Association of gut microbiome with COPD in Japanese male residents: the SESSA study

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The microbial composition of the gut may be altered by smoking but may not directly contribute to the development of COPD. Presence of gut *Prevotella* may be a risk factor for COPD.
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Abstract

Background Altered gut microbiota may contribute to COPD development or progression. Herein, we investigated the association of gut microorganisms with COPD, taking into account the impact of smoking status.

Methods This cross-sectional observational study was a part of the Shiga Epidemiological Study of Subclinical Atherosclerosis, a population-based cohort study of Japanese men aged 46–76 years, conducted from 2010 to 2016. The gut microbiome, determined using 16S rRNA gene sequencing, was compared among 99 never-smokers, 306 non-COPD ever-smokers and 76 patients with COPD while adjusting for age, body mass index, ethanol consumption and treatment for type 2 diabetes mellitus.

Results The abundance of phylum Firmicutes was comparable between patients with COPD and non-COPD ever-smokers but tended to be higher in never-smokers. Similarly, the α- and β-diversity analysis showed similarity between patients with COPD and non-COPD ever-smokers, which tended to differ from never-smokers. Discriminant analysis identified the genus *[Prevotella]* to be more prevalent in patients with COPD than in never-smokers or non-COPD ever-smokers. *Post hoc* analysis confirmed similarity of gut microbiome between COPD Global Initiative for Chronic Obstructive Lung Disease (GOLD) I and non-COPD ever-smokers, which was different from GOLD II.

Conclusion Smoking may alter the overall gut microbial composition, but gut microbial composition itself may not play a role in the development of COPD. Rather, specific gut bacteria, such as *[Prevotella]*, could be a risk factor for the development of COPD; this may be a potential therapeutic target.

Introduction

COPD, characterised by persistent respiratory symptoms and airflow limitation [1], is a leading cause of morbidity and mortality worldwide [2]. COPD is typically caused by tobacco smoke and other noxious particles or gases. However, genetic factors [3], autoimmunity [4] and airway microbiota [5] may also contribute to the pathogenesis of COPD, since 25% of ever-smokers (current and former smokers) develop COPD [6]. At present, the pathogenesis of COPD has not been fully clarified.

The human gut is colonised by many microorganisms, which, through interaction with each other and with the host, form a microbial community termed the microbiota [7]. Imbalance of the gut microbiota, or gut dysbiosis, is caused by numerous factors, including host factors such as medication use and tobacco smoking [8–10]. Gut dysbiosis may cause disorders such as obesity, diabetes or inflammatory bowel

disease [7]. Recently, several studies have evaluated the association between the gut microbiome and COPD, hypothesising that an altered gut microbiota contributes to the development or progression of COPD [11–13]. However, these studies either excluded non-COPD participants or included both never-and ever-smokers as the non-COPD controls. Therefore, the effects of COPD and smoking history could not be evaluated separately.

The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) was a prospective cohort study of a relatively large random sample from a general population of Japanese men living in Kusatsu City, Shiga, Japan [14, 15]. Although SESSA was originally conducted to investigate atherosclerosis, it also included information regarding pulmonary function and the gut microbiome. Utilising these data, we conducted the present study to examine the association of gut microorganisms in patients with COPD in comparison to that in never-smokers and ever-smokers without COPD.

Materials and methods

Participants

This cross-sectional observational study was conducted as a part of a SESSA follow-up survey. The SESSA participants underwent physical measurements and interviews regarding smoking from 2010 to 2014, pulmonary function tests from 2014 to 2015, and provided faecal samples from 2015 to 2016. Participants who underwent both pulmonary function and gut microbiology tests were considered eligible.

This study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Shiga University of Medical Science (approval no. G2008-061). All participants provided written informed consent to participate in this study.

General characteristics

The following information collected from the participants was analysed: age, height, weight, body mass index (BMI), smoking habits and amount of ethanol consumption per week. In addition, participants were asked if they were under treatment for type 2 diabetes mellitus (T2DM).

Pulmonary function tests

Pulmonary function tests were performed according to the recommendations from the American Thoracic Society/European Respiratory Society [16]. The predicted values for spirometry were calculated using the formula provided in the Japanese Respiratory Society guidelines [17]. Patients with COPD were identified based on smoking exposure and airflow limitation of forced expiratory volume in 1 s (FEV_1) over forced vital capacity <70% after bronchodilator use according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1].

For the *post hoc* analysis, we subgrouped patients with COPD based on GOLD stages; GOLD I, FEV_1 ⩾80% predicted; GOLD II, FEV_1 ⩾50% and <80% predicted; GOLD III, FEV_1% ⩾30% and <50% predicted; and GOLD IV, FEV_1 <30% predicted.

Gut microbiome estimation

Participants collected their faeces in brush-type faecal collection containers (TechnoSuruga Laboratory Co, Shizuoka, Japan) [18]. DNA was extracted from stool samples using the bead-beating method [19]. Subsequently, the bacterial 16S ribosomal RNA gene V3-V4 region was amplified using the polymerase chain reaction method, and DNA sequencing was performed on a MiSeq System (Illumina, Inc., San Diego, CA, USA).

The output of the sequencer was processed using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), Sickle (https://github.com/najoshi/sickle), FLASH [20] and the UCHIME algorithm of USEARCH [21]. We identified operational taxonomic units (OTUs) on Qiime [22] with Greengenes (version 13_8) [23] as the reference database. The microbiome data of each participant was sampled to equalise to 9247 reads. Intra-individual diversity indices of the gut microbiome (α-diversity index), *i.e.*, number of unique OTUs, Chao1 and Shannon diversity index, were calculated using the R vegan package (https://cran.r-project.org/web/packages/vegan/index.html).

