Distribution of Porphyromonas gingivalis fimA and mfa1 fimbrial genotypes in subgingival plaques

Keiji Nagano, Yoshiaki Hasegawa, Yura Iijima, Takeshi Kikuchi, Akio Mitani

1 Department of Microbiology, School of Dentistry, Aichi Gakuin University, Nagoya, Japan
2 Department of Periodontology, School of Dentistry, Aichi Gakuin University, Nagoya, Japan

Corresponding Author: Keiji Nagano
Email address: nagano@dpc.agu.ac.jp

Background. Strains of periodontal disease-associated bacterium Porphyromonas gingivalis have different pathogenicity, which can be attributed to clonal genetic diversity. P. gingivalis typically expresses two types of fimbriae, FimA and Mfa1, which comprise six (I, Ib, II, III, IV, and V) and two (mfa53 and mfa70) genotypes, respectively. This study was conducted to investigate the distribution of the two fimbrial genotypes of P. gingivalis in clinical specimens.

Methods. Subgingival plaques were collected from 100 participants during periodontal maintenance therapy and examined for P. gingivalis fimbrial genotypes by direct PCR and/or DNA sequencing. We also analyzed the relationship between fimbrial genotypes and clinical parameters of periodontitis recorded at the first medical examination.

Results. Both fimbrial types could be detected in 63 out of 100 samples; among them, fimA genotype II was found in 33 samples (52.4%), in which the mfa70 genotype was 1.75 times more prevalent than mfa53. The total detection rate of fimA genotypes I and Ib was 38.1%; in these samples, the two mfa1 genotypes were observed at a comparable frequency. In two samples positive for fimA III (3.2%), only mfa53 was detected, whereas in four samples positive for fimA IV (6.3%), the two mfa1 genotypes were equally represented, and none of fimA V-positive samples defined the mfa1 genotype. No associations were found between clinical parameters and fimbrial subtype combinations.

Discussion. Both P. gingivalis fimbrial types were detected at various ratios in subgingival plaques, and a tendency for fimA and mfa1 genotype combinations was observed. However, there was no association between P. gingivalis fimbrial genotypes and periodontitis severity.
Distribution of *Porphyromonas gingivalis* fimA and mfa1 fimbrial genotypes in subgingival plaques

Keiji Nagano\(^1\)*, Yoshiaki Hasegawa\(^1\), Yura Iijima\(^1\), Takeshi Kikuchi\(^2\) and Akio Mitani\(^2\)

Keiji Nagano\(^1\) (correspondence)
Affiliation Department of Microbiology, School of Dentistry, Aichi Gakuin University
Address 1-100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464-8650, Japan
E-mail nagano@dpc.agu.ac.jp

Yoshiaki Hasegawa\(^1\)
Affiliation Department of Microbiology, School of Dentistry, Aichi Gakuin University
Address 1-100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464-8650, Japan
E-mail yhase@dpc.agu.ac.jp

Yura Iijima\(^1\)
Affiliation Department of Microbiology, School of Dentistry, Aichi Gakuin University
Address 1-100 Kusumoto-cho, Chikusa-ku, Nagoya, Aichi 464-8650, Japan
E-mail ag13d006@sdent.agu.ac.jp

Takeshi Kikuchi\(^2\)
Affiliation Department of Periodontology, School of Dentistry, Aichi Gakuin University
Address 2-11 Suemori-dori, Chikusa-ku, Nagoya, Aichi 464-8651, Japan
E-mail tkikuchi@dpc.agu.ac.jp

Akio Mitani\(^2\)
Affiliation Department of Periodontology, School of Dentistry, Aichi Gakuin University
Address 2-11 Suemori-dori, Chikusa-ku, Nagoya, Aichi 464-8651, Japan
E-mail minita@dpc.agu.ac.jp
Abstract

Background. Strains of periodontal disease-associated bacterium Porphyromonas gingivalis have different pathogenicity, which can be attributed to clonal genetic diversity. P. gingivalis typically expresses two types of fimbriae, FimA and Mfa1, which comprise six (I, Ib, II, III, IV, and V) and two (mfa53 and mfa70) genotypes, respectively. This study was conducted to investigate the distribution of the two fimbrial genotypes of P. gingivalis in clinical specimens.

Methods. Subgingival plaques were collected from 100 participants during periodontal maintenance therapy and examined for P. gingivalis fimbrial genotypes by direct PCR and/or DNA sequencing. We also analyzed the relationship between fimbrial genotypes and clinical parameters of periodontitis recorded at the first medical examination.

