[24-26] The method of using the damaged-gene score has been used in a previous study on drug responsiveness to damaged genes.[27]

Analytic validation

Analytic validation was performed for RNA microarray and DNA exome sequencing by Quantitative TaqMan PCR and SNP Type Assay, respectively.

Quantitative TaqMan PCR

Gene expression profiles were validated by Quantitative TaqMan PCR. Validation was performed by Macrogen (Seoul, South Korea). From the genes showing a significant correlation between expression level and treatment response, we arbitrarily selected 28 (see Supplementary Table E1 for the full names of the selected genes), and endogenous controls were GAPDH and ACTB. The comparative Ct method was performed for relative quantification. Then we performed a Pearson correlation analysis to evaluate the accuracy of the RNA microarray results. The higher the correlation between the RNA microarray and quantitative TaqMAN PCR results, the more reliable the microarray results were considered to be. The medians (ranges) of the Pearson coefficients were 0.853 (0.792–0.921) and 0.835 (0.638–0.953) for the genes associated with the LABA treatment response and LAMA treatment response, respectively.

SNP Type Assay

Exome sequencing was validated using SNP Type Assay (Fluidigm, San Francisco, CA, USA). The SNP Type Assay sequencing was performed by a specialized company (DNA Link, Inc., Seoul, South Korea). Mutant alleles detected by whole exome next-generation sequencing and SNP Type Assay were compared for all genotypes of damaged variants of the 4 and 3 genes used in the prediction models for LABA and LAMA treatment, respectively. Genotype was classified as either homozygous or heterozygous in alleles. A total of 33 variants of genes used in the prediction model were found to be completely identical in the two methods.

Statistical analysis

In addition to age, the following 13 clinical traits were chosen for the analysis: body mass index, smoking status (ex-smoker versus current smoker), intensity of smoking (pack-years of cigarettes smoked), CAT score, mMRC dyspnea scale score, pre- and post-bronchodilator FEV_1 (% predicted value), bronchodilator reversibility to 200 μg of salbutamol (%predicted value), diffusing capacity (% of predicted value), total lung capacity (% of predicted value), ratio of inspiratory capacity to total lung capacity, residual volume (% of predicted value), and residual volume/total lung capacity. We reduced this number from 13 to 6, using a factor

analysis, as shown in Supplementary Table E2. Body mass index, intensity of cigarette smoking, CAT score, post-bronchodilator FEV_1, BDR, RV/TLC were chosen.

The association between gene expression level and treatment response (i.e., FEV_1 changes after 4 weeks of long-acting bronchodilator treatment) was assessed using a Pearson's correlation analysis, identifying the 28 genes with the highest correlation with improvement in FEV_1 values. The 28 genes were further assessed by linear regression models, leaving 7 and 9 genes that showed a statistically significant association with improvement in FEV_1 after the LABA and LAMA treatment, respectively. Factor analysis was then done to reduce the number of the genes, with 3 and 4 genes selected for the prediction model for LABA and LAMA treatment response, respectively (Supplementary Figure E1). The damaged-gene scores were also assessed using the same method, ultimately identifying 4 and 3 genes for the LABA and LAMA models, respectively (Supplementary Figure E2).

Multivariate linear regression analysis was performed to build models to predict changes in FEV_1 after treatment, using the aforementioned 6 clinical traits, plus age, level of gene expression, and damaged-gene scores. The prediction performance was assessed using the R^2 value.

SPSS, version 21 (IBM Corporation, Armonk, NY, USA), was used for statistical analysis.

Results

Baseline clinical traits

Of the 79 patients screened, 65 were included in the final analysis (Figure 2). There was only 1 female patient, and we decided not to include the female patient due to the possibility of confounding. The baseline clinical traits are shown in Table 1. The mean (±standard deviation) age was 67.4 (±8.2) years. Ex-smokers accounted for 69.2% of the patients, whereas the rest were current smokers. The mean number of pack-years was 41.9 (±19.2). More than three-quarters of participants (78.4%) reported grade 1 dyspnea. The mean CAT score was 9.9±6.2, and the mean post-bronchodilator FEV_1 was 69.8% of the predicted value. Thirty-three patients were randomized to receive the LAMA first, and the rest (32 patients) were given the LABA first.

Response to and compliance with bronchodilator treatment

Of all of the study patients, 34 showed a significant response (FEV_1 increase >100 ml) to the LABA and 33 patients to the LAMA. The mean ± SD change in FEV_1 after LABA and LAMA treatment was 118 ± 186 mL and 112 ± 192 mL, respectively. About one-quarter of patients (17/65) showed a significant treatment response to both types of bronchodilators. Conversely, 15 patients had no response to either type.

All 34 LABA responders had good compliance; of the non-responders, 3 patients (9.7%) were categorized as poor compliance, while the remaining 28 patients (90.3%) had good compliance. Of the 33 LAMA responders, 3 patients (9.1%) had poor compliance, while the remaining 30 patients (90.9%) had good compliance; all 32 non-responders had good compliance.

Correlation between clinical traits and FEV_1 changes after bronchodilator treatment

Age and the 6 clinical characteristics prespecified by the factor analysis were analyzed to evaluate potential correlations with FEV_1 changes following the 4-week treatment with long-acting bronchodilators (Table 2). FEV_1 changes after beta-2 agonist treatment were significantly correlated with BDR (R^2 = 0.396, p = 0.001), and were not statistically

significantly correlated with the other characteristics. No significant correlations with FEV_1 changes after muscarinic antagonist treatment were found.

Prediction models for long-acting bronchodilator treatment response using clinical traits

The clinical traits were used in the linear regression to build prediction models for the treatment response to the bronchodilators. Among the 6 models built for each bronchodilator, we chose one which consists of variables with p-value < 0.05. The model of post-bronchodilator FEV_1 and BDR could predict FEV_1 response after the LABA treatment with statistical significance (R^2 = 0.231, p < 0.00) (Table 3-A). Likewise, CAT score and BDR could predict FEV_1 response after the LAMA treatment (R^2 = 0.121, p = 0.021) (Table 3-B).