Statistical analysis

Summary statistics were evaluated using the Wilcoxon rank-sum and Chi-squared tests. To compare the overall gut microbial compositions between participants with and without COPD, we performed principal coordinate analysis and permutational analysis of variance (ANOVA) based on Bray–Curtis dissimilarity using the R vegan package. To identify gut microorganisms whose abundance in patients with COPD was

different from never-smokers or non-COPD ever-smokers, we used sparse partial least-squares discriminant analysis (sPLS-DA) [24]. We selected 10 genera whose absolute values of loadings were highest and further compared them using the Wilcoxon rank-sum test. p-values were corrected for multiple comparison by the method of Benjamini and Hochberg. For the same purpose, we also used the analysis of composition of microbiomes (ANCOM) and considered W/(number of genera tested – 1) > 0.70 as statistically significant [25]. We used the R packages mixOmics for sPLS-DA (www.bioconductor.org/packages/release/bioc/html/mixOmics.html) and analysis of composition of microbiomes with bias correction (ANCOMBC) for ANCOM (www.bioconductor.org/packages/release/bioc/html/ANCOMBC.html).

When appropriate, we performed analyses while adjusting for age, BMI, amount of ethanol consumption per week (grammes per week) and treatment for T2DM. We used ANOVA to determine whether adding smoking status, *i.e.* current or former smokers, to a model improved the model fitting by testing significance of reduction in sum of squared residuals. p-values <0.05 were considered statistically significant. Data handling, plotting and statistical analyses were performed using R version 4.3.2 (www.r-project.org/) and RStudio version 2023.3.1.446 (Posit Software, Boston, MA, USA) with the tidyverse package (www.tidyverse.org/).

Additional methodological details are provided in the supplementary material.

Results

Study profile

Figure 1 presents the participant screening process. Of the 853 SESSA study participants, 495 underwent both pulmonary function tests and faecal examination. Of these, 14 participants were excluded because information on either ethanol consumption or T2DM treatments was not available. Subsequently, 481 participants were included in the analysis, comprising 99 never-smokers, 306 non-COPD ever-smokers and 76 patients with COPD.

Table 1 shows the characteristics of the study participants. All participants were men, reflecting the participant recruitment strategy of SESSA. There were no significant differences in age. BMI tended to be lower in patients with COPD compared with never-smokers and non-COPD ever-smokers. Patients with COPD and non-COPD ever-smokers consumed a similar amount of ethanol, which was significantly greater than that consumed by never-smokers. Patients with COPD were more likely to be current smokers

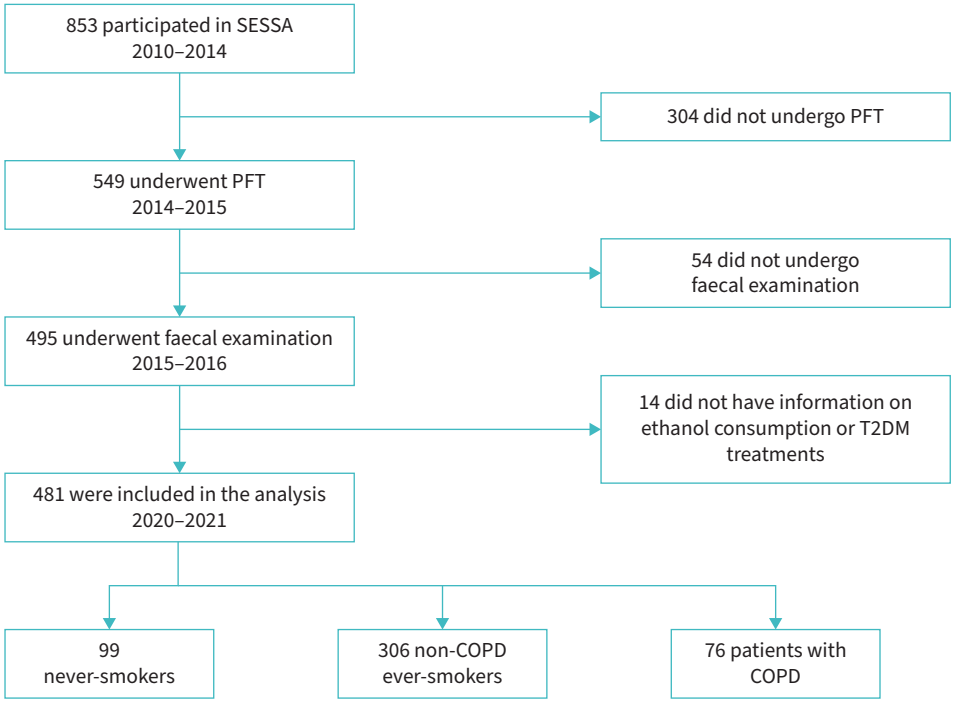

FIGURE 1 Study participant screening process. SESSA: Shiga Epidemiological Study of Subclinical Atherosclerosis; PFT: pulmonary function test; T2DM: type 2 diabetes mellitus.

