Dysbiosis of Oral Microbiota Associated with Palmoplantar Pustulosis

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Abstract

Background: Dysbiosis of oral microbiota is implicated not only in oral inflammatory lesions, but also in a variety of extraoral diseases. The etiology of palmoplantar pustulosis (PPP) remains unclear; however, it has been suggested that chronic inflammation caused by periodontopathic bacterial infection may play a role. Objectives/Methods: To determine whether patients with PPP have altered diversity and composition of oral microbiota, we conducted the 16S rDNA analysis using saliva samples collected from 21 outpatients with PPP and 10 healthy individuals. Results: We found that the proportion of bacteria in the phylum Proteobacteria was significantly lower in PPP patients ($p = 0.025$). At the genus level, patients with PPP had a significantly lower abundance of \textit{Neisseria} ($p = 0.014$), which best accounted for the observed decrease in Proteobacteria. We also identified multiple minor genera and species that were represented at a significantly higher level in the PPP group, several of which have been associated with periodontal diseases. Conclusion: Our results suggest a possible link between PPP and dysbiosis of oral microbiota, particularly the lower abundance of \textit{Neisseria}, the most predominant genus of Proteobacteria in healthy oral microbiota. Probiotics that improves oral dysbiosis may be beneficial for patients with PPP as an adjunctive therapy.

Introduction

The human oral cavity harbors more than 700 different species of resident microorganisms and is the second most diverse microbial community in the body. The growth of periodontal pathobionts, local odontogenic infection, and chronic inflammation have been linked to changes in the diversity and composition (dysbiosis) of the oral microbiota [1, 2]. In addition, oral dysbiosis may also contribute to a variety of systemic or autoimmune diseases [3–6], including atherosclerosis [7, 8], coronary artery disease [9, 10], diabetes mellitus [11, 12], rheumatoid arthritis [13, 14], systemic lupus erythematosus [15, 16], and Sjögren’s syndrome [17, 18]. The oral microbiome serves as a source of pathogenic microorganisms, bioactive metabolites, and proinflammatory molecules for extraoral tissues.
Palmoplantar pustulosis (PPP) is a refractory skin disorder in which crops of multiple sterile pustules appear recurrently and bilaterally on the palms and soles. The pustules form erythematous, scaly plaques with chronic pain and/or itch, which seriously impairs the quality of life of the patients. The etiological factors for PPP remain unclear; however, it has been suggested that smoking and dental metal hypersensitivity trigger and/or exacerbate PPP [19–22]. Furthermore, accumulating evidence suggests that chronic odontogenic infection, in particular periapical lesions, has a close pathogenetic relationship with PPP [23–25].

To evaluate the role of oral microbiota in the pathogenesis of PPP, we conducted a comparative analysis of oral microbial profiles in patients with PPP and healthy individuals. The results of this study highlight specific oral dysbiosis in patients with PPP and suggest the possibility of adjunctive oral probiotics that improves the taxonomic composition of the oral microbial community in combination with the current pharmacotherapy for PPP.

**Materials and Methods**

For further details, see the online supplementary material (see www.karger.com/doi/10.1159/000511622 for all online suppl. material,) (Fig. 1). The main characteristics of PPP patients are summarized in online supplementary Table 1.

**Results**

*Periodontal Health Status of the Subjects*

Periodontal disease (probing depth ≥ 4 mm) was found in 42.1% of the PPP patients and half of the healthy controls (HC), and the difference was not significant (*p* = 0.714 in two-tailed Fisher’s exact test). No significant difference in the number (5.75 [PPP] vs. 2.00 [HC], *p* = 0.0789 in two-tailed Welch’s *t* test) and the mean depth (4.41 mm [PPP] vs. 4.00 mm [HC], *p* = 0.0931 in two-tailed Welch’s *t* test) of periodontal pockets between PPP and HC was found. We thus reasoned that the enrolled PPP and HC subjects had a similar periodontal health status.

