Porphyromonas gingivalis: a clonal pathogen?

Diversities in housekeeping genes and the major fimbriae gene

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The introduction of multilocus sequence typing (MLST) in infectious disease research has allowed standardized typing of bacterial clones. Through multiple markers around the genome, it is possible to determine the sequence type (ST) of bacterial isolates to establish the population structure of a species. For the periodontal pathogen, *Porphyromonas gingivalis*, the MLST scheme has been established at www.pubmlst.org/pgingivalis, and data from the database indicate a high degree of genetic diversity and a weakly clonal population structure comparable with *Neisseria menigitidis*. The major fimbriae (FimA) have been held responsible for the adhesive properties of *P. gingivalis* and represent an important virulence factor. The *fimA* genotyping method (PCR based) indicate that *fimA* genotype II, IV and Ib are associated with diseased sites in periodontitis and tissue specimens from cardiovascular disease. *fimA* genotyping of the isolates in the MLST database supports the association of genotypes II and IV with periodontitis. As a result of multiple positive PCR reactions in the *fimA* genotyping, sequencing of the *fimA* gene revealed only minor nucleotide variation between isolates of the same and different genotypes, suggesting that the method should be redesigned or re-evaluated. Results from several investigations indicate a higher intraindividual heterogeneity of *P. gingivalis* than found earlier. Detection of multiple STs from one site in several patients with refractory periodontitis, showed allelic variation in two housekeeping genes indicating recombination between different clones within the periodontal pocket.

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The development in high throughput sequencing of DNA through the last decades and subsequent full genome sequencing of bacterial species accelerated the possibility for microbiologists to investigate genetic variation of bacterial strains by different typing techniques (1, 2). Three classes of genes can be identified in prokaryotes: informational, core housekeeping, and hypervariable genes. Informational genes are essential for central metabolism and are related to transcription, translational, and regulatory pathways, whereas the core housekeeping genes are ubiquitous and coding for other essential proteins. Both classes evolve at a slow rate and are necessary for survival of the cell. On the contrary, the hypervariable genes encode for surface proteins interacting with the host, and for pathogenic species they may be associated with virulence. This class of genes evolve at an accelerated rate compared to the former due to the necessity for the bacterial cell to adapt to variable host conditions (1). Thus, studies of variation in the two different groups of genes (housekeeping and hypervariable) may reflect different aspects of characterization of pathogenic bacterial strains.

Housekeeping genes

*Multilocus enzyme electrophoresis, population genetic models*

Before nucleotide sequencing was available, genetic variation could be investigated within a bacterial population by multilocus enzyme electrophoresis (MLEE). The technique was based on variation in electrophoretic mobility of the products of several housekeeping genes, where the mobility variants of the enzymes were directly equated with alleles at the corresponding locus. The variants were used to define an allelic profile of each isolate where the acquired electrophoretic types had the
The main advantage of being directly related to the variation at the chromosome.

Large-scale MLEE surveys of many pathogens performed in the 1980s suggested that most bacterial populations were highly clonal (Fig. 1) because the majority of isolates seemed to belong to a small number of clusters of closely related genotypes (3, 4). Furthermore, the repeated recovery of identical isolates from diverse geographic origin, many years apart, was also interpreted as a low rate of homologous recombination in natural bacterial populations, also supporting the clonal population structure paradigm (4). In this model, vertical descent of genetic information from mother to daughter cells occurs, and the changes are only caused by point mutations in the chromosome. Based on the MLEE investigations, the clonal paradigm remained central for the understanding of genetic variation of bacterial populations until the beginning of the 1990s. However, as early as in 1986, results from a MLEE study of isolates of *Neisseria meningitidis* from subjects with systemic disease and healthy carriers gave clear indications of a substantial rate of recombination within isolates of the species (7).

Horizontal exchange of genetic material in prokaryotes can occur through several mechanisms: Transduction refers to horizontal transfer of DNA among bacteria that have the common property of being infected by a specific bacterial virus; transformation refers to bacterial uptake of DNA from the environment, whereas conjugation involves a cell to cell contact-dependent horizontal gene transfer. When recombinational events occur to a large extent with rearrangement of the bacterial chromosome, in addition to the vertical descent of genetic information, the population structure becomes non-clonal, panmictic or free recombining (Fig. 1). In a non-clonal population structure model, genetic variation is randomly assorted and with no evidence of lineage structure and no congruence of gene trees (5). With the accumulation of MLEE data and later the increasing use of nucleotide sequencing, it became evident that genetic variation at housekeeping loci in many bacterial species accumulated as frequently by homologous recombination as by point mutation (3, 5, 8). It also became evident from these data, in particular from those on *N. meningitidis*, that closely related clones, designated clonal complexes, could arise from an underlying recombining background population, defining another term, the epidemic population structure (4–6, 9, 10) (Fig. 1). These data and conclusions made the basis for modern population genetics in prokaryotes.

