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Original Article

Relationship between gut microbiota composition and sensitization to inhaled allergens

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JCP	Japanese cedar pollen
HD	house dust
rRNA	ribosomal ribonucleic acid
DNA	deoxyribonucleic acid
AD	atopic dermatitis

ABSTRACT

Background: An imbalance in gut microbiota is implicated in several pathological conditions, including allergic diseases. This study investigates the association between gut microbiota composition and sensitization to two inhaled antigens.

Methods: The study comprised 1109 local residents who had participated in the Iwaki Health Promotion Project in 2016. Blood samples were analyzed for levels of antigen-specific IgE against Japanese cedar pollen (JCP) and house dust (HD1). Fecal samples were analyzed for bacterial 16S rRNA (ribosomal ribonucleic acid) using next generation sequencing. The percent composition of gut microbes was compared between patients sensitized and unsensitized group for JCP and HD1 to determine whether the rate of sensitization to inhaled antigens associates with specific bacterial orders composing the gut microbiota.

Results: In participants aged 20–49 years, the percent composition of Bacteroidales was significantly higher among participants sensitized to JCP than in those unsensitized. The percent composition of Lactobacillales was significantly higher in participants unsensitized to HD group than in those sensitized to that antigen. In addition, participants with low Bacteroidales and high Bifidobacteriales or Lactobacillales has low sensitization rates to HD compared with high Bacteroidales and low Bifidobacteriales or Lactobacillales.

Conclusions: The presence of bacteria of order Lactobacillales, Bifidobacteriales, and Bacteroidales in the gut microbiota may affect sensitization to inhaled allergens.

Introduction

The importance of gut microbiota to human health has been the focus of many recent studies. Conventional studies of gut microbiota use culture-based analysis. Because most bacteria in the intestine are anaerobic and difficult to cultivate, this method can provide only limited information regarding gut microbiota. As an alternative, genome analysis using PCR can identify gut microbes through analysis of the 16S rRNA gene.[1] Studies using this method have identified 500–1000 bacterial species in the human intestine, with a total bacterial count of 100 trillion—nearly 10 times the number of human somatic cells.[2,3] Next generation sequencing now allows for the processing of a large number of samples in a short time. Together, these new techniques have provided data indicating a relationship between an imbalance of gut microbiota (dysbiosis) and conditions such as diabetes, arteriosclerosis, malignant tumors, autoimmune diseases, inflammatory bowel diseases, and allergic diseases.[3,4]

During the past decades, prevalence of allergic diseases such as allergic rhinitis, bronchial asthma, and atopic dermatitis is rapidly increasing in developed countries, including Japan.[5] This finding has been attributed to the hygiene hypothesis and changes in gut microbiota due to changing eating habits and lifestyle.[6–11] The gut microbiota is known to play an important role in health, producing vitamins and short-chain fatty acids and regulating the immune system.[12] Therefore, several studies have been conducted on the prevention and treatment of allergic diseases by balancing the gut microbiota. It is reported that the intake *Bifidobacterium* or *Lactobacillus* as probiotics reduces the symptoms of allergic rhinitis, atopic dermatitis (AD), and bronchial asthma.[6,13–19] However, few

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studies have investigated how the gut microbiota is involved in sensitization to inhaled allergens. This study investigates the association between the gut microbiota and sensitization to an inhaled antigen in a study of participants in the Iwaki Health Promotion Project of 2016, a community-based program aimed at improving the average life expectancy by providing general health checkups.