Prediction models for long-acting bronchodilator treatment response using gene expression and damaged-gene scores

Three gene expressions were identified as having significant correlation with FEV_1 changes after LABA treatment: CLN8, PCSK5, and SKP2. For LAMA treatment, 4 (C1orf115, PRKX, KIAA1618, and RHOQ) were found to be associated with significant changes in FEV_1 values. Supplementary Figure E3-A and E3-B shows the microarray results. The linear regression model built for the prediction of FEV_1 changes following treatment, using the gene expressions, is shown in Table 4. The R^2 of the models for LABA and LAMA treatment were 0.158 and 0.404, respectively.

The analysis of damaged-gene scores yielded 4 and 3 genes, respectively, for the LABA and LAMA models. EPG5, FNBP4, SCN10A, and SPTBN5 were found to be associated with prediction of LABA response, whereas FBN3, FDFT1, and ZBED6 were found to predict LAMA treatment response (Supplementary Figure E4-A and E4-B). They were used in the prediction models, as shown in Table 5 (R^2 = 0.318 and 0.199 for the LABA and LAMA treatment, respectively).

Prediction models for long-acting bronchodilator treatment response using gene expressions and damaged-gene scores in addition to clinical traits

The clinical traits, gene expressions, and damaged-gene scores were included together to

build the final prediction models for treatment response (i.e., 2 clinical and 7 genetic traits were all put together in the linear regression model) (Table 6). When the clinical traits, gene expressions, and damaged-gene scores were combined together in the prediction model, the predictability was highest (R^2 = 0.512 for LABA and 0.575 for LAMA treatment; both p-values < 0.001).

Discussion

In this multicenter, prospective study, we built prediction models for treatment response to long-acting bronchodilators using the pretreatment traits of the patients, before the treatment was initiated. In total, 2 clinical traits and 7 genes were found to be correlated with the FEV_1 changes and were included in the model. The clinical traits alone were not enough to predict the treatment response; however, when gene expressions and damaged-gene scores were added, the predictability increased.

In this study, the efficacy of the treatment, defined as FEV_1 changes after bronchodilator treatment, was similar to the results of previous studies.[28, 29] However, individual treatment response varied. About a quarter of the patients had a response to both bronchodilators, whereas the rest responded only to one or the other, or to none. Heterogeneity in the phenotypes of COPD patients is thought to be one of the reasons for the varied responsiveness to bronchodilator treatment,[13] and genetic variations in the beta-2 adrenergic receptors[30] and muscarinic receptors may play a role.[17] No conclusive measure exists to predict who will respond to one class or the other.

Bronchodilator reversibility was included in both of the final prediction models for the two types of treatment (Table 3-A and Table 3-B). It is reasonable to assume that long-acting beta-2 agonist treatment response is partially predicted by BDR, a response to the short-acting beta-2 agonist that acts on the same beta receptors to dilate the airways. Although anticholinergics act on different receptors (muscarinic receptors), BDR was also associated with the FEV_1 changes in response to the muscarinic antagonist treatment (Table 3-B). Previous reports showed signal interactions between muscarinic and adrenergic receptors, which might explain this.[31-33] Despite this theoretical background and the correlation shown in our study, however, bronchodilator reversibility was not enough to predict the treatment responses to long-acting bronchodilators.

Of note, CAT score was statistically significant in predicting the treatment response to a muscarinic antagonist in the multivariate analysis (Table 3-B). The mechanism of the relationship is unknown, because no previous studies have evaluated the relationship between CAT score and the treatment response to anticholinergic agents. One plausible

explanation is that one of the items included in the CAT evaluates the amount of phlegm, which is possibly an important indicator of increased cholinergic tone.[34] With respect to the symptoms, we further analyzed the relationship between long-acting bronchodilator treatment responses and the changes in the patients' symptoms, such as cough, dyspnea, sputum, and CAT score. A significant association was found between improvement in FEV_1 following the LAMA treatment and symptom improvement (i.e., CAT score change); FEV_1 change after LABA treatment did not show a significant association with symptom changes (Supplementary Table E3). This finding hints at an association between CAT score and LAMA treatment response, although the specific mechanisms are yet to be determined.

Bronchodilator reversibility to a short-acting beta agonist generally decreases as FEV_1 decreases,[16, 35] suggesting that lower lung function is related to the poorer bronchodilator response. However, post-bronchodilator FEV_1 at baseline was found to be inversely correlated with response to beta-2 agonist treatment in our study (Table 6). This may be due to our inclusion of only patients with moderate COPD and similar lung functioning. Moreover, there is a study that showed a similar result, in which higher pre-bronchodilator FEV_1 was related to lower bronchodilator reversibility in patients with COPD.[36]

Our study employed a novel approach by using gene analysis and clinical traits together to build a prediction model for long-acting bronchodilator treatment response. We used peripheral blood to investigate genetic traits. Sampling lung tissue is invasive and peripheral blood has been utilized as a noninvasive alternative.[37-40] The rationale for the use of peripheral blood is COPD is a systemic disease.[41] Moreover, some overlapping gene expressions were found between blood and lung tissue[37] or alveolar macrophage[38] in previous studies. Thus, we hypothesized that gene expressions and changes in the DNA sequencing identified from peripheral blood may reflect those of the lung. The genes found to be highly correlated with FEV_1 changes following treatment with bronchodilators in our study were rather unexpected. Although beta2-adrenergic receptor gene (ADRB2), for example, has been identified with genetic variations[18, 42, 43] and suggested to be one of the markers that partially explain variable responses to a short-acting beta agonist,[30] its role as the sole genetic determinant of a response to bronchodilators is not conclusive.[44, 45] Also, the

genetic effects on long-acting bronchodilator treatment responses have not been determined.[46] It is difficult to explain the association between the treatment response and the genes found in this study, since the biologic functions of some genes have not been confirmed. In addition, this result might have been derived by chance following the tests in which numerous genes were analyzed at once. Even though the true influence of these genes is unclear, there may be some plausible explanations. For example, SCN10A is known to encode proteins associated with voltage-gated sodium channels that are required in excitable cells including muscles.[47] Because bronchial smooth muscles constrict and relax in response to voltage changes, we may assume a damage in this gene can have a reduced bronchodilator response. RHOQ may have a role in trafficking of CFTR which is found in epithelial cells[48] and thought to be associated with thickness of mucus and airway clearance. Thus, expression of RHOQ may be related with airway clearance, explaining the association with the treatment response to a LAMA.