**Multilocus sequence typing**

The introduction of the multilocus sequence typing (MLST) method in infectious disease research has allowed the identification of bacterial clones in a standardized manner, by making data comparable between laboratories, using high throughput sequencing of fragments of housekeeping genes as determinants. The method derived from MLEE, with use of multiple markers, scattered physically around the genome, requires knowledge of approximately 450 bp fragments of at least seven ubiquitous, core housekeeping genes (1, 11). Gene fragments are amplified by PCR and sequenced for determination of the nucleotide sequence. Thus, the MLST detects allelic variation at multiple housekeeping loci accumulating slowly in the bacterial population. The discriminatory power is achieved by the analysis of the DNA sequence of fragments detecting the degree of genetic diversity within the population of interest. The combination of the different alleles for a selected strain will assign the strain to a certain sequence type (ST) number according to earlier recordings of other STs.

Soon after the introduction of the MLST for *N. meningitidis* (11), other schemes were developed for several prokaryotic (6) and eukaryotic pathogens such as *Candida albicans* (12) and *Candida glabrata* (13).
then MLST schemes have also been created for numerous bacteria, among them *Streptococcus pneumoniae*, *Campylobacter jejuni*, *Streptococcus pyogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Haemophilus influenzae* and *Bacillus cereus* (14–20).

MLST data generated throughout the last decade have contributed to both epidemiologic surveillance and fundamental studies of pathogen biology (2, 8, 21). Thus, MLST is a well-established and good method to detect genetic diversity of bacterial pathogens and well suited to study large strain collections of broad origin for long-term molecular epidemiology. Furthermore, the method has provided numerous insights into epidemiology and population genetics of bacterial populations (2). An overview of the MLST schemes for pathogenic organisms can be accessed at several MLST websites existing on the Internet (http://pubmlst.org/databases.shtml), and MLST databases are hosted at several institutions around the world (the University of Oxford, UK (http://pubmlst.org) (22); Imperial College, London, UK (http://www.mlst.net/databases) (9); the Environmental Resarch Institute, Cork, Ireland, (http://mlst.uc.ie), at the Pasteur Institute, Paris, France (http://www.pasteur.fr/recherche/genopole/PAF/mlst), at the University of Oslo, Oslo, Norway (http://mlstoslo.uio.no), and at The University of Hong Kong.

The largest MLST database, for *Neisseria* spp. (http://pubmlst.org/neisseria), contains at date 8,972 distinct allelic profiles or STs, differentiated among 18,584 isolates.

However, recent advances in technology have further improved DNA sequence capability and increased the discriminatory power of sequence-based data beyond the MLST method, in bacterial populations where levels of nucleotide diversity are too low. These improvements have resulted in the detection of single nucleotide polymorphisms, small insertions, deletions, and other detailed genetic variation (2, 23).

The periodontal pathogen *Porphyromonas gingivalis*

*Porphyromonas gingivalis* is a small black-pigmented anaerobic rod-shaped bacteria, belonging to the oral microbiota. Most commonly, it can be detected in subgingival biofilms in coaggregation with other species, among them *Fusobacterium* spp. and *Streptococcus gordongii* (24, 25). It is one of the main periodontal pathogens involved in the initiation and progression of periodontal diseases and can be detected in patients with various forms of periodontitis (26–30). Furthermore, it belongs to the ‘red complex’ bacteria together with *Treponema denticola* and *Tannerella forsythia* recognized as a microbial indicator of progression of chronic periodontitis (31, 32).

For a long time, it was assumed to be related only to diseased periodontal sites because it was not possible to culture from healthy gingival sulci. However, improved techniques have made detection possible also in healthy sites (33–35) as well as in other parts of the oral cavity (lateral part and dorsum of the tongue), located to niches with growth conditions favoring its special requirements (36).

The species harbors a number of virulence factors, e.g. fimbriae, lipopolysaccharide, collagenase, and cysteine proteinases (gingipains) (27, 28, 37–41) and is capable of invading host tissue cells that may protect it from the host’s immune system. Furthermore, new investigations have also shown in vitro that the organism can survive intracellularly in a nutritionally rich environment (28, 42, 43) and can be transmitted between different types of host cells such as epithelial, endothelial, and smooth muscle cells (43, 44).