Results. Both fimbrial types could be detected in 63 out of 100 samples; among them, fimA genotype II was found in 33 samples (52.4%), in which the mfa70 genotype was 1.75 times more prevalent than mfa53. The total detection rate of fimA genotypes I and Ib was 38.1%; in these samples, the two mfa1 genotypes were observed at a comparable frequency. In two samples positive for fimA III (3.2%), only mfa53 was detected, whereas in four samples positive for fimA IV (6.3%), the two mfa1 genotypes were equally represented, and none of fimA V-positive samples
defined the *mfa1* genotype. No associations were found between clinical parameters and fimbrial subtype combinations.

**Discussion.** Both *P. gingivalis* fimbrial types were detected at various ratios in subgingival plaques, and a tendency for *fimA* and *mfa1* genotype combinations was observed. However, there was no association between *P. gingivalis* fimbrial genotypes and periodontitis severity.
Introduction

Periodontal diseases are developed because of colonization of the subgingival area by multiple bacterial species (Page & Kornman 1997). Socransky et al. have determined that three bacterial species, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, are mostly responsible for the development and advancement of periodontitis, and proposed to call them the “red complex” (Socransky et al. 1998). Among these species, *P. gingivalis*, a gram-negative anaerobic bacterium forming characteristic black-pigmented colonies on blood agar, has been extensively studied for its pathogenicity (Gibbons & Macdonald 1960; Macdonald & Gibbons 1962; Macdonald et al. 1960), and accumulated evidence indicates its critical role in periodontitis (Lamont & Jenkinson 1998; Socransky & Haffajee 2002). Furthermore, although the proportion of *P. gingivalis* in the periodontal biofilm is low, it could lead to dysbiosis at the periodontal site, which prompted Hajishengallis et al. to call *P. gingivalis* a keystone pathogen (Hajishengallis et al. 2011). Still, it is well known that *P. gingivalis* can also be detected in healthy people (Amano et al. 2004; Griffen et al. 1998; Haffajee et al. 1998; Teanpaisan et al. 1996; Ximenez-Fyvie et al. 2000), suggesting that its presence may not necessarily cause periodontitis. This discrepancy is suggested to be attributed to heterogenic virulence of *P. gingivalis* (Griffen et al. 1999; Igboin et al. 2009; Tribble et al. 2013), which shows a high degree of genetic clonal diversity (Enersen 2011).

Although *P. gingivalis* expresses a number of potential virulence factors (How et al. 2016), fimbriae, filamentous proteinaceous appendages on the bacterial surface, are one of the most important because they play a pivotal role in *P. gingivalis* colonization through association with other bacteria and host tissues (Hospenthal et al. 2017; Lamont & Jenkinson 2000). *P. gingivalis*
generally expresses two distinct types of fimbriae: FimA and Mfa1 (Yoshimura et al. 2009). FimA fimbriae are primarily composed of polymers of the FimA protein encoded by the *fimA* gene (Dickinson et al. 1988; Yoshimura et al. 1984), whereas Mfa1 fimbriae are mostly composed of the Mfa1 protein encoded by the *mfa1* gene (Hamada et al. 1996). In addition, several minor accessory components are incorporated into the respective fimbriae (Hasegawa et al. 2013; Nishiyama et al. 2007).

Based on *fimA* sequence variability, the gene is classified into six genotypes (I, Ib, II, III, IV, and V) (Amano et al. 2004; Nakagawa et al. 2002b), and the encoded proteins exhibited distinct antigenicity, with the exception of subtypes I and Ib (Nagano et al. 2013; Nakagawa et al. 2002b). Several studies indicate that strains with *fimA* genotype II are the most prevalent in patients with periodontitis, whereas those with genotype I are predominantly detected in healthy individuals (Amano et al. 2004; Enersen et al. 2013; Kuboniwa et al. 2010; Missailidis et al. 2004; Miura et al. 2005), indicating that the genotype-II *P. gingivalis* may have higher pathogenicity compared with genotype-I bacteria. Furthermore, genotype-II strains showed higher adhesion and invasion ability in human epithelial cells (Nakagawa et al. 2002a) and in a mouse abscess model (Nakano et al. 2004). However, other reports indicated that *fimA* genotypes were not associated with adhesion to and invasion of host cells (Inaba et al. 2008; Umeda et al. 2006); moreover, there are studies showing that genotype-II strains had rather low rates of adhesion to and invasion of epithelial cells (Eick et al. 2002) and induced significantly less alveolar bone resorption in mice compared to genotype-I strains (Wang et al. 2009). Collectively, these data indicate that *P. gingivalis* pathogenicity cannot be defined based on the *fimA* genotype.
In contrast to \textit{fimA}, there are few clinical data regarding \textit{mfa1} genotypes. Recently, we found that the \textit{mfa1} gene had at least two variants (Nagano et al. 2015), encoding proteins with molecular weights about 70 kDa [67 (Arai et al. 2000; Hamada et al. 1996) or 75 kDa (Park et al. 2005)] and 53 kDa (Arai et al. 2000; Nagano et al. 2015), hereafter called Mfa$^{70}$ and Mfa$^{53}$ ($mfa^{70}$ and $mfa^{53}$, respectively, for the genes). In this study, we investigated the distribution of \textit{P. gingivalis mfa1} as well as \textit{fimA} genotypes in clinical specimens.

