Note

Gut microbial composition of elderly women born in the Japanese longevity village Ogimi

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Ogimi is one of Japan's longevity villages and is located in Okinawa Prefecture. In this study, we focused on the elderly women living in the village, classified them into two groups based on whether or not they lived in Ogimi during the first 3 years of their lives, and compared the gut microbiota between the two groups. There were no differences in alpha and beta diversity; however, we found that the elderly women who lived in Ogimi during the first 3 years of their lives had a higher rate of *Akkermansia muciniphila* colonization in their guts.

Key words: microbiota, *Akkermansia muciniphila*, longevity, early-life environment

It is believed that human health is determined by the complex interactions between different factors, including genetics, epigenetics, diet, activity level, and lifestyle. In recent years, many studies have shown that the human microbial community formed in the gastrointestinal tract is another factor involved in our health. The formation of the gut microbiota begins immediately after birth, and the delivery method (i.e., vaginal delivery or cesarean section) and food source in infancy (formula or breast milk) influence the initial composition of the microbiota [1–4]. An adult-like gut microbiota is established in the first 3 to 5 years of life, and it remains stable throughout a person's lifetime, but it may change depending on both environmental and host factors, such as lifestyle, diet, antibiotics, genetics, gut senescence, and immune senescence [5–7]. Thus, the composition and relative abundance of different bacterial taxa in the human adult gut microbiota differ from country to country and between the elderly and young adults [8–10].

Okinawa Prefecture is one of the 47 prefectures of Japan and is located in the southernmost part of the country. Since the prefecture was an independent country before 1875, called the Ryukyu Kingdom, its unique cultural lifestyle and food still remain, especially in the northern area of the main island of Okinawa. Furthermore, it has been reported that the genetic background differs between the Ryukyu people (called Ryukyuans), who live mainly in Okinawa, and the Japanese people living on the mainland of Japan [11]. Ogimi is a village located in the northern region of Okinawa's main island and has a population of approximately 3,000. The village is known as a village of longevity in Japan, and thus the lifestyle and foods of the elderly living in the village have been researched [12, 13]. However, the gut microbiota of the residents has not yet been researched. According to data published by the Ministry of Health, Labour and Welfare (https://www.mhlw.go.jp/toukei/saikin/hw/life/ckts15/index.html), the average lifespan of women living in the village is longer than the average Japanese woman's lifespan, but that of the men living in the village is shorter than the average Japanese man's lifespan; thus, we focused on the elderly women living in Ogimi and investigated the profile of the gut microbiota in them. The study protocol was approved by the Ethical Committee for Research in Humans of Shiba Palace Clinic (Tokyo, Japan) in compliance with the Declaration of Helsinki (permission No. ASHB_rn-20876). Informed consent was provided by all participants prior to enrollment. In total, 60 Japanese women who were over 70 years of age and living in Ogimi as of August 2016 were enrolled in this study. Since the human gut microbiota is established during the first 3 to
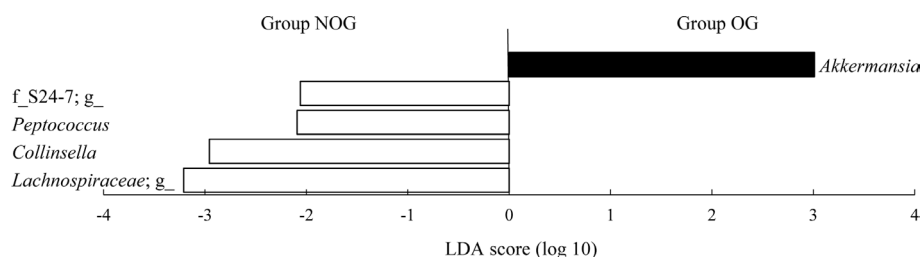

Fig. 1. Most likely bacterial genera to explain differences between groups.

Three known and two unidentified genera were detected by the LEfSe analytic method (log-linear discriminant analysis [LDA] score >2). Bacterial taxa significantly enriched in group OG is shown on the right side, and those in group NOG are shown on the left side.