Preliminary analysis at genus level showed that *Streptococcus* and *Lactobacillus* tend to reduce HD sensitization (unpublished data presented in the congress of the International Society of Inflammation and Allergy of the Nose in 2018). *Lactobacillus* and *Streptococcus* are lactic acid bacteria and are classified as Lactobacillales at the order level, which is the higher phylogenetic classification. Therefore, we investigated the effects of 48 orders of bacteria composing the gut microbiota on host sensitization to HD and JCP, two major allergens in Japan.[5]

Methods

Subjects

We have conducted the health survey 'Iwaki Health Promotion Project' annually since 2005. This project targets people ≥20 years of age who live in the Iwaki District of Hirosaki City, Aomori Prefecture, Japan. Invitation letters for this survey were sent to approximately 10 000 people to recruit participants. A total of 1145 volunteers participated in this project in 2016. Volunteers who lacked data regarding blood or/and stool samples were excluded, and eventually the date of 1109 volunteers were used for the present cross-sectional study. Data collection for this study and project was approved by the Ethics Committee of Hirosaki University School of Medicine (Authorization number: 2016-085), and all participants gave written informed consent before participating.

Physical characteristics

Subject date of birth and sex were indicated on the questionnaire. Height and weight were measured, and the body mass index (BMI) was calculated as kg/m^2.

Blood analysis

Peripheral blood samples were collected from participants and measured eosinophil count, total cholesterol, and triglycerides. The total IgE and IgE specific for JCP and HD1 were determined using the immuno CAP system. If the antigen-specific IgE score ≥1 (0.35 kU/L), we defined that they were sensitized to the antigen.

Extraction of DNA (deoxyribonucleic acid) from fecal samples

Each subject promptly transferred two to three grams of fresh feces to a storage container (TechnoSuruga Laboratory Co.) containing a stock guanidine thiocyanate solution (100 mM Tris−HCl [pH 9.0], 40 mM Tris−EDTA [pH 8.0], and 4 M guanidine thiocyanate). Specimens were stored at room temperature. Fecal sample suspensions were milled with zirconia beads at 5 m/s for 2 min using the FastPrep 24 Instrument (MP Biomedicals, Santa Ana, CA, USA). DNA was extracted from 200-μL aliquots of the samples using the automated Magtration System 12Gc with MagdDEA DNA 200 as the reagent.

Table 1
Number of participants by age.

Age(years)	20−29	30−39	40−49	50−59	60−69	70−79	≥80
Men	23	81	77	84	109	47	17
Women	34	97	112	128	182	94	24
Total	57	178	189	212	291	141	41

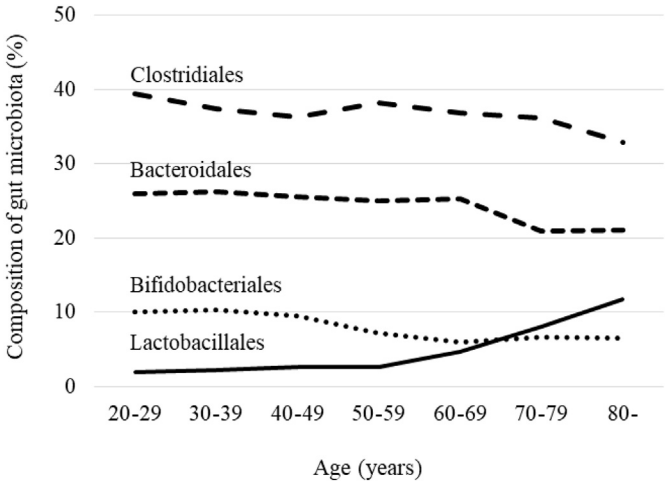

Fig. 1. The percent composition of representative bacterial orders by host age group. Note that the composition of the gut microbiota fluctuated greatly after 50 years of age.

Analysis of gut microbiota by next generation sequencing

The sequence of the V3−V4 region of 16S rDNA was used to identify bacteria. Using an Illumina MiSeq system (Illumina, San Diego, USA), bacterial sequences were detected and identified using Metagenome@KIN software (World Fusion Co, Japan) analysis software. Analysis was conducted for 97% sequence similarity using the TechnoSuruga Lab Microbial Identification database DB-BA10.0 (TechnoSurgara laboratory, Japan). Bacterial orders represented in the gut microbiota are expressed as % composition (the number of leads of each bacterial gene divided by the total number of leads).