Clinical traits such as CAT scores and spirometry results are easy to obtain and are noninvasive. However, clinical traits were not enough to predict the treatment responses to long-acting bronchodilators (R^2 = 0.231 and 0.121, for the LABA and LAMA model, respectively). When only genetic data were used to build the prediction model, the predictability slightly increased. In the final model we built, using the 2 clinical traits, gene expressions, and damaged-gene scores together, the predictability was highest, at R^2 = 0.512 and 0.575 for the LABA and LAMA model, respectively. Given the recent advances in COPD genetics and the ongoing active research, our method of considering genetic data in addition to clinical traits may be a guide for further studies.

There are some limitations to our study. First, the results might have been influenced by the small number of included patients. However, some variables were demonstrated to be statistically significant, with a reasonable explanation, such as BDR. Second, as mentioned before, it is difficult to explain how the genes found in this study function as predictors of the treatment response. Nevertheless, the genes were found through the use of rigorous methods; professionals performed the RNA microarray and exome sequencing. Also, analytic validation was done to ensure the accuracy of the results. The specific roles of the genes remain a subject for further research. Third, the model did not

undergo clinical validation, and further studies with larger numbers of patients are needed.

In spite of the limitations, our study has value in that it is the first study that included the genetic traits as well as the clinical traits of the patients in building a prediction model for long-acting bronchodilator treatment responses. In addition, the gene analyses were performed by professionals with reliable accuracy, and the data underwent analytic validation. Although how the genes identified in this study are involved in COPD treatment responsiveness remains unknown, the prediction model may provide a guide for future research to incorporate genetic data and clinical traits in predicting the treatment response to long-acting bronchodilators.

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Table1. Baseline clinical characteristics of study patients

Traits	n = 65
Age, years, mean ± SD	67.4 ± 8.2
Smoking status	
Ex-smoker, n (%)	45 (69.2)
Current smoker, n (%)	20 (30.8)
Cigarette smoking, pack-years, mean ± SD	41.9 ± 19.2
mMRC dyspnea grade, n (%)	
0	2 (3.1)
1	51 (78.4)
2	10 (15.4)
3	2 (3.1)
COPD assessment test, total score, mean ± SD	9.9 ± 6.2
Body mass index, kg/m^2, mean ± SD	24.2 ± 2.6
Post-bronchodilator FEV_1, %pred., mean ± SD	69.8 ± 9.6
Bronchodilator reversibility[a], %pred. value, mean ± SD	4.5 ± 4.6
Diffusing capacity, %pred., mean ± SD	73.6 ± 16.1
Total lung capacity, %pred. value, mean ± SD	93.6 ± 14.2
RV/TLC, %, mean ± SD	35.1 ± 10.1

Abbreviations: SD, standard deviation; mMRC, modified Medical Research Council; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in one second; RV, residual volume; TLC, total lung capacity.

[a]Bronchodilator reversibility is defined by a FEV_1 change after inhalation of 200 μg salbutamol (%pred.)

Table 2. Pearson correlation between clinical characteristics and FEV_1 changes after 4-week of bronchodilator treatment

Clinical characteristics	FEV_1 changes after LABA		FEV_1 changes after LAMA	
	Correlation coefficient[a]	p-value	Correlation coefficient[a]	p-value
Age	0.012	0.926	-0.021	0.867
Body mass index	-0.027	0.830	0.084	0.510
Cigarette smoking, pack-years	0.005	0.970	-0.157	0.215
CAT score	-0.174	0.165	0.242	0.054
Post-bronchodilator FEV_1	-0.240	0.056	-0.128	0.318
Bronchodilator reversibility[b]	0.396	0.001	0.227	0.073
RV/TLC	0.199	0.113	0.126	0.321

Abbreviations: FEV_1, forced expiratory volume in one second; LABA, long-acting beta agonist; LAMA, long-acting muscarinic antagonist; CAT, chronic obstructive pulmonary disease assessment test; RV, residual volume; TLC, total lung capacity.

[a]Pearson correlation analysis

[b]Bronchodilator reversibility is defined by a FEV_1 change after inhalation of 200 ug salbutamol (%pred.)

Table 3-A. Linear regression models for predicting FEV_1 changes after LABA, using clinical traits

Models	Variables							R^2
1	Age	BMI	Intensity of smoking	CAT score	Post-BD FEV_1	BDR[a]	RV/TLC	0.281
2	Age		Intensity of smoking	CAT score	Post-BD FEV_1	BDR	RV/TLC	0.281
3	Age			CAT score	Post-BD FEV_1	BDR	RV/TLC	0.279
4	Age			CAT score	Post-BD FEV_1	BDR		0.268
5				CAT score	Post-BD FEV_1	BDR		0.261
6					Post-BD FEV_1	BDR		0.231

Abbreviations: FEV_1, forced expiratory volume in one second; LABA, long-acting beta-2 agonist; BMI, body mass index; CAT, chronic obstructive pulmonary disease assessment test; BD, bronchodilator; BDR, bronchodilator reversibility; RV, residual volume; TLC, total lung capacity.

[a]Bronchodilator reversibility is defined by a FEV_1 change after inhalation of 200 μg salbutamol (%pred.)