5 years of life, we classified the women into two groups based on whether they were living in Ogimi during the first 3 years of their lives (group OG, n=40) or not (group NOG, n=20) and compared the gut microbiotas between the two groups. The areas where they lived from their birth to the present are shown in Supplementary Table 1. The mean age ± standard deviation (SD) of group OG was 83.8 ± 6.8 years, and that of group NOG was 81.3 ± 6.1 years (p=0.16, student's t-test). The gut microbiota of all participants were characterized by Illumina MiSeq sequencing of the V4 region of the bacterial 16S rRNA gene using DNA extracted from fecal samples. These analyses were carried out as previously described [14]. In total, 3,558,508 high-quality filtered sequences were obtained, and we first compared the Chao1, Shannon diversity index, and phylogenetic distance (PD) whole tree between groups OG and NOG. All data were expressed as the mean ± SD, and p-values were calculated by the two-sample t-test using Monte Carlo permutations. The results revealed no significant differences (Chao1, 1,468 ± 495 for group OG and 1,563 ± 452 for group NOG, p=0.62; Shannon diversity index, 5.81 ± 1.00 for group OG and 5.99 ± 0.97 for group NOG, p=0.50; PD whole tree, 38.68 ± 11.94 for group OG and 40.39 ± 11.64 for group NOG, p=0.62). Then, principal coordinate analysis based on the unweighted or weighted UniFrac distance was performed, and no significant differences were observed between the two groups (p=0.36 and 0.39, respectively, with p-values determined by permutational multivariate analysis of variance). These results showed that there were no differences between groups OG and NOG in the overall gut microbiota. In studies which focused on the gut microbiota of centenarians or elderly people living in longevity areas, differences were observed in the diversity of the gut microbiota, and this conflicts with our results [15–18]. The previous studies grouped subjects according to age and/or living area, which may have contributed to the differences found in their gut microbiotas. However, our study compared two groups that differed in terms of their early-life environments but matched in terms of age, gender, and current living area. This might explain why no differences were observed in our microbial diversity analysis.

The microbial compositions of both groups at the genus level and adjusted p-values of the Mann-Whitney U test with Benjamini-Hochberg false discovery rate correction (q-value) are shown in Table 1. No differences were observed in the relative abundances of each genus between two groups if q-value <0.05 was considered to indicate statistical significance. However, when our microbiota data were analyzed with the linear discriminant analysis effect size algorithm (LEfSe) (http://huttenhower.sph.harvard.edu/galaxy/), 5 genera were found to be significantly different between the groups (Fig. 1). *Akkermansia* was more abundant in group OG, and *Lachnospiraceae*;g_, *Collinsella*, *Peptococcus*, and f_S24_7;g_ were more abundant in group NOG. *Akkermansia muciniphila*, which was isolated from human feces in 2004 [19], is expected to be a next-generation probiotic. The administration of live *A. muciniphila* partially protected against high-fat diet-induced obesity and insulin resistance in mice [20], and oral administration of pasteurized *A. muciniphila* significantly improved insulin sensitivity and reduced plasma total cholesterol in humans [21]. To determine the cell counts and detection rate of *A. muciniphila* in groups OG and NOG, we calculated the cell counts of *A. muciniphila* in fecal samples by quantitative PCR using *A. muciniphila*-specific primers [22]. Quantitative PCR amplification and detection were performed on a LightCycler 480 Instrument II (Roche Diagnostics K.K., Tokyo, Japan) using SYBR Premix Ex Taq II (Takara Bio Inc., Kusatsu, Japan). The standard curve was generated using a synthesized partial 16S rRNA gene of *A. muciniphila* and serial 10-fold diluents. The partial 16S rRNA gene was a 429 bp fragment that contained the region from the forward primer annealing position to the reverse primer annealing position (329 bp) and 50 bp up- and downstream of that region. The fragment was synthesized by Thermo Fisher Scientific K.K. (Tokyo, Japan). Due to *A. muciniphila* having 3 copies of the 16S rRNA gene, copy numbers measured by the quantitative PCR were divided by 3 to calculate the cell count of *A. muciniphila*. *A. muciniphila* was detected in 27 of 40 group OG fecal samples and in 8 of 20 group NOG fecal samples. The detection rate of *A. muciniphila* in group OG was significantly higher than that in group NOG (Table 2). Also, the cell count of *A. muciniphila* was higher in group OG than in group NOG (Table 2). However, when the cell counts of *A. muciniphila* were compared using only the data from participants in which *A. muciniphila* was detected, no significant difference was observed between the groups (Table 2). These results indicated that the elderly women who lived in Ogimi during the first 3 years of their lives had a higher rate of *A. muciniphila* colonization in their guts than those who were born in other regions and moved to Ogimi.