Data analysis

We examined the percent composition of the represented bacterial orders and rates of sensitization to HD1 and JCP by age group. Statistical analysis was used to determine the relationship between

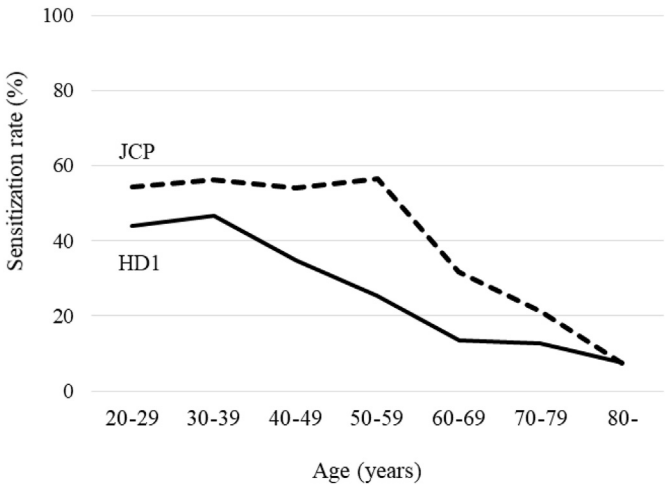

Fig. 2. Sensitization rates against HD and JCP by age.

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gut microbiota and sensitization to HD1 and JCP. Multivariate analysis of factors affecting sensitization was performed. Lactobacillales, Bifidobacteriales, and Bacteroidales were divided into low and high groups based on the median. We combined two bacterial orders selected out of these three bacterial orders and made four groups. We compared the counts of eosinophils, total IgE, and IgE specific for HD1 and JCP among the four groups.

Statistical analysis

Comparison of gut microbiota between sensitized and unsensitized groups was performed using the two-sample t-test or Welch's two-sample t-test. Multiple logistic regression analysis was performed using sensitization to JCP and HD1 as the dependent variable. Independent variables were age, sex, BMI, eosinophil, total cholesterol, triglyceride, and presence of major four bacterial orders (Bacteroidales, Bifidobacteriales, Clostridiales, and Lactobacillales). Multiple comparison tests between 4 groups with 2 bacterial orders were performed using Tukey's method. $p < 0.05$ was considered significant for all tests. All analyses were conducted using the Statistical Package for Social Sciences (SPSS ver. 25.0, IBM Armonk, NY, USA).

Results

Number of participants by age

The age group with the largest number of participants was 60–69 years. Throughout the age group, more number of women participated than men (Table 1).

Percent composition of bacterial orders and sensitization rates to JCP and HD by age

The percent composition of the 4 major bacterial orders (Clostridiales, Bacteroidales, Bifidobacteriales, and Lactobacillales) of the 48 bacterial orders represented according to host age is shown in Figure 1. The percent composition of Bacteroidales and Bifidobacteriales decreased and that of Lactobacillales increased with increasing age. The sensitization rates to both JCP and HD also changed with age (Fig. 2). The rate of sensitization to JCP was high in participants through their 50s, decreasing after the age of 60. Similarly, the rate of sensitization to HD was high in participants through their 30s, decreased after the age of 40. Therefore, to reduce the influence of age, the following analysis was performed