TABLE 1 Characteristics of participants in this analysis

Characteristic	Never-smokers	Non-COPD ever-smokers	COPD	p-value
Participants n	99	306	76	
Age years	66.3±7.9	66.6±7.1	66.9±6.3	0.9
Height cm	165.9±5.6	166.4±5.7	166.9±5.0	0.5
Weight kg	65.1±9.1	65.3±9.7	63.5±8.5	0.3
BMI kg·m^{-2}	23.6±2.7	23.5±3.0	22.8±2.7	0.086
Smoking status				<0.001
Current	0 (0)	67 (22)	27 (36)	
Former	0 (0)	239 (78)	49 (64)	
Never	99 (100)	0 (0)	0 (0)	
Smoking history pack-years	0.0±0.0	30.4±23.1	45.2±30.6	<0.001
Ethanol consumption g·week^{-1}	91.6±112.7	173.3±183.5	157.1±168.1	<0.001
Under treatment for T2DM	11 (11)	46 (15)	4 (5.3)	0.063
FVC % predicted	95.5±13.3	93.9±13.6	99.4±17.8	0.012
FEV_1 % predicted	90.4±12.7	89.1±12.6	77.7±15.1	<0.001
FEV_1/FVC %	77.0±5.6	77.0±5.1	63.4±6.3	<0.001

Data are presented as mean±SD or number (percentage in the corresponding group). BMI: body mass index; T2DM: type 2 diabetes mellitus; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s.

with a higher smoking index compared to non-COPD ever-smokers. Patients with COPD were less likely to be undergoing treatment for T2DM compared with never-smokers and non-COPD ever-smokers. FEV_1 values were lower in patients with COPD than in non-COPD participants, as expected. In the COPD group, 33 patients were stage I, 40 were stage II, two were stage III and one was stage IV, based on the GOLD classification [1].

Diversities of gut microbiome

At the phylum level, the abundance of Firmicutes differed significantly among the three groups (p=0.025 by the ANCOVA model with adjustment for age, BMI, amount of ethanol consumption and treatment for T2DM; figure 2). The abundance of Firmicutes tended to be less in patients with COPD and non-COPD ever-smokers compared with never-smokers (p=0.074 for patients with COPD and p=0.026 for non-COPD smokers in comparison to never-smokers by *post hoc* Tukey test) but was comparable between patients with COPD and non-COPD ever-smokers (p=0.96).

α-Diversity indices, number of unique OTUs, Chao1 index and Shannon index, were significantly lower in patients with COPD and tended to be lower in non-COPD ever-smokers than never-smokers. These indices did not significantly differ between patients with COPD and non-COPD ever-smokers (figure 3 and supplementary tables S1–S6). Including current smoking status, *i.e.* either current or former smokers, in the statistical model to compare patients with COPD and non-COPD ever-smokers did not improve the statistical model fitting (p=0.25, 0.36 and 0.53 by ANOVA for the models to predict number of species, Chao-1 and Shannon index, respectively), indicating that current smoking status had no significant effects on these diversity indices.

In the β-diversity analysis, the gut microbial composition of the three groups tended to be different (p=0.057 by the permutational ANOVA after adjustment for age, BMI, amount of ethanol consumption and treatment for T2DM; figure 4 and supplementary table S7). Pairwise analysis showed that the composition of patients with COPD and non-COPD ever-smokers was similar (p=0.377 between patients with COPD and non-COPD ever-smokers) but differed from that of never-smokers (p=0.057 between never-smokers and non-COPD ever-smokers, and p=0.023 between never-smokers and patients with COPD, respectively, by permutational ANOVA adjustment for age, BMI, amount of ethanol consumption and treatment for T2DM; supplementary tables S8–S10).

Identification of genera with different abundances in patients with COPD compared to never-smokers or non-COPD ever-smokers

To identify genera whose abundance differed between patients with COPD and never-smokers, we performed sPLS-DA and chose the 10 genera with the highest absolute loadings on the axis to differentiate these two groups (figure 5a). Among them, the abundance of genera *Anaerostipes*, *Bulleidia*, *Bilophila*, *Dehalobacterium* and *Ruminococcus* was significantly lower in patients with COPD, while genus *[Prevotella]*,

