Association between oral frailty and *Prevotella* percentage in the oral microbiota of community-dwelling older adults who participated in the CHEER Iwamizawa project, Japan

Running Title: Oral frailty *Prevotella* oral microbiota

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Data availability statement

The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to ethical and legal restrictions imposed by the Hokkaido University Ethics Committee.

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Conflict of Interest

None.

Ethics Approval.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Hokkaido University Faculty of Dental Medicine Clinical/Epidemiological Research Institutional Review Board (2020 no. 9).

Patient consent:

The principal investigator explained the nature of the study to the participants in advance, both orally and in writing, and all participants provided written informed consent.

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Abstract

Background

Prevotella bacteria are associated with inherent diseases of the oral cavity, such as periodontal disease, and systemic diseases. Oral frailty (OF) has been associated with nursing necessity and death. However, the relationship between OF and oral microbiota has not been fully clarified.

Objective

This cross-sectional study investigated the association between OF and *Prevotella* percentage in the oral

microbiota of community-dwelling older adults.

Methods

Oral bacteria species from saliva were identified in 208 community-dwelling older individuals aged ≥60 years in Japan. The proportion of *Prevotella* in the oral microbiota was classified into three tertile groups, and its relationship with each test item for OF (number of remaining teeth, masticatory performance, oral diadochokinesis, tongue pressure, difficulties eating tough foods, difficulties swallowing tea or soup, number of applicable OF judgement items, and existence of OF) was examined using ordinal logistic regression analysis.

Results

The *Prevotella* proportions were classified into lower, middle, and upper groups, comprising 70, 69, and 69 participants, respectively. The three groups showed a significant relationship between the number of remaining teeth (odds ratio [OR]: 0.946, 95% confidence interval [CI]: 0.915–0.977), masticatory performance (OR: 0.897, 95% CI: 0.844–0.953), number of applicable OF judgement items (OR: 1.477, 95% CI: 1.14–1.915), and existence of OF (OR: 4.194, 95% CI: 1.519–11.576).

Conclusion

The proportion of *Prevotella* in oral microbiota was high in individuals with OF. Among the older adults, the type of oral microbiota and systemic diseases may be related to the examination and management of oral function decline.

Keywords: cross-sectional study, *Prevotella* , microbiota, frailty, aged, community

Introduction

The oral cavity harbors an estimated 500–700 bacterial species, each fostering distinct bacterial communities on the surfaces of teeth, periodontal pockets, saliva, tongue, and buccal mucosa.[1]

Prevotella is a ubiquitous, anaerobic gram-negative bacterium residing not only in the oral cavity, but also on the skin and in the digestive tract. Its association extends beyond oral diseases, such as periodontal disease, to include various systemic conditions such as inflammatory autoimmune diseases and rheumatoid arthritis.[2] *Prevotella* is prevalent among older adults and has emerged as one of the bacteria causing aspiration pneumonia.[3]

Oral frailty, assessed comprehensively using diminished tooth count, restricted tongue movement and muscle strength, and impaired mastication and swallowing functions, has recently gained prominence. A prior study has indicated substantially higher incidences of frailty and sarcopenia during a 2-year period in older individuals with oral frailty. [4] Those with oral frailty had a notable rise in long-term care needs and mortality over a period of approximately 4 years.[4] In addition, physical frailty and sarcopenia caused by oral frailty are reported to be associated with the progression of diabetes and its complications.[5] Oral frailty has also been linked to malnutrition and heart disease.[6] Furthermore, an association between oral frailty and

pneumonia development leading to hospitalization has been reported.[7]

These results suggest that oral frailty may be related to the deterioration of general health conditions through the disruption of oral microbiota; however, the relationship between oral frailty and oral microbiota has not been fully clarified. Therefore, we hypothesized that oral frailty is related to oral microbiota, considering that among oral microbiota, bacterial flora deterioration related to *Prevotella* represents oral environment deterioration, which is associated with undernutrition, decreased immunity, and risk of aspiration pneumonia; thus, it is considered to be associated with the worsening of the general health condition. This study investigated the association between oral frailty and *Prevotella* proportion in the oral microbiota of community-dwelling older adults.