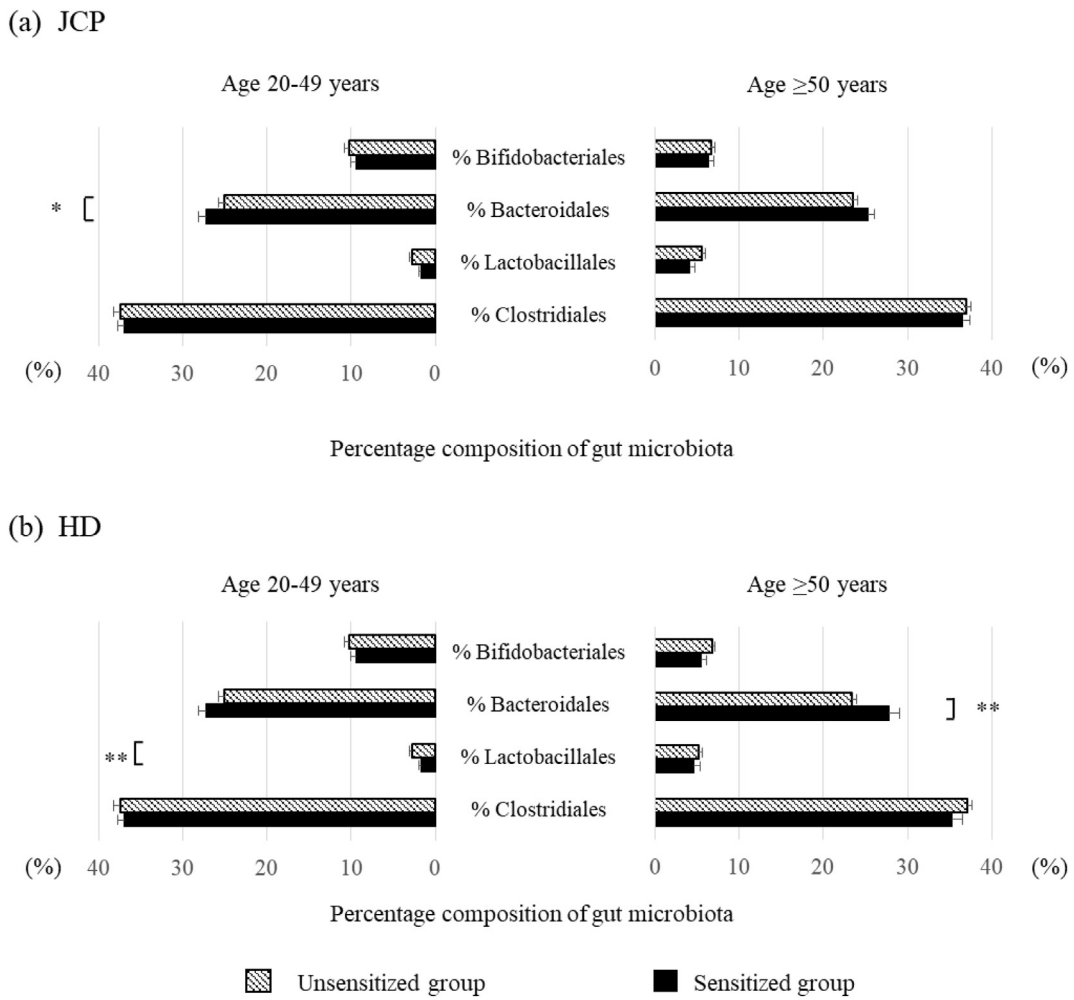

Fig. 3. Comparison of the percent composition of bacterial orders between sensitized and unsensitized participants by age group (20–49 and ≥ 50 years). **(a)** JCP, **(b)** HD. Only 4 representative bacterial orders (Clostridiales, Bacteroidales, Bifidobacteriales, and Lactobacillales) out of 48 are shown. No significant differences were observed in sensitization rates for the undisplayed bacterial orders. Bars indicate the mean secretion % ± Standard deviation. $^{*}p < 0.05$; $^{**}p < 0.01$.

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with the subjects divided into age groups of 20–49 years and ≥50 years.