Table 3-B. Linear regression models for predicting FEV_1 changes after LAMA, using clinical traits

Models	Variables							R^2
1	Age	BMI	Intensity of smoking	CAT score	Post-BD FEV_1	BDR[a]	RV/TLC	0.205
2		BMI	Intensity of smoking	CAT score	Post-BD FEV_1	BDR	RV/TLC	0.202
3		BMI	Intensity of smoking	CAT score	Post-BD FEV_1	BDR		0.200
4		BMI		CAT score	Post-BD FEV_1	BDR		0.178
5		BMI		CAT score		BDR		0.158
6				CAT score		BDR		0.121

Abbreviations: FEV_1, forced expiratory volume in one second; LAMA, long-acting muscarinic antagonist; BMI, body mass index; CAT score, chronic obstructive pulmonary disease assessment test score; BD, bronchodilator; RV, residual volume; TLC, total lung capacity.

[a]Bronchodilator reversibility (BDR) is defined by a FEV_1 change after inhalation of 200 μg salbutamol (%pred.)

Table 4. Linear regression models for predicting FEV_1 changes after LABA and LAMA, using gene expressions

	LABA (R^2=0.158, p=0.018)			LAMA (R^2=0.404, p <0.001)	
	Regression coefficient	p-value		Regression coefficient	p-value
CLN8	-176.7	0.136	C1orf115	235.9	0.006
PCSK5	-247.4	0.045	KIAA1618	143.1	0.047
SKP2	188.6	0.176	PRKX	-320.3	0.014
			RHOQ	256.8	0.000

Abbreviations: FEV_1, forced expiratory volume in one second; LABA, long-acting beta-2 agonist; LAMA, long-acting muscarinic antagonist.

Table 5. Linear regression models for predicting FEV_1 changes after LABA and LAMA, using damaged-gene scores

	LABA (R^2=0.318, p=0.000)			LAMA (R^2=0.199, p=0.007)	
	Regression coefficient	p-value		Regression coefficient	p-value
EPG5	126.9	0.059	FBN3	−165.4	0.020
FNBP4	−135.7	0.051	FDFT1	122.0	0.123
SCN10A	−106.0	0.034	ZBED6	254.7	0.057
SPTBN5	−170.7	0.014			

Abbreviations: FEV_1, forced expiratory volume in one second; LABA, long-acting beta-2 agonist; LAMA, long-acting muscarinic antagonist.

Table 6. Linear regression models for predicting FEV_1 changes after LABA and LAMA, using clinical traits, gene expressions, and damaged-gene scores

	LABA (R^2=0.512, p=0.000)			LAMA (R^2=0.575, p=0.000)	
	Regression coefficient	p-value		Regression coefficient	p-value
Post-BD FEV_1	−4.9	0.031	CAT score	−2.3	0.602
BDR	9.4	0.058	BDR	6.8	0.224
CLN8	68.9	0.275	C1orf115	268.7	0.004
PCSK5	−104.6	0.136	KIAA1618	34.9	0.675
SKP2	−115.2	0.018	PRKX	−159.9	0.233
EPG5	−191.8	0.007	RHOQ	272.0	0.000
FNBP4	−97.3	0.352	FBN3	−162.0	0.016
SCN10A	−150.3	0.179	FDFT1	92.1	0.235
SPTBN5	145.5	0.256	ZBED6	274.5	0.014

Abbreviations: FEV_1, forced expiratory volume in one second; LABA, long-acting beta-2 agonist; LAMA, long-acting muscarinic antagonist; BD, bronchodilator; BDR, bronchodilator reversibility is defined by a FEV_1 change after inhalation of 200 μg salbutamol (%pred.); CAT, chronic obstructive pulmonary disease assessment test.

Figure 1. Study design

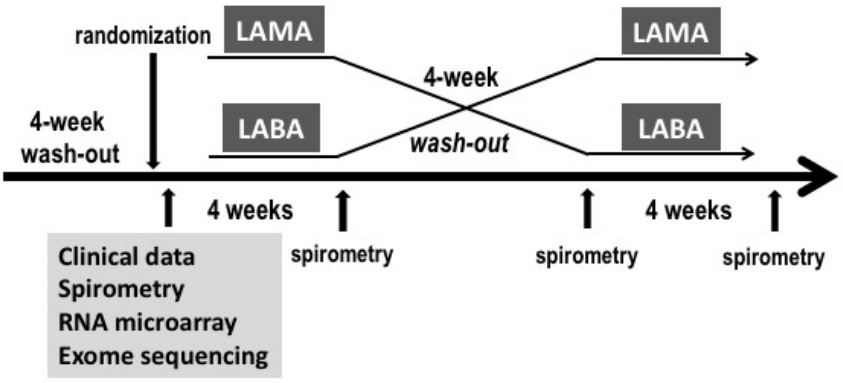
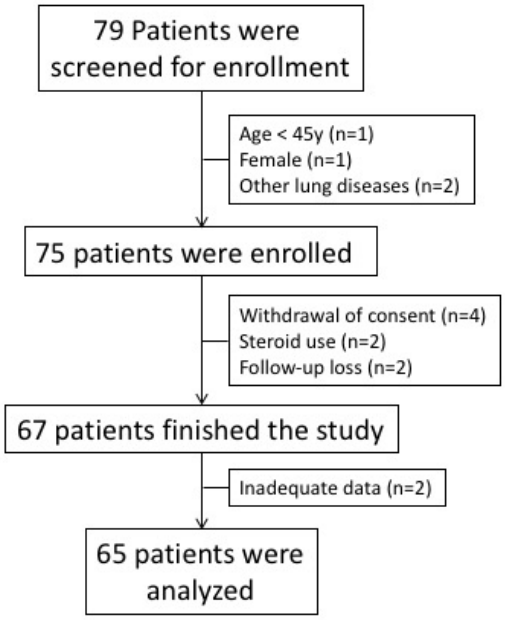