Materials and Methods

Study design

This was a cross-sectional study of community-dwelling older adults in Japan.

Participants

In I-city, a local city of Hokkaido located in north Japan, citizens ≥ 60 years of age were recruited to participate in a general wellness checkup. City staff explained this to members of the city senior citizens' club and invited them to participate. In addition, an article on participant recruitment was posted in the public relations department of the city, and leaflets were posted at the main facilities in the city to recruit

participants. This study included 232 participants (0.7% of I-city residents aged ≥ 60 years) who participated in health checkups conducted in October 2020 and whose health status was measured. They excluded those with incomplete questionnaire responses, those who had taken antibiotics within the prior month, and those who had not had an oral function assessment.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Hokkaido University Faculty of Dental Medicine Clinical/Epidemiological Research Institutional Review Board (2020 No. 9)

Health assessments

Basic characteristics

Information on age, sex, smoking status, medical history (malignancies, stroke, ischemic heart disease, arrhythmia, hypertension, hyperlipidemia/dyslipidemia, diabetes, hyperuricemia/gout, gastric/duodenal ulcers, liver disease, chronic obstructive pulmonary disease, tuberculosis, kidney disease, thyroid disease, collagen disease, allergies, depression, dementia, Parkinson's disease, prostate disease, gynecologic diseases), and medications were collected from a self-administered questionnaire. Skeletal muscle index (SMI) was measured using the Body Component Analyzer InBody (InBody Japan Inc, Tokyo, Japan) by one investigator who had been previously trained on the method.

Oral health indicator assessments

Objective assessment

Oral function evaluation and a survey on the use of dentures were conducted by 10 dentists who had received prior training and standardized the criteria.

The measurement items included number of remaining teeth, masticatory ability, tongue pressure, and oral diadochokinesis (ODK), which evaluates articulatory oral motor skills of the lip and tongue dorsum.

The evaluation methodology and cutoff values (data in parentheses) for each parameter were established based on a report by Tanaka et al.[4]:

1 Number of remaining teeth: the number of teeth erupted into the oral cavity was calculated, excluding those with residual roots and severe periodontitis (< 20)

2 Masticatory performance: color-determining gum (XYLITOL; Lotte, Tokyo, Japan) that changes from green to red when masticated and mixed with saliva was administered. Chewing ability was measured as a marker of general masticatory performance.[4] The gum was chewed for 1 min and then spat out. Color was measured at five locations using an absorptiometer (Color Reader CR -20; KONICAMINOLTA, Tokyo, Japan), and the average value of red absorbance (a * value) was defined as masticatory performance. This study used free chewing to measure masticatory performance based on the report by Tanaka et al.[4] (men, < 14.2; women, < 10.8).

3 Tongue pressure: it was measured using a tongue pressure meter (JMS tongue pressure measurement instrument; GC, Tokyo, Japan). The balloon of the tongue pressure probe was inserted between the tongue and palate, the tongue was raised, pressure was applied for approximately 7 s by pushing with

maximum force, and the measurements were recorded. This was measured three times, and the average value was defined as the tongue pressure (men, < 27.4 kPa; women, < 26.5 kPa).

4 ODK: measurements were performed using an oral function-measuring device (Kenkou-kun Handy; Takei Scientific Instruments, Kamo City, Nigata, Japan). The participants were taught to repeat/ta/continuously for 5 s as fast as possible, and the number of sounds per second was recorded. We measured each syllable once based on a previous report[4] (men, < 5.2 times/s; women, < 5.4 times/s).

Subjective assessment

1 Based on the October 2020 survey, the respondents who replied "yes" to the inquiry, "Do you have any difficulty eating tough foods compared to 6 months ago?" were determined to have diminished masticatory performance.

2 Respondents acknowledging "yes" to the query, "Have you choked on your tea or soup recently?" were categorized as having compromised swallowing function.

Oral frailty was determined based on the results of these four objective and two subjective items. Those who had three or more items were determined to have oral frailty.