The relationship between gut microbiota and sensitization to HD and JCP

Only two bacterial orders, Bacteroidales and Lactobacillales, of the 48 orders were associated with sensitization to JCP and HD (Fig. 3). The presence of Bacteroidales was significantly higher in the group sensitized to JCP than in the unsensitized group (age 20–49 years). Similarly, the presence of Bacteroidales was significantly higher in the group sensitized to HD than in the unsensitized group (age ≥ 50 years). The presence of Lactobacillales was significantly lower in the group sensitized to HD than in the unsensitized group (age 20–49 years). No other bacterial order was associated with sensitization to JCP or HD in either age group.

Multivariate analysis of factors associated with sensitization to JCP and HD

Table 2 shows the results of multiple logistic regression analysis. In the sensitized group of JCP (age 20–49 years), Bacteroidales were significantly high, and Bifidobacteriales tended to be low (Table 2a). On the other hand, in the sensitized group of HD, Lactobacillales were significantly low (age 20–49 years) and Bacteroidales tended to be high (age ≥ 50 years) (Table 2b).

The results in both univariate analysis (Fig. 3) and multivariate analysis (Table 2) suggest that Bacteroidales may have a promotive effect and Lactobacillales may have a suppressive effect on allergen sensitization.

Table 2
Multivariate analysis of factors associated with sensitization to JCP and HD.

(a) JCP				
Age group	20–49		>50	
	OR (95% CI)	p	OR (95% CI)	p
Age	0.998 (0.970–1.028)	0.918	0.914 (0.893–0.935)	0.000**
Sex	0.691 (0.449–1.063)	0.092	0.862 (0.593–1.255)	0.439
Body Mass Index	1.020 (0.962–1.080)	0.510	0.997 (0.943–1.055)	0.920
Eosinophil	1.121 (1.029–1.222)	0.009**	1.115 (1.035–1.201)	0.004**
Total cholesterol	0.999 (0.993–1.005)	0.662	0.999 (0.993–1.004)	0.610
Triglyceride	1.001 (0.997–1.004)	0.718	1.001 (0.998–1.004)	0.453
Bifidobacteriales	0.971 (0.940–1.001)	0.062	0.995 (0.970–1.021)	0.705
Bacteroidales	1.026 (1.003–1.050)	0.028*	1.001 (0.985–1.018)	0.890
Lactobacillales	0.980 (0.928–1.034)	0.464	1.005 (0.982–1.028)	0.690
Clostridiales	1.012 (0.990–1.036)	0.285	0.997 (0.979–1.015)	0.743
(b) HD				
Age group	20–49		>50	
	OR (95% CI)	p	OR (95% CI)	p
Age	0.968 (0.939–0.997)	0.029*	0.941 (0.915–0.967)	0.000**
Sex	0.864 (0.560–1.333)	0.509	0.632 (0.402–0.996)	0.048*
Body Mass Index	1.025 (0.967–1.086)	0.411	1.018 (0.949–1.091)	0.622
Eosinophil	1.106 (1.023–1.195)	0.011*	1.042 (0.951–1.142)	0.381
Total cholesterol	1.001 (0.995–1007)	0.713	0.998 (0.992–1.005)	0.610
Triglyceride	1.001 (0.998–1.004)	0.707	1.001 (0.997–1.004)	0.714
Bifidobacteriales	1.002 (0.972–1.032)	0.914	0.987 (0.954–1.022)	0.461
Bacteroidales	1.006 (0.983–1.029)	0.620	1.018 (0.997–1.039)	0.095
Lactobacillales	0.913 (0.847–0.983)	0.016*	1.016 (0.987–1.045)	0.282
Clostridiales	0.998 (0.975–1.021)	0.835	0.999 (0.977–1.022)	0.953

OR, Odds ratio; 95% CI, 95% confidence interval.
*$p < 0.05$; **$p < 0.01$.

