Doctor of Philosophy

Study about novel treatment strategy for emphysema utilizing gut-lung axis and microbiome technique in the view of fecal microbiota transplantation and probiotics

분변미생물이식 및 프로바이오틱스 관점에서 마이크로바이옴 기술을 활용한 폐기종의 새로운 치료 전략 모색에 대한 연구

The Graduate School

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Study about novel treatment strategy for emphysema utilizing gut-lung axis and microbiome technique in the view of fecal microbiota transplantation and probiotics

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Study about novel treatment strategy for emphysema utilizing gut-lung axis and microbiome technique in the view of fecal microbiota transplantation and probiotics

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is manageable, but incurable disease by the standard treatment based on the current practice. As microbiome modulation methods, probiotics administration and fecal microbiota transplantation (FMT) has showed the clinical potentials in various diseases. Therefore, we aimed to investigate the beneficial effect of FMT and specific bacteria administration as probiotics on the emphysema development.

Methods: We investigated the clinical characteristics, microbiome, and metabolome in eligible smokers with significant smoking history, defined by more than 30 pack*year smoking history. Sputum and fecal samples were collected for microbiome and metabolome analysis and FMT. The pharmaceutical meta-analytical screening (PMAS) system, which mimicked the intestinal environment, was utilized to screen the candidate bacteria. An emphysema mouse model was used to investigate the effect of FMT and administration of candidate bacteria.

Results: Seventy-eight eligible smokers were classified into 19 smokers in non-emphysema, 26 smokers in non-severe emphysema, and 33 smokers in severe emphysema groups. Three groups presented distinct microbiome and metabolome characteristics. In an emphysema mouse model, FMT from non-emphysema and non-severe emphysema group decreased the emphysematous change compared to smoking only group. Administration of HEM20792, which increases the acetic acid and propionic acid levels in the PMAS system, also attenuated emphysematous change compared to smoking only group.

Conclusions: FMT from non-emphysema and non-severe emphysema groups and administration of HEM20792 as probiotics attenuated the emphysema development and showed clinical potential as novel treatment strategy for emphysema utilizing gut-lung axis and microbiome technique.

Keywords: Emphysema, Microbiome, Fecal microbiota transplantation, Probiotics

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive and irreversible airflow limitation caused by interaction among genetic and environmental factors and one of the high burden diseases in the world [1]. The mainstay of treatment for COPD has been using the bronchodilator inhaler [2]. However, efficacy of bronchodilator inhalers is limited and do not change the disease course. Therefore, aims of COPD treatment in international guidelines are to reduce symptoms and risk of exacerbation, not prevent COPD development and restore lung function [1,3]. This limitation has highlighted the need of studies for alternative treatment methods.

The interactions between the host and microbiome could have effect on the immunomodulation and various organs in human [4,5]. There is sufficient evidence that dysbiosis in gut microbiome could be related to various diseases [6-8]. In this point, the microbiome has gained attention as a fascinating therapeutic alternative option that have potential to complement standard treatments for various chronic diseases including lung disease, as evidenced by recent studies [9,10].

After the emergence of the concept of the gut-lung axis, the role of the gut microbiome in respiratory diseases is also receiving attention and being actively studied [11]. Several studies have reported that the gut microbiome may influence the development and course of chronic respiratory diseases through various mediators, although there is no firm evidence related to clear mechanism about gut-lung axis [12-14]. In this context, the therapeutic application of gut microbiomes modulation for chronic respiratory diseases is considered as a suitable option to overcome the current limitations of respiratory disease treatments. In the previous our studies, high fiber diet modification, as prebiotics, and short chain fatty acids administration, as postbiotics, attenuated emphysema development in the mice [15,16]. If we identify the beneficial effects of specific bacteria species that mitigates the development of emphysema, this result implies the clinical potential of gut microbiome modulation in the view of probiotics aspects.

We tried to screen the potentially beneficial bacteria species and to investigate the beneficial effects against smoking exposure in this study. For this aim, the microbiome and metabolome among non-emphysema, non-severe emphysema and severe emphysema groups

were compared. After that, fecal materials from each group of patients were transplanted into germ-free mice to investigate the impact of gut microbiome modulation on the development of emphysema. Finally, we aim to select bacterial species that most closely reproduced the short chain fatty acids (SCFAs) level change after fecal microbiota transplantation (FMT) and transplant them into emphysema mouse model to investigate the effects on the development of emphysema.

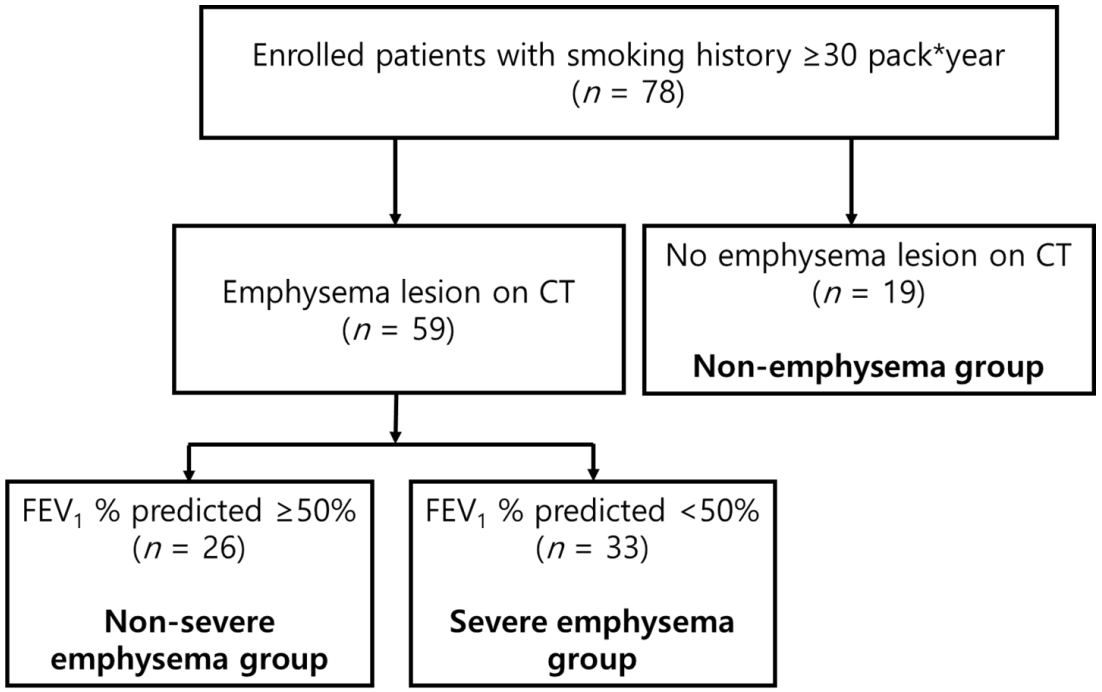
Results

1. Characteristics of smokers with/without emphysema

We recruited 78 smokers with a smoking history of 30 pack-years or more (**Figure 1**). We firstly classified smokers according to existence of emphysematous lesions on CT scans. Among smokers with emphysema, we classified them into severe emphysema group and non-severe emphysema group based on the forced expiratory volume in 1 sec (FEV_1) % predicted value of 50% as the criterion. 78 smokers were finally classified into non-emphysema group (n = 19), non-severe emphysema group (n = 26), and severe emphysema group (n = 33). **Table 1** presented baseline characteristics of study populations. There was no difference of age, male proportion, smoking amount, and COPD exacerbation history in the previous year. However, patients in severe emphysema group presented the significant lower body mass index, results of pulmonary function test, and COPD assessment test score, compared to the other groups.

Study population donated fecal samples for this study, and we measured the levels of six SCFAs (acetic acid, butyric acid, isobutyric acid, isovaleric acid, propionic acid and valeric acid) from the fecal samples. **Table 2** showed fecal SCFA levels in each group. We found that acetic acid level was significantly decreased in non-severe (53.03 ± 23.16 umol/g) and severe emphysema groups (60.37 ± 20.42 umol/g) compared to non-emphysema group (71.01 ± 18.10 umol/g, P = 0.006). Although there was no statistical significance, propionic acid and butyric acid were also lower in non-severe and severe emphysema groups than non-emphysema group (P = 0.059 in propionic acid and P = 0.056 in butyric acid).

Figure 1. Flowchart of 78 patients' classification



Abbreviation: *CT*, computed tomography; *FEV_1*, forced expiratory volume in 1 sec

Table 1. Baseline characteristics of study participants

	Total (n = 78)	Non-emphysema (n = 19)	Non-severe emphysema (n = 26)	Severe emphysema (n = 33)	P value
Male, n (%)	72 (92.3%)	18 (94.7%)	24 (92.3%)	30 (90.9%)	0.883
Age, years	66.1 ± 6.1	64.6 ± 6.1	66.9 ± 6.2	66.3 ± 6.1	0.419
Body mass index, kg/m^2	22.6 ± 3.4	24.9 ± 2.8	23.9 ± 3.2	20.3 ± 2.5	<0.001
Current smoker, n (%)	67 (85.9%)	12 (63.2%)	23 (88.5%)	32 (97.0%)	0.003
Smoking amount, pack*years	42.3 ± 16.4	43.2 ± 17.2	40.8 ± 10.9	43.0 ± 19.7	0.761
Exacerbation in the previous year	6 (7.7%)	0 (0.0%)	2 (13.3%)	4 (26.7%)	0.112
COPD assessment test score	15.8 ± 9.1	9.7 ± 4.3	14.5 ± 9.8	23.2 ± 6.7	<0.001
Pulmonary function test					
FEV_1 measured, L	1.91± 0.98	2.78 ± 0.54	2.54 ± 0.59	0.92 ± 0.28	<0.001
FEV_1 % predicted	61.7 ± 29.4	87.1 ± 13.2	82.4 ± 15.9	30.8 ± 8.2	<0.001
FVC measured, L	3.60 ± 0.76	3.92 ± 0.57	4.01 ± 0.68	3.08 ± 0.61	<0.001
FVC % predicted	85.4 ± 14.2	89.3 ± 10.0	93.9 ± 13.2	76.5 ± 12.0	<0.001
FEV_1/FVC	51.1 ± 20.3	70.9 ± 7.9	63.5 ± 10.3	29.9 ± 7.0	<0.001
DL_{CO} measured, mL/min/mmHg	12.2 ± 6.3	18.0 ± 3.6	15.3 ± 4.8	6.2 ± 2.6	<0.001
DL_{CO} % predicted	57.7 ± 28.0	82.9 ± 14.1	72.2 ± 20.7	31.0 ± 12.8	<0.001

Data are presented as mean ± standard deviation or number (%). The Kruskal-Wallis test for the continuous variables and the χ^2 for the categorical variables were used for analysis.

Abbreviation: *COPD* chronic obstructive pulmonary disease; *DLco* carbon monoxide diffusion in the lung; *FEV_1* forced expiratory volume in 1 sec; *FVC* forced vital capacity

Table 2. Fecal short chain fatty acid levels in study population

	Non-emphysema (*n* = 19)	Non-severe emphysema (*n* = 26)	Severe emphysema (*n* = 33)	*P* value
Acetic acid (umol/g)	71.01 ± 18.10	53.03 ± 23.16	60.37 ± 20.42	0.006
Propionic acid (umol/g)	18.59 ± 5.72	16.76 ± 14.63	15.55 ± 7.01	0.059
Isobutyric acid (umol/g)	1.46 ± 0.98	1.58 ± 0.98	1.61 ±1.06	0.871
Butyric acid (umol/g)	13.51 ± 5.33	9.87 ± 4.27	10.66 ± 5.59	0.056
Isovaleric acid (umol/g)	2.05 ± 1.83	2.39 ± 1.87	2.49 ± 1.84	0.558
Valeric acid (umol/g)	1.79 ± 1.19	1.84 ± 1.36	1.88 ± 1.63	0.971