Assessment of Prevotella percentage in oral microbiota

The participants chewed a saliva testing gum (Sulliver gum alpha; Tokyo Tooth Material, Tokyo, Japan) for 2 min. The expelled saliva was placed in a sterile plastic tube, collected, and weighed. In the saliva collection, it was confirmed in the advance interview sheet that no toothbrushing, eating or drinking were done 30 min

before the saliva collection. The collected saliva samples were pelleted by centrifugation at the Oral Microbiome Center (Kagawa, Japan) and frozen until further extraction. The saliva pellets were mechanically crushed using MORA beads (Cosmo Bio Inc., Tokyo, Japan), and a genomic DNA purification kit (Genfind V2); Nippon Genetics Co., Ltd., Tokyo, Japan) was used to extract and purify DNA. Amplification of the 16 S rRNA gene V3-V4 region was performed using primer sequences 341 F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNCCTACGGGNGGCWGCAG-3′), 806 R (5′-GTCTCGTGGCTCGGAGATGTGTATAAGAGACAGNGACTACHVGGGTATCTAATCC-3′). After confirmation of amplification, the primers were removed by purification using AmPure XP (Nippon Genetics Co., Ltd., Tokyo, Japan). Then, polymerase chain reaction (PCR) was carried out using primer sequences F (5′-AATGATACGGCGACCACCGAGATCTACACTATAGCCT TCGTCGGCAGCGTC-3′) and R (5′-CAAGCAGAAGACGGCATACGAGAT CTAGTACG GTCTCGTGGGCTCGG -3′), and after purification of the PCR product, it was quantified using electrophoresis and Qubit, and the concentration of each sample was adjusted to be equal. The base sequence was analyzed using Qiime2, the bacterial species were identified, and the composition proportion of the bacterial species was calculated. Referring to the report by Yamashita et al.,[8] the main bacterial species were identified, and the proportion of bacterial flora was examined. We focused on *Prevotella*, as its proportion in all participants was normally distributed.

Statistical analyses

After a Kolmogorov–Smirnov test for normality, the proportion of *Prevotella* in the saliva was classified into three groups by order of decreasing proportion: upper, middle, and lower groups, using tertile ranges. No previous study has grouped and compared one type of bacteria; therefore, we used tertiles, which are often applicable in the absence of previous research (Figure 1).

We compared the three *Prevotella* tertile range groups using a one-way analysis of variance and Kruskal–Wallis tests for continuous variables and χ^2 tests for the categorical variables age,[9,10] sex,[11] SMI,[12] collagen disease,[2,13] smoking,[14] ischemic heart disease,[15,16] diabetes,[15,17] and denture use,[18] which have been associated with *Prevotella* in previous studies. The continuous variables were age and SMI. The categorical variable is sex, collagen disease, smoking, ischemic heart disease, diabetes, and denture use. Similarly, we compared the three *Prevotella* tertile range groups using the Jonckheere–Terpstrak test for continuous variables and the Cochran–Amitage test for categorical variables. We compared each examination item of oral frailty, the number of applicable oral frailty judgement items, and the existence of oral frailty.

Ordinal logistic analysis was performed using the three *Prevotella* groups as dependent variables and six oral frailty test items(number of remaining teeth, masticatory performance, ODK for “ta”, tongue pressure, difficulties eating tough foods (yes), difficulties swallowing tea or soup (yes)), number of applicable oral frailty judgement items, and existence of oral frailty as independent variables one by one. Covariates adjusted in this study included age, sex, SMI, smoking, ischemic heart disease, diabetes, and denture wear. Age, sex, SMI, diabetes, and heart disease have been reported to be associated with

Prevotella.[9-12,15-17] We also adjusted for smoking because it is linked to periodontal disease[14], which is also associated with *Prevotella*.[2] In addition, denture wear was adjusted because *Prevotella*[18] is frequently detected in people with an extended use of complete dentures, denture use is related to items of oral frailty such as masticatory performance, and tooth loss due to periodontal disease is related to *Prevotella*. There were no missing values in the analyzed data, and all statistical analyses were performed using SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA). Statistical significance level was set at 5%.

Results

The total population of I-city is 77,492 persons, and the population over 60 years old comprises 44.5% (34,463 persons) (data as of October 2020). A total of 232 participants were included in this study, out of which 208 (58 men and 150 women, mean age 74.3 ± 6.1 years) were analyzed. Twenty-four participants were excluded because they had incomplete answers to the questionnaire (11), had taken antibiotics within the prior month (9), or did not undergo an oral function evaluation (4) (Figure 2).