\textbf{Materials & Methods}

\textbf{Participants}

A total of 100 patients, who visited Aichi Gakuin University Dental Hospital at Nagoya, Japan, for periodontal treatment from September 2016 to March 2017, participated in this study. The study was approved by the institutional review board (Aichi Gakuin University, School of Dentistry, Ethics Committee, approval numbers 460 and 478), and written informed consent was obtained from all participants.

\textbf{Clinical oral examination, and consolidation and maintenance treatments}

Among the 100 participants, 81 could be examined for clinicopathological parameters of periodontitis at the first visit. Clinical oral examination was performed according to the guidelines published by the Japanese Society of Periodontology (Periodontology 2015). Probing pocket depth
(PD) and bleeding on probing (BOP) analyzed in six sites per tooth (buccal-mesial, mid-buccal, buccal-distal, lingual-mesial, mid-lingual and lingual-distal) for all remaining teeth. The PD and BOP values were utilized to calculate periodontal inflamed surface area (PISA) and periodontal epithelial surface area (PESA), which reflect the surface area of bleeding epithelium and total pocket epithelium (in mm$^2$), respectively, using a free spreadsheet (downloaded from www.parsprototo.info) (Nesse et al. 2008; Nesse et al. 2009). Consolidation and maintenance treatments mainly consisted of professional scaling and cleaning. Patients visited the hospital for consultation every one to six months.

Collection of subgingival plaques

Subgingival plaque samples were collected by a sterile hand scaler and transferred in either 1 ml of sterile reduced transfer fluid (RTF) consisting of 0.01% dithiothreitol in PBS, pH 7.4 or 1 ml of distilled water. The samples were immediately placed at 4°C and analyzed within 4 h.

Isolation and identification of black-pigmented bacteria

The samples collected in RTF were thoroughly suspended, serially diluted, and aliquots spread on blood agar consisting of Brucella HK agar (Kyokuto Pharmaceutical Industrial Co., Ltd, Tokyo, Japan), 5% laked rabbit blood, and 100 μg/ml kanamycin, as anaerobic bacteria, including $P. gingivalis$, are typically kanamycin resistant (Jouseimies-Somer et al. 2002). Plates were cultured at 37°C under anaerobic conditions for a week, and the emerged black-pigmented colonies were streaked on fresh plates to ensure isolation of single clones, which were then subjected to species identification. For this, genomic DNA was purified using Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) and analyzed for 16S rRNA-encoding genes by
polymerase chain reaction (PCR) using primers (5′-GAAGAGTTTGATCMTGGCTCAGATTG-3′ and 5′-TACGGYTACCTTGTTACGACTTCAC-3′) slightly modified from the universal primers 27F and 1492R (Frank et al. 2008). PCR products were subjected to DNA sequencing by a dye-terminator method and sequencing reads were analyzed by the BLAST search (https://blast.ncbi.nlm.nih.gov/). Bacterial species were identified if samples showed the lowest expectation (E) value (i.e., the highest probability) in the list of BLAST results. Most of E values were zero, whereas the highest was $3 \times 10^{-66}$, i.e. were sufficiently low to identify bacterial species.

Genotyping of fimA and mfa1

FimA genotypes were determined by PCR, sequencing, and BLAST analysis. Plaque samples in RTF or water were directly used as PCR templates. Primers for PCR (5′-AGCTTGTAACAAAGACAACGAGGCAG-3′, and 5′-GAGAATGAATACGGGGAGTGGAGCG-3′) and sequencing (5′-AGCTTGTAACAAAGACAACGAGGCAG-3′) were designed for common fimA regions based on fimA sequencing data for 84 P. gingivalis strains (Nagano et al. 2013). PCR-amplified fragments of a predicted size (around 1.2 kb) were sequenced by the dye-terminator method and the fimA genotype was determined by BLAST analysis.