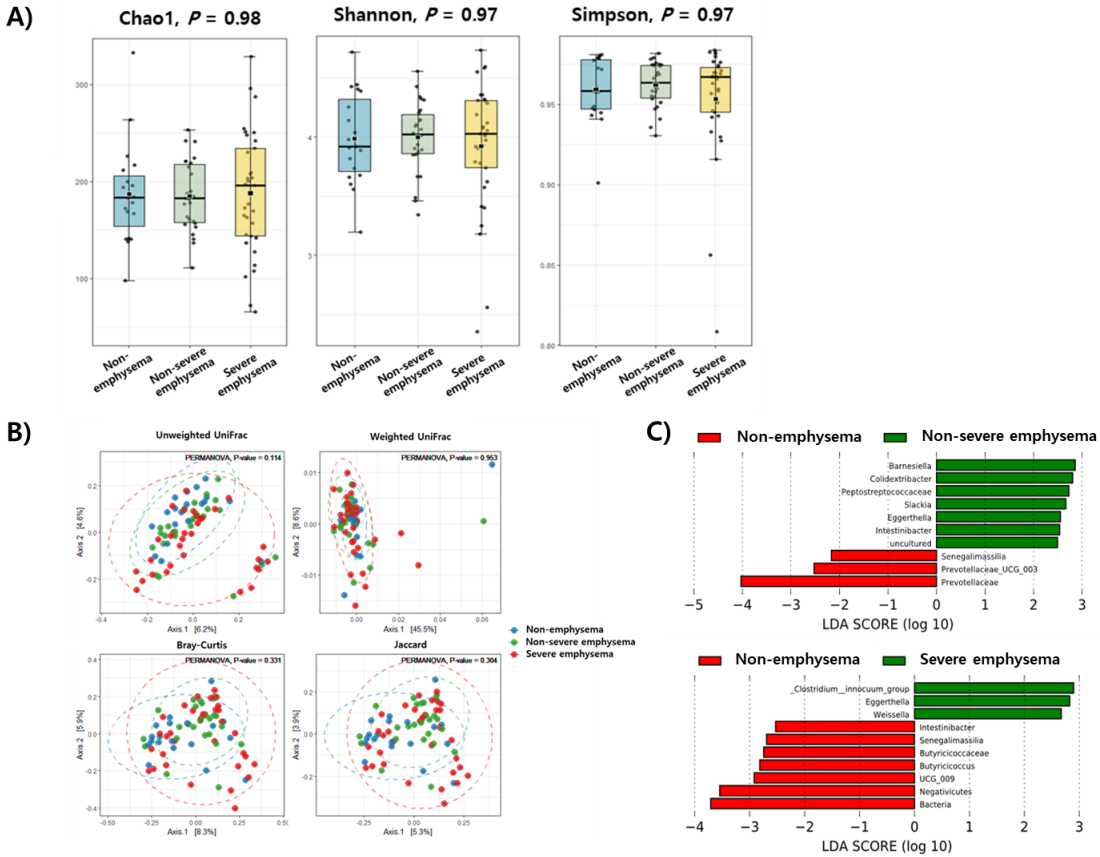
Data are presented as mean ± standard deviation. The Kruskal-Wallis test was used for analysis.

2. Microbiome analysis of study population

Figure 2 describes the characteristics of gut microbiome of three groups. Three groups did not show significant differences in the alpha-diversity indices such as the Chao1, Shannon, and Simpson Index and beta-diversity indices such as the unweighted UniFrac, weighted UniFrac, Bray-Curtis, and Jaccard Index (**Figure 2-A, B**). The principal coordinates analysis (PCoA) plots are presented in **Figure 2-B**. The linear discriminant analysis (LDA) effect size method investigated the bacterial groups that were differentially abundant, defined by LDA Score of >2.0, between the two groups (**Figure 2-C**). In non-severe emphysema group, seven bacteria, including *Barnesiella*, *Colidextribacter* and *Peptostreptococcaceae*, were enriched compared to non-emphysema group. On the contrary, *Senegalimassilia* and *Prevotellaceae* were enriched in non-emphysema group. In severe emphysema group, *Clostridium*, *Eggerthella*, and *Weissella* were abundant compared to non-emphysema group. Non-emphysema group presented significant enrichment of *Intestinibacter*, *Senegalimassilia* and *Butyricicoccaceae*. Even with similar smoking amount, these results implied that the individual microbiome characteristics could have effect on the emphysema development and the degree of pulmonary function impairment.

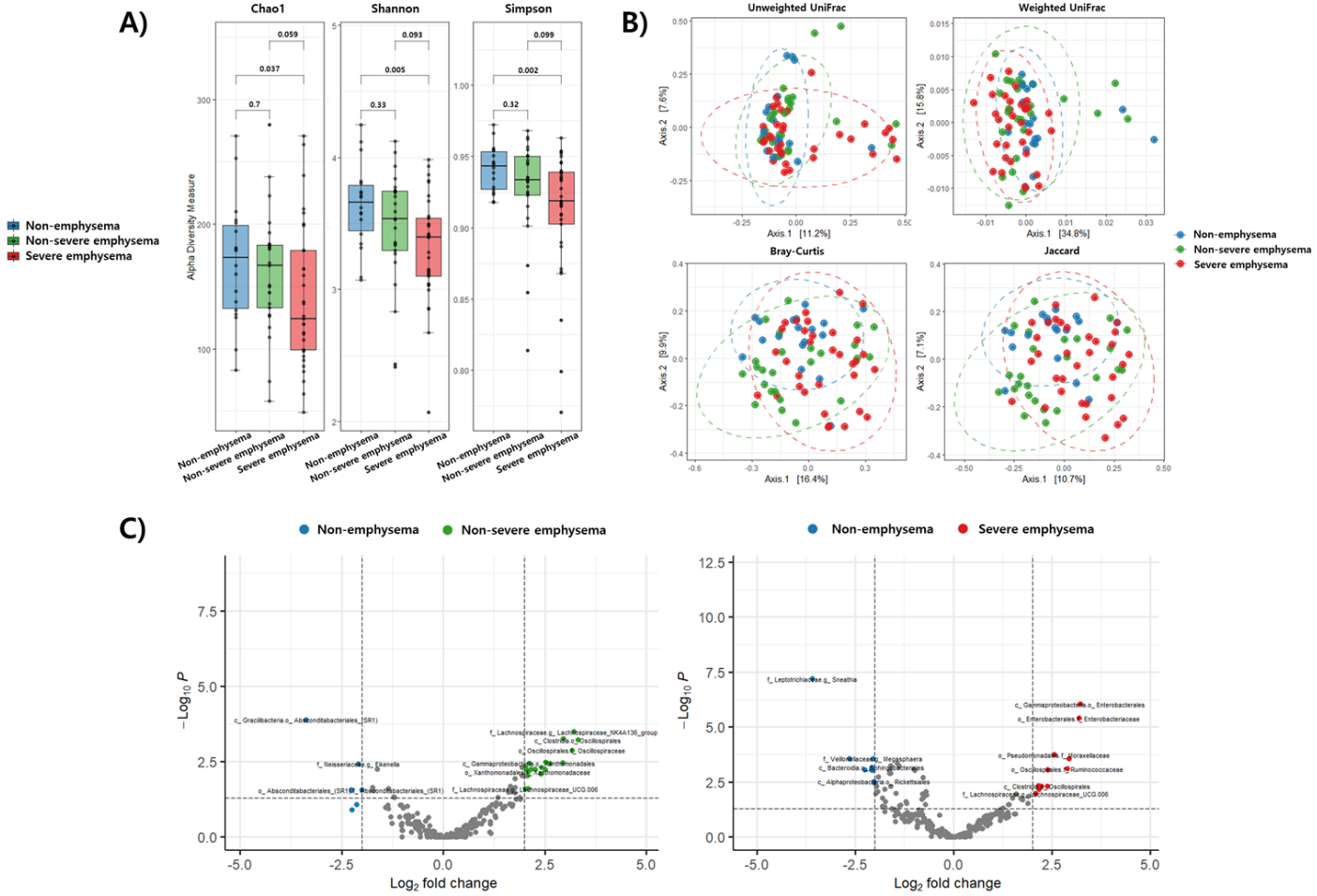
Figure 3 showed the characteristics of sputum microbiome of three groups. The alpha-diversity of severe emphysema group was significantly decreased compared to non-emphysema group (**Figure 3-A**). The beta diversity was presented in **Figure 3-B.** Compared to non-emphysema group, non-severe and severe emphysema group showed the significant difference in Bray-Curtis and Jaccard analysis. The bacterial species, which showed the significant difference compared to non-emphysema group, in non-severe and severe emphysema group in **Figure 3-C** (Volcano plot).

Figure 2. Characteristics of gut microbiota of three groups.



(A) Boxplots of alpha-diversity index (Chao 1, Shannon, and Simpson index) (B) Beta diversity (PCoAs of unweighted UniFrac, weighted UniFrac, Bray-Curtis, and Jaccard index) (C) Linear discriminant analysis effect size method for microbiome analysis.

Figure 3. Characteristics of sputum microbiota of three groups.



(A) Boxplots of alpha-diversity index (Chao 1, Shannon, and Simpson Index) (B) Beta diversity (PCoAs of unweighted UniFrac, weighted UniFrac, Bray-Curtis, and Jaccard index) (C) Volcano plot shows differential abundance, non-emphysema-enriched as blue dots, non-severe emphysema-enriched as green dots, and severe emphysema enriched as red dots.

3. Emphysema development after fecal microbiota transplantation from distinct donors

We conducted the first experiments to investigate the impact of fecal microbiome from each group on the development of emphysema, by utilizing FMT from two representative subjects in each group into germ-free emphysema mouse models. We selected two representative subjects in the view of the microbiome analysis. Each representative subject is indicated in **Figure 2-B**. In **Table 3**, clinical characteristics of fecal donors are presented.

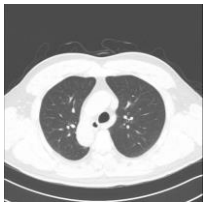
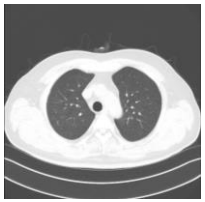
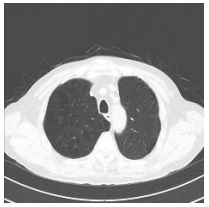
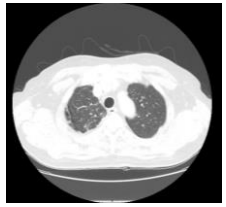
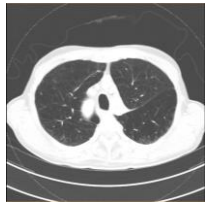
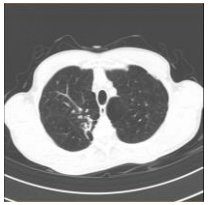
Figure 4-A illustrated the process of the experiment. Mice were classified into five groups. Mice in the control group was exposed to filtered air. Smoking exposed groups were further divided into four groups. Smoking only group was exposed to smoking without FMT. Mice in the non-emphysema, non-severe emphysema, and severe emphysema groups were exposed to smoking and receiving FMT from representative donors of each group. Fresh feces from each donor were transplanted into a germ-free mouse through oral gavage at one-week intervals, a total of three times. Germ-free mice were exposed to smoking or filtered air for 4 weeks. Compared to control group, the relative body weight of the smoking exposed groups did not present constant increase during the study period (**Figure 4-B**). Smoking exposure is known to result in emphysematous change, measured by the mean linear intercept (MLI) indicating the mean interalveolar septal wall distance. **Figure 4-C** illustrated increased MLI in smoking exposed groups than control group. Compared to germ-free mice in smoking only group, germ-free mice in non-emphysema and non-severe emphysema group showed significantly decreased MLI, not in severe emphysema group. In pathology, lung parenchymal structure was concordant with MLI (**Figure 4-D**). Within smoking exposed groups, inflammatory cell infiltration had a trend to be increased in the bronchoalveolar lavage fluid (BALF) compared to control group (**Figure 4-E**). However, this trend was attenuated after FMT from subjects, especially in severe emphysema group. The mRNA expression of proinflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interferon gamma (IFN)-γ were elevated in smoking exposed groups compared to control group (**Figure 4-F**). However, each mediator presented different response to FMT from each group. T cell proportion, indicated by $CD3^+$, were increased. Furthermore, the number of $CD4^+IL\text{-}17^+IFN\text{-}\gamma^-$, $CD4^+IL\text{-}17^-IFN\text{-}\gamma^+$ and $CD8^+IL\text{-}17^-IFN\text{-}\gamma^+$ were significantly higher in the

lung tissue of the severe emphysema group compared with the control group, and the latter two T cell types were elevated compared to the smoking only group (**Figure 4-G**). The protein levels of TNF-α and IL-1β were decreased in the BALF in the non-emphysema group compared with the smoking only group (**Figure 4-H**). On the contrary, IL-4 and IL-12p70 level were elevated in non-emphysema group compared to smoking only group.

Figure 5 showed the characteristics of fecal microbiome of FMT experiment. The alpha-diversity group was significantly different between non-emphysema and severe emphysema groups (**Figure 5-A**). **Figure 5-B** illustrated change of the fecal microbiome beta diversity of human, germ-free mouse before and after FMT. The bacterial species, which showed the significant difference compared to non-emphysema group, in non-severe and severe emphysema group in **Figure 5-C** (Volcano plot).