The number of participants was 70 (33.7%), 69 (33.2%), and 69 (33.2%) in the lower, middle, and upper groups, respectively. The mean percentages of *Prevotella* in the three groups were 11.9%, 22.4%, and 34.6%, respectively. Oral frailty was present in 17 (8.2%) participants. When the factors reported to be associated with *Prevotella* (age, sex, SMI, smoking, collagen disease, Ischemic heart disease, diabetes, removable denture use) were compared among the three groups, significant differences were found only for

sex (Table 1).

In addition, the three *Prevotella* groups showed significant differences in the number of remaining teeth, masticatory performance, number of applicable oral frailty judgement items, and existence of oral frailty (Table 2).

Ordinal logistic regression analysis showed significant associations between the three *Prevotella* groups and the number of remaining teeth (odds ratio [OR]: 0.946, 95% confidence interval [CI]: 0.915–0.977), masticatory performance (OR: 0.897, 95% CI: 0.844–0.953), number of applicable oral frailty judgement items (OR: 1.477, 95% CI: 1.14–1.915), and existence of oral frailty (OR: 4.194, 95% CI: 1.519–11.576) (Table 3).

Discussion

To our knowledge, this was the first study to demonstrate the association between oral frailty and a high proportion of *Prevotella* in the oral microbiota.

Previous studies have shown that oral frailty increases physical frailty, sarcopenia, long-term care needs, and mortality,[4] and that oral microbiota is associated with general health conditions, including the development of aspiration pneumonia.[19] The relationship between oral frailty and oral microbiota clarified in this study may be a potential mechanism for the relationship between oral frailty and general health conditions.

The conventional measures against oral frailty are caries treatment, root canals, prosthetic treatments such as dentures, crown prostheses, and implants, and oral function exams along with oral function training, as necessary.[20] These conventional measures against oral frailty are performed to improve oral cavity function and general health conditions.[20] The results of this study suggest that oral function improvement as a countermeasure against oral frailty may be associated with oral microbiota improvement.[20] Deterioration of oral microbiota exacerbates oral diseases, such as periodontal disease and dental caries, and adversely affects the general health conditions, such as aspiration pneumonia and rheumatoid arthritis.[13,21] Therefore, examination and management of oral function may not only relate to the nature of oral microbiota in older adults, but may also influence aspiration pneumonia and systemic diseases associated with the oral microbiota.[13,21,22]

Prevotella prevalence increases with age,[10] and these bacteria can be swallowed and spread throughout the body, affecting various organs.[23] In addition, the main causative bacteria of aspiration pneumonia are the bacteria indigenous to the oral cavity, and among them, *Prevotella* is frequently isolated.[3] *Prevotella* is frequently detected in the oral microbiota of patients with oral cancer.[24] In the previous study by Yamashita et al.,[8] the bacterial microbiota type of saliva was classified into type I and type II, with type I implicated in more pneumonia deaths among the facility residents. The genus *Prevotella* has been identified as the major type I bacteria. Kageyama et al.[25] indicated that the risk of death by pneumonia in nursing home residents was high for oral microbiota type I. Furthermore, the Tohoku Medical Megabiobank Project

reported that bacterial microbiota types containing a large proportion of *Prevotella* were associated with a high risk of pneumonia mortality.[26] The results of these previous studies suggest that a high proportion of *Prevotella* is associated with worsening oral microbiota and pneumonia risk, and the association with oral frailty further supports the need for countermeasures.

In this study, the proportion of *Prevotella* increased as the number of teeth decreased. The masticatory ability of those with few remaining teeth is reduced.[27] Thus, it is considered that when masticatory function decreases, the proportion of *Prevotella* increases in those with missing teeth due to serious oral diseases such as periodontal disease. However, because a decrease in the number of teeth leads to a reduction in the foci of periodontal disease, the relationship between oral function and number of teeth, *Prevotella* proportion, and periodontal disease status should be carefully examined in the future. In this study, eight participants had edentulous upper and lower jaws, among whom four individuals had a higher percentage of *Prevotella* than the average of all participants. The percentage of participants with oral frailty was 25% in those with lower-than-average proportion of *Prevotella*, whereas the proportion of those with oral frailty was 50% in those with higher-than-average proportion of *Prevotella*. Since there are reports that *Prevotella* is frequently detected in people who use dentures for a long time[18] and that the mucosa under the denture base is an anaerobic environment,[28] the increase in *Prevotella* proportion in these individuals may be more affected by dentures and oral dysfunction than by periodontal disease.