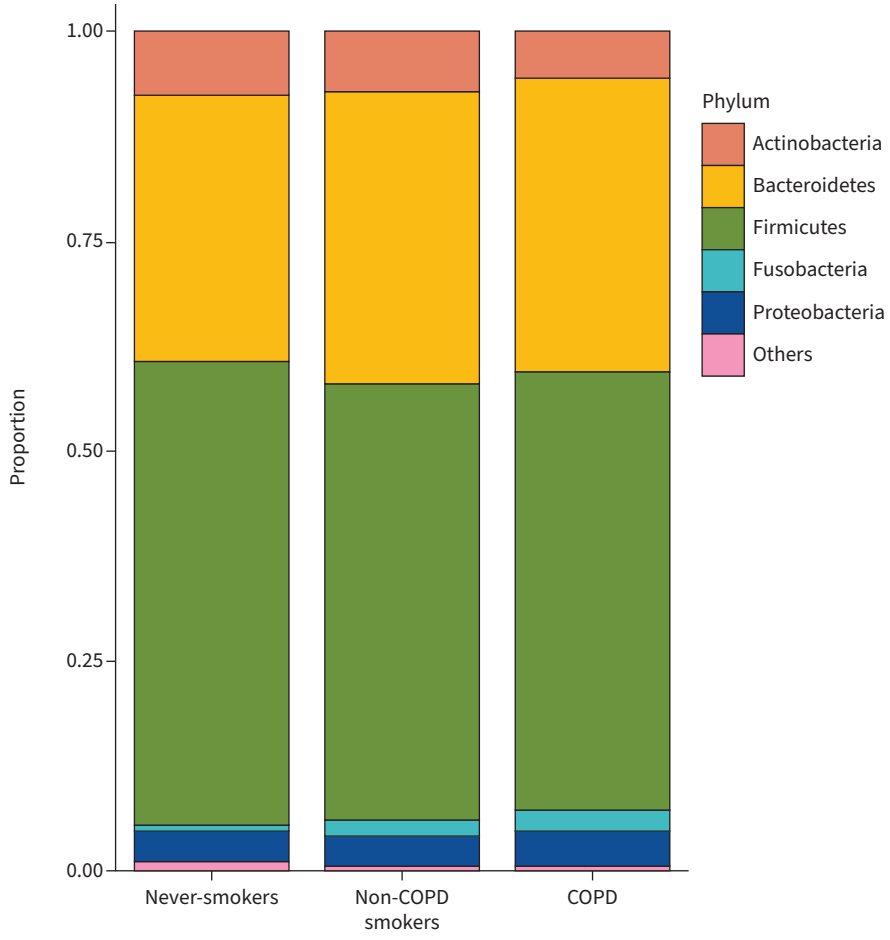

FIGURE 2 Composition of gut microbiome at the phylum level. Proportion of gut microbiome at the phylum level in each participant was averaged for never-smokers (n=99), non-COPD ever-smokers (n=306) or patients with COPD (n=76).

which was proposed to be affiliated with the family *[Paraprevotellaceae]* by the Greengenes database, was significantly more abundant in patients with COPD compared to never-smokers. The ANCOM model with adjustment for age, BMI, amount of ethanol consumption and treatment for type 2 diabetes did not detect any genera whose abundance was significantly different between patients with COPD and never-smokers.

While the similarity of α- and β-diversities in the gut microbiome between patients with COPD and non-COPD ever-smokers indicated that the abundance of most of the components of the gut microbiota was similar between these two groups, there was still a possibility that the abundance of a few might be different. Thus, we applied the same methods as above to compare patients with COPD and non-COPD ever-smokers. Among the genera with top 10 absolute loadings to differentiate these two groups using sPLS-DA, genus *Catenibacterium* and genus *[Prevotella]* were significantly more abundant in patients with COPD than in non-COPD ever-smokers (figure 5b). The ANCOM model with the adjustment detected genus *[Prevotella]* to be significantly different between these two groups (supplementary table S11).

When we examined the prevalence of gut *[Prevotella]* in the three groups, the gut *[Prevotella]* was more frequently present in the gut of patients with COPD compared to never-smokers and non-COPD ever-smokers (34.2%, 16.2% and 16.0%, respectively; $p<0.001$ by Chi-squared test; figure 6).

Post hoc *analysis comparing gut microbiome among patients with COPD GOLD I, GOLD II and non-COPD ever-smokers*

To evaluate whether the gut microbial composition changes with progression of COPD, we performed a subgroup analysis dividing patients with COPD based on GOLD stages. Among 76 patients with COPD,

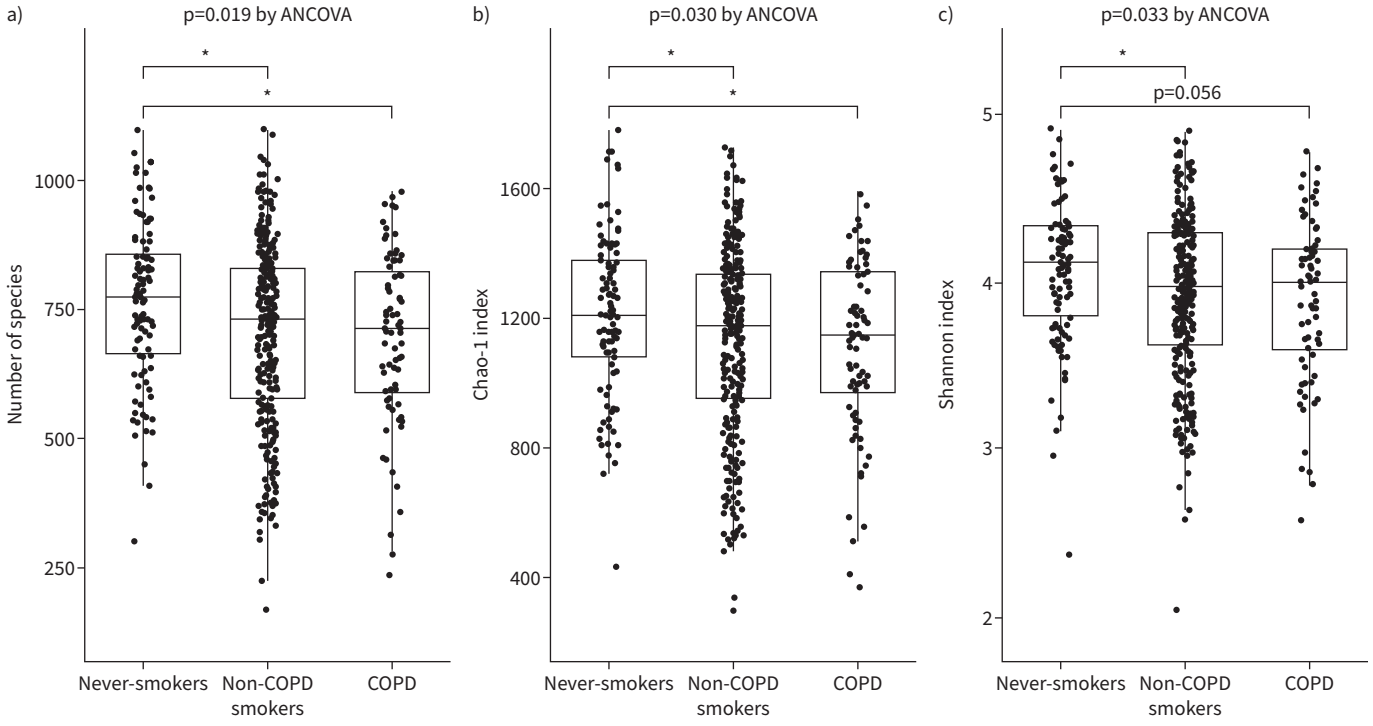

FIGURE 3 α-diversity indices of the gut microbiome. a) The number of species, b) Chao-1 index and c) Shannon index of the gut microbiome from each participant were compared among never-smokers (n=99), non-COPD ever-smokers (n=306) and patients with COPD (n=76). Group difference was tested using the analysis of covariance (ANCOVA) model, and pairwise comparison was performed using the Tukey honestly significant difference test adjusting for age, body mass index, ethanol consumption and treatment for type 2 diabetes mellitus. *: p<0.05.