The SCFAs levels in the fecal samples of germ-free mice after smoking were presented in **Figure 6**. Acetic acid level was lower in severe emphysema group than non-emphysema group. Propionic acid and butyric acid did not show the significant difference among groups.

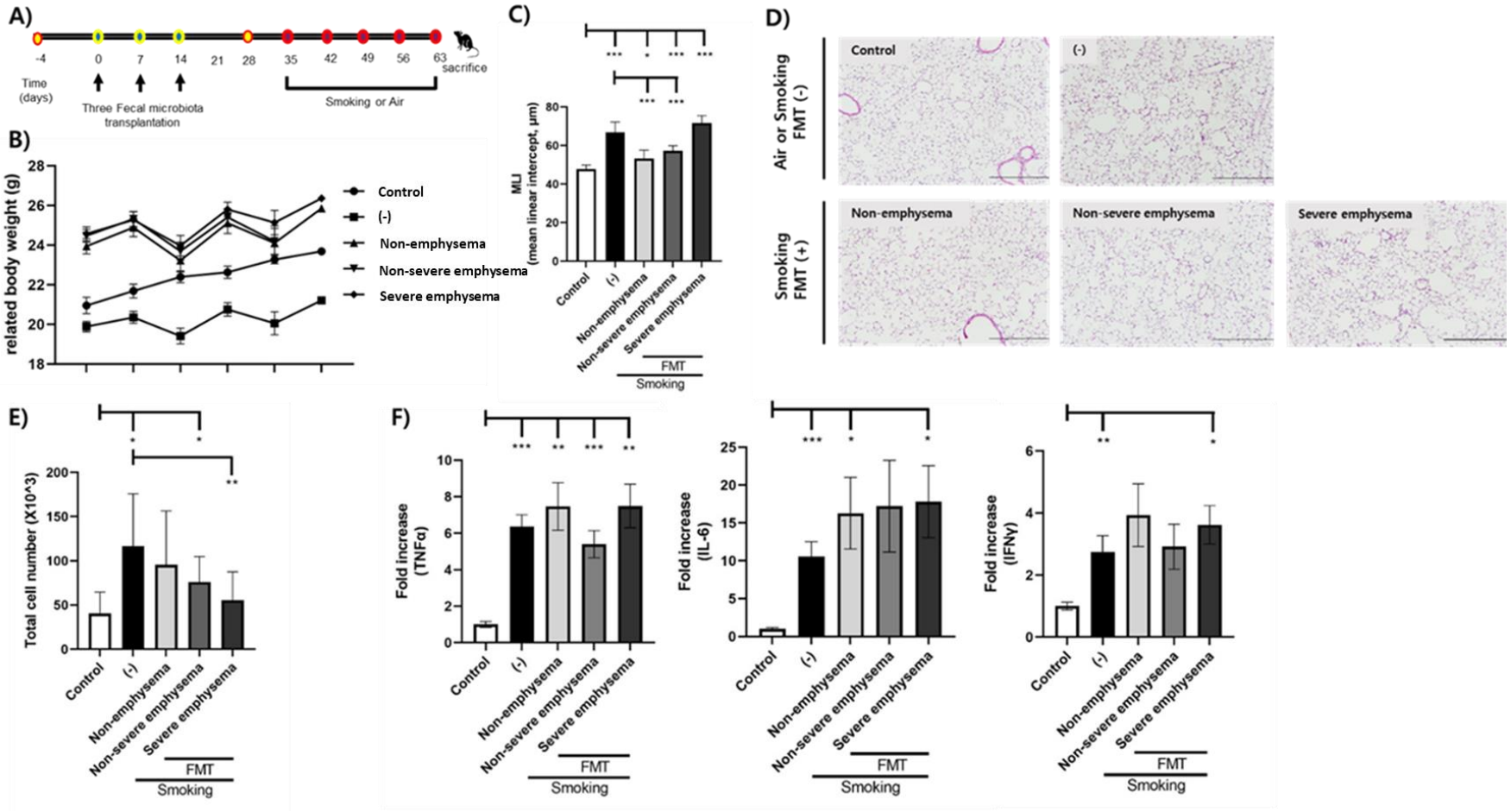
Table 3. Clinical characteristics of donors for fecal microbiota transplantation

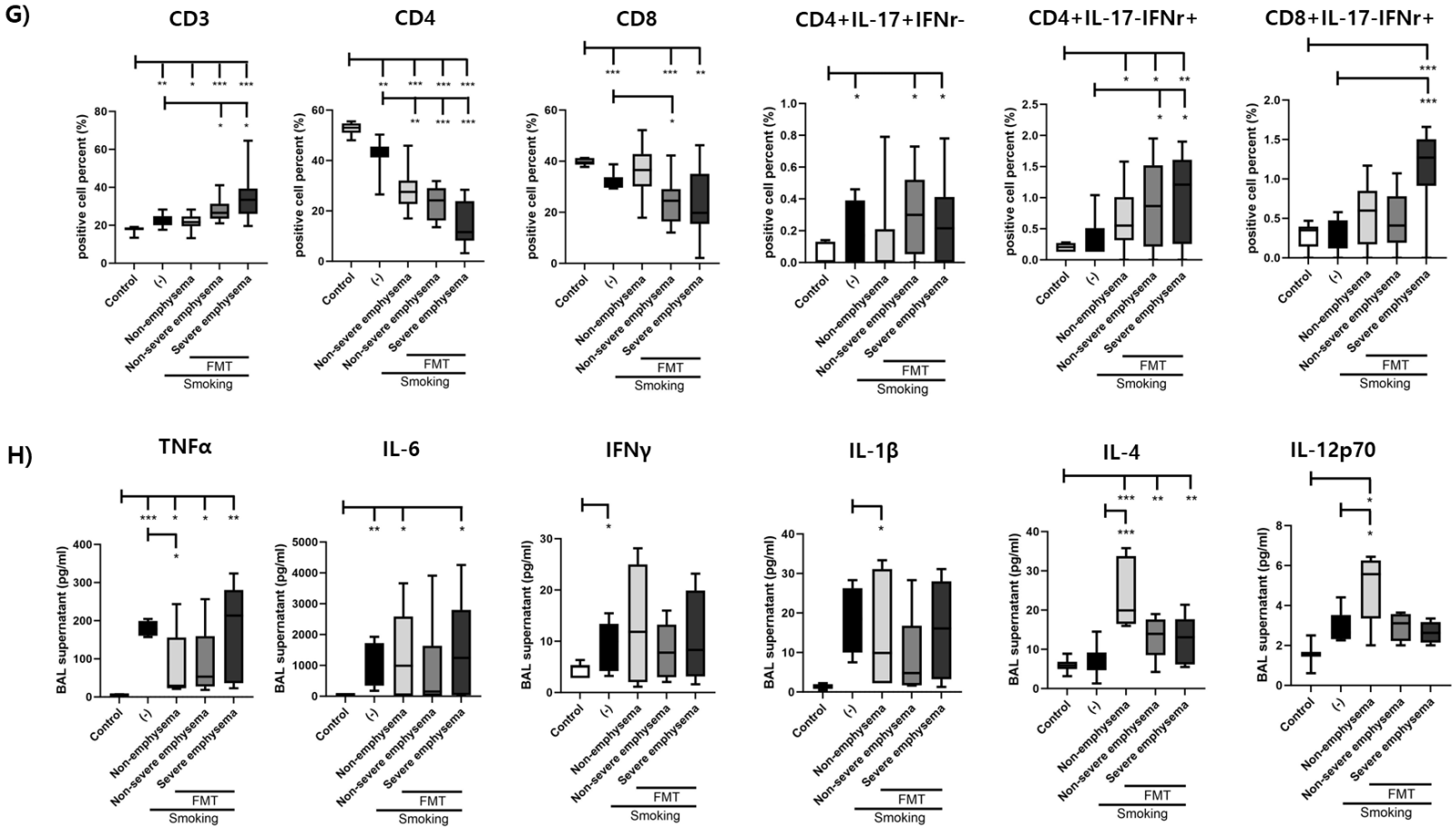
	Non-emphysema group		Non-severe emphysema group		Severe emphysema group	
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
CT imaging						
Sex/Age	Male/72yrs	Male/62yrs	Male/65yrs	Male/70yrs	Male/61yrs	Male/68yrs
Body mass index, kg/m^2	28.7	27.1	22.5	28.7	18.5	17.8
Smoking status	Ex-smoker	Ex-smoker	Current smoker	Ex-smoker	Ex-smoker	Ex-smoker
Smoking amount, pack*years	50	60	25	53	40	40
Pulmonary function test						
FEV_1 measured, L	2.67	3.08	2.99	3.04	0.68	0.82
FEV_1 % predicted	89	92	97	85	22	31
FVC measured, L	3.58	3.86	4.01	3.77	3.06	3.21
FVC % predicted	80	85	96	72	71	91
FEV_1/FVC	75	80	75	81	22	26
DL_{CO}, mL/min/mmHg	19.8	20.4	15.5	22.4	2.9	6.3

DL_{CO} % predicted	98	90	75	91	14	36

Abbreviation: *CT*, computed tomography; *DLco,* carbon monoxide diffusion in the lung; FEV_1, forced expiratory volume in 1 sec; *FVC*, forced vital capacity

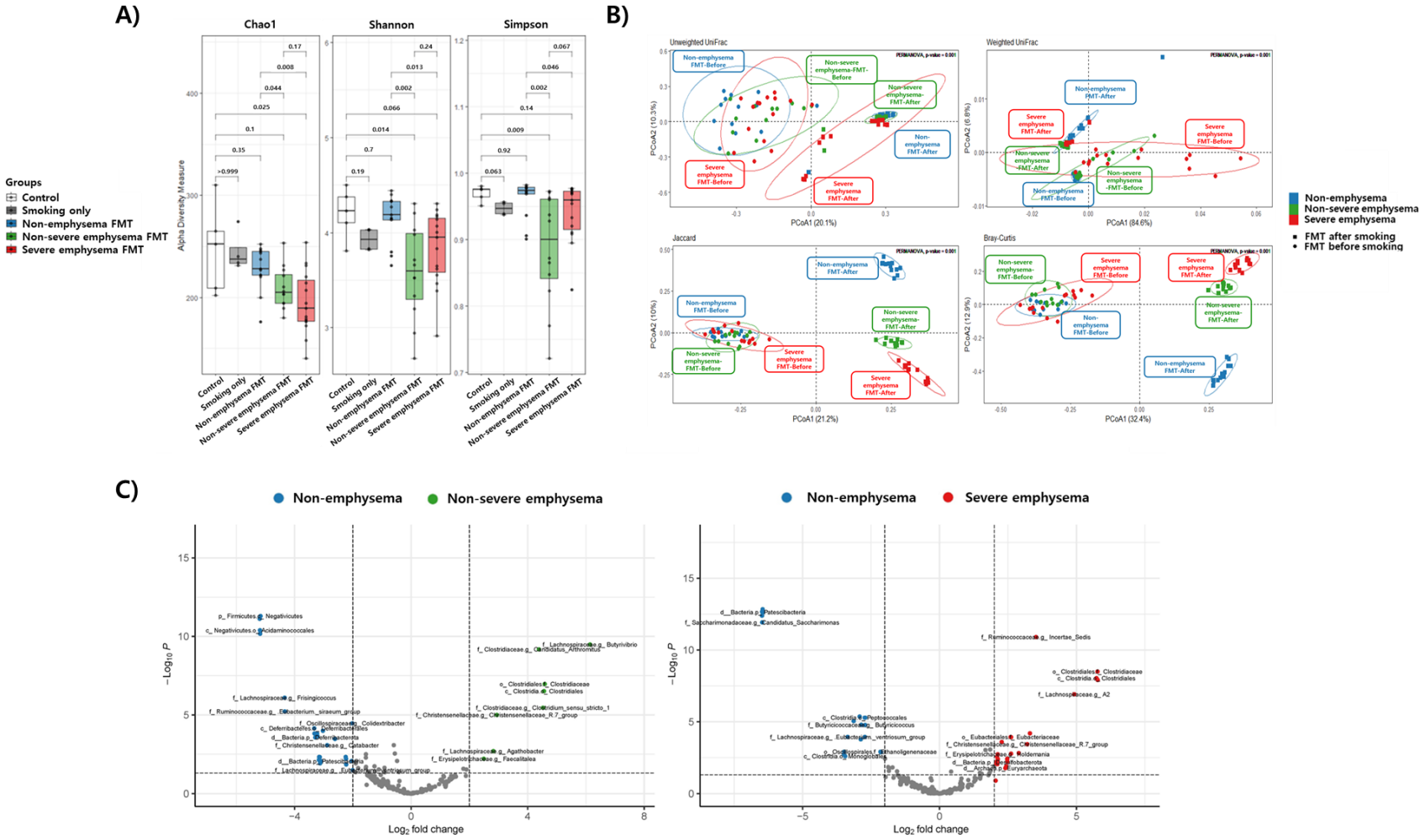
Figure 4. Emphysema development in germ-free mice as FMT from donors of three groups