As a result of the color change gum test, which is an objective evaluation of masticatory function,

a high proportion of *Prevotella* was related to reduced masticatory function. Saliva quantity reduces when masticatory function decreases,[29] and it is considered that the self-cleaning action of saliva decreases because of the decline in masticatory function, leading to the deterioration of oral microbiota. However, the proportion of *Prevotella* was not related with the subjective evaluation "Do you have any difficulty eating tough foods compared to 6 months ago?" Nevertheless, the results of subjective and objective chewing abilities are not always concurrent.[30] Since this was a subjective evaluation, it is possible that many of those who answered "No" to this question began to eat soft foods and did not find hard foods difficult to eat, even though their masticatory function actually decreased.

In the present study results, the *Prevotella* proportion was not related to "Have you choked on your tea or soup recently?" Although previous studies have shown an association between dysphagia assessed by a self-administered questionnaire and a water drinking test and an increase in *Prevotella* in community-dwelling older adults,[31] this study relied on only a subjective assessment using a questionnaire. Thus, it is possible that those who answered "No" to this question actually had decreased swallowing function but no subjective symptoms. The participants were younger than in a previous study,[31] which could be a reason for the absence of a relationship between the proportion of *Prevotella* and dysphagia.

In the comparison between the three *Prevotella* groups and various associated factors, only sex showed a significant difference. The lack of significant differences in the other items may be due in part to the low

prevarication of each item. Smoking, diabetes, heart disease, and the presence of dentures have been associated with *Prevotella* in previous studies;[14-18] however, the mean age of the participants in this study was higher than that in previous studies.[14-18] Given that bacterial microbiota is strongly affected by age,[10] the difference may not have been significant.

This study analyzed the results of health awareness check-ups conducted by I-city to promote health among citizens. Instead of a study in which the sample size was calculated and participants were recruited, the participants participated freely, and the number of participants could not be predicted in advance. As a result of the data aggregation, the *Prevotella* proportion in the oral microbiota had a normal distribution, and approximately 70 persons were in each tertile range group. The adjustment factors for oral frailty and *Prevotella* comprised seven items, and the sample size was sufficient. Moreover, the number of participants was larger than that in previous studies on *Prevotella*.[16-18,31] Compared to four previous studies on oral frailty[4,32–34] conducted in different regions of Japan, the average age of the participants had little difference; however, the prevalence of oral frailty in this study was as low as 8.2%. This may have been influenced by the fact that the participants were older adults with a high health consciousness, exemplified by their participation in health education medical examination.

In addition, when comparing each component of oral frailty with that reported in two previous studies,[4,32] except for the proportion of those who answered “yes” to “Have you choked on your tea or soup recently?” the proportion of those who answered “yes” to “Do you have any difficulty eating tough foods

compared to 6 months ago?" was low, and the mean of the measured values for each item was high. Therefore, when evaluating the results of this study, the high oral function in the population should be considered.

This study has some limitations. First, the study did not consider dental caries, periodontal disease, or denture status(size, usage status). Since it was difficult to consider diurnal variation and the effect of meals on saliva collection in this large-scale study, countermeasures such as confirmation that tooth brushing, eating, and drinking did not occur at least 30 minutes before saliva collection were applied. These factors also affect oral microbiota and function, and their validity should be investigated in future studies. In the analyses, collagen diseases related to *Prevotella* were excluded from the adjustment factors because only one participant had collagen diseases. In addition, as *Prevotella* is related to periodontal disease,[10] it seems necessary to examine dementia,[35] which is also related to periodontal disease. However, no participant was diagnosed with dementia in this study. Finally, this was a cross-sectional study; therefore, we could not examine the causality of the results. We are following the present participants, and future studies will longitudinally examine the decline in oral function and changes in oral microbiota, including *Prevotella*.