Mfa1 genotypes were determined by PCR using two primer sets (5′-GAGCATTGCTCTCATTGGGCTTTG-3′ and 5′-CATCAGAAAAGGCAGCGTAAGCTG-3′, and 5′-GAGCATTGCTCTCATTGGGCTTTG-3′ and 5′-
TTAGGTATTGGCGACGTTCTCCTTG-3'), which yielded mfa\textsuperscript{53} and mfa\textsuperscript{70} fragments of 410 bp and 830 bp, respectively.

**Statistical analysis**

The data were expressed as the mean ± SEM. Differences between groups were analyzed by the nonparametric Kruskal-Wallis H test, and were considered statistically significant at $P < 0.01$. The chi square test is used to determine if there is a relationship in the genotype distribution ($P < 0.01$).

**Results**

**Isolation of \textit{P. gingivalis}**

The first 73 dental plaque samples were collected in RTF to isolate \textit{P. gingivalis} by a culture method. Although black-pigmented colonies were obtained from the majority of samples, 16S rDNA sequencing analysis showed that they were mostly formed by \textit{Prevotella} species, and the isolation rate of \textit{P. gingivalis} was only 5.5%. Therefore, we decided to examine fimbrial genotypes by direct PCR; in addition, the collection solution was changed to water, did not affect experimental results, but slightly improved detectability.

**Distribution of \textit{fimA} and \textit{mfa1} genotypes**

The distribution of fimbrial \textit{fimA} and \textit{mfa1} genotypes is summarized in Table 1. Among the 100 samples, both fimbrial types were detected in 63 and a single type in 15 samples, whereas 22 had
no fimbrial genes. *FimA* genotype II was the most prevalent and detected in 33 of the 63 samples positive for both fimbrial genes (52.4%), followed by genotypes Ib and I detected in 27.0% and 11.1% samples, respectively, whereas the frequency of the other *fimA* genotypes was low.

Although there was no statistically significant difference in combination of the *fimA* and *mfa1* genotypes, the following tendency was observed. The *mfa*\(^{53}\) and *mfa*\(^{70}\) genotypes were detected at comparable frequencies (44.4% and 55.6%, respectively) and each of them showed almost the same frequency in samples positive for *fimA* genotypes I, Ib, and IV. However, the prevalence of *mfa*\(^{70}\) was 1.75 times higher than that of *mfa*\(^{53}\) in genotype-II positive samples, whereas only *mfa*\(^{53}\) was detected in the two genotype III-positive samples, and no *mfa1* genes were found in genotype V-positive samples.

**Relationship between clinical parameters and fimbrial genotypes**

We also examined the association of the fimbrial genotypes with clinical characteristics of periodontitis (maximal and mean PD values, and BOP, PISA, and PESA values) (Table 2). However, no statistically significant differences in periodontitis severity were observed depending on the fimbrial genotypes.

**Discussion**

In this study, we first attempted to isolate *P. gingivalis* from dental plaque samples by a culture method, because we thought that analysis of chromosomal DNA purified from isolated bacterial
clones by PCR would provide unequivocal fimbrial genotyping results. However, *P. gingivalis* was rarely isolated by the culture method. On the other hand, direct PCR detected either *fimA* or *mfa1* in 78% samples, indicating that *P. gingivalis* was present with high frequency in patients receiving periodontal maintenance therapy, although its proportion among dental plaque bacteria was low.

*FimA* genotypes have been determined by PCR using genotype-specific primers (Amano et al. 2004; Nakagawa et al. 2002b); in addition, restriction enzyme digestion is used to discriminate genotypes I and Ib (Nakagawa et al. 2002b), which, however, may not be necessary for the analysis of the entire *fimA* gene, because genotypes I and Ib cannot be clearly discriminated [Fig. S1 in Supporting Information and (Nagano et al. 2013)]. Furthermore, immunological analysis did not detect any differences in antigenicity between *FimA* I and Ib fimbriae (Nagano et al. 2013; Nakagawa et al. 2002b). Therefore, we do not discuss differences between genotypes I and Ib here.