*PPP Patients Have Dysbiosis of Oral Microbiota*

In next-generation sequencing-based 16S rDNA amplicon analysis, averages (SD) of 17,621 (4,683; PPP) and 20,805 (4,985; HC) clean reads were obtained per sample, clustered into operational taxonomic units with 97% similarity, and assigned taxonomically. Of 198 operational taxonomic units (OTUs) obtained, 103 OTUs (52.0%) were assigned taxonomically at the genus level and 42 OTUs (21.2%) at the species level. A total of 14 phyla, 22 classes, 36 orders, 59 families, 96 genera, and 42 species were represented in the saliva samples (online suppl. Table 2). Generally, biodiversity of a microbial community has been assessed from 2 aspects: species diversity within a single ecosystem (α-diversity) and differences in overall diversity between different environments (β-diversity). There was no significant difference in the standard estimates of α-diversity between PPP patients and HC, as measured by species richness (*p* = 0.521 in two-tailed Welch’s *t* test; Fig. 2a), evenness (*p* = 0.601 in two-tailed Welch’s *t* test; Fig. 2b), and diversity (*p* = 0.998 in two-tailed Welch’s *t* test; Fig. 2c; *p* = 0.381 in two-tailed Welch’s *t* test; Fig. 2d). However, β-diversity analysis revealed a clear separation between the PPP and HC microbial communities at the phylum and the genus levels (Fig. 2e, f), suggesting that PPP patients and HC have distinct oral microbial compositions.
Decreased Proportion of Proteobacteria in PPP
We next investigated the taxonomic composition of the oral microbiota of PPP patients and HC. At the phylum level, a significant decrease in the proportion of Proteobacteria ($p = 0.0251$ in two-tailed Welch’s $t$ test) and a significant increase in the proportion of Synergistetes ($p = 0.0485$ in two-tailed Welch’s $t$ test) were observed in patients with PPP (Fig. 3a–c). Proteobacteria, along with Bacteroidetes and Firmicutes, was the most predominant phylum in HC; however, in patients with PPP, the relative abundance of Proteobacteria was decreased to nearly half that of Bacteroidetes and Firmicutes (Fig. 3a). Hierarchical cluster analysis revealed that PPP and HC were separated into 4 different clusters based on the phylum composition of the oral microbiota: one HC-specific cluster characterized by a higher proportion of Proteobacteria and 3 PPP clusters with higher relative abundance of Bacteroidetes or Firmicutes (Fig. 3d).

Reduced Abundance of Neisseria in PPP
The differences in oral microbiota composition were further confirmed at the genus level. We found that patients with PPP had a significantly reduced abundance of the genus *Neisseria* than HC did ($p = 0.0140$ in two-tailed Welch’s $t$ test; Fig. 4a, b). *Neisseria* was the most predominant genus within Proteobacteria in healthy oral microbiota (Fig. 4a), and this reduction accounted for a large percentage of the observed decrease in Proteobacteria in PPP patients (Fig. 4b). Furthermore, the relative abundance of the rare genera within Firmicutes and Synergistetes was significantly increased in PPP patients: *Dialister* ($p = 0.0356$ in two-tailed Welch’s $t$ test), *Schwartz-
Proteobacteria
Bacteroidetes
Fusobacteria
Firmicutes
Actinobacteria
Synergistetes
Chloroflexi
Cyanobacteria
Elusimicrobia
Spirochaetes
Others

PPP HC

Relative abundance, %

$p = 0.0251$

PPP
HC

Proteobacteria

$p = 0.0485$

PPP
HC

Synergistetes

Abundance, %

Dissimilarity

(For legend see next page.)
Fig. 3. Taxonomic composition analysis of oral microbiomes of the PPP and HC groups at the phylum level. a The relative abundances of oral bacterial phyla of PPP and HC are represented by pie charts. The phyla representing <0.5% of the total oral microbes of HC are included in “others.” b–e Box-and-whisker plots show the comparisons of the relative abundance of the phyla with significant differences between PPP and HC. The mean values and outliers are shown by cross marks and open circles, respectively.