The effect of the combination of two bacterial orders on the allergic factors

Multiple comparison analysis of two of the three bacterial orders (Bacteroidales, Lactobacillales, and Bifidobacteriales) observed to be associated with sensitization to JCP and/or HD was carried out for each age group. No significant correlation was observed between any of the combinations and allergic factors including the sensitization rate for JCP, total IgE, and the number of eosinophils. However, significant correlations were observed between several combinations of the bacterial orders with HD sensitization. Among participants aged 20–49 years, the rate of sensitization to HD was significantly low level in participants with low Bacteroidales and high Lactobacillales compared to those with high Bacteroidales and low Lactobacillales (Fig. 4a). The rate of sensitization to HD was significantly low level in participants with high Bifidobacteriales and high Lactobacillales compared to those with low Bifidobacteriales and low Lactobacillales (Fig. 4b). Among participants aged ≥50 years, the rate of sensitization to HD was low level in those with low Bacteroidales and high Bifidobacteriales compared to those with high Bacteroidales and low Bifidobacteriales (Fig. 4c).

Discussion

This study investigates the association between gut microbiota composition and sensitization to several inhaled antigens. We observed that among patients aged 20–49 years, the percent composition of Bacteroidales was significantly higher in those sensitized to JCP than in those unsensitized. The percent composition of Lactobacillales was significantly higher in participants unsensitized to HD than in those sensitized to that antigen. The percent composition of Bacteroidales was significantly higher in HD-sensitized than in unsensitized participants aged ≥50 years. Participants with low Bacteroidales and high Bifidobacteriales or Lactobacillales has low level of sensitization rates to HD compared with high Bacteroidales and low Bifidobacteriales or Lactobacillales. Thus, the fraction of bacteria of order Lactobacillales, Bifidobacteriales, and Bacteroidale in the gut microbiota may affect sensitization to inhaled allergens.

Previous studies showed that gut microbiota composition is different between patients with allergic diseases and those with non-allergic diseases. For example, children with allergic diseases in Estonia and Sweden were less often colonized with lactobacilli ($p < 0.01$) than children with non-allergic diseases.[20] Watanabe *et al.* reported that the percentages of *Bifidobacterium* were significantly lower in patients with AD (age < 20 years) compared to those in healthy subjects.[21] Furthermore, as AD symptoms became more severe, the counts of *Bifidobacterium* decreased. Recent studies also suggested the potential prevention and treatment of such allergic conditions by controlling the balance of gut microbiota. The symptoms of allergic rhinitis, AD, and bronchial asthma were reported to be alleviated by the oral administration of *Bifidobacterium* and *Lactobacillus* as probiotics.[6,13–18]

In the present study, although Bifidobacteriales alone were not associated with allergen sensitization, the rate of sensitization to HD was significantly low level in participants with high Bifidobacteriales and Lactobacillales compared to those with low Bifidobacteriales and Lactobacillales. The rate of sensitization to HD was significantly elevated in those with high Bacteroidales and low Bifidobacteriales compared to those with low Bacteroidales and high Bifidobacteriales. These results indicate that symbiosis of Lactobacillales and Bifidobacteriales may suppress allergen sensitization whereas Bacteroidales may promote allergen sensitization. Furthermore, this relation seems to be stronger for sensitization to HD than JCP. These results are consistent with