In contrast, genotype II (and possibly IV) could be further divided into two or more groups [Fig. S1 in Supporting Information and (Nagano et al. 2013)]. Regarding *mfa1*, two genotypes are currently known: *mfa*\textsuperscript{53} and *mfa*\textsuperscript{70}. However, in 12% of *fimA*-positive specimens, *mfa1* was not detected, suggesting that existence of additional *mfa1* genotypes. Therefore, reclassification of *fimA* and *mfa1* genotypes would be needed in the future.

In this study, we observed that in samples positive for *fimA* genotype II, *mfa*\textsuperscript{70} genotype was detected 1.75 times more frequently compared to *mfa*\textsuperscript{53}, and in the previous study, where we analyzed 84 *P. gingivalis* strains stocked in our laboratory, the frequency of *mfa*\textsuperscript{70} detection among *fimA* II strains was 3.6 times higher than that of *mfa*\textsuperscript{53} [Appendix Table 2 of reference (Nagano et al. 2013)].
al. 2015)]. These findings indicate that mfa70 is the major mfa1 genotype in P. gingivalis strains positive for fimA II. On the other hand, in this study, the two mfa1 genotypes had almost the same detection rate in samples positive for fimA I (including I and Ib), whereas our previous results indicate that mfa70 detection frequency was 2.3 times higher compared to that of mfa53 in fimA-I strains (Nagano et al. 2015). Among fimA IV-positive samples, the detection rate of each mfa1 genotype was the same in this study, and in our previous study, mfa53 and mfa70 genotypes were detected in four and two samples, respectively (Nagano et al. 2015). Taken together, these results suggest that strains with fimA genotypes I and IV tend to have either the same frequency of mfa1 genotypes or slightly higher prevalence of mfa70. Although we found only two genotype III-positive samples in this study, both had mfa53, which was consistent with our earlier findings that 12 out of 13 genotype-III strains carried mfa53 (Nagano et al. 2015). In this study, mfa1 was not detected in genotype V-positive samples, which, however, were all found mfa53-positive in our previous study (Nagano et al. 2015). These results indicate that genotype-III and -V strains almost exclusively carry mfa53. Thus, there is a tendency for correlation between the two fimbrial types in P. gingivalis: fimA II strains preferably carry mfa70, whereas fimA I/IV strains may have both mfa1 genotypes in equal proportions, and fimA III/V strains mostly carry mfa53. However, the reason for such correlations is unknown because there is a wide distance between the two genetic loci. There are polymorphisms in other P. gingivalis genes (Enersen 2011). Thus, the ragA gene, which encodes a major outer membrane protein and is located downstream of the mfa1 gene, exhibits four genetic variants (Hall et al. 2005; Liu et al. 2013); besides, genetic variability has also been reported for capsular antigens (Laine et al. 1996; Laine et al. 1997). In future studies, it will be interesting to find out whether these genetic polymorphisms are correlated with those in the fimA and mfa1 genes.
We did not observe statistically significant associations between clinical parameters of periodontitis and the distribution of fimbrial genotypes. However, there was a time lapse between periodontal examination and sample collection, and it was possible that *P. gingivalis* clones were replaced during that interval; still, the chances for such clonal change may be low because it is reported that *P. gingivalis* showed high clonal stability (Valenza et al. 2009; van Winkelhoff et al. 2008). In addition, we would like to note that most similar studies have the same problems which are inherent to this type of clinical research, because generally the treatment for chronic periodontitis takes a long time. Therefore, it is necessary to develop an appropriate study design for examining the relationship between bacterial genotypes and clinical symptoms of periodontitis.

**Conclusions**

There was a tendency in the distribution of fimbrial genotypes *fimA* (I–V) and *mfa1* (*mfa*<sup>53</sup> and *mfa*<sup>70</sup>) among patients with periodontitis. However, we did not observe any associations between fimbrial genotypes and the severity of the disease.

**Acknowledgements**

We sincerely thank dental doctors in Aichi Gakuin University Dental Hospital for their cooperation in sample collection.
References

Amano A, Nakagawa I, Okahashi N, and Hamada N. 2004. Variations of Porphyromonas gingivalis fimbriae in relation to microbial pathogenesis. *J Periodontal Res* 39:136-142.

Arai M, Hamada N, and Umemoto T. 2000. Purification and characterization of a novel secondary fimbrial protein from *Porphyromonas gingivalis* strain 381. *FEMS Microbiol Lett* 193:75-81.