Figure 2. Study flow



Appendix

Method for RNA preparation and processing

The total RNA was amplified and purified using an Ambion Illumina RNA amplification kit (Ambion, Austin, USA) after extraction from leukocytes using Trizol (Invitrogen Life Technologies, Carlsbad, USA), to yield biotinylated cRNA according to the manufacturer's instructions. Of the total RNA, 550 ng was reverse-transcribed to cDNA using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, transcribed in vitro, and labeled with biotin-NTP. The cRNA was quantified using the ND-1000 Spectrophotometer (NanoDrop, Wilmington, USA), and 750 ng of labeled cRNA was hybridized to each human HT-12 expression version 4 bead array for 16–18 hours at 58°C, according to the manufacturer's instructions (Illumina, Inc., San Diego, USA). The array signal was detected using Amersham Fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK). The arrays were scanned with an Illumina Bead Array Reader confocal scanner. Array data export processing and analysis were performed using Illumina BeadStudio, version 3.1.3 (Gene Expression Module, version 3.3.8). All data analysis and visualization of differentially expressed genes was conducted using R, version 2.4.1 (www.r-project.org).

Method for DNA preparation and processing

DNA was extracted from leukocytes for whole exome next-generation sequencing. The Agilent SureSelect Target Enrichment protocol for Illumina paired-end sequencing library (version B.3, June 2015) was used together with 200 ng input formalin-fixed, paraffin-embedded DNA. In all cases, the SureSelect Human All Exon V5 probe set was used. The quantification of DNA and the quality of the DNA was measured by PicoGreen and Nanodrop. Fragmentation of 1 μg of genomic DNA was performed using adaptive focused acoustic technology (Covaris, Massachusetts, USA). The fragmented DNA was repaired, and Agilent adapters were then ligated to the fragments. The adapter-ligated product was PCR amplified. The final purified product was then quantified using qPCR according to the qPCR Quantification Protocol Guide, and qualified using the Caliper LabChip High Sensitivity DNA kit (PerkinElmer). For exome capture, 250 ng of the DNA library was mixed with hybridization buffers, blocking mixes, RNase block and 5 μl of SureSelect all exon capture library, according to the standard Agilent SureSelect Target Enrichment protocol. Hybridization to the capture baits was conducted at 65°C using the heated thermal cycler lid option at 105°C for 24 hours on the PCR machine. The captured DNA was then amplified.

Supplementary Table E1. Names of 28 genes selected for Quantitative TaqMan PCR

ACSF2	Acyl-CoA Synthetase Family Member 2
ADSS	Adenylosuccinate Synthase
AGPAT4	1-Acylglycerol-3-Phosphate O-Acyltransferase 4
ANKRD57	Ankyrin Repeat Domain 57
APTX	Aprataxin
ATP6AP1	ATPase H+ Transporting Accessory Protein 1
ATPAF1	ATP Synthase Mitochondrial F1 Complex Assembly Factor 1
ATRN	Attracting
BACE2	Beta-Site APP-Cleaving Enzyme 2
BLVRA	Biliverdin Reductase A
C1orf115	Chromosome 1 Open Reading Frame 115
C5orf37	Chromosome 5 Open Reading Frame 37
C9orf46	Chromosome 9 Open Reading Frame 46
CACNA2D2	Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 2
CISD1	CDGSH Iron Sulfur Domain 1
CLC	Charcot-Leyden Crystal Galectin
CLN8	CLN8, Transmembrane ER And ERGIC Protein
DDRGK1	DDRGK Domain Containing 1
DTD1	D-Tyrosyl-TRNA Deacylase 1
EIF2AK2	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 2
EPG5	Ectopic P-Granules Autophagy Protein 5 Homolog
F2R	Coagulation Factor II Thrombin Receptor
FBN3	Fibrillin 3
FDFT1	Farnesyl-Diphosphate Farnesyltransferase 1
FNBP4	Formin Binding Protein 4
FOLR3	Folate Receptor 3
FOXK1	Forkhead Box K1
IFITM3	Interferon Induced Transmembrane Protein 3

KIAA1618 (RNF213)	Ring Finger Protein 213
LRRCC1	Leucine Rich Repeat And Coiled-Coil Centrosomal Protein 1
PCSK5	Proprotein Convertase Subtilisin/Kexin Type 5
PRKX	Protein Kinase, X-Linked
RHOQ	Ras Homolog Family Member Q
SCN10A	Sodium Voltage-Gated Channel Alpha Subunit 10
SKP2	S-Phase Kinase Associated Protein 2
SPTBN5	Spectrin Beta, Non-Erythrocytic 5
TNRC6A	Trinucleotide Repeat Containing 6A
UHMK1	U2AF Homology Motif Kinase 1
ZBED6	Zinc Finger BED-Type Containing 6

Supplementary Table E2. Result of a factor analysis

	Factor					
	1	2	3	4	5	6
Body mass index	-0.09	0.35	**0.66**	0.32	0.01	0.15
Smoking status (ex-smoker vs. current smoker)	0.13	0.17	-0.47	0.55	-0.32	0.23
Amount of cigarette smoking	0.24	0.28	0.20	0.45	-0.30	**-0.47**
CAT score	0.20	0.09	-0.41	0.12	**0.63**	0.40
mMRC dyspnea scale	0.19	-0.25	0.43	-0.23	0.43	-0.27
FEV_1 %pred.	-0.70	0.61	-0.16	-0.09	0.18	-0.23
Post-bronchodilator FEV_1 %pred.	-0.59	**0.65**	-0.12	-0.39	-0.04	-0.08
Bronchodilator response %pred.	0.35	-0.01	0.11	**-0.60**	-0.48	0.35
Diffusing capacity %pred.	-0.32	0.48	0.36	0.19	0.15	0.32
Total lung capacity %pred.	0.57	0.67	-0.20	-0.24	-0.03	-0.12
IC/TLC	-0.58	-0.03	0.28	-0.01	-0.15	0.46
Residual volume %pred.	0.85	0.42	0.13	-0.05	0.03	0.12
RV/TLC	**0.86**	0.22	0.15	0.01	0.15	0.09