only three were in GOLD III or IV. Therefore, we excluded patients with COPD GOLD III or IV, and compared gut microbiome among patients with COPD GOLD I, GOLD II and non-COPD ever-smokers. The characteristics of study participants in this *post hoc* analysis are shown in supplementary table S12.

α-Diversity indices, number of unique OTUs, Chao1 index and Shannon index, were similar among these three groups (supplementary figure S1 and tables S13–S15). In the β-diversity analysis, the gut microbial composition of the three groups tended to be different (p=0.067 by the permutational ANOVA after adjustment for age, BMI, amount of ethanol consumption and treatment for T2DM; supplementary figure S2 and table S16). Pairwise analysis showed that the gut microbial composition of patients with GOLD I and non-COPD ever-smokers was similar (p=0.833 by permutational ANOVA with adjustment, supplementary table S17), but the composition of patients with GOLD II was likely different from that of patients with GOLD I or non-COPD ever-smokers (p=0.051 between GOLD II and GOLD I, and p=0.015 between GOLD II and non-COPD ever-smokers, respectively, by permutational ANOVA with adjustment; supplementary tables S18 and S19). The prevalence of gut *[Prevotella]* increased as COPD progressed (supplementary figure S3).

Discussion

In the present study, we used the SESSA data of a random sample from male residents in Kusatsu City, Shiga, Japan, and showed that gut microbiome composition of patients with COPD was different from that of never-smokers and similar to that of non-COPD ever-smokers. In addition, discriminant analysis revealed that several genera, especially genus *[Prevotella]*, differed in abundance among patients with COPD, never-smokers and non-COPD ever-smokers. To our knowledge, this is the first study which assessed the association between gut microbiome and COPD separately from smoking history.

A few previous studies have investigated the gut microbiome in patients with COPD. Bowerman *et al.* [11] compared 28 patients with COPD (n=4, 11, 8 and 11 in GOLD stages I, II, III and IV, respectively) and 29 healthy controls (18 never-smokers and 11 ever-smokers) and found that β-diversity analysis showed a significant difference in gut microbial components between these two groups. Chiu *et al.* [12] assessed the gut microbiome in 60 patients with COPD (n=20, 20 and 20 in GOLD stages I, II and III/IV, respectively)

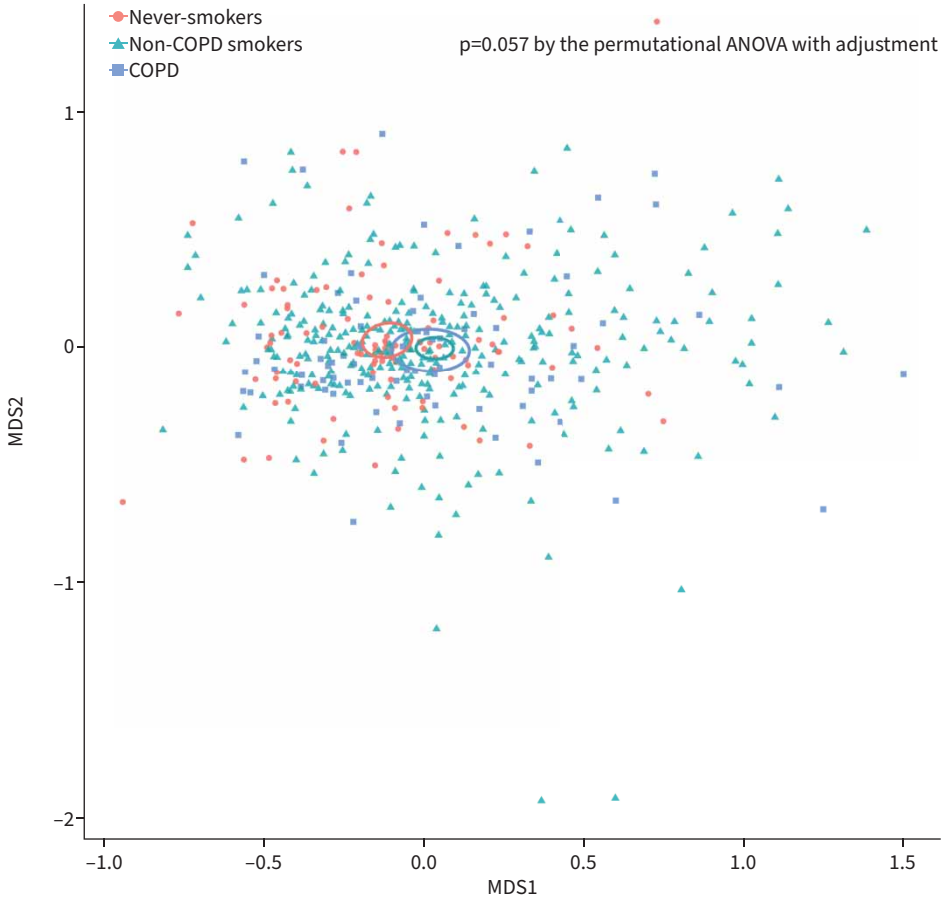

FIGURE 4 Multi-dimensional scaling (MDS) plot of gut microbiome by Bray–Curtis dissimilarity. Red circles, green triangles and blue squares indicate never-smokers, non-COPD ever-smokers and patients with COPD, respectively. Ellipses indicate standard errors of the centroids of groups. Statistical analyses were performed using the permutational ANOVA while adjusting for age, body mass index, ethanol consumption and treatment for type 2 diabetes mellitus.