Dickinson DP, Kubiniec MA, Yoshimura F, and Genco RJ. 1988. Molecular cloning and sequencing of the gene encoding the fimbrial subunit protein of *Bacteroides gingivalis*. *J Bacteriol* 170:1658-1665.

Eick S, Rodel J, Einax JW, and Pfister W. 2002. Interaction of *Porphyromonas gingivalis* with KB cells: comparison of different clinical isolates. *Oral Microbiol Immunol* 17:201-208.

Enersen M. 2011. *Porphyromonas gingivalis*: a clonal pathogen?: Diversities in housekeeping genes and the major fimbriae gene. *J Oral Microbiol* 3.

Enersen M, Nakano K, and Amano A. 2013. *Porphyromonas gingivalis* fimbriae. *J Oral Microbiol* 5.

Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, and Olsen GJ. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 74:2461-2470.
Gibbons RJ, and Macdonald JB. 1960. Hemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. *J Bacteriol* 80:164-170.

Griffen AL, Becker MR, Lyons SR, Moeschberger ML, and Leys EJ. 1998. Prevalence of *Porphyromonas gingivalis* and periodontal health status. *J Clin Microbiol* 36:3239-3242.

Griffen AL, Lyons SR, Becker MR, Moeschberger ML, and Leys EJ. 1999. *Porphyromonas gingivalis* strain variability and periodontitis. *J Clin Microbiol* 37:4028-4033.

Haffajee AD, Cugini MA, Tanner A, Pollack RP, Smith C, Kent RL, Jr., and Socransky SS. 1998. Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. *J Clin Periodontol* 25:346-353.

Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskin MA, McIntosh ML, Alsam A, Kirkwood KL, Lambris JD, Darveau RP, and Curtis MA. 2011. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* 10:497-506.

Hall LM, Fawell SC, Shi X, Faray-Kele MC, Aduse-Opoku J, Whiley RA, and Curtis MA. 2005. Sequence diversity and antigenic variation at the *rag* locus of *Porphyromonas gingivalis*. *Infect Immun* 73:4253-4262.

Hamada N, Sojar HT, Cho MI, and Genco RJ. 1996. Isolation and characterization of a minor fimbria from *Porphyromonas gingivalis*. *Infect Immun* 64:4788-4794.
Hasegawa Y, Nagano K, Ikai R, Izumigawa M, Yoshida Y, Kitai N, Lamont RJ, Murakami Y, and Yoshimura F. 2013. Localization and function of the accessory protein Mfa3 in *Porphyromonas gingivalis* Mfa1 fimbriae. *Mol Oral Microbiol* 28:467-480.

Hospenthal MK, Costa TRD, and Waksman G. 2017. A comprehensive guide to pilus biogenesis in Gram-negative bacteria. *Nat Rev Microbiol* 15:365-379.

How KY, Song KP, and Chan KG. 2016. *Porphyromonas gingivalis*: an overview of periodontopathic pathogen below the gum line. *Front Microbiol* 7:53.

Igboin CO, Griffen AL, and Leys EJ. 2009. *Porphyromonas gingivalis* strain diversity. *J Clin Microbiol* 47:3073-3081.

Inaba H, Nakano K, Kato T, Nomura R, Kawai S, Kuboniwa M, Ishihara K, Ooshima T, and Amano A. 2008. Heterogenic virulence and related factors among clinical isolates of *Porphyromonas gingivalis* with type II fimbriae. *Oral Microbiol Immunol* 23:29-35.

Jouseimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, and Finegold SM. 2002. *Anaerobic Bacteriology Manual* Star Publishing Company.

Kuboniwa M, Inaba H, and Amano A. 2010. Genotyping to distinguish microbial pathogenicity in periodontitis. *Periodontol 2000* 54:136-159.

Laine ML, Appelmelk BJ, and van Winkelhoff AJ. 1996. Novel polysaccharide capsular serotypes in *Porphyromonas gingivalis*. *J Periodontal Res* 31:278-284.
Laine ML, Appelmelk BJ, and van Winkelhoff AJ. 1997. Prevalence and distribution of six capsular serotypes of *Porphyromonas gingivalis* in periodontitis patients. *J Dent Res* 76:1840-1844.

Lamont RJ, and Jenkinson HF. 1998. Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiol Mol Biol Rev* 62:1244-1263.

Lamont RJ, and Jenkinson HF. 2000. Subgingival colonization by *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 15:341-349.