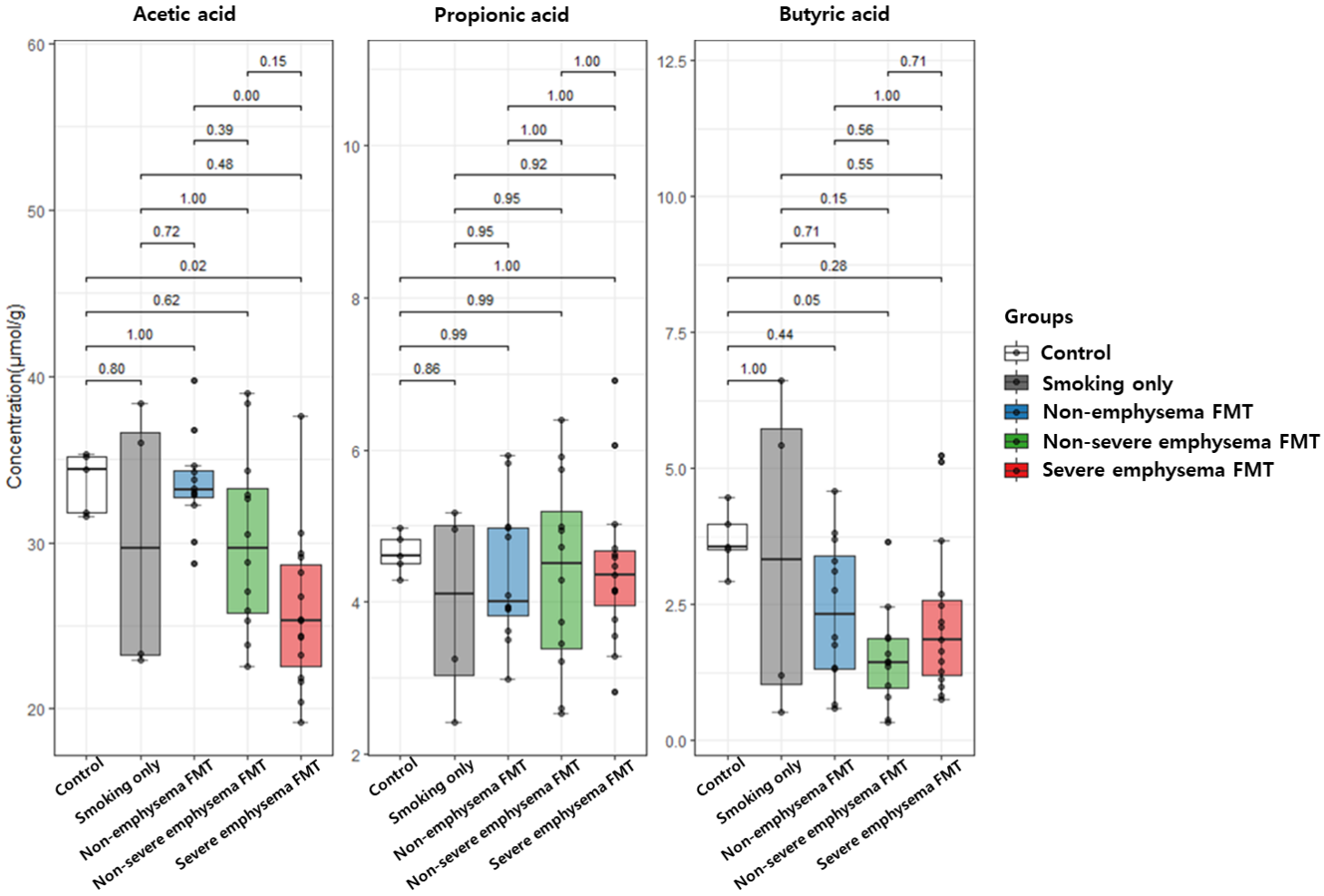
(A) Experimental scheme. Mice received the FMT weekly for three weeks. After that, they were exposed to cigarette smoke for 4 weeks. (B) Body weight changes. (C) The MLI of lung tissues from each group. (D) H&E staining of lung lobes. Representative sections from five independent experiments are shown. Scale bar=250 μm (magnification: ×100). (E) Total number of cells in the BALF infiltrating the airways in each group. (F) Relative mRNA levels of TNF-α, IL-6 and IFN-γ in lung tissues. (G) The percentage of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+IL\text{-}17^+$ T cells, $CD4^+IFN\text{-}\gamma^+$ T cells, $CD8^+IFN\text{-}\gamma^+$ T cells in lung tissue of each group are shown (H) The levels of the cytokines in the BALF were measured using ELISA. $^*P$ <0.05, $^{**}P$ <0.01 and $^{***}P$ <0.001. (-) indicates smoking only group.

Figure 5. Feces microbiome in germ-free mice as FMT from donors of three groups



(A) Boxplots of alpha-diversity index (Chao 1, Shannon, and Simpson index) (B) Beta diversity (PCoAs of unweighted UniFrac, weighted UniFrac, Jaccard, and Bray-Curtis index) is presented. Non-emphysema group is indicated in blue, non-severe emphysema group in green, and severe emphysema group in red. Beta diversity before smoking exposure is represented by circular dots, and beta diversity after smoking exposure is represented by square dots. (C) Volcano plot shows differential abundance, non-emphysema-enriched as blue dots, non-severe emphysema-enriched as green dots, and severe emphysema enriched as red dots.

Figure 6. SCFAs levels in fecal sample of germ-free mice as FMT from donors three groups



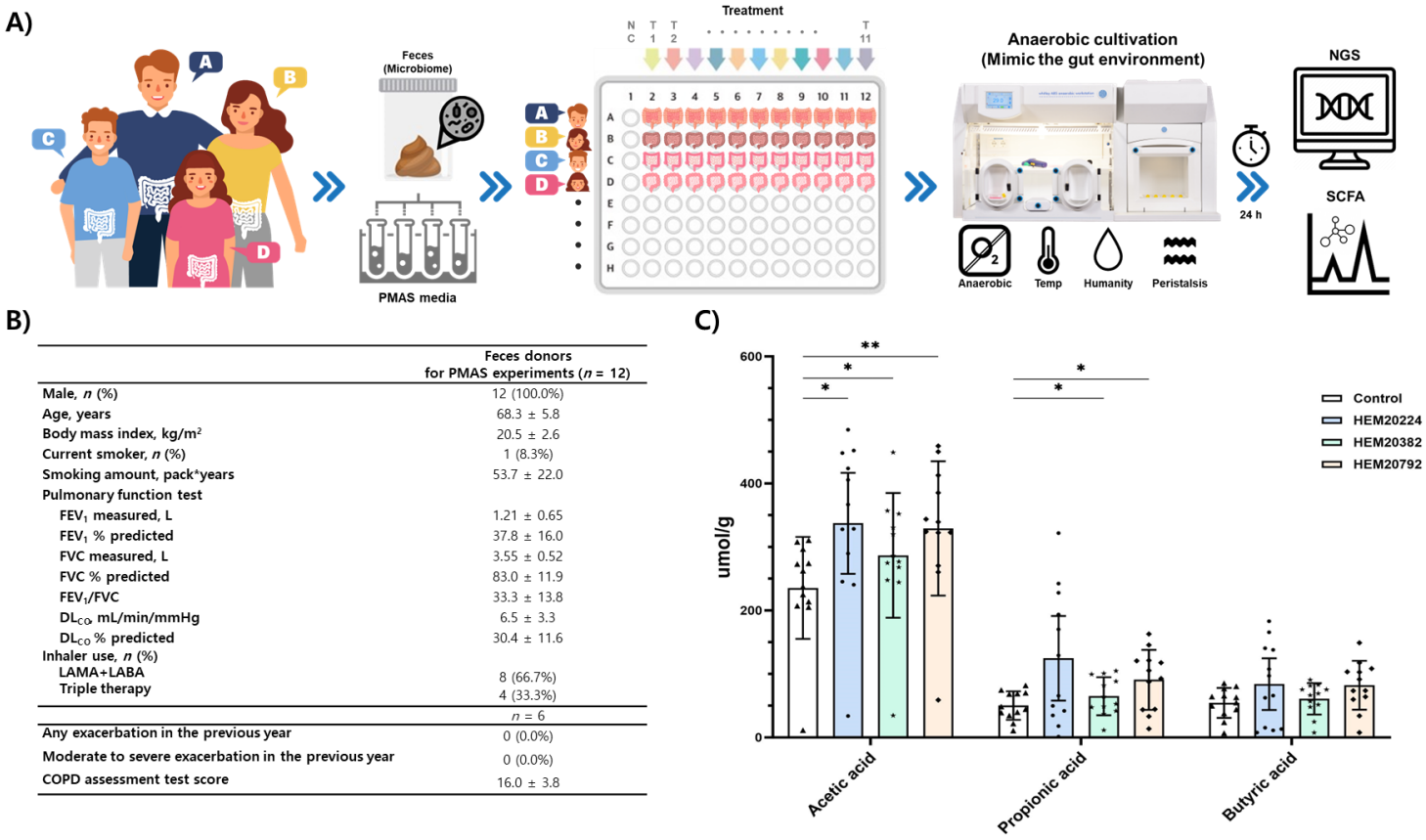
4. Screening of therapeutic bacteria through the pharmaceutical meta-analytical screening system

Pharmaceutical meta-analytical screening (PMAS) system was utilized to screen candidate bacteria species, by identifying the target SCFAs (acetic acid, propionic acid, and butyric acid) change. **Figure 7-A** briefly illustrates the process of PMAS system. Detailed methods of PMAS system were described in Methods and Materials section. We selected three species for screening by PMAS system: HEM20224, HEM20382, and HEM20792.

Among enrolled patients, we screened the 12 fecal donors for screening process by PMAS system. **Figure 7-B** presented baseline characteristics of 12 donors. 12 male donors were 68.3 years old and ex-smoker except one. Mean value of FEV_1 and DL_{CO} % predicted values were 37.8 ± 16.0 % and 30.4 ± 11.6 %, respectively. Two thirds used dual bronchodilators and the remained donors were prescribed dual bronchodilators and inhaled corticosteroid. There was no history of exacerbation in the previous year.

Figure 7-C presented the SCFAs production in the PMAS system. HEM20382 and HEM20792 showed the significant increase in acetic acid (337.2 ± 125.2 umol/g in HEM20224 *vs.* 286.7 ± 98.2 umol/g in HEM20382 *vs.* 329.1 ± 105.8 umol/g in HEM20792 *vs.* 235.5 ± 80.2 umol/g in control) and propionic acid (124.7 ± 104.8 umol/g in HEM20224 *vs.* 64.9 ± 29.9 umol/g in HEM20382 *vs.* 90.8 ± 47.3 umol/g in HEM20792 *vs.* 50.3 ± 22.5 umol/g in control) level compared to control group. HEM20224 presented the highest mean value in acetic acid and propionic acid among groups. Although there was no significant difference in butyric acid levels among four groups (84.0 ± 63.9 umol/g in HEM20224 *vs.* 60.8 ± 24.7 umol/g in HEM20382 *vs.* 82.2 ± 38.4 umol/g in HEM20792 *vs.* 54.4 ± 23.5 umol/g in control), similar trends were also observed. Based on these results, we selected HEM20224 and HEM20792 as candidate species for bacterial species gavage experiment.

Figure 7. Screening of therapeutic bacteria through the pharmaceutical meta-analytical screening system



	Feces donors for PMAS experiments (n = 12)
Male, n (%)	12 (100.0%)
Age, years	68.3 ± 5.8
Body mass index, kg/m²	20.5 ± 2.6
Current smoker, n (%)	1 (8.3%)
Smoking amount, pack*years	53.7 ± 22.0
Pulmonary function test	
FEV_1 measured, L	1.21 ± 0.65
FEV_1 % predicted	37.8 ± 16.0
FVC measured, L	3.55 ± 0.52
FVC % predicted	83.0 ± 11.9
FEV_1/FVC	33.3 ± 13.8
DL_{CO}, mL/min/mmHg	6.5 ± 3.3
DL_{CO} % predicted	30.4 ± 11.6
Inhaler use, n (%)	
LAMA+LABA	8 (66.7%)
Triple therapy	4 (33.3%)
	n = 6
Any exacerbation in the previous year	0 (0.0%)
Moderate to severe exacerbation in the previous year	0 (0.0%)
COPD assessment test score	16.0 ± 3.8

(A) A schematic diagram of the PMAS system's process and (B) clinical characteristics of feces donors for PMAS experiments. (C) Boxplots of SCFAs level after PMAS experiments. *P <0.05, **P <0.01 and ***P <0.001.