Fig. 4. Taxonomic composition analysis of the oral microbiomes of the PPP and HC groups at the genus level. a Comparisons of the genus-level abundance within Proteobacteria of PPP and HC are shown in bar plots. The genera representing <0.1% of the total oral microbes of HC are included in “others.” b–e Comparisons of the relative abundance of the genera with significant differences between PPP and HC. The genera within Proteobacteria (b), Firmicutes (c, d), and Synergistetes (e) are shown in box-and-whisker plots. The mean values and outliers are shown by cross marks and open circles, respectively.

Relative abundance of the genera with significant differences between PPP and HC. The genera within Proteobacteria (b), Firmicutes (c, d), and Synergistetes (e) are shown in box-and-whisker plots. The mean values and outliers are shown by cross marks and open circles, respectively.
ia \( (p = 0.0351 \text{ in two-tailed Welch’s } t \text{ test}) \) and TG5 \( (p = 0.0461 \text{ in two-tailed Welch’s } t \text{ test}) \), all of which have been associated with periodontal disease and endodontic lesions (Fig. 4c–e) [26–28].

**Higher Proportion of Periodontopathic Bacterial Species in PPP Patients**

We found 11 species with distinct relative abundance between PPP patients and HC (Table 1). Several of these species, such as *Prevotella* sp., *Dialister* sp., *Schwartzia* sp., and TG5 sp., were members of genera that are closely associated with odontogenic infection [26–29]. Three species were detected only in patients with PPP, 2 of which (unidentified species within the family Veillonellaceae and *Desulfobulbus* sp.) belong to taxa known as periodontal pathobionts [28, 30]. The overall results are illustrated schematically in Figure 5.

**Post Hoc Power Analysis**

Twenty-one PPP patients and 10 HC were enrolled in this study. In the analysis of Proteobacteria, the mean proportion and the SD were 20.0 and 9.56 (PPP) versus 29.7 and 9.92 (HC), respectively. The effect size \( (d) \) was calculated to be 1.00. Similarly, in the analysis of *Neisseria*, the mean proportion and the SD were 9.82 and 6.23 (PPP) versus 19.7 and 9.50 (HC), respectively. The effect size \( (d) \) was calculated to be 1.33. On the basis of these statistics, we obtained the statistical power values of 0.711 (Proteobacteria) and 0.917 (*Neisseria*).

**Discussion/Conclusion**

A significantly lower proportion of Proteobacteria was observed in PPP patients and appears largely attributable to the reduction of the genus *Neisseria*, although no specific species responsible for the decrease in *Neisseria* was identified in this study. Notably, an abundance of *Neisseria* is related to healthy periodontal conditions [31–34]. Most species within *Neisseria*, except for patho-

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**Table 1.** The species with distinct relative abundance between PPP and HC groups.

| Phylum          | Class            | Order            | Family            | Genus       | Species          | Relative abundance (PPP), % | Relative abundance (HC), % | Abundance ratio (PPP/HC) | p value (Welch’s t test) |
|-----------------|------------------|------------------|-------------------|-------------|------------------|-----------------------------|---------------------------|--------------------------|-------------------------|
| Bacteroidetes   | Bacteroidia      | Bacteroidiales   | Prevotellaceae    | Prevotella  | Not identified   | 2.026                       | 1.918                     | 1.05                     | 0.009                   |
|                 |                  |                  |                   |             | Not identified   | 6.490                       | 3.286                     | 1.98                     | 0.000                   |
| Firmicutes      | Bacilli          | Gemellales       | Gemellaceae       | Not identified | Not identified | 0.139                       | 0.263                     | 0.52                     | 0.039                   |
|                 | Clostridia       | Lachnospiraceae  | Paraproteobacillaceae | Not identified | Not identified | 0.015                       | 0.001                     | 4.75                     | 0.024                   |
|                 | Clostridia       | Veillonellaceae  | Veillonellaceae   | Not identified | Not identified | 0.172                       | 0.061                     | 2.83                     | 0.036                   |
|                 | Clostridia       | Veillonellaceae  | Schwartzia sp.    | Schwartzia  | Not identified   | 0.012                       | 0.000                     | 4.51                     | 0.035                   |
|                 | Clostridia       | Veillonellaceae  | TG5 sp.           | TG5         | Not identified   | 0.007                       | 0.000                     | 4.51                     | 0.041                   |