and found no differences in α- and β-diversities among these three groups. Of note, they did not compare patients with COPD to either never- or non-COPD ever-smokers. Li *et al.* [13] compared patients with COPD in GOLD stages I/II (n=67) and III/IV (n=32) with 73 healthy non-COPD participants (38 never-smokers, 15 former smokers and 20 current smokers). They showed that the number of unique OTUs in the gut microbiome was significantly lower in patients with GOLD III/IV compared to healthy participants or patients with GOLD I/II but was similar between healthy participants and patients with GOLD I/II. In their β-diversity analysis, the three groups did not have significantly different gut microbial components. Since these studies either excluded non-COPD participants or included combined never- and ever-smokers as a non-COPD control group, the association of COPD with the gut microbiome could not be assessed separately from the effect of smoking, although smoking is known to cause COPD as well as alter the gut microbiota [8–10, 26]. In our analysis, we compared the gut microbiome between patients with COPD, never-smokers and non-COPD ever-smokers with a relatively large number of participants (n=494) and showed that the gut microbial composition was similar between patients with COPD and non-COPD ever-smokers and was different from that of never-smokers. Since patients with COPD in our cohort were mostly in GOLD stages I and II, our findings may be consistent with the results by Li *et al.* in that patients with early-stage COPD and non-COPD ever-smokers were similar in terms of gut microbial composition. Combining the results of the current and previous reports, the overall gut microbial composition appears to be altered by smoking but may not be a direct driver of the development of COPD. Rather, the overall gut microbial composition may change with COPD progression. This notion was further supported by our *post hoc* analysis, which showed no differences between patients with GOLD I and non-COPD ever-smokers in α- and β-diversities of gut microbiome, but gut microbial composition, *i.e.* β-diversity, in patients with GOLD II was likely different from patients with GOLD I or non-COPD