Because of the large pathogenic potential of *P. gingivalis*, it is referred to as a molecular vampire (41). In addition to being a major periodontal pathogen, it is suggested to be a mediator in the development of cardiovascular disease by spreading from the periodontal pocket to the blood stream (45–49).

The major fimbriae (FimA)

*P. gingivalis*’ ability to adhere to salivary components, host cells, solid surfaces, and bacterial cells is facilitated by its fimbriae (42, 48–51). These are curly and filamentos appendages arranged peritrichously on the cell surface (50) and are essential for the early establishment of infection by its adhesive properties. The fimbriae have been classified into major and minor types (50–54) and have been extensively studied throughout the last decades. It is constructed from a fimbrillin monomer subunit protein of approximately 43 kDa as the main building block (50), coded from the *fimA* gene. FimA has been held directly responsible for many of the adhesive properties of the organism because of specifically binding to and activation of various host cells (human epithelial, endothelial, spleen, and peripheral blood monocytes), resulting in the release of cytokines and several adhesion molecules (28, 30, 42). The *fimA* gene occurs as a single copy in the chromosome (29, 51, 53, 55), and it has been classified into six types based on variation in nucleotide sequence (genotype I, Ib, II, III, IV, and V) (49, 55–58).

Several clinical studies have indicated that the variation of the *fimA* gene is related to the virulence of different strains of the species. In chronic periodontitis, *P. gingivalis* isolates with *fimA* genotypes II, IV, and Ib are significantly more prevalent than isolates with other genotypes (56–61). Studies concerning the pathogenic potential of distinct genotypes also indicate that genotype II is more prevalent in patients suffering from aggressive periodontitis (62). In contrast, isolates with *fimA* genotype I are most prevalent among *P. gingivalis* positive healthy adults, followed by genotype III and V (28).
Furthermore, it has been demonstrated that recombinant FimA protein corresponding to fimA genotype II has greater ability to adhere to and invade human epithelial cells than the FimA protein corresponding to other genotypes (63). The pathogenic difference between different genotypes has also been verified in animal models supporting the hypothesis of variable virulence because fimA II, Ib, and IV types are causing stronger infectious symptoms and inflammatory changes than strains harboring genotypes I and III (51, 64–66). Furthermore, mutants where the type I gene was substituted with type II showed an enhanced bacterial adhesion/invasion of epithelial cells and vice versa (67). However, a study of adhesion and invasion abilities of different fimA genotypes of P. gingivalis in KB cells (originally derived from epidermal carcinoma of the mouth) did not indicate any significant difference between different genotypes, suggesting that other characteristics besides the variation of the fimA gene also could be responsible for the observations (10, 51, 68). In addition, Inaba et al. (69) reported a heterogenic genotype II strains, indicating that variation in pathogenic potentials and invasive efficiency could be related to gingipain activities and correlated to extracellularly secreted gingipains.

Frequently detected fimA type II and IV clones from cardiovascular specimens of P. gingivalis-positive patients also indicate a possible involvement of these in the initiation and progression of cardiovascular disease (70). Recent results (49) also indicate that fimA II, IV, and Ib genotypes may be involved in the formation of aortic aneurysms, and that the major characteristics of aortic tissue with P. gingivalis infection were smooth muscle cell proliferation in association with intense, local inflammation.

Results from studies of periradicular infections also indicate an association between P. gingivalis-specific fimA genotypic clones (types II, IV, and Ib) in primary endodontic infections as well as chronic, apical periodontitis (71, 72).

**MLST – P. gingivalis**

The genetic diversity among strains of bacteria is a reflection of nucleotide sequence differences in their chromosomal genes. Before 2003, several molecular typing methods (MLEE, random amplified polymorphic DNA fingerprinting, and ribotyping) were used to characterize P. gingivalis strains (73–76). The results of these studies were contradictory when it came to population structure of the species. However, in 2003, MLST was first introduced for the species (77). To test the conclusions from this study which indicated a panmictic population structure of the organism, we investigated 38 isolates from various geographical origins (78). In additional studies (79), the overall results of the MLST analysis represented a total of 84 isolates from human periodontitis with an even broader geographic origin. Due to the limited variation of one selected gene (nah) observed both by Koehler et al. (77) and by Enersen et al. (78), the MLST results presented in Enersen et al. (79) were based on a seven loci scheme without the nah gene. The proposed MLST modification in this paper with exclusion of the nah gene, is an example of how important the selection of the right genes is for keeping the discriminatory power of the method. The modified MLST scheme forms the basis for the MLST website, http://pubmlst.org/pgingivalis, set up in collaboration with Dr. K. Jolly, University of Oxford and professor D. A. Caugant, National Institute of Public Health, Oslo (79).