**Fig. 5.** Schematic summarizing the shift in oral bacteria in PPP patients. Taxa in each taxonomic hierarchy are shown by circles. The average relative abundance of the taxon in PPP (the numerical value in or below the circle) is represented by the size of the circle. Changes in the relative abundance of the taxa between PPP and HCC are expressed as the base 2 logarithm of the ratio of the average relative abundance (PPP/HCC). The weight of the outline for each circle represents the statistical significance of the taxon (two-tailed Welch’s t test) between PPP and HC.

(For figure see next page.)
Significance (Welch’s t test)  

- $p < 1$  
- $p < 0.1$  
- $p < 0.05$  

Relative abundance in PPP, %  

- <0.1  
- 0.3  
- 1  
- 3  
- 10  
- 30  
- 100  

$n$-fold change  

log$_{2}$(PPP/HC)  

- Not present in HC  
- 3  
- 2  
- 1  
- 0  
- -0.5  
- -1  

Kingdom  

- Bacteria  

Phylum  

- Firmicutes  

- Bacteroidetes  

Class  

- Bacteroidia  

- Clostridia  

- Bacilli  

Order  

- Bacteroidales  

- Clostridiales  

- Gemellales  

Family  

- Prevotellaceae  

- Peptostreptococcaceae  

- Veillonellaceae  

- Lachnospiraceae  

Genus  

- Prevotella  

- Catonella  

- Clostridium  

- Actinobacillus  

- Filifactor  

- Dialister  

- Schwartzia  

- Desulfobulbus  

- Neisseria  

Species  

- sp.  

- TM7  

- TM7-3  

- (Welch’s t test)  

- $p < 1$  

- $p < 0.1$  

- $p < 0.05$  

- <0.1  

- 0.3  

- 1  

- 3  

- 10  

- 30  

- 100  

- Not present in HC  

- 3  

- 2  

- 1  

- 0  

- -0.5  

- -1  

- log$_{2}$(PPP/HC)
genic *N. gonorrhoeae* and *N. meningitides*, are nonpathogenic commensal bacteria in the human oral cavity. We speculate that the reduction of resident *Neisseria* permits the colonization and/or growth of other bacteria directly involved in PPP pathogenesis; these may include the periodontal pathogenic taxa that were found to be increased in PPP patients in this study. *Neisseria* is a dominant producer of acetaldehyde in the oral cavity [35, 36]. Alternatively, *Neisseria* is an important contributor to the oral bacteria-mediated reduction of nitrate (NO$_3^-$) to nitrite (NO$_2^-$) [37, 38], which can be converted to physiologically active nitric oxide (NO) in the stomach. Dysregulation of this NO$_3^-$-NO$_2^-$-NO pathway may cause PPP.

The phylum-level analysis also suggested that PPP was associated with a significantly higher proportion of Synergistetes, implicated in periodontitis and peri-implantitis [39–41]. The genus TG5, which was found to be significantly increased in PPP patients, has a close phylogenetic relationship with cluster A species, a periodontopathic bacterial group within the phylum Synergistetes [42–44]. Synergistetes has a potential role in oral dysbiosis through the generation of cyclodipeptide metabolites with quorum-sensing and/or bactericidal/bacteriostatic activity [45]. This induction of dysbiosis by Synergistetes may also be associated with PPP.