In the Japanese elderly, the relative abundance of *Akkermansia* has been reported to be in the range of 0 to 0.79% [23]. The relative abundance of this bacteria in the group OG ranged from 0 to 13.6%, and the mean was 1.2%. For the group NOG, relative abundance ranged from 0 to 2.3%, and the mean was 0.2%. Due to differences in methodology of microbiota analysis,

Table 1. Relative abundance of genera in feces from groups OG and NOG

Taxa[a]	Relative abundance (mean ± SD, %)		q-value[b]
	Group OG	Group NOG	
f__*Lachnospiraceae*;g__*Blautia*	14.9 ± 11.8	18.3 ± 15.1	0.91
f__*Streptococcaceae*;g__*Streptococcus*	13.9 ± 13.4	8.3 ± 9.4	0.73
f__*Bifidobacteriaceae*;g__*Bifidobacterium*	9.6 ± 12.4	9.7 ± 12.2	0.94
f__*Ruminococcaceae*;g__*Faecalibacterium*	8.4 ± 7.4	9.6 ± 9.4	0.94
f__*Lactobacillaceae*;g__*Lactobacillus*	7.4 ± 13.7	5.7 ± 13.2	0.91
f__*Lachnospiraceae*;g__*Coprococcus*	6.9 ± 5.8	7.7 ± 5.7	0.91
f__*Enterobacteriaceae*;g__*Escherichia*	4.1 ± 10.1	1.1 ± 2.7	0.84
f__*Lachnospiraceae*;g__[*Ruminococcus*]	3.0 ± 2.5	3.4 ± 2.4	0.87
f__*Ruminococcaceae*;g__*Ruminococcus*	2.4 ± 2.7	3.0 ± 3.4	0.94
f__*Enterobacteriaceae*;Other	2.3 ± 4.6	3.1 ± 4.8	0.91
f__*Lachnospiraceae*;g__	2.0 ± 2.3	3.5 ± 2.6	0.21
f__*Coriobacteriaceae*;g__*Collinsella*	1.8 ± 2.6	2.5 ± 2.0	0.29
f__*Lachnospiraceae*;g__*Dorea*	1.7 ± 1.7	1.9 ± 1.3	0.84
f__*Erysipelotrichaceae*;g__[*Eubacterium*]	1.7 ± 3.4	2.5 ± 3.4	0.73
o__*Clostridiales*;f__;g__	1.5 ± 3.2	2.3 ± 4.9	0.91
f__*Lachnospiraceae*;g__*Roseburia*	1.4 ± 2.0	1.6 ± 2.1	0.84
f__*Bacteroidaceae*;g__*Bacteroides*	1.4 ± 3.0	0.7 ± 0.7	0.94
f__*Clostridiaceae*;g__SMB53	1.3 ± 2.2	1.2 ± 1.8	0.91
f__*Clostridiaceae*;g__*Clostridium*	1.3 ± 2.6	0.9 ± 0.9	0.91
f__*Verrucomicrobiaceae*;g__*Akkermansia*	1.2 ± 2.7	0.2 ± 0.5	0.21
f__*Lachnospiraceae*;Other	1.0 ± 1.2	1.0 ± 0.9	0.91
f__*Ruminococcaceae*;g__*Oscillospira*	0.8 ± 1.0	1.0 ± 1.2	0.98
f__*Ruminococcaceae*;g__	0.8 ± 1.2	0.9 ± 0.9	0.84
f__*Methanobacteriaceae*;g__*Methanobrevibacter*	0.7 ± 2.2	0.5 ± 1.1	0.84
f__*Erysipelotrichaceae*;g__	0.6 ± 1.0	1.1 ± 2.1	0.84
f__*Enterococcaceae*;g__*Enterococcus*	0.5 ± 1.5	0.2 ± 0.4	0.94
f__*Ruminococcaceae*;Other	0.5 ± 0.5	0.4 ± 0.3	1.00