Liu Y, Zhang Y, Wang L, Guo Y, and Xiao S. 2013. Prevalence of *Porphyromonas gingivalis* four *rag* locus genotypes in patients of orthodontic gingivitis and periodontitis. *PLoS One* 8:e61028.

Macdonald JB, and Gibbons RJ. 1962. The relationship of indigenous bacteria to periodontal disease. *J Dent Res* 41:320-326.

Macdonald JB, Gibbons RJ, and Socransky SS. 1960. Bacterial mechanisms in periodontal disease. *Ann N Y Acad Sci* 85:467-478.

Missailidis CG, Umeda JE, Ota-Tsuzuki C, Anzai D, and Mayer MP. 2004. Distribution of *fimA* genotypes of *Porphyromonas gingivalis* in subjects with various periodontal conditions. *Oral Microbiol Immunol* 19:224-229.
Miura M, Hamachi T, Fujise O, and Maeda K. 2005. The prevalence and pathogenic differences of *Porphyromonas gingivalis fimA* genotypes in patients with aggressive periodontitis. *J Periodontal Res* 40:147-152.

Nagano K, Abiko Y, Yoshida Y, and Yoshimura F. 2013. Genetic and antigenic analyses of *Porphyromonas gingivalis* FimA fimbriae. *Mol Oral Microbiol* 28:392-403.

Nagano K, Hasegawa Y, Yoshida Y, and Yoshimura F. 2015. A major fimbrilin variant of Mfa1 fimbriae in *Porphyromonas gingivalis*. *J Dent Res* 94:1143-1148.

Nakagawa I, Amano A, Kuboniwa M, Nakamura T, Kawabata S, and Hamada S. 2002a. Functional differences among FimA variants of *Porphyromonas gingivalis* and their effects on adhesion to and invasion of human epithelial cells. *Infect Immun* 70:277-285.

Nakagawa I, Amano A, Ohara-Nemoto Y, Endoh N, Morisaki I, Kimura S, Kawabata S, and Hamada S. 2002b. Identification of a new variant of *fimA* gene of *Porphyromonas gingivalis* and its distribution in adults and disabled populations with periodontitis. *J Periodontal Res* 37:425-432.

Nakano K, Kuboniwa M, Nakagawa I, Yamamura T, Nomura R, Okahashi N, Ooshima T, and Amano A. 2004. Comparison of inflammatory changes caused by *Porphyromonas gingivalis* with distinct *fimA* genotypes in a mouse abscess model. *Oral Microbiol Immunol* 19:205-209.
Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, and Vissink A. 2008. Periodontal inflamed surface area: quantifying inflammatory burden. *J Clin Periodontol* 35:668-673.

Nesse W, Linde A, Abbas F, Spijkervet FK, Dijkstra PU, de Brabander EC, Gerstenbluth I, and Vissink A. 2009. Dose-response relationship between periodontal inflamed surface area and HbA1c in type 2 diabetics. *J Clin Periodontol* 36:295-300.

Nishiyama S, Murakami Y, Nagata H, Shizukuishi S, Kawagishi I, and Yoshimura F. 2007. Involvement of minor components associated with the FimA fimbriae of *Porphyromonas gingivalis* in adhesive functions. *Microbiology* 153:1916-1925.

Page RC, and Kornman KS. 1997. The pathogenesis of human periodontitis: an introduction. *Periodontol 2000* 14:9-11.

Park Y, Simionato MR, Sekiya K, Murakami Y, James D, Chen W, Hackett M, Yoshimura F, Demuth DR, and Lamont RJ. 2005. Short fimbriae of *Porphyromonas gingivalis* and their role in coadhesion with *Streptococcus gordonii*. *Infect Immun* 73:3983-3989.

Periodontology TJS. 2015. *JSP Clinical Practice Guideline for the Periodontal Treatment, 2015*. Tokyo: Ishiyaku Publishers, Inc.

Socransky SS, and Haffajee AD. 2002. Dental biofilms: difficult therapeutic targets. *Periodontol 2000* 28:12-55.
Socransky SS, Haffajee AD, Cugini MA, Smith C, and Kent RL, Jr. 1998. Microbial complexes in subgingival plaque. *J Clin Periodontol* 25:134-144.

Teanpaisan R, Douglas CW, Eley AR, and Walsh TF. 1996. Clonality of *Porphyromonas gingivalis, Prevotella intermedia* and *Prevotella nigrescens* isolated from periodontally diseased and healthy sites. *J Periodontal Res* 31:423-432.