Although we could not find the causality between oral dysbiosis and PPP in this study, our findings suggest the possibility of oral probiotics that supplements *Neisseria* and/or improves oral dysbiosis as an add-on therapy. In fact, previous randomized controlled trials have demonstrated that oral probiotics using lozenges containing probiotic bacterial strains has beneficial effects on oral microbiome [46–48].

The small numbers of patients and controls examined in this study may limit its conclusions and generalizability, although the sample size yielded statistical power values at the recommended levels in a post hoc power analysis. In addition, we could find no evidence to suggest the causal relationship between oral dysbiosis and PPP in this study. Further confirmation studies with larger cohorts are needed to establish the causality. Mechanistic investigation will also be essential for a better understanding of the impact of periodontal disease and oral microbiome on PPP. Oral dysbiosis can affect the gut microbiota directly through saliva and indirectly through blood flow [49]; however, there have been no reports on the intestinal microbiome of PPP patients. Further comparative studies are required to elucidate whether PPP patients have specific dysbiosis of gut microbiota.

In conclusion, we found dysbiosis of oral microbiota, particularly, the significant decrease in the genus *Neisseria* and the concomitant increase in bacteria within the periodontopathic taxa, in the PPP cohort.

**Key Message**

Microbiome analysis reveals altered composition of oral microbiota in patients with palmoplantar pustulosis.

**Statement of Ethics**

This case-control observational study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the strengthening of the reporting of observational studies in epidemiology (STROBE) guidelines. Ethical approval for this study was obtained from the Institutional Review Board of Takanawa Clinic (approval No.: 2016-1). A signed informed consent form was obtained from each participant prior to inclusion in this study.

**Conflict of Interest Statement**

Y.K., Y.S., K.K., M.M., and K.A. are employees of Takanawa Clinic (Tokyo, Japan). T.A. and T.N. have advisory roles in conducting clinical research in Takanawa Clinic.

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**Author Contributions**

Y.K., Y.S., K.K., M.M., and K.A. contributed to conception and design, contributed to data analysis and interpretation, and critically revised the manuscript. T.A. contributed to conception and design and critically revised the manuscript. T.N. contributed to conception and design, contributed to data analysis and interpretation, and drafted the manuscript. All authors have read and approved the final paper.
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References

1 Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017 Jun;3(1):17038.
2 Rosier BT, Marsh PD, Mira A. Resilience of the oral microbiota in health: mechanisms that prevent dysbiosis. J Dent Res. 2018 Apr; 97(4): 371–80.
3 Graves DT, Corrêa JD, Silva TA. The oral microbiota is modified by systemic diseases. J Dent Res. 2019 Feb;98(2):148–56.
4 Jia G, Zhi A, Lai PF, Wang G, Xia Y, Xiong Z, et al. The oral microbiota – a mechanistic role for systemic diseases. Br Dent J. 2018 Mar; 224(6): 447–55.
5 Nikitakis NG, Papaioannou W, Sakkas LI, Kousvelari E. The autoimmunity–oral microbiome connection. Oral Dis. 2015 Oct;21(8): 895–905. 
6 Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partially normalized after treatment. Nat Med. 2015 Aug;21(8): 895–905.
7 Corrêa JD, Calderaro DC, Ferreira GA, Mendonça SM, Fernandes GR, Xiao E, et al. Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status. Microbiome. 2017 Mar;5(1): 34.
8 Koren O, Spor A, Felin J, Fåk F, Stombaugh J, Regezi JA, et al. Altered mucosal microbiome diversity and disease severity in Sjögren syndrome. Sci Rep. 2016 Apr;6(1): 23561.
9 van der Meulen TA, Harmens H, Bootsm A, Spijkervet F, Kroese F, Vissink A. The microbiome–systemic diseases connection. Oral Dis. 2016 Nov;22(8): 719–34.
10 Pietiäinen M, Liljestrand JM, Kopra E, Pussinen PJ. Mediators between oral dysbiosis and cardiovascular diseases. Eur J Oral Sci. 2011 Mar; 119(Suppl 1): 4592–8.
11 Hyvärinen K, Mäntylä P, Buhlin K, Paju S, Nieminen M, Sinisalo J, et al. A common peri-odontal pathogen has an adverse association with both acute and stable coronary artery disease. Atherosclerosis. 2012 Aug;223(2): 478–84.
12 Pietiäinen M, Liljestrand JM, Kopra E, Pussinen PJ. Mediators between oral dysbiosis and cardiovascular diseases. Eur J Oral Sci. 2018 Oct;126(Suppl 1): 26–36.
13 Long J, Cai Q, Steinwandel M, Hargreaves MK, Bordenstein SR, Blot WJ, et al. Association of oral microbiome with type 2 diabetes risk. J Periodontal Res. 2017 Jun; 52(3): 636–43.
14 Tam J, Hoffmann T, Fischer S, Bornstein S, Grässler J, Noack B. Obesity alters composition and diversity of the oral microbiota in patients with type 2 diabetes mellitus independently of glycemic control. PLoS One. 2018 Oct;13(10):e0204724.
15 Scher JU, Ubeda C, Equinda M, Khanin R, Buiskool Y, Viale A, et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. Arthritis Rheum. 2012 Oct; 64(10): 3083–94.
16 Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partially normalized after treatment. Nat Med. 2015 Aug;21(8): 895–905.
Koyanagi T, Sakamoto M, Takeuchi Y, Okumura M, Izumi Y. Analysis of microbiota associated with peri-implantitis using 16S rRNA gene clone library. J Oral Microbiol. 2010 May;2.