Conclusions

This study revealed that the proportion of *Prevotella* in the oral microbiota was high in individuals with oral frailty. The results of this study suggest that examination and management of oral function may relate not

only to the nature of oral microbiota in older adults, but also to systemic diseases associated with oral microbiota.

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Variables	Overall	Lower tertile	Median	Upper tertile	p-value
	(n = 208)	(n = 70)	(n = 69)	(n = 69)	
	mean±SD n(%)				
Age, years	74.3±6.1	73.6±6.7	73.9±6.2	75.3±5.4	0.208
Sex, male, n(%)	58(27.9)	27(38.6)	19(27.5)	12(17.4)	0.021*
SMI	6.2±0.9	6.3±0.9	6.3±0.9	6.0±0.9	0.102
Smoking, n (%)	10(4.8)	2(1.0)	4(1.9)	4(1.9)	0.645
Collagen disease (rheumatoid arthritis), n (%)	1(0.5)	0	0	1(1.4)	0.366
Ischemic heart disease, n(%)	8(3.8)	3(4.3)	4(5.8)	1(1.4)	0.403
Diabetes, n(%)	24(11.5)	7(10.0)	10(14.5)	7(10.1)	0.643
Removable denture use, n(%)	103(49.5)	29(41.4)	33(47.8)	41(59.4)	0.099

Categorical variables are denoted by n (%); continuous variables are indicated by means and standard deviations for those with a normal distribution and medians (Q1, Q3) for those without a normal distribution.

Q1, first quartile; Q3, third quartile; SD, standard deviation; SMI, skeletal muscle mass index. *p<0.05

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Table 2. Comparison of the three *Prevotella* tertile groups ranges and oral frailty test items

	Overall	Lower	Median	Upper	p-value

Variables		tertile		tertile	
	(n = 208)	(n = 70)	(n = 69)	(n = 69)	
	mean±SD n(%), median (Q1, Q3)				
Number of remaining teeth, n	22 (15, 26)	24 (18, 26)	23 (17, 26)	20 (10, 24.5)	<0.001†**
Masticatory performance	22.4±4.6	23.1±4.6	23.2±4.0	20.6±4.6	<0.001†**
ODK for “ta” syllable, times/s	6.3±0.7	6.4±0.7	6.2±0.7	6.3±0.9	0.795†
Tongue pressure, kPa	34.8±7.2	34.8±6.8	35.2±6.5	34.3±8.3	0.868†
Difficulties eating tough foods (yes)	34(16.3)	11(15.7)	10(14.5)	13(18.8)	0.621‡
Difficulties swallowing tea or soup (yes)	69(33.2)	20(28.6)	21(30.4)	28(40.6)	0.134‡
Oral frailty (number of applicable items)	1(0,2)	1(0,2)	1(0,1)	1(1,2)	0.010†**
Oral frailty	17(8.2)	2(2.9)	4(5.8)	11(15.9)	0.005‡**

Categorical variables are denoted by n(%); continuous variables are indicated by means and standard deviations for those with a normal distribution and medians (Q1, Q3) for those without a normal distribution**.** ODK, oral diadochokinesis; Q1, first quartile; Q3, third quartile; SD, standard deviation.

†Jonckheere–Terpstra test; ‡Cochran–Amitage test; **p<0.01 ***p<0.001

Table 3. Relationship between the three *Prevotella* tertile groups ranges and oral function

	Crude			Adjusted		
	OR	**95% CI**		**OR**	**95% CI**	
Number of remaining teeth, n	0.942	0.912	0.974	0.946	0.915	0.977
Masticatory performance	0.897	0.844	0.953	0.897	0.844	0.953
ODK for “ta” syllable, times/s	0.874	0.621	1.231	0.865	0.614	1.218
Tongue pressure, kPa	0.994	0.959	1.03	0.992	0.957	1.028
Difficulties eating tough foods (yes)	1.176	0.593	2.331	1.191	0.601	2.359
Difficulties swallowing tea or soup (yes)	1.488	0.87	2.544	1.509	0.883	2.58
Oral frailty (number of applicable items)	1.478	1.14	1.916	1.477	1.14	1.915
Oral frailty	4.157	1.506	11.479	4.194	1.519	11.576

Crude: an ordinal logistic regression analysis was performed by entering the three *Prevotella* tertile groups as the dependent variable and oral frailty test items and presence/absence of judgment as independent variables.