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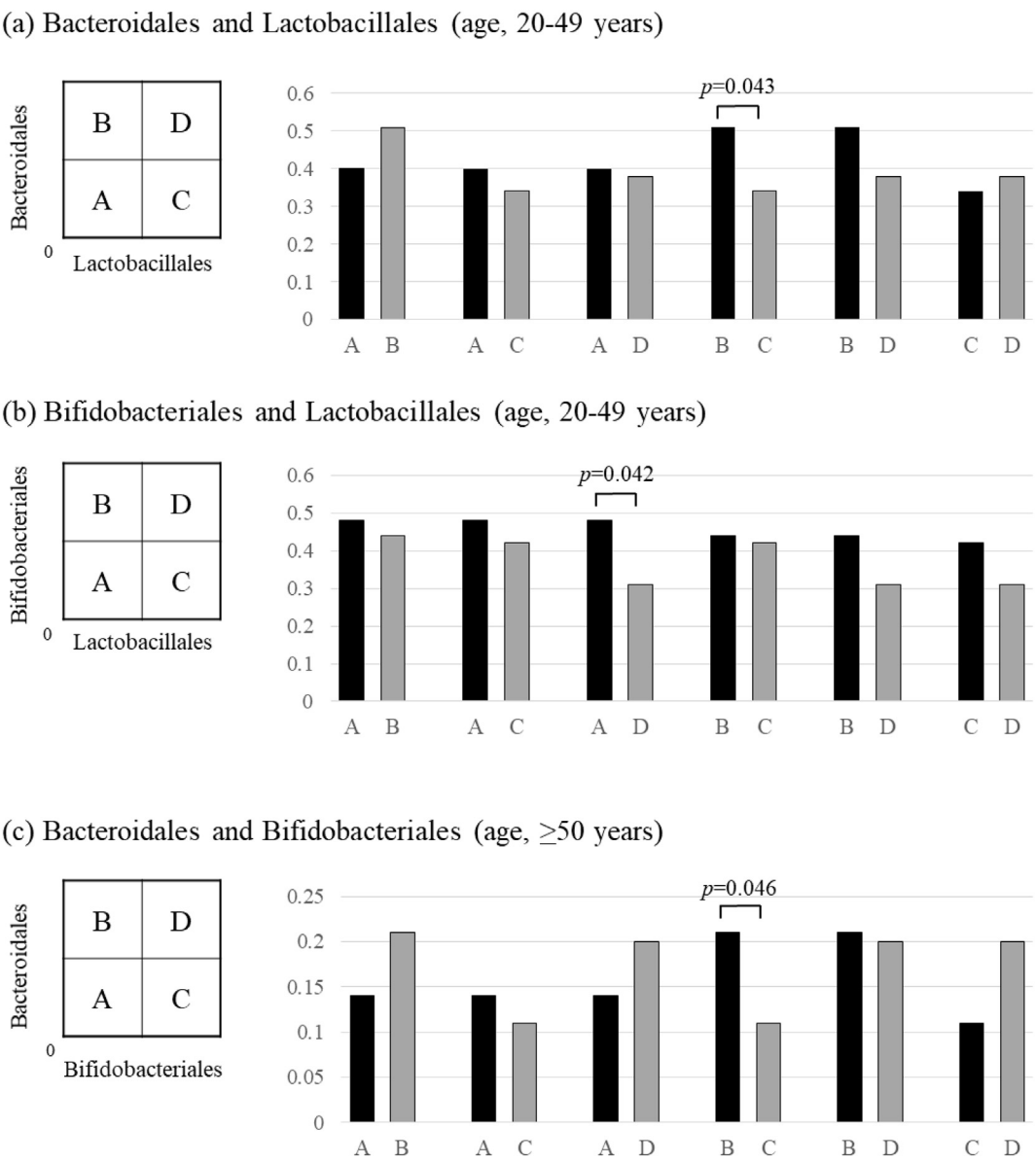

Fig. 4. Comparison of sensitization rates to HD between 4 groups divided by high and low presence of two bacterial orders. **(a)** Bacteroidales and Lactobacillales (age 20–49 years). The rate of sensitization to HD was significantly low level in participants with low Bacteroidales and high Lactobacillales (C) compared to those with high Bacteroidales and low Lactobacillales (B). **(b)** Bifidobacteriales and Lactobacillales (age 20–49 years). The rate of sensitization to HD was significantly low level in participants with high Bifidobacteriales and high Lactobacillales (D) compared to those with low Bifidobacteriales and low Lactobacillales (A). **(c)** Bacteroidales and Bifidobacteriales (age ≥ 50 years). The rate of sensitization to HD was low level in those with low Bacteroidales and high Bifidobacteriales (C) compared to those with high Bacteroidales and low Bifidobacteriales (B).