You M, Mo S, Watt RM, Leung WK. Prevalence and diversity of Synergistetes taxa in periodontal health and disease. J Periodontal Res. 2013 Apr;48(2):159–68.

Yu XL, Chan Y, Zhuang LF, Lai HC, Lang NP, Lacap-Bugler DC, et al. Distributions of Synergistetes in clinically-healthy and diseased periodontal and peri-implant niches. Microb Pathog. 2016 May;94:90–103.

Belibasakis GN, Oztürk VO, Emingil G, Bostanci N. Synergistetes cluster A in saliva is associated with periodontitis. J Periodontal Res. 2013 Dec;48(6):727–32.

Hugenholtz P, Hooper SD, Kyrpides NC. Focus: synergistetes. Environ Microbiol. 2009 Jun;11(6):1327–9.

Vartoukian SR, Palmer RM, Wade WG. Cultivation of a Synergistetes strain representing a previously uncultivated lineage. Environ Microbiol. 2010 Apr;12(4):916–28.

Marchesan JT, Morelli T, Moss K, Barros SP, Ward M, Jenkins W, et al. Association of Synergistetes and cyclopentapeptides with periodontitis. J Dent Res. 2015 Oct;94(10):1425–31.

Mayanagi G, Kimura M, Nakaya S, Hirata H, Sakamoto M, Benno Y, et al. Probiotic effects of orally administered Lactobacillus salivarius WB21-containing tablets on periodontopathic bacteria: a double-blinded, placebo-controlled, randomized clinical trial. J Clin Periodontol. 2009 Jun;36(6):506–13.

Iniesta M, Herrera D, Montero E, Zurbriggen M, Matos AR, Marín MJ, et al. Probiotic effects of orally administered Lactobacillus reuteri-containing tablets on the subgingival and salivary microbiota in patients with gingivitis. A randomized clinical trial. J Clin Periodontol. 2012 Aug;39(8):736–44.

Alanzi A, Honkala S, Honkala E, Varghese A, Tolvanen M, Söderling E. Effect of Lactobacillus rhamnosus and Bifidobacterium lactis on gingival health, dental plaque, and periodontopathogens in adolescents: a randomised placebo-controlled clinical trial. Benef Microbes. 2018 Jun;9(4):593–602.

Olsen I, Yamazaki K. Can oral bacteria affect the microbiome of the gut? J Oral Microbiol. 2019 Mar;11(1):1586422.