5. Emphysema development after bacterial species gavage

We performed the pilot study to investigate adequate concentration of candidate bacteria. We daily administrated HEM 20792 at concentration of $1*10^8$, $1*10^9$, and $1*10^{10}$ CFU with inulin 5mg (**Figure 8-A**). Within $1*10^9$ CFU/mouse/day group, inflammatory cell infiltration had a trend to be decreased in the lung tissue compared to $1*10^8$ CFU/mouse/day group and $1*10^{10}$ CFU/mouse/day group (**Figure 8-B**). The trend was shown that the mRNA expression of proinflammatory cytokines such as TNF-α, IL-6, IFN-γ, IL-1β and IL-18 were decreased in $1*10^9$ CFU/mouse/day group compared to $1*10^8$ CFU/mouse/day group and 1*1010 CFU/mouse/day group (**Figure 8-C**). From these results, we decided that $1*10^9$ CFU/mouse/day group was adequate for the effectiveness candidate bacteria.

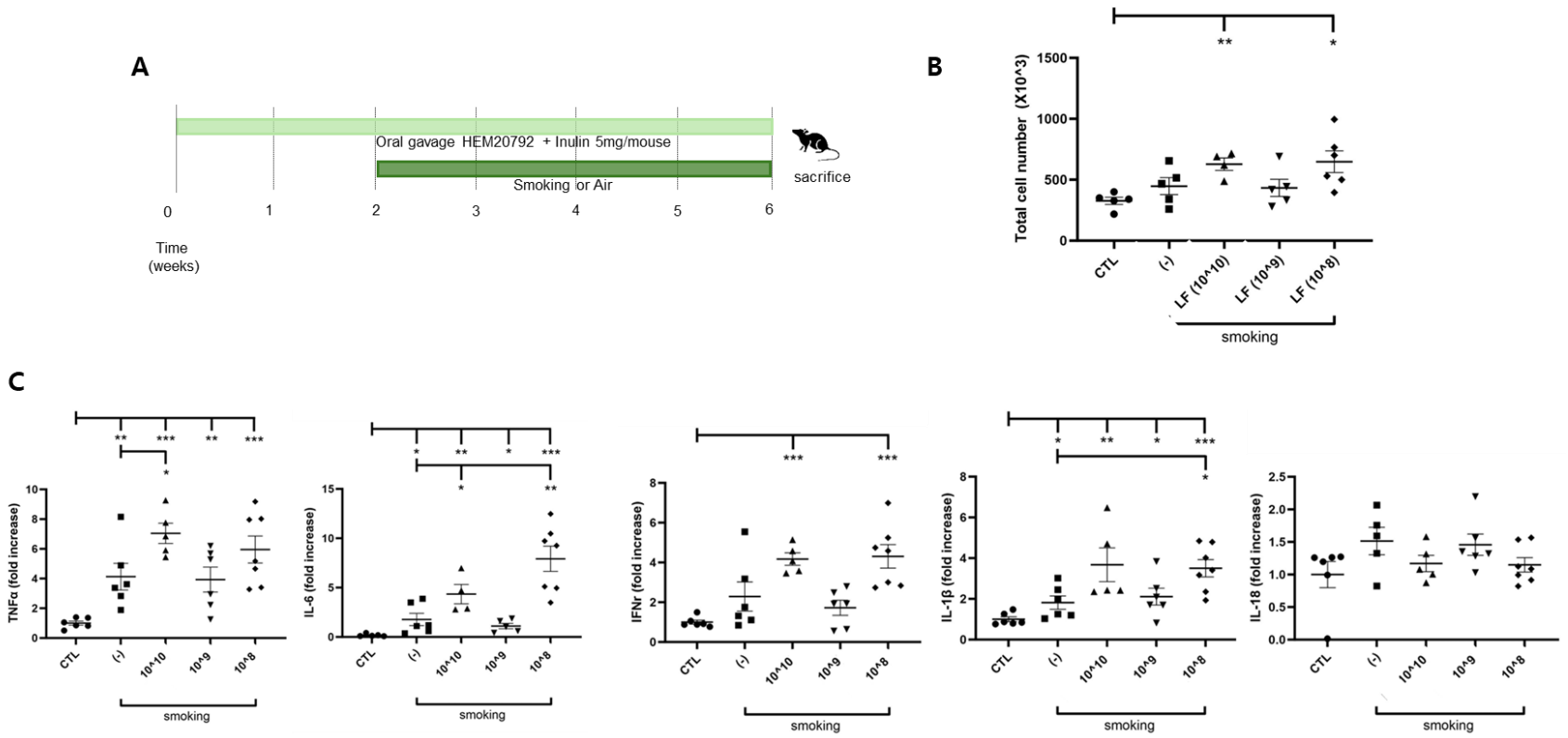
We conducted the additional experiments to investigate the impact of HEM20224 and HEM20792 administration, based on screening results of the PMAS system. **Figure 9-A** briefly illustrated the process of the additional experiment. HEM20224 and HEM20792 ($1 * 10^9$ CFU/mouse/day) were administrated with inulin (20mg/mouse). Mice were exposed to smoking or filtered air for 4 weeks after 2 weeks from administration of candidate bacterial species. **Figure 9-B** presented MLI value were significantly decreased in HEM20792 group compared to other groups. **Figure 9-C** also showed the similar result with MLI. With smoking exposure, the number of inflammatory cell (lymphocyte and neutrophil) was increased in the BALF compared to control group (**Figure 9-D, E**). The mRNA expression of proinflammatory cytokines such as TNF-α, IL-6, IFN-γ and IL-1β were elevated in smoking exposed groups compared to control group (**Figure 9-F**). T cell proportion was decreased in smoking exposed group compared to control group. the proportion of macrophage and $CD8^+IL\text{-}17^+IFN\text{-}\gamma^-$ were significantly decreased in the lung tissue of the HEM20792 group compared with the smoking only group (**Figure 9-G**). The protein levels of IL-23 in the BALF in the HEM20792 group was increased compared to control group (**Figure 9-H**). The protein levels of IL-6, IL-1β, IL-18 and GM-CSF were elevated in HEM20224 group compared to control group.

Figure showed the characteristics of fecal microbiome of candidate bacteria administration experiment. The alpha-diversity compared to smoking only group was not significantly different in HEM20224 and HEM20792 groups (**Figure 10-A**). Figure 5-B

illustrated change of the fecal microbiome beta diversity of each group (**Figure 10-B**). After administration of HEM20224, Relative abundance of HEM20224 in cecum and fecal sample was increased in HEM20224 groups (**Figure 11**). However, HEM20792 in all groups was not detected in both cecum and fecal samples after HEM20792 administration.

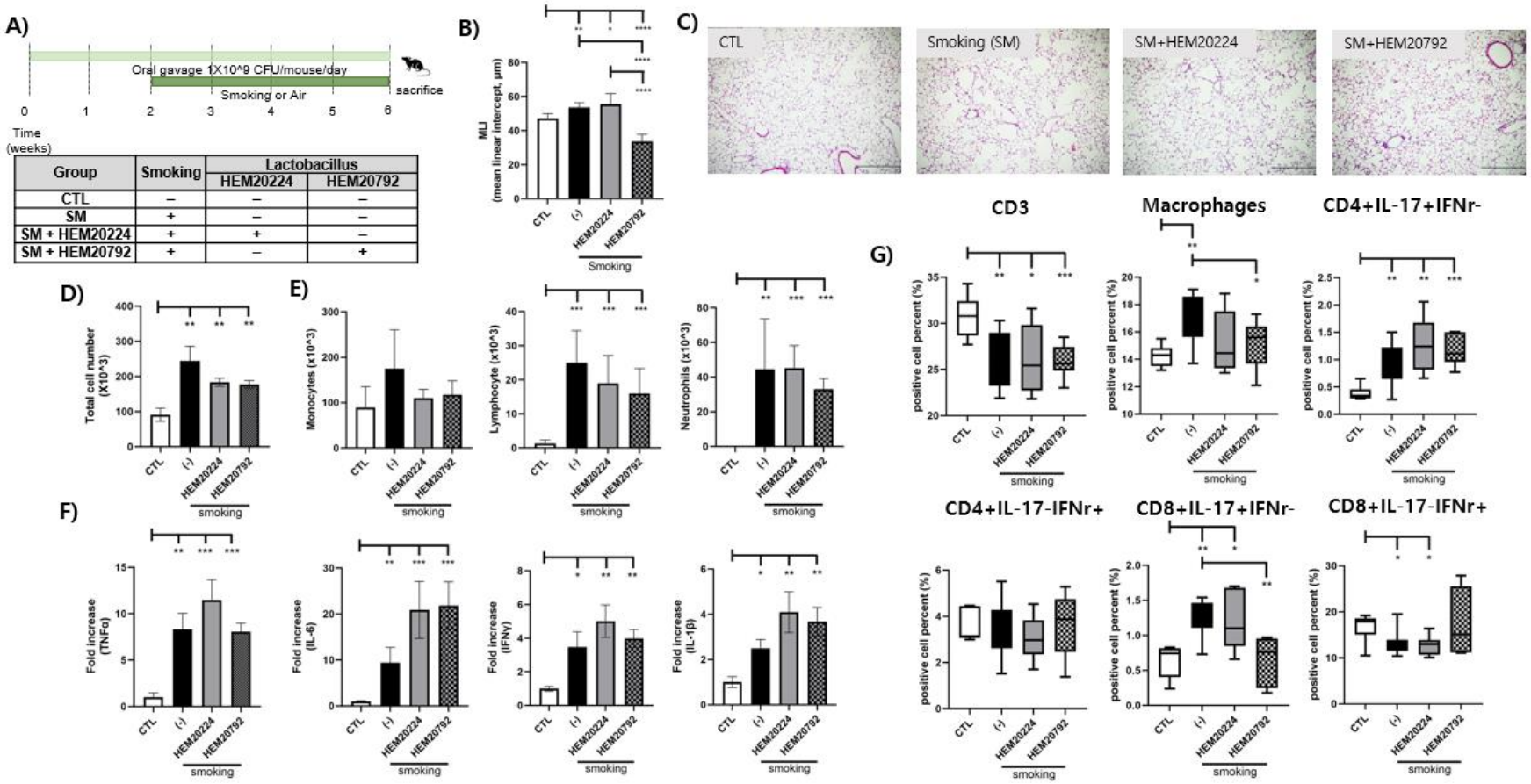
The SCFAs levels in fecal sample after HEM20224 and HEM20792 in smoking exposed mice were presented in **Figure 12**. Although there was no significant difference, acetic acid, propionic acid, and butyric acid level presented increased trend in HEM20224 and HEM20792 group compared to smoking only group.