Tribble GD, Kerr JE, and Wang BY. 2013. Genetic diversity in the oral pathogen *Porphyromonas gingivalis*: molecular mechanisms and biological consequences. *Future Microbiol* 8:607-620.

Umeda JE, Missailidis C, Longo PL, Anzai D, Wikstrom M, and Mayer MP. 2006. Adhesion and invasion to epithelial cells by *fimA* genotypes of *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 21:415-419.

Valenza G, Veihelmann S, Peplies J, Tichy D, Roldan-Pareja Mdel C, Schlagenhauf U, and Vogel U. 2009. Microbial changes in periodontitis successfully treated by mechanical plaque removal and systemic amoxicillin and metronidazole. *Int J Med Microbiol* 299:427-438.

van Winkelhoff AJ, Rijnsburger MC, and van der Velden U. 2008. Clonal stability of *Porphyromonas gingivalis* in untreated periodontitis. *J Clin Periodontol* 35:674-679.
Wang M, Liang S, Hosur KB, Domon H, Yoshimura F, Amano A, and Hajishengallis G. 2009. Differential virulence and innate immune interactions of Type I and II fimbrial genotypes of Porphyromonas gingivalis. Oral Microbiol Immunol 24:478-484.

Ximenez-Fyvie LA, Haffajee AD, and Socransky SS. 2000. Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol 27:648-657.

Yoshimura F, Murakami Y, Nishikawa K, Hasegawa Y, and Kawaminami S. 2009. Surface components of Porphyromonas gingivalis. J Periodontal Res 44:1-12.

Yoshimura F, Takahashi K, Nodasaka Y, and Suzuki T. 1984. Purification and characterization of a novel type of fimbriae from the oral anaerobe Bacteroides gingivalis. J Bacteriol 160:949-957.
Table 1 (on next page)

Genotype distribution of fimbriae-encoding genes *fimA* and *mfa1*
Table 1  Genotype distribution of fimbriae-encoding genes *fimA* and *mfa1*.

|       | *mfaA* | *mfaB* | *Undetermined* | Total (%) |
|-------|--------|--------|----------------|-----------|
|       | *mfaA* | *mfaB* |                |           |
| I     | 4      | 3      | (1)            | 7 (11.1)  |
| Ib    | 8      | 9      | (3)            | 17 (27.0) |
| I + Ib| 12     | 12     | (4)            | 24 (38.1) |
| II    | 12     | 21     | (6)            | 33 (52.4) |
| III   | 2      | 0      | (0)            | 2 (3.2)   |
| IV    | 2      | 2      | (1)            | 4 (6.3)   |
| V     | 0      | 0      | (1)            | 0 (0)     |
| Undetermined | (0) | (3) | - | - |
| Total (%) | 28 (44.4) | 35 (55.6) | - | 63 (100) |

Genes encoding both fimbrial types were determined in 63 of 100 samples. Samples marked “undetermined” were not included in the total numbers.
Table 2 (on next page)

Clinicopathological parameters of 81 study participants
### Table 2  Clinicopathological parameters of 81 study participants.

| fimA  | mfa1 | Females (n) | Males (n) | Age (years) | PD (mm) | BOP (%) | PISA (mm$^2$) | PESA (mm$^2$) |
|-------|------|-------------|-----------|-------------|---------|---------|---------------|---------------|
|       |      | Mean ±     | Max ±     |             |         |         |               |               |
| Untyped | 22  | 9           |           | 60.5 ± 2.5  | 3.22 ± 0.16 | 8.94 ± 0.51 | 44.2 ± 6.7 | 703 ± 135 | 1640 ± 114 |
|        | 53  | 5           | 3         | 51.5 ± 2.91 | 7.88 ± 0.61 | 38.9 ± 10.7 | 462 ± 136 | 1436 ± 168 |
| I + Ib | 70  | 9           | 2         | 59.1 ± 3.73 | 9.40 ± 0.85 | 73.1 ± 13.0 | 1016 ± 244 | 1951 ± 191 |
| II    | 70  | 8           | 10        | 59.6 ± 3.50 | 9.67 ± 1.08 | 49.3 ± 9.2  | 889 ± 155 | 1839 ± 156 |
| III   | 70  | 0           | 0         | -           | -        | -        | -             | -             |
| IV    | 70  | 2           | 0         | 68.0 ± 2.60 | 6.00 ± 2.60 | 52.0 ± 5.6  | 519 ± 193 | 1395 ± 918 |
| V     | 70  | 0           | 0         | -           | -        | -        | -             | -             |