f__*Erysipelotrichaceae*;g__*Catenibacterium*	0.5 ± 1.4	0.6 ± 1.4	0.91
f__*Leuconostocaceae*;g__	0.5 ± 1.6	0.7 ± 2.1	0.85
f__*Coriobacteriaceae*;g__	0.4 ± 0.5	0.6 ± 0.5	0.53
o__*Clostridiales*;Other;Other	0.4 ± 1.0	0.4 ± 0.7	0.91
f__*Ruminococcaceae*;g__*Butyricicoccus*	0.3 ± 0.7	0.5 ± 0.5	0.53
o__RF39;f__;g__	0.3 ± 3.2	0.4 ± 4.9	0.91
f__[*Mogibacteriaceae*];g__	0.3 ± 0.3	0.3 ± 0.3	0.94
f__*Lachnospiraceae*;g__*Anaerostipes*	0.3 ± 0.5	0.2 ± 0.4	1.00
f__*Lachnospiraceae*;g__*Clostridium*	0.2 ± 0.6	0.1 ± 0.3	0.73
f__*Turicibacteraceae*;g__*Turicibacter*	0.2 ± 0.6	0.1 ± 0.2	0.91
f__[*Mogibacteriaceae*];g__*Mogibacterium*	0.2 ± 0.7	0.2 ± 0.5	0.91
f__*Rikenellaceae*;g__	0.2 ± 0.4	0.1 ± 0.1	1.00
f__*Porphyromonadaceae*;g__*Parabacteroides*	0.2 ± 0.5	0.1 ± 0.1	0.91
f__*Peptostreptococcaceae*;g__*Clostridium*	0.2 ± 0.7	0.6 ± 2.6	0.94
f__*Prevotellaceae*;g__*Prevotella*	0.1 ± 0.6	0.1 ± 0.2	0.53
f__*Erysipelotrichaceae*;g__*Bulleidia*	0.1 ± 0.8	0.0 ± 0.1	0.73
f__*Christensenellaceae*;g__	0.1 ± 0.3	0.1 ± 0.2	0.91
f__*Veillonellaceae*;g__*Dialister*	0.1 ± 0.5	0.0 ± 0.0	0.97
f__*Veillonellaceae*;g__*Megamonas*	0.1 ± 0.7	0.0 ± 0.1	1.00
f__*Veillonellaceae*;g__*Veillonella*	0.1 ± 0.4	0.0 ± 0.1	0.80
f__*Methanobacteriaceae*;g__*Methanosphaera*	0.1 ± 0.4	0.1 ± 0.4	0.84
f__*Lachnospiraceae*;g__*Lachnospira*	0.1 ± 0.3	0.3 ± 1.0	0.84
f__*Coriobacteriaceae*;g__*Eggerthella*	0.1 ± 0.2	0.1 ± 0.2	0.85
f__*Enterobacteriaceae*;g__	0.1 ± 0.2	0.0 ± 0.1	0.84
f__*Erysipelotrichaceae*;g__*Clostridium*	0.1 ± 0.2	0.1 ± 0.2	0.84
f__*Desulfovibrionaceae*;g__*Desulfovibrio*	0.1 ± 0.2	0.0 ± 0.1	0.84
f__*Coriobacteriaceae*;g__*Adlercreutzia*	0.1 ± 0.1	0.1 ± 0.1	0.84
f__*Enterobacteriaceae*;g__*Citrobacter*	0.1 ± 0.1	0.1 ± 0.3	0.84
f__*Coriobacteriaceae*;Other	0.1 ± 0.2	0.0 ± 0.0	0.84
o__*Lactobacillales*;Other;Other	0.1 ± 1.0	0.0 ± 0.7	0.91
f__*Veillonellaceae*;g__*Megasphaera*	0.1 ± 0.2	0.0 ± 0.2	1.00
f__*Leuconostocaceae*;g__*Leuconostoc*	0.1 ± 0.2	0.1 ± 0.2	0.91
f__*Streptococcaceae*;g__*Lactococcus*	0.1 ± 0.1	0.3 ± 0.8	0.84
f__*Erysipelotrichaceae*;g__*Coprobacillus*	0.1 ± 0.1	0.0 ± 0.0	0.98
f__*Clostridiaceae*;g__	0.0 ± 0.0	0.1 ± 0.4	0.84
f__*Peptococcaceae*;g__*Peptococcus*	0.0 ± 0.0	0.1 ± 0.3	0.21