Figure 8. Pilot study to investigate the adequate concentration of candidate bacteria

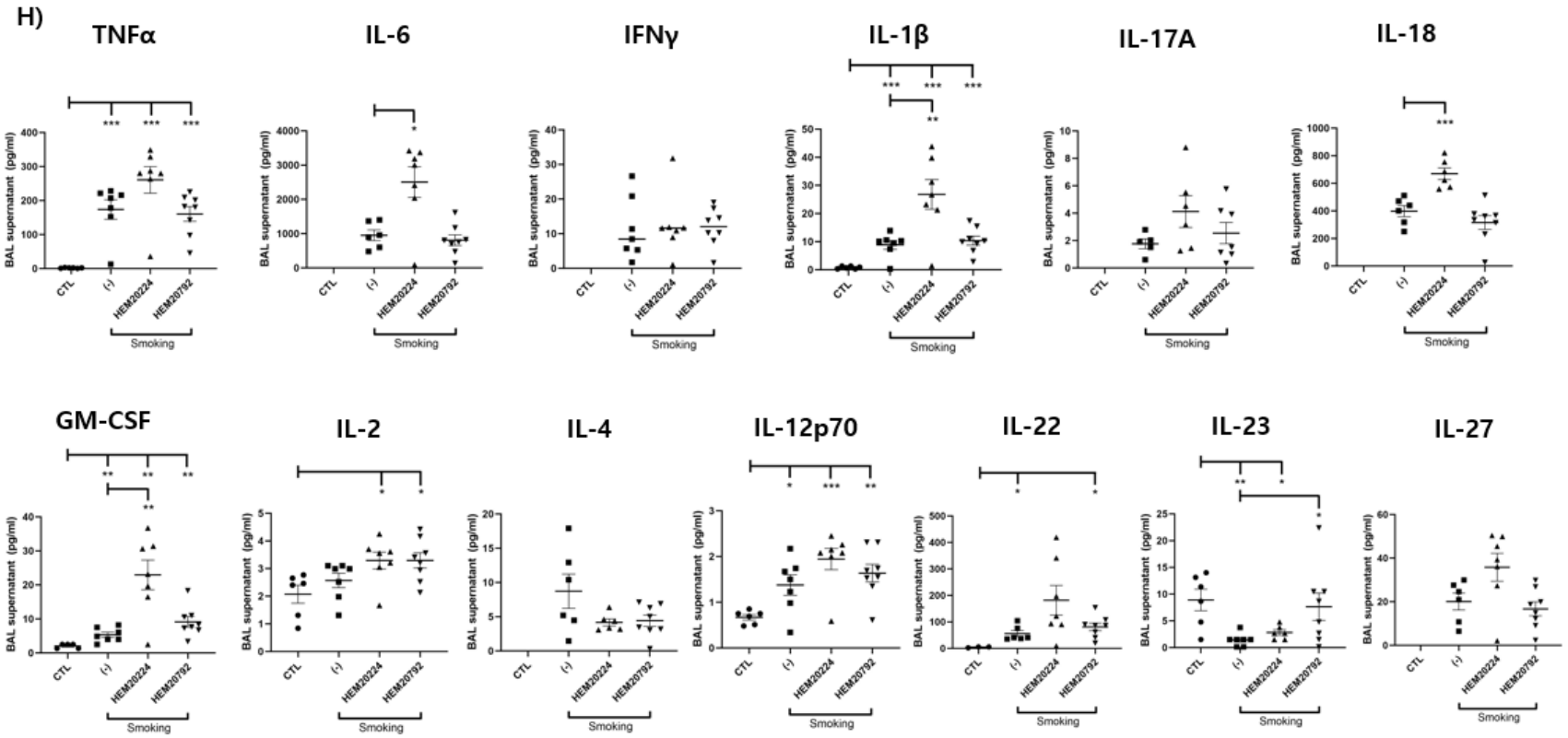


(A) Experimental scheme. Mice received the oral gavage with candidate bacteria and inulin. After that, they were exposed to cigarette smoke for 4 weeks. (B) Total number of cells in the BALF infiltrating the airways in each group. (C) Relative mRNA levels of TNF-α, IL-6, IFN-γ, IL-1β and IL-18 in lung tissues. **P* <0.05, ***P* <0.01 and ****P* <0.001. (-) indicates smoking only group.

Figure 9. The impact of candidate bacterial administration in smoking exposed emphysema model



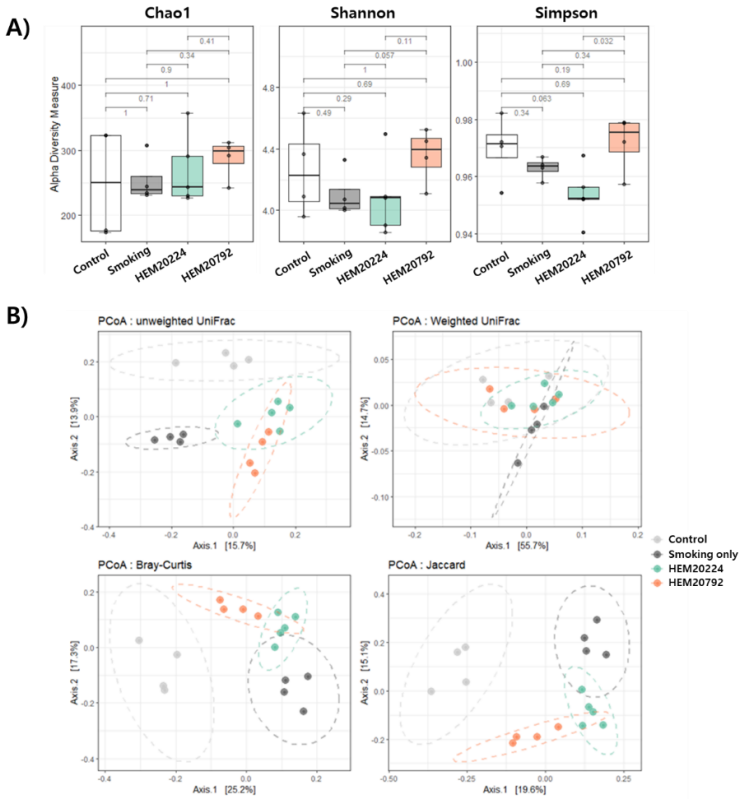
Group	Smoking	Lactobacillus	
		HEM20224	HEM20792
CTL	–	–	–
SM	+	–	–
SM + HEM20224	+	+	–
SM + HEM20792	+	–	+



(A) Experimental scheme. Mice received the oral gavage with $1*10^9$ CFU/mouse/day candidate bacteria. After that, they were exposed to cigarette smoke for 4 weeks. (B) The MLI of lung tissues from each group. (C) H&E staining of lung lobes. Representative sections from five independent experiments are shown. Scale bar=250 μm (magnification: ×100). (D) Total number of cells in the BALF infiltrating the airways in each group. (E)

Differential cell numbers in the BALF in each group. (F) Relative mRNA levels of TNF-α, IL-6, IFN-γ and IL-1β in lung tissues. (G) The percentage of $CD3^+$ T cells, macrophages, $CD4^+IL\text{-}17^+$ T cells, $CD4^+IFN\text{-}\gamma^+$ T cells, $CD8^+IL\text{-}17^+$ T cells, and $CD8^+IFN\text{-}\gamma^+$ T cells in the lung tissue of each group are shown. (H) The levels of the cytokines in the BALF were measured using ELISA. **P* <0.05, ***P* <0.01 and ****P* <0.001. (-) indicates smoking only group.

Figure 10. Feces microbiome after HEM20224 and HEM20792 administration in smoking exposed mice



(A) Boxplots of alpha-diversity index (Chao 1, Shannon, and Simpson index) (B) Beta diversity (PCoAs of unweighted UniFrac, weighted UniFrac, Bray-Curtis, and Jaccard index) is presented. Control group is indicated in grey, Smoking only group in black, HEM20224 group in green and HEM20792 group in orange color.

Figure 11. Relative abundance of HEM20224 after HEM20224 administration in smoking exposed mice

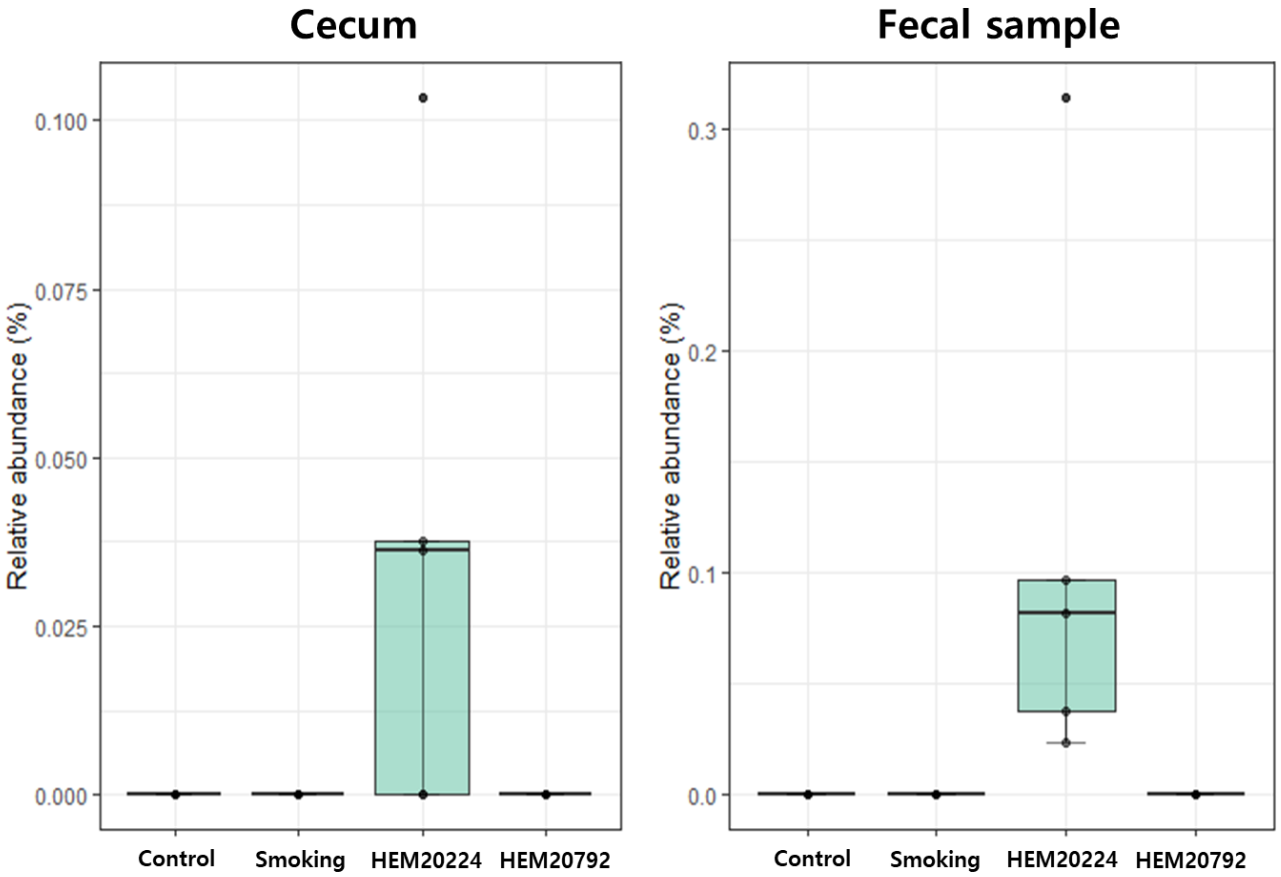
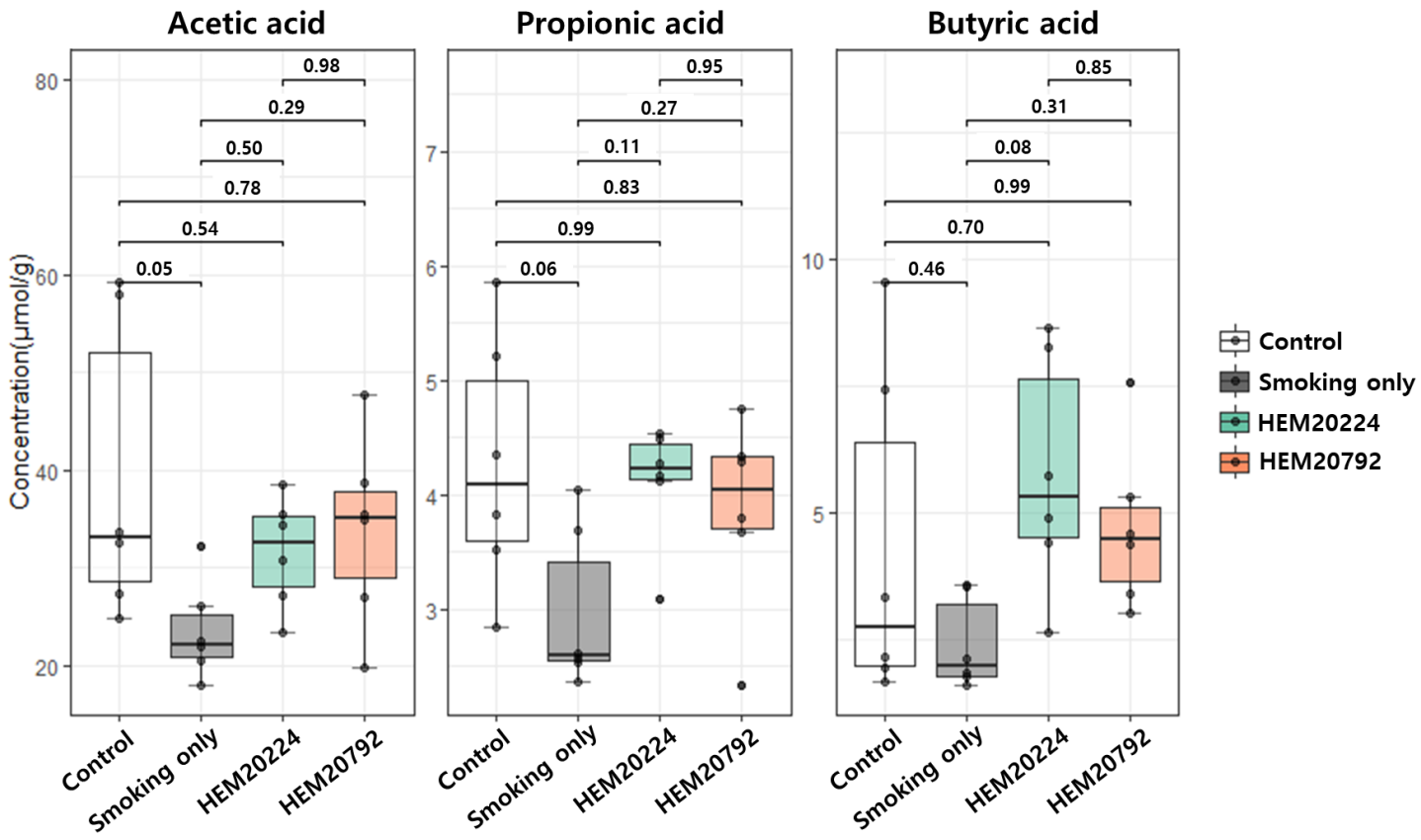