Adjusted: Covariates included age, sex, SMI (skeletal muscle mass index), smoking, ischemic heart disease, diabetes, and denture use.

The three *Prevotella* tertile groups were used as the dependent variable, and six items from the oral frailty

test items, number of items, and oral frailty test as independent variables. Confounding factors: age, years; sex, male, n(%); SMI; smoking, n(%); ischemic heart disease, n(%); diabetes, n(%); and removable denture use, n(%). CI , confidence interval; ODK, oral diadochokinesis; OR, odds ratio.

Figure Legends

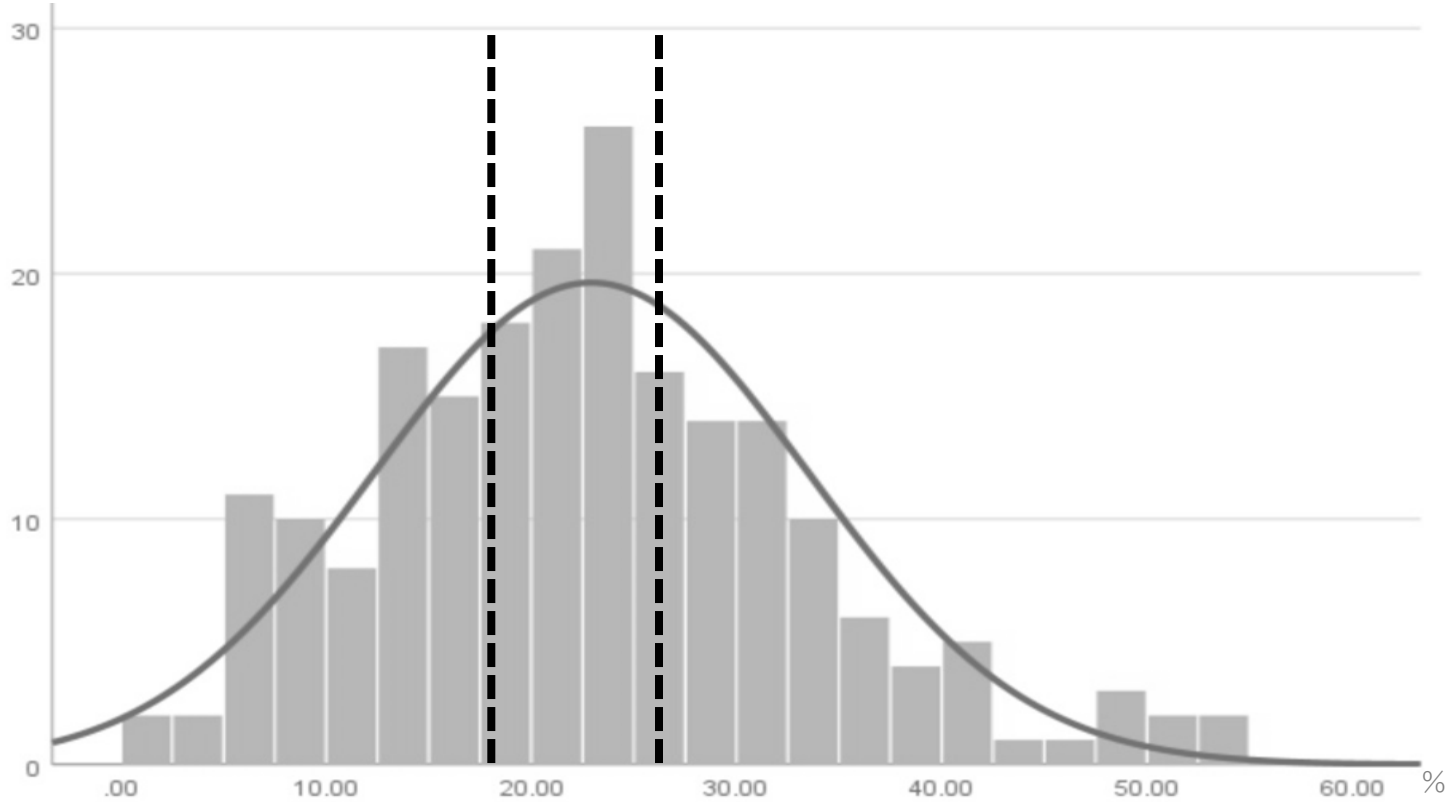
Figure 1. Classification of the three *Prevotella* tertile groups