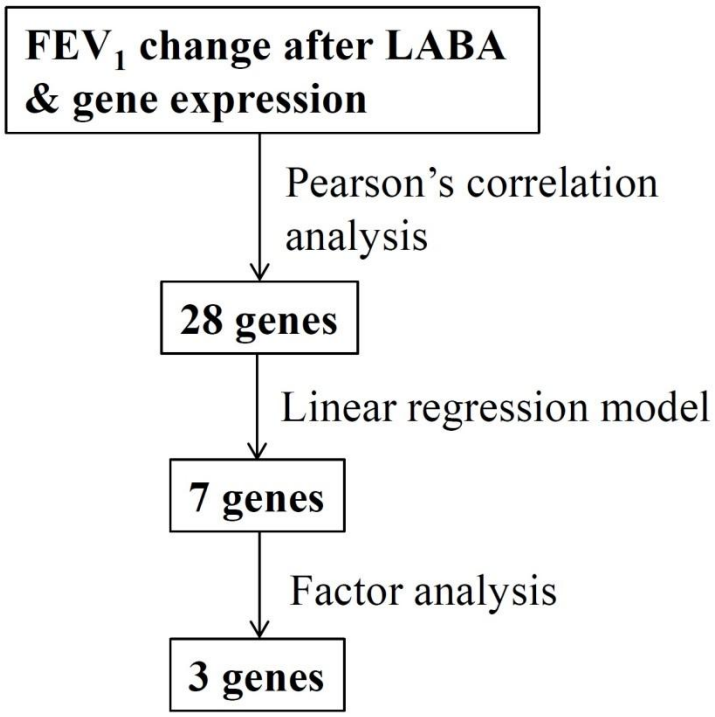
Abbreviations: CAT, chronic obstructive pulmonary disease assessment test; mMRC, modified Medical Research Council, FEV_1, forced expiratory volume in one second; IC, inspiratory capacity; TLC, total lung capacity; RV, residual volume.

Supplementary Table E3. Pearson correlation analysis between changes of symptoms and FEV1 changes after 4-week long-acting bronchodilator treatment

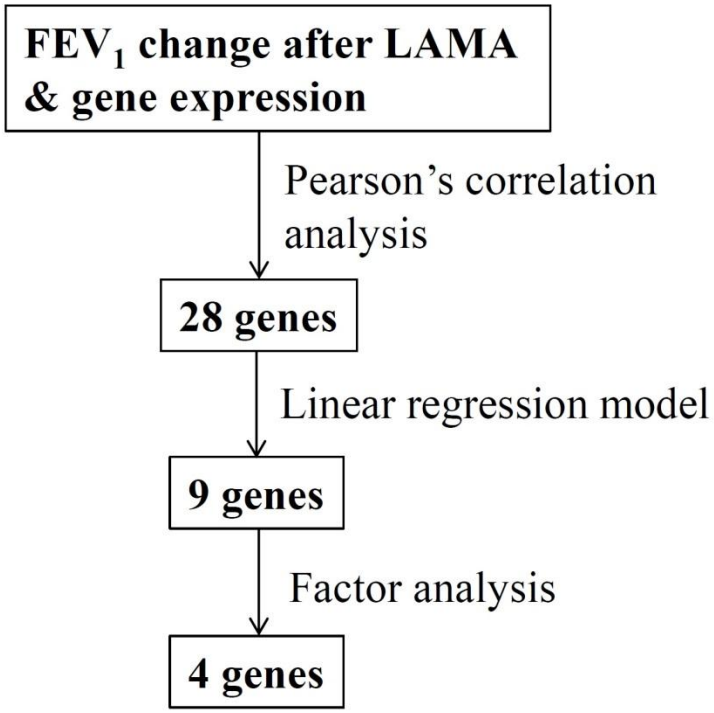
	FEV_1 change after LABA (mL)		FEV_1 change after LAMA (mL)	
	R	p-value	R	p-value
CAT score change[a]	-0.070	0.580	-0.322	0.009
Cough (VAS) change[a]	-0.014	0.914	-0.285	0.023
Sputum (VAS) change[a]	0.013	0.918	-0.142	0.262
Dyspnea (Borg) change[a]	0.020	0.872	0.052	0.682
Dyspnea-12 change[a]	-0.110	0.382	-0.063	0.620

[a]Change was calculated by a score after the bronchodilator treatment – a score before the bronchodilator treatment.

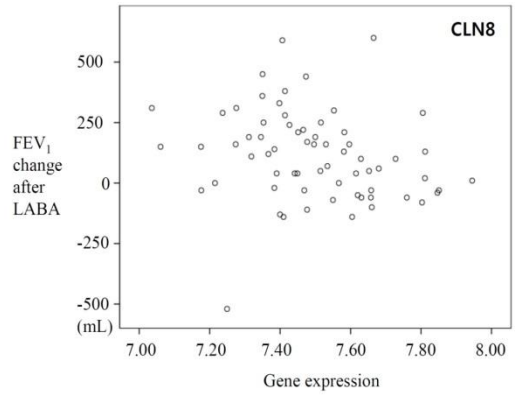
Supplementary Figure E1.

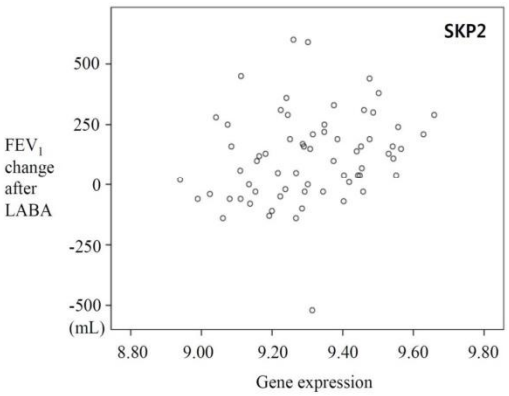


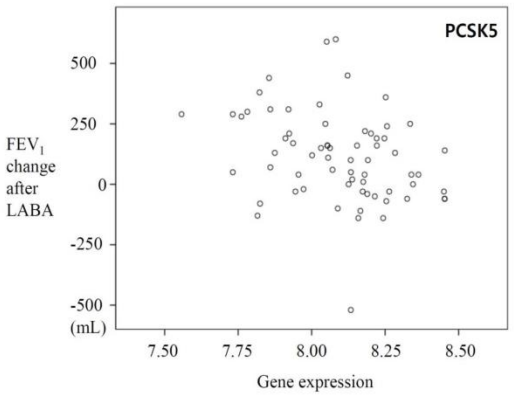
Supplementary Figure E2.