Figure 12. SCFAs levels in fecal sample after HEM20224 and HEM20792 in smoking exposed mice



Discussion

Smoking exposure is regarded as one of the most important risk factors for emphysema development [1,2]. Despite similar smoking amount, 24.4% of subjects did not develop emphysema. Of the 59 remained smokers who developed emphysema, thirty-three patients showed the severe decline in lung function, defined by FEV_1. In this study, we investigated gut microbiome and metabolome of smokers in non-emphysema, non-severe emphysema, and severe emphysema groups. Compared to smoker in non-emphysema group, emphysema patients showed the significantly decreased acetic acid level and decreased trend of propionic acid, and butyric acid levels. FMT from non-emphysema and non-severe emphysema group reduced the emphysematous change in emphysema mouse model. In the PMAS system, HEM20224 and HEM20792 increased acetic acid levels. After administration of HEM20792, emphysematous changes of mice were attenuated in this study. Therefore, our results indicated that gut microbiome and metabolome might play a significant role in the pathogenesis of emphysema and HEM20792 could be the fascinating candidate option for emphysema prevention. As we know, there are only limited studies to show the clinical potentials of specific bacterial species in chronic disease, especially in emphysema.

In this study, 78 subjects were classified into three groups based on the presence and severity of emphysema. Emphysema severity is assessed by various parameters including emphysema severity index, DLco, quantification of emphysema extent on chest CT [17-19]. On the contrary, FEV_1 is universally utilized to evaluated the severity of COPD, including emphysema and chronic bronchitis [1]. Although emphysema extent is more related to DL_{CO} than FEV_1 [19], we could not collect the information related to DLco in all patients and did not quantify the extent of emphysema on chest CT in this study. However, FEV_1 is also a factor that reflects the severity in emphysema patients, which is an indication for interventions such as lung volume reduction [1]. Analyses of microbiome and metabolome among three groups revealed significant differences in the fecal acetic acid levels, beta-diversity in fecal microbiome, and alpha-diversity in sputum microbiome. This result is concordant with the other previous studies reporting that changes in the microbiome and increased levels of SCFAs can delay the onset of emphysema [12,15,20,21]. Especially, acetic acid, propionic acid and butyric

acid, which were decreased in non-severe and severe emphysema groups in this study, are focused on the candidate key mediators of gut-lung axis [11]. These results lead to hypothesis that gut microbiome and metabolome from non-emphysema and non-severe emphysema groups could be associated with the attenuation of the emphysema development other groups.

Germ-free mice receiving FMT from non-emphysema and non-severe emphysema groups showed the attenuated emphysematous change, indicated by MLI, compared with smoking only group. This result implied that factors included in fecal material from each patient group might affect emphysema development. Studies have also reported that the characteristics of FMT donors can influence the disease severity of recipients in other diseases [22,23]. We investigated several factors known to be associated with the development of emphysema. Among them, fecal acetic acid level was significantly different between FMT groups from non-emphysema and severe emphysema group. Several studies reported that higher fecal SCFAs level were associated with alleviated severity of various diseases in the mouse models [24-28]. SCFAs produced by gut microbiota perform the crucial role as a mediator between gut microbiome and specific organs, including lung [11,29,30]. The changes in SCFA levels could be altered due to change of gut microbiome induced by FMT [11,31,32]. However, FMT to alter the gut microbiome is not easy to be performed in the real practice. Therefore, we hypothesized that administration of candidate bacterial species could lead to comparable therapeutic effects if candidate bacterial species change the SCFA levels. Although the fecal acetic acid level in the non-severe emphysema group was lower than that in the severe emphysema group, FMT from non-severe emphysema group also showed improvement in MLI in the FMT experiment. This result implied that therapeutic effects of FMT was not only related to SCFAs level. SCFAs levels are easily affected by several factors, including diet, gut microbiome, colonic environment, and gut motion [33]. Additionally, not only SCFAs but also factors including secondary bile acids are known to be important mediators of the gut-other organs axis [34-36].

To investigate mechanisms for clinical effects of FMT, we performed the microbiome and metabolome analysis and measurement of the various pro- and anti-inflammatory cytokines, cell levels in lung tissue and BALF. In the investigations, trends of TNF-α and IL-1β level, which are known as representative pro-inflammatory cytokine, were in line with

trends of MLI. TNF-α, IL-1β level and MLI presented decreased trends in non-emphysema and non-severe emphysema groups compared to smoking only and severe emphysema groups. TNF-α and IL-1β level is known to be increased in emphysema and related to the emphysema development [37-40]. In mice with non-emphysema and non-severe emphysema FMT group showing attenuated emphysema development, TNF-α and IL-1β level were decreased compared to mice in only smoking exposed group and severe emphysema group. IL-4 and IL-12p70 level was increased in mice with non-emphysema group, not in non-severe emphysema group. IL-4 was known as anti-inflammatory cytokine suppressing the production of pro-inflammatory cytokine [41]. IL-12p70 is a heterodimer of IL-12p40 and IL-12p35 [42]. In general, IL-12p70 is known to promote inflammatory reaction. However, IL-12p70 showed the anti-inflammatory features in heart allograft rejection rate models via production of IFN-γ and nitric oxide [43]. This result was disagreed with the previous studies reporting imbalance between IL-4 and IL-12 results in type 1 or type 2 inflammation [44]. Therefore, further studies might be required to prove the association between the change of these cytokines level and emphysema development.

To screen the candidate bacterial species, we utilized the PMAS system. As we described in Methods and Materials, this system mimicked the intestinal environment, including *enterococcus*, temperature, humidity, and motion. This system is designed to select the bacterial species that induce desirable effects and has been patented in the US [45]. For screening test utilizing the PMAS system, 12 fecal donors were recruited and had mean FEV_1 % predicted value 37.8 ± 16.0% with airflow limitation, accordant to criteria for severe emphysema group. In a previous FMT experiment, the severe emphysema group exhibited characteristics similar to the smoking only group, rather than the non-emphysema or non-severe emphysema groups. Therefore, fecal donors complied with severe emphysema criteria was adequate for the objective of the PMAS system, which aims to screen the candidate bacteria species capable of restoring these characteristics. We selected three candidate bacterial species, which are detected in Kimchi, Korean traditional fermented food [46]. Several studies reported that SCFAs might play a role to attenuate the various pro-inflammatory cytokines related response, including TNF-α and IL-1β [47]. Therefore, we focused on the change of SCFAs level after administration of candidate bacterial species to the PMAS system. In the

PMAS system, three candidate bacterial species significantly increased acetic acid level. Propionic acid level was also increased in three candidate bacterial species, but not significant in HEM20224. Regardless of statistical significance, mean value was highest in HEM20224, followed by HEM20792, and HEM20382. Based on the results, we selected HEM20224 and HEM20382.

To modulate the gut microbiome, four ways were suggested, administration of prebiotics, probiotics, postbiotics and FMT [48]. We additionally investigated the effect of administration of probiotics (candidate bacterial species) into emphysema mouse model in this study. Probiotics have effects on the host via inhibitory action against pathogens, restore of gastrointestinal tract barrier, immunomodulating effects, and change of gut-organ axis mediator production [49]. Effective probiotics have the advantage of potentially providing more lasting effects by producing beneficial mediators, compared to the administration of prebiotics or postbiotics. After administration of HEM20792, MLI value was significantly decrease in this study. Attenuated emphysematous change was also observed by high fiber diet (prebiotics) and administration of SCFAs (postbiotics) in our previous studies [15,16]. This result indicated that HEM20792 had clinical potential against emphysema development. HEM20792 group presented increased trend of acetic acid, propionic acid and butyric acid levels compared to smoking only group, although there was no statistical significance. This result was consistent with change of SCFAs level, which were decreased in the emphysema, in the investigation of 78 smokers and the FMT experiment. The levels of SCFAs might be associated with the development and severity of emphysema.

In the experiment related to administration of candidate bacterial species, we found decreased macrophage and $CD8^{+}IL\text{-}17^{+}IFN\text{-}\gamma^{-}$ T cell in the lung tissue and increased IL-23 level in BALF. Macrophage is known to produce pro-inflammatory cytokine and proteases involved in emphysema pathogenesis [50,51]. In COPD patients, the level of dual polarized macrophage is increased [50]. M1 macrophage induced inflammatory reaction for pathogen clearance [52]. On the contrary, M2 macrophage presented resolving process against damaged cells. C1q is involved in this process and induced Matrix Metalloproteinases 9, which is related to emphysema development [52,53]. One study showed that butyrate and propionate attenuated M2 polarization in macrophage via G-protein-coupled receptor 43 activation and histone

deacetylase inhibition [54]. The results of previous studies indicated that macrophage might play the essential role in the emphysema development. Therefore, further studies might be required to investigate this point. $CD8^{+}IL\text{-}17^{+}IFN\text{-}\gamma^{-}$ T cell was also associated with the emphysema development [55,56]. IL-23 has a pro-inflammatory property and increase the level of lung matrix metalloproteinases-9, which contributes the emphysema development [57-59]. Therefore, increased IL-23 level did not explain the attenuated emphysema development. Additionally, IL-23 is also related to development of various chronic disease [60,61]. Therefore, further studies might be required to confirm the meaning of increased IL-23 levels after administration of HEM20792.

There are several limitations in this study. Firstly, we classified patients with emphysema into two groups based on the FEV_1. However, FEV_1 showed weak correlation with the extent of emphysema. Classification based on the other indicators, including DLco, emphysema severity index and quantification of extent of emphysema, could show the different result. Therefore, the results of this study need to be validated in the patients classified on the other indicators for emphysema severity. We observed the relationship between FMT from non-emphysema group and non-severe emphysema group, HEM20792 administration and attenuation of emphysema development. However, we did not concordant change related to factors related to inflammatory process. Additionally, the respiratory and gut microbiomes are easily changeable, and their results can vary depending on various confounders and covariates. Although we observed the change of diversity after FMT, we could not determine whether the FMT or administered HEM20792 directly produced beneficial effects on emphysema, or their interaction with other factors might be associated with the beneficial effects. Additionally, the fecal level of HEM20792 was not increased after administration of HEM20792. Therefore, we could not convince that decreased MLI in HEM20792 group was directly resulted from HEM20792 administration or via other factors transitionally induced by HEM20792 administration. further studies might be required to clarify the efficacy and mechanism of HEM20792 as probiotics and FMT.

Conclusion

This study indicated that individual characteristics in microbiome and metabolome could be related to development emphysema and degree of pulmonary function impairment. FMT from non-emphysema and non-severe emphysema groups and administration of HEM20792 attenuated the emphysema development. These results implied clinical potential of FMT and administration of HEM20792 against emphysema development as novel treatment strategy for emphysema utilizing gut-lung axis and microbiome technique. Further studies in humans might be required to provide clinical evidence of FMT and HEM20792 administration as the alternative treatments for emphysema development.

Methods and Materials

1. Study populations

Subjects older than 18 years with a smoking history of ≥20 pack*years were screened for this study. Among them, we enrolled those with informed consent to participate in the study and to provide fecal sample between January 2020 and April 2021.

Eligible subjects were classified into three groups. At first, we classified eligible subjects into two groups by existence of emphysema on chest CT scan at the time of study enrollment. Two pulmonologists independently reviewed existence of emphysema on the chest CT images. If disagreement was observed between them, a consensus decision was achieved after discussion. After that, we more classified subjects with emphysema into two groups based on the FEV_1 <50% of predicted value. Finally, subjects were classified into non-emphysema, non-severe emphysema, and severe emphysema groups. We excluded individuals with a history of antibiotic exposure within 2 months and underlying diseases such as malignancy, congestive heart failure or diabetes mellitus on insulin therapy. The study was approved by the Institutional Review Boards (IRB no. 2020-0314). All participants provided written informed consent.