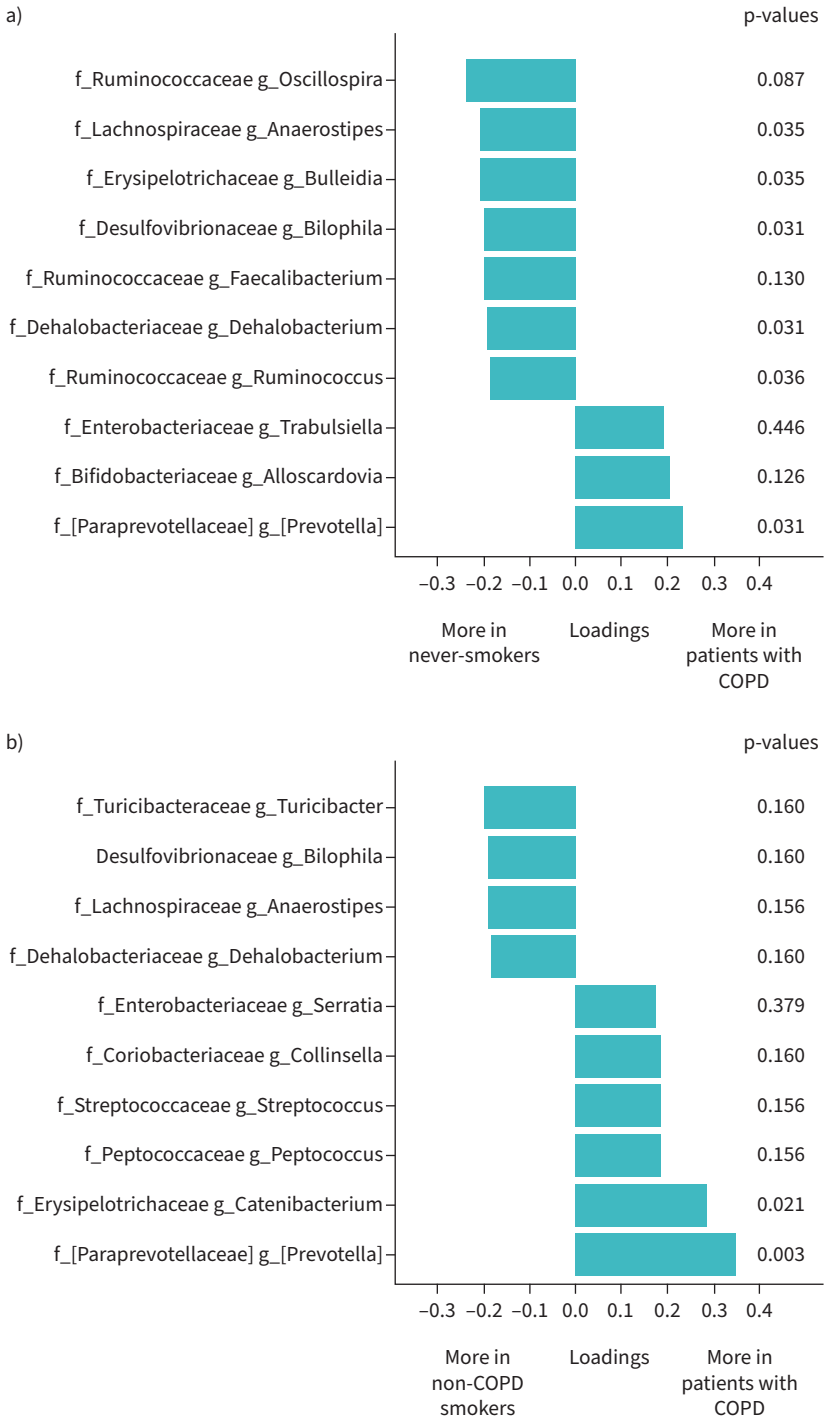

FIGURE 5 10 genera with highest absolute loadings using sparse partial least-squares discriminant analysis (sPLS-DA) to discriminate the gut microbiome of patients with COPD from either a) never-smokers or b) non-COPD ever-smokers. Statistical analyses were performed using Wilcoxon tests and adjusted using the Benjamini–Hochberg procedure for the multiple comparisons.

ever-smokers. Disagreement between our *post hoc* analysis and the results of Li *et al.* [13] may be attributed to the sample size, or the study population.

In our discriminant analysis, we found genus *[Prevotella]* was more prevalent in the gut of patients with COPD compared to never-smokers or non-COPD ever-smokers. *Prevotella* is an obligately anaerobic

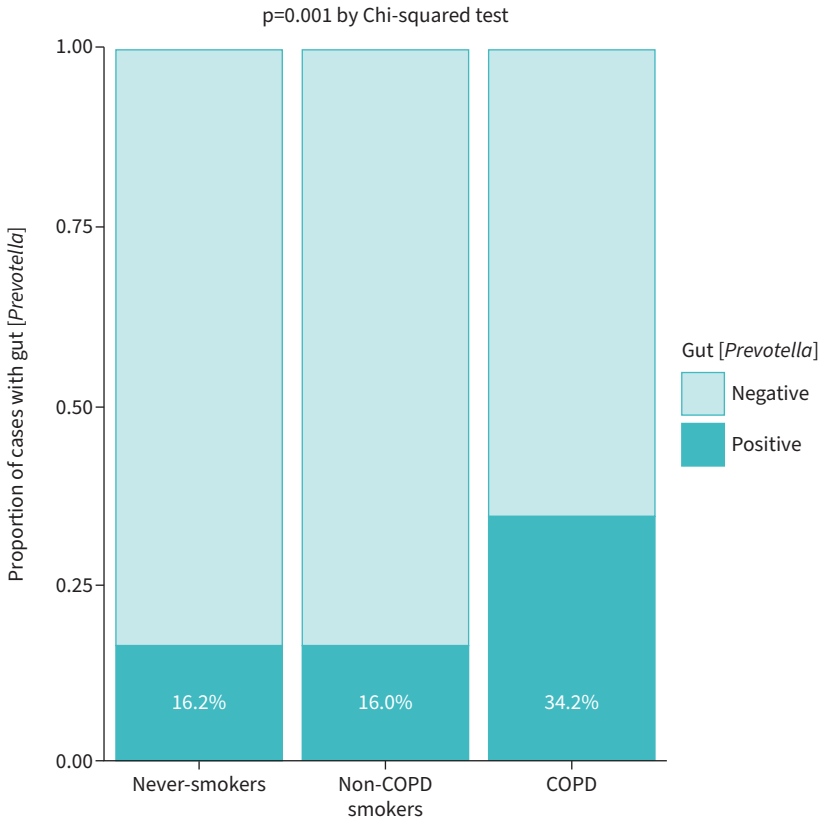

FIGURE 6 Prevalence of genus *[Prevotella]* in the stool samples. Statistical analysis was performed using the Chi-squared test.

Gram-negative bacteria that belongs to the phylum Bacteroidetes. Gut *Prevotella* has been linked to inflammatory disorders such as rheumatoid arthritis [27] and human immunodeficiency virus infection [28, 29]. Li *et al*. reported that patients with COPD were more likely to have a *Prevotella*-dominated enterotype compared to healthy controls. Furthermore, mice that have undergone faecal transplants from patients with COPD showed elevated inflammatory cytokines in the lungs. When these mice were exposed to biomass smoke, they developed emphysematous changes as well as airway remodelling [13]. These results suggest that gut microbiota rich in *Prevotella* may promote systemic inflammation and COPD development in people exposed to noxious substances. Since our finding was about the genus *[Prevotella]*, which is proposed to be a member of family *[Paraprevotellaceae]* by the Greengenes database, genus *Prevotella* and genus *[Prevotella]* may have different functions. Further studies are necessary to confirm the significance of genus *Prevotella*.

This study has several limitations. First, the cross-sectional, observational design complicated speculation about the causal relationship between gut microbiota and COPD. Second, we did not have complete information about other factors that may affect the gut microbiota, such as history of gastrointestinal diseases, episodes of infectious disease and COPD exacerbations, history of medication usage, especially antibiotics and corticosteroids which are used to treat COPD exacerbations, and dietary habits. Furthermore, information on whether participants received treatment for COPD, such as inhaled corticosteroids or bronchodilators, was not available. Few studies have shown that the gut microbiota was altered during COPD exacerbation [30, 31]. Regarding past exacerbation events and gut microbiome, only one small sized analysis with 28 patients with COPD by Bowerman *et al*. [11] was performed and showed that there were no differences in microbial components between stable and frequent exacerbators. To date, no study has been conducted to reveal the association of gut microbiota with future risk of COPD exacerbations. Further studies are needed to clarify these points. Third, participation in this study was limited only to Japanese men, making it difficult to generalise the results to women and other ethnicities. The relationship between gut microbiota and COPD in these populations remains to be investigated. Fourth, although the sample size of patients with COPD in this study was relatively larger than that of

previous studies, it might be limited in terms of broadly generalising the results of this study; therefore, further investigations with larger sample sizes are necessary.