Prevotella in saliva showed a normal distribution. The proportion of *Prevotella* in saliva was classified into three groups in order of decreasing proportion: upper group, middle group, and lower group using tertile ranges.

Figure 2. Flowchart of the study participant selection criteria

A total of 208 participants (58 men and 150 women, mean age 74.3 ± 6.1 years) were included in the analysis, excluding 24 participants who had incomplete answers to the questionnaire (11), had taken antibiotics within the prior month (9), or did not undergo an oral function evaluation (4)

Figure 1. Classification of the three *Prevotella* tertile groups



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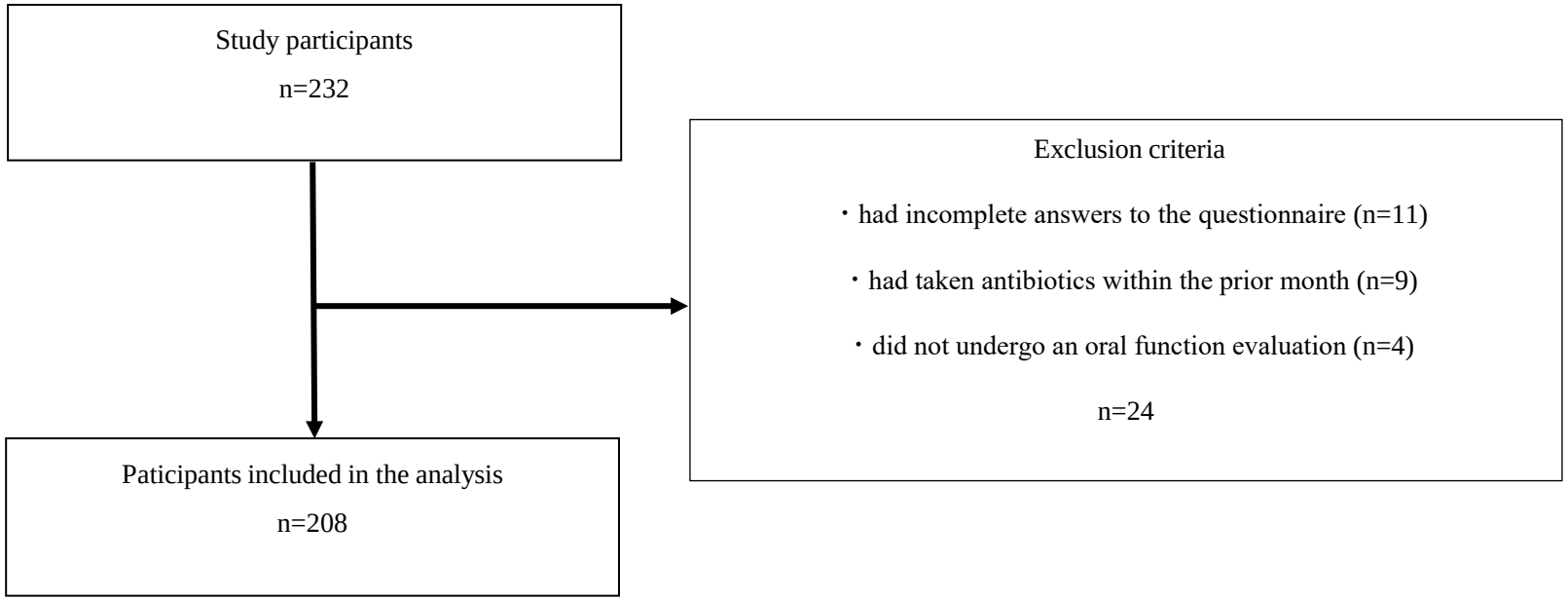

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