2. Animal models

All animal care and experimental procedures were approved by Institutional Animal Care and Use Committee of Asan Medical Center (approval number 2022-13-126). Male seven-week-old C57BL/6 mice (Orient Bio, Seongnam, Republic of Korea) were randomly classified into five group in FMT experiment: control, smoking exposure, smoking exposure with FMT from non-emphysema subjects, smoking exposure with FMT from non-severe emphysema subjects and smoking exposure with FMT from severe emphysema subjects. In bacterial species administration experiment, mice were randomly classified into four groups: control, smoking exposure, smoking exposure, and oral gavage with HEM20224, and smoking exposure and oral gavage with HEM20792 groups. Mice in groups with smoking exposure were exposed to cigarettes smoking in a whole-body apparatus in 5 days/week during 4 weeks, using 12 commercial cigarettes per day (4 cigarettes/session, 3 sessions/day, 8.0 mg tar/cigarette, and

0.70mg nicotine/cigarette, Camel, R. J. Reynolds Tobacco Company, Winston-Salem, NC, USA) [15,16,62]. The control group inhaled only clean-room air from the cages.

3. Study design for animal experiments

Study 1 (Figure 2-A). The effect of FMT from each fecal sample donors on the development of emphysema was determined in five groups (*n* = mice/group); control, CS exposure only, CS exposure with FMT from non-emphysema, CS exposure with FMT from non-severe emphysema, and CS exposure with FMT from severe emphysema groups.

Study 2 (Figure 4-A). The effect of oral gavage with bacterial species on the development of emphysema was determined in four groups (*n* = mice/group); control, CS exposure only, CS exposure with HEM20224 oral gavage, and CS exposure with HEM20792 oral gavage groups.

4. Fecal microbiota transplantation

Fresh fecal samples were collected from two representative donors in each group and resuspended in 5 mL of PBS. 40 μm pore-size nylon filters were used to remove large particulate and fibrous matter. Homogenates was centrifuged for 2 min and transplanted into recipient mouse through oral gavage with 200μL resuspended feces at three times, once a week.

5. Bacterial species gavage

We screened and elected to gavage two bacterial species that produce key SCFAs including acetic acid, propionic acid, and butyric acid, by pharmaceutical meta-analytical screening system. HEM20224 and HEM20792 (HEM pharma Inc., Suwon, Republic of Korea) were gavage via oral gavage at $1 * 10^9$ CFU/mouse/day for the entire 6 weeks of bacterial species administration experiment. Control mice received sterile medium.

6. Separation and preparation of samples

After end of the experimental period, all mice were anesthetized by inhaled isoflurane and euthanized. After euthanasia, blood samples were collected by heart puncture. To collect BALF, trachea was catheterized and instilled with 1.5 mL of PBS. To separate supernatants and cellular components, BALF were centrifuged at 2,200 rpm, for 5 mins at 4°C. The cell pellet

was suspended in PBS, attached to a slide, and stained with Diff-Quick (Sysmex, Kobe, Japan). After bronchoalveolar lavage, lungs were harvested for histology. After ligating the right main bronchus, the left lung lobe inflated by 0.5% low melting point agarose at a constant pressure of 15 cmH_2O and fixed in 10% formalin. Remained right lobes were collected, immediately frozen, and stored at -80°C for further analysis. Among them, right postcaval lobe was utilized for quantitative real-time PCR analysis.

7. Histopathological and mean linear intercept assessment

After fixation, each lung lobe was separated and paraffinized. Paraffinized lobes were embedded, and 5 μm sections were taken. These sections were stained with hematoxylin and eosin for measurement the MLI, indicating severity of emphysematous changes [63]. To assess MLI, the mean inter-alveolar septal wall distance was calculated by the number of interruptions in 1 mm lines of the alveolar wall. Four lines were drawn in each field, and at least five random fields per mouse were examined.

8. Measurement of cytokine levels

The levels of TNF-α, IFN-γ, IL-1, IL-4, IL-6 and IL-12p70 in the serum and BALF samples were measured using a commercially available ELISA kit (R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

9. Quantitative real-time PCR analysis

Total RNA was extracted from lung tissue using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1μg) was used to cDNA synthesis by using the primeScript RT Master Mix kit (Takara, Kyoto, Japan). Transcript were quantified by real-time PCR using sequence-specific primers for TNF-α, IL-6, IFN-γ, and IL-1β. Amplification reactions were performed with Advanced Universal SYBR Green Supermix kit and the CFX Connect real-time PCR system (Biorad, Hercules, California, USA). The target gene expression levels were normalized to 18 S RNA as an endogenous control gene. The equation 2-ΔΔCT was used to calculate the relative changes.

10. Measurement of short chain fatty acids

Standard metabolites and internal standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were purchased from J. T. Baker (Center Valley, PA, USA). 10-20 mg of feces were freeze-dried for 24 hours using a Benchtop manifold freeze drier and stored at -80℃ until analysis.

Fecal samples were shaken with 150μL of internal standard solution (1mM of propionic acid (C3)-d_6 in water) in vortex apparatus and centrifuged at 13,200 rpm for 10 min at 4 ℃. Subsequently, the supernatant was collected. 100μL of 20 mM TPP and 100μL of 20 mM DPDS in acetonitrile and 100μL of 20 mM AABD-SH in dichloromethane were added to the supernatant. The solution was incubated and vortexed for 10 min at room temperature. After that, the incubated solution was dried under vacuum for storage. Dried samples were prepared with 20μL methanol for Liquid Chromatography-Tandem Mass spectrometry (LC-MS/MS) analysis.

An LC-MS/MS system included 1290 HPLC (Agilent Technologies, Glostrup, Denmark), Qtrap 5500 (ABSciex, Framingham, MA), and a reverse-phase column (Pursuit 5 C18 150 × 2.0 mm, Agilent Technologies). Mass spectrometry was run by using the positive ion mode with turbo ion-spray voltage of 5500V, generated by 20 psi curtain gas, 50 psi drying gas and nebulizer gas at 400°C. The LC separation used mobile phase A (0.1 % formic acid in water) and mobile phase B (0.1 % formic acid in acetonitrile) at 500 μl/min and 40 °C. The separation gradient was as follows: 30% of B at 0 min, 50% of B for 30 min, 50 to 30% of B for 0.1 min, and then 30 % of B for 4.9 min. 15 V of collision energies were used for multiple reaction monitoring of each SCFA. LC-MS/MS data were analyzed with Analyst 1.5.2 software (ABSciex).

Extracted ion chromatogram (EIC) corresponding to the specific transition for each metabolite was used for quantitation. Area under the curve of each EIC was normalized to that of EIC of internal standard.

11. DNA extraction

DNA was extracted from the fecal samples using a DNA isolation kit (MO BIO, Carlsbad,

California, USA) following the manufacturer's instructions. Further analysis methods were described in the online supplemental file. DNA was amplified with 16S_V3_F and 16S_V4_R primers specific to the V3-V4 hypervariable regions of the 16S rDNA gene (Supplementary Table S1). The libraries were prepared using PCR products in accordance with the MiSeq System Guide (Illumina, USA) and quantified using the QIAxpert System (QIAGEN, Germany). Each amplicon was quantified, and an equimolar ratio was pooled and sequenced on a MiSeq (Illumina) in accordance with the manufacturer's instructions. Microbiota bacterial composition analysis was performed, following the procedure previously described.

12. Analysis of bacterial composition in the microbiota

Bacterial genomic DNA was amplified by using 16S_V3_F and 16S_V4_R primers, which specific for V3-V4 hypervariable regions of 16S rDNA gene. The libraries were constructed using polymerase chain reaction products in accordance with MiSeq System guide (Illumina, USA) and quantified using a QIAxpert (QIAGEN, Germany). Each amplicon was pooled at equimolar concentrations and sequenced on a MiSeq (Illumina, USA) according to the manufacturer's protocol.

End reads, consistent with the adapter sequences, were matched by cutadapt version 1.1.654 and incorporated into FASTQ files. FASTQ files were merged with CASPER, and verified by Phred (Q) score. Only reads between 350 and 550 were used. Chimeric sequences were detected by VSEARCH against the SILVA gold database. After this process, the reads were congregated into Operational Taxonomic Units (OTUs) using VSEARCH, considering 97% sequence similarity as threshold. The congregated OTUs were classified based on the SILVA 128 database with UCLUST (parallel_assign_taxonomy_uclust.py script on QIIME version 1.9.1).

In order to investigate diversity, we calculated alpha diversity (Chao1 Index, the Shannon Index, and the Gini-Simpson Index) and beta diversity (Principal Coordinate Analysis plot on unweighted UniFrac). Difference in bacterial taxa level among groups were assessed by linear discriminant analysis (LDA) effect size (LEfSe) using Limma R package. In the LEfSe analysis, genera with LDA value >2 and P <0.05 were regarded as significant.

13. Personalized Pharmaceutical Meta-Analytical Screening System

The personalized PMAS system imitated the intestinal environment of each person. The imitated factors included enterococcus, temperature, humidity, and motion. For PMAS experiments, 12 feces donors were selected. Fecal samples and PMAS medium are mixed at a ratio of 1:12 and homogenized using a stomacher. Residue is removed by filter. The mixture is matured in an anaerobic chamber for 4 hours. After aforementioned process, the mixture from each subject is subsequently distributed into the horizontal rows of 96 well plates. Candidate species are suspended in PBS, homogenized in preset concentration and amount. Using the measured CFU values of the candidate species, an equivalent CFU is dispensed into the vertical column plate wells containing the mixture. Following continuous anaerobic incubation at 37 for 24 hours in PMAS system similar to the intestinal environment. SCFAs were examined from the supernatant collected by centrifugation. By identifying the target SCFAs change, it is possible to screen for bacterial species as improvement candidates.

14. Statistical analysis

Clinical data are presented as mean ± standard deviation for continuous variables and or number (%) for categorical variables. The $\chi 2$ or Fisher's exact test, Student's *t*-test, the Kruskal-Wallis test and the one-way ANOVA, followed by a Tukey's test, using SPSS software version 24.0 (IBM, Armonk, NY, USA). Differences between groups were compared using one-way ANOVA, followed by post hoc Tukey's test for multiple comparisons, and Student's t-test for two groups using GraphPad Prism software (Version 5.01, GraphPad, San Diego, CA, USA). Statistical significance was accepted for P value<0.05.

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국문요약

배경

만성 폐쇄성 폐질환은 현재의 진료지침에 근거한 표준 치료에 의해 관리할 수는 있지만, 완치는 어려운 질병입니다. 최근, 프로바이오틱스 투여와 대변미생물이식과 같이 마이크로바이옴에 변화를 주는 방법은 다양한 질병에서 효과를 보여주었습니다. 우리는 본 연구에서 대변미생물이식과 특정 박테리아를 투여하는 것이 폐기종 발달에 미치는 효과를 조사하고자 하였습니다

연구방법

우리는 30 갑년 이상의 흡연력을 가진 흡연자들을 대상으로 임상 특성과 마이크로바이옴 및 메타볼롬의 특성을 조사하였습니다. 마이크로바이옴 및 메타볼롬 분석과 대변미생물이식을 위해 흡연자들로부터 가래 및 대변 검체를 수집하였습니다. 장 환경을 모방한 약물 메타분석 스크리닝 시스템(pharmaceutical meta-analytical screening system, PMAS system)을 사용하여 후보 박테리아를 선별했습니다. 대변미생물이식 및 후보 박테리아 투여의 효과를 조사하기 위하여, 연구진들은 폐기종 쥐 모델을 사용하였습니다.

결과

78 명의 흡연자는 비폐기종 그룹의 19 명, 비중증 폐기종 그룹의 26 명, 중증 폐기종 그룹의 33 명으로 분류되었습니다. 세 그룹 간에는 뚜렷한 마이크로바이옴 및 메타볼롬 특성 차이를 보여주었습니다. 폐기종 쥐 모델에서 비폐기종 및 비중증 폐기종 그룹의 대변미생물이식은 흡연군에 비해 폐기종 변화를 감소시켰습니다. PMAS 시스템에서 아세트산과 프로피온산 수준을 증가시키는 HEM20792 균주의 투여 또한 흡연군에 비해 폐기종 변화를 완화시켰습니다.

결론

비폐기종 및 비중증 폐기종 그룹으로부터의 대변미생물이식과 프로바이오틱스로서 HEM20792 투여는 폐기종 발생을 완화시켰습니다. 이는 장-폐축 및 마이크로바이옴 기술을 활용한 폐기종의 새로운 치료 전략으로서 임상 잠재력을 본 연구에서 보여주었다고 할 수 있습니다.

중심단어: 폐기종, 마이크로바이옴, 대변미생물이식, 프로바이오틱스

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