Conclusions

Our results from this population-based study of Japanese men indicated that the overall gut microbial composition may be affected by smoking but may not play a role in the development of COPD. In addition, specific gut bacteria, such as *[Prevotella]*, could be a risk factor for the development and progression of COPD, and this may be a potential therapeutic target to prevent COPD.

Provenance: Submitted article, peer reviewed.

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Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest: The authors declare that they have no competing interests.

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Ethics statement: This study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Shiga University of Medical Science (approval number G2008-061). All participants provided written informed consent to participate in this study.

References

1 Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management and Prevention of COPD. 2023 Report. Date last accessed: 2 September 2023. https://goldcopd.org/2023-gold-report-2/
2 Roth GA, Abate D, Abate KH, *et al.* Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; 392: 1736–1788.
3 Cho MH, McDonald MLN, Zhou X, *et al.* Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *Lancet Respir Med* 2014; 2: 214–225.
4 Lee SH, Goswami S, Grudo A, *et al.* Antielastin autoimmunity in tobacco smoking-induced emphysema. *Nat Med* 2007; 13: 567–569.
5 Sze MA, Dimitriu PA, Suzuki M, *et al.* Host response to the lung microbiome in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015; 192: 438–445.
6 Løkke A, Lange P, Scharling H, *et al.* Developing COPD: a 25 year follow up study of the general population. *Thorax* 2006; 61: 935–939.
7 Hou K, Wu ZX, Chen XY, *et al.* Microbiota in health and diseases. *Signal Transduct Target Ther* 2022; 7: 135.
8 Tomoda K, Kubo K, Asahara T, *et al.* Cigarette smoke decreases organic acids levels and population of bifidobacterium in the caecum of rats. *J Toxicol Sci* 2011; 36: 261–266.
9 Biedermann L, Brülisauer K, Zeitz J, *et al.* Smoking cessation alters intestinal microbiota: insights from quantitative investigations on human fecal samples using FISH. *Inflamm Bowel Dis* 2014; 20: 1496–1501.
10 Lee SH, Yun Y, Kim SJ, *et al.* Association between cigarette smoking status and composition of gut microbiota: population-based cross-sectional study. *J Clin Med* 2018; 7: 982.
11 Bowerman KL, Rehman SF, Vaughan A, *et al.* Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. *Nat Commun* 2020; 11: 5886.

12 Chiu YC, Lee SW, Liu CW, *et al.* Comprehensive profiling of the gut microbiota in patients with chronic obstructive pulmonary disease of varying severity. *PLoS One* 2021; 16: e0249944.
13 Li N, Dai Z, Wang Z, *et al.* Gut microbiota dysbiosis contributes to the development of chronic obstructive pulmonary disease. *Respir Res* 2021; 22: 274.
14 Kadota A, Miura K, Okamura T, *et al.* Carotid intima-media thickness and plaque in apparently healthy Japanese individuals with an estimated 10-year absolute risk of CAD death according to the Japan atherosclerosis society (JAS) guidelines 2012: The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA). *J Atheroscler Thromb* 2019; 26: 746.
15 Miyazawa I, Ohkubo T, Kadowaki S, *et al.* Change in pericardial fat volume and cardiovascular risk factors in a general population of Japanese men. *Circ J* 2018; 82: 2542–2548.
16 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.
17 Sasaki H, Nakamura M, Kida K, *et al.* Reference values of spirograms and arterial blood gas partial pressure in Japanese. *J Jap Respir Soc* 2001; 39: S1–S17.
18 Hosomi K, Ohno H, Murakami H, *et al.* Method for preparing DNA from feces in guanidine thiocyanate solution affects 16S rRNA-based profiling of human microbiota diversity. *Sci Rep* 2017; 7: 1–10.
19 Takahashi S, Tomita J, Nishioka K, *et al.* Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS One* 2014; 9: e105592.
20 Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 2011; 27: 2957–2963.
21 Edgar RC, Haas BJ, Clemente JC, *et al.* UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011; 27: 2194–2200.
22 Caporaso JG, Kuczynski J, Stombaugh J, *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; 7: 335–336.
23 DeSantis TZ, Hugenholtz P, Larsen N, *et al.* Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006; 72: 5069–5072.
24 Cao KAL, Boitard S, Besse P. Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. *BMC Bioinformatics* 2011; 12: 253.
25 Mandal S, Van Treuren W, White RA, *et al.* Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis* 2015; 26: 27663.
26 Antinozzi M, Giffi M, Sini N, *et al.* Cigarette smoking and human gut microbiota in healthy adults: a systematic review. *Biomedicines* 2022; 10: 510.
27 Alpizar-Rodriguez D, Lesker TR, Gronow A, *et al.* Prevotella copri in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis* 2019; 78: 590–593.
28 Dillon SM, Lee EJ, Kotter CV, *et al.* Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T-cell activation in untreated HIV-1 infection. *Mucosal Immunol* 2016; 9: 24–37.
29 Dillon SM, Lee EJ, Kotter CV, *et al.* An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunol* 2014; 7: 983–994.
30 Wu Y, Luo Z, Liu C. Variations in fecal microbial profiles of acute exacerbations and stable chronic obstructive pulmonary disease. *Life Sci* 2021; 265: 118738.
31 Sun Z, Zhu QL, Shen Y, *et al.* Dynamic changes of gut and lung microorganisms during chronic obstructive pulmonary disease exacerbations. *Kaohsiung J Med Sci* 2020; 36: 107–113.