previous studies on gut microbiota and the mechanism of allergy. *Lactobacillus* is recognized by dendritic cells through M cells of Peyer's patches in the small intestine and promotes differentiation of naive T cells to Th1 cells by producing IL-12.[22] *Lactobacillus* also modulates the Th1/Th2 balance and reduces allergic symptoms. In addition, *Lactobacillus* induces Treg by promoting IL-10 and TGF-β production and inhibiting that of IL-6.[23–27] Treg acts directly on Th2 cells, mast cells, and eosinophils, and suppresses allergic reactions.[28,29] This immunoregulatory action of Lactobacillales may underlie our observation that participants with gut microbiota rich in Lactobacillales exhibited a lower rate of sensitization to HD.

Bifidobacterium is reported to be effective as a probiotic against allergic diseases. In a previous study, the administration of *Bifidobacterium breve* reduced the total IgE and IL-4 production and improved the Th1/Th2 balance in mice.[30] *Bifidobacterium infantis* is reported to suppress the secretion of IL-13 and total IgE and inhibit allergic inflammation.[6]

Although the mechanism whereby Bacteroidales affects the immune response is still unknown, the percentage of Bacteroidales in the gut microbiota is reported to be high in people with allergic diseases such as asthma, rhinitis, eczema, and food allergy.[31,32] This observation may be explained by two possibilities. First, Bacteroidales may promote Th2 differentiation. Second, this phenomenon may be caused not by the high composition of Bacteroidales in the gut microbiota but rather the concomitant low fraction of Lactobacillales and Bifidobacteriales in sensitized participants. Further studies are needed to investigate the effects of Bacteroidales on the mechanism of allergy.

Interestingly, we observed that the gut microbiota was more strongly associated with sensitization to HD than to JCP in participants younger than 50 years old. Atopic dermatitis is reported to be

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influenced by childhood gut microbiota,[21] and children with atopic dermatitis and food allergies are susceptible to allergic rhinitis.[33–35] HD-related perennial allergic rhinitis begins in childhood. Allergy to JCP also occurs in childhood, but the number of JCP patients is increasing further in adulthood.[5] Moreover, the results of our airborne pollen survey show that the amount of JCP scattering has been increasing significantly for a quarter of a century (data not shown). Therefore, gut microbiota may influence HD allergy in childhood, and JCP sensitization might be more greatly affected by the increase in JCP scattering than gut microbiota.

Our results indicate that Lactobacillales have a stronger influence on sensitization in allergic rhinitis than do Bifidobacteriales. Lactobacillales reside mainly in the small intestine, while Bifidobacteriales reside in the large intestine. Because the intestinal immune system develops in the small intestine, Lactobacillales may have a greater influence on sensitization than do Bifidobacteriales. However, the diverse gut microbiota is very important for suppressing allergic disease.[32] Our results also indicate that high level of both Bifidobacteriales and Lactobacillales is associated with suppression of HD sensitization. Thus, we suggest that the presence of multiple bacterial species that produce lactic acid and acetic acid in the intestine might be effective in preventing allergic sensitization. Our results may provide epidemiological support for reports that *Bifidobacterium* and *Lactobacillus* are effective against allergic rhinitis as probiotics.

To the best of our knowledge, this is the first report that investigates the relationship between gut microbiota composition and sensitization to inhaled allergens. We believe this study has high epidemiological value. However, several limitations should be addressed. Since this study did not consider nasal symptoms, we have not investigated the relationship between gut microbiota and the onset or prevalence of nasal allergy. This project conducts health checkups for residents in the same area every year, so we would like to longitudinally examine the effects of gut microbiota on the onset of allergic rhinitis. To that end, we would like to identify the specific gut bacteria involved in the development and prevention of allergic rhinitis.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

AN and AM designed the study and wrote the manuscript. SG and JT contributed to the analysis of the data. KS, KI, and SNperformed data collection. All authors have read this paper and agreed to submit the manuscript.

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