Supplementary Figure E3-A.

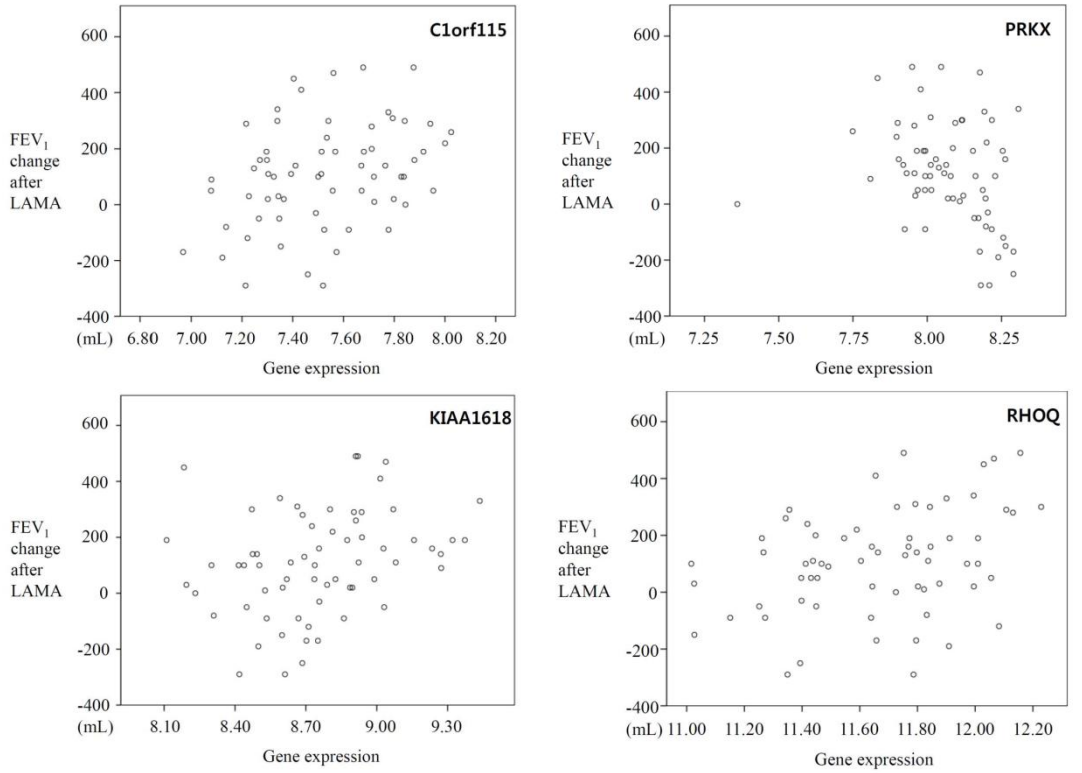






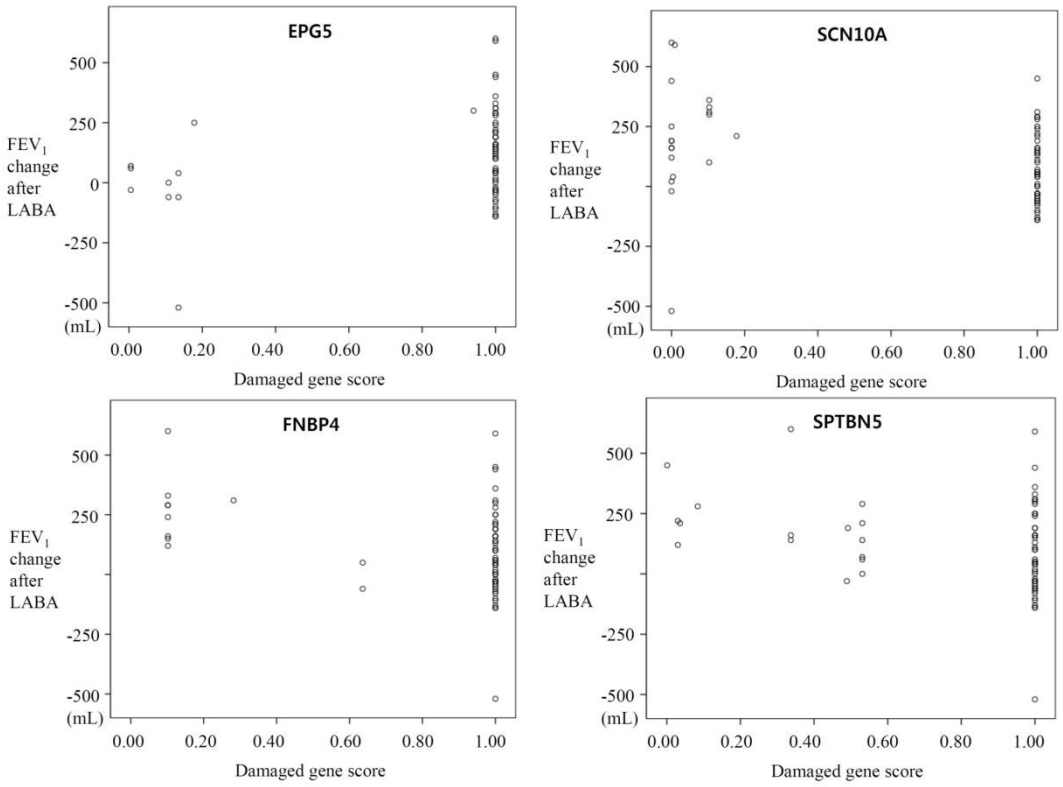
$\Delta FEV_1 = -176.7(CLN8) - 247.4(PCSK5) + 188.6(SKP2)$

Supplementary Figure E3-B.