[a] One hundred five genera with mean relative abundances over 0.01% in at least one group were compared. The table shows 63 genera having mean relative abundances of over 0.1% in at least one group.
[b] q-values indicate adjusted p-values of the Mann-Whitney *U* test with Benjamini-Hochberg false discovery rate correction.

Table 2. Number of samples in which *A. muciniphila* was detected by qPCR and cell counts of *A. muciniphila* in groups OG and NOG

Participant	Sample group	No. of detected samples/ total no. of samples (%)	χ^2 test [a]	Log no. of cells (mean ± SD)/g feces	Mann-Whitney U test [a]
All participants	OG	27/40 (67.5)	0.042	5.33 ± 4.03	0.040
	NOG	8/20 (40.0)		2.97 ± 3.89	
A. muciniphila detected participants	OG (n=27)	-	-	7.90 ± 1.81	0.550
	NOG (n=8)			7.43 ± 1.77	

[a] p-values are shown.

such as differences in sample processing, DNA extraction, and subsequent processing, it is difficult to compare previously reported results with our results. However, elderly women who lived in Ogimi during the first 3 years of their lives might have more *Akkermansia* in their guts than Japanese elderly living in other locations.

A recent study showed that the composition of the gut microbiota is different between people living in the same city due to differences in ethnic background affecting their genetics, cultural habits, and early-life environments [24]. We did not investigate the genetic background of our participants; however, their family names indicated that more than 90% of them were Ryukyuan, suggesting that genetic background would not play a part in the differences between the two groups in this study. In particular, *A. muciniphila* colonizes the human gut within a year of birth [22], suggesting that the area where a person lives in early life contributes to the differences in colonization rate of this bacteria. We concluded that the differences in *A. muciniphila* colonization rate between groups OG and NOG may have been due to whether the participants lived in Ogimi during the first few years of their lives. We attempted to determine the factors affecting the gut microbiota in our participants, but the living environment, lifestyle, and food in the village have changed in the past 70 to 90 years. Therefore, the reasons behind the high rate of *A. muciniphila* colonization in the women who lived in Ogimi in early life are unclear.

In a Chinese study, the relative abundance of *Akkermansia* was significantly lower in a centenarian group than in an 85 to 99 years old group [16]. In contrast, an Italian study and a South Korean study both showed that the abundance of *Akkermansia* was higher in a centenarian group than in an elderly group [15, 17]. Whether *Akkermansia* is related to longevity is unclear; however, it is notable that the elderly women who were born in Ogimi have a higher rate of *A. muciniphila* colonization than other women. A follow-up study is needed to determine whether our participants who had *A. muciniphila* in their guts live longer.

DATA AVAILABILITY

16S rRNA gene sequence data have been deposited into the DNA Data Bank of Japan (DDBJ) under the accession no. DRA010457.

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