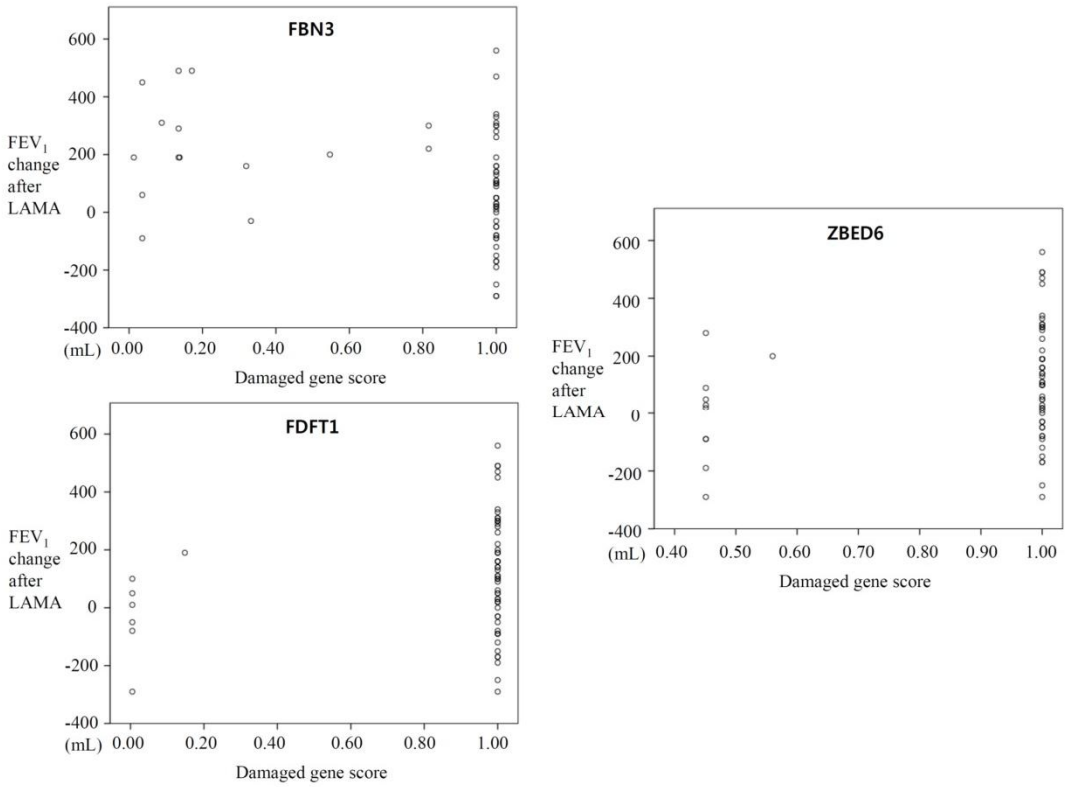
$\Delta FEV_1 = 235.9(C1orf115) + 143.1(KIAA1618) - 320.3(PRKX) + 256.8(RHOQ)$

Supplementary Figure E4-A.



$\triangle FEV_1$= 126.9(EPG5) –135.7(FNBP4) –106.0(SCN10A) –170.7(SPTBN5)

Supplementary Figure E4-B.



$$\Delta FEV_1 = -165.4(FBN3) + 122.0(FDFT1) + 254.7(ZBED6)$$

국문요약

만성폐쇄성폐질환 환자에서 기관지 확장제에 대한 치료 반응 예측모델

만성폐쇄성폐질환의 가장 중요한 치료제는 기관지 확장제이다. 어떤 특성을 가진 환자가 어떤 기관지 확장제에 더 잘 반응할 것인지 예측할 수 있는 방법은 현재까지 알려진 것이 없다. 이 연구에서는 기관지 확장제에 대한 치료 반응을 예측하는 모델을 만들고자 하였다.

이 연구는 안정시 만성폐쇄성폐질환 환자 65 명에게 4 주간 흡입지속성베타-2 작용제(LABA)와 흡입지속성항콜린제(LAMA)를 무작위 순서로 각각 4 주간 투여한 전향적 연구로서, 각 약제 사이에는 4 주간의 약제 세척기를 두었다. 환자들의 임상 특성과 31,426 개 유전자의 발현 정보, 6,464 유전자의 damaged-gene score 를 분석하였다. 모델을 만드는 데는 선형회귀분석을 사용하였다.

LABA 에 대한 치료반응을 예측하는 임상 특성으로는 기관지 확장제 투여후 FEV_1 과 가역성 기류제한(bronchodilator reversibility)이 확인되었다. 이에 더해 3 유전자의 발현 정도(CLN8, PCSK5, SKP2) 및 4 유전자의 damaged-gene score (EPG5, FNBP4, SCN10A, SPTBN5)가 모델에 포함되어 R^2 = 0.512 ($p < 0.001$)를 보여주었다. LAMA 의 경우 임상특성으로는 COPD assessment test score 와 가역성 기류제한이 임상특성으로, 그리고 4 유전자의 발현 정도(C1orf115, KIAA1618, PRKX, RHOQ) 및 3 유전자의 damaged-gene score (FBN3, FDFT1, ZBED6)가 모델에 포함되어 R^2 = 0.575 ($p < 0.001$)를 보여주었다. 임상 특성만으로 모델을 만들었을 때는, LABA 모델의 경우 R^2 = 0.231, LAMA 모델의 경우 R^2 = 0.121 로 예측력이 약했다.

임상 특성에 유전자 발현 정도와 damaged gene score 를 추가하는 것이 기관지 확장제에 대한 치료 반응을 예측하는 데 있어서 도움을 준다.