**Population structure**

The evaluation of a new molecular technique on isolates that have been studied earlier with other techniques is important for assessing the discriminatory power of the new method and for testing the strength of the conclusions made earlier. In this comparison, the conclusion from Enersen et al. (79) is in accordance with earlier studies (73, 74), indicating that genetic exchange through recombination may not be as extensive in P. gingivalis as suggested by other investigators (76, 77).

Several methods can be used in the analysis of data in population genetic studies. The MLST analysis software S.T.A.R.T. 2 (Sequence Type Analysis and Recombinational Tests) is a single package available at the http://www.pubmlst.org (80). The standard MLST statistics, number of polymorphic sites, average G+C contents, allele frequencies, phylogenetic trees and the calculation of pairwise ratios of non-synonymous to synonymous substitutions (dN/dS) can be calculated with the software, as well as the index of association (78–80).

The index of association, I_A, has been used to statistically estimate the degree of association between alleles at different loci using one representative of each ST in a bacterial population (5). Originally, from MLEE data the I_A value for Salmonella species was calculated to be 1, a fully clonal species, while Neisseria gonorrhoeae was estimated to be a panmictic species with I_A close to 0 (5). Index of association can also be used in MLST calculations (5, 22) (http://pubmlst.org/software/analysis/start/manual/index_of_association.shtml).

For the P. gingivalis isolates in the MLST database, I_A is calculated to be 0.3704 (81), indicating a significant linkage disequilibrium pointing to a weakly clonal population structure. The standardized index of association (I_A^* ) (82) (Haubold and Hudson, 2000) which is a result of further development of the simple I_A calculation, takes into account the number of genes investigated in the MLST and gives an even better estimate of linkage equilibrium than the simple I_A value (0.062). Further...
support for a weakly clonal population structure is verified when comparing the corresponding $I_S$ value estimated for the human pathogen *N. meningitidis* (0.14) with the value estimated for *N. gonorrhoeae* (0.005), characterized as a panmictic species undergoing extensive recombination (5).

According to population genetic theory (83), the recovery of strains with identical or similar genotypes sampled in different geographic areas as well as no evidence of intragenic recombination (no mosaic genes) support a significant degree of clonality of the species. It is reasonable to assume that the explanation for the results in our MLST investigations (79) could be the inclusion of a larger strain population with a much broader geographic origin, comparable to the studies by Loos et al. and Menard and Mouton (73, 74), than in the study by Koehler et al. (77).

At the moment, there are 138 STs in the MLST database for *P. gingivalis* including the strains collected from patients with ‘refractory’ periodontitis (79, 81, 84). The UPGMA dendrogram presented in Fig. 2 shows the genetic relationships among all the STs (one representative of each) in the database, and 30 clusters were identified. Though the overall number of isolates of *P. gingivalis* analyzed by MLST still is small compared to databases of other bacterial pathogens, the dendrogram verifies clusters of identical or closely related STs from diverse geographic origins, which may be sampled years apart. This is an observation that further supports the substantial clonality and a low rate of homologous recombination for the species.

Split decomposition analysis (85) is a tool for graphical illustration of whether recombination plays a role in the evolutionary history of the separate genes investigated in the MLST. When utilizing the allelic profiles of the isolates (seven gene fragments) (79) the obtained splitstree graph visualizes the degree of recombination between the isolates in the respective bacterial populations. The algorithm utilized in the analysis creates a tree-like structure where the descent is clonal and bush-like structure when recombination plays a major role in the evolutionary history of the genes investigated. The test can be performed for all isolates with each of the gene fragments individually or for the concatenated allele sequences. Based on the allelic profiles of 138 STs in the MLST database (Fig. 3), this analysis also strongly indicates the clonality of *P. gingivalis* and is in accordance with the results from the other presented methods. However, it is important to emphasize that our results altogether only reflect a small population of *P. gingivalis* strains.

**Clonal heterogeneity within individuals, recombination**

The general acceptance that periodontal patients harbor a single clonal type of *P. gingivalis* (86–88) changed when Loos et al. (86) discovered by DNA fingerprinting that one out of nine patients was infected with two clonal types. Later, similar findings (intraindividual detection of two or three clones) were interpreted as transient coinfections (89, 90) of additional clones. The studies by Enersen et al. (78, 79), through MLST analysis, revealed an even larger clonal heterogeneity within different periodontitis patients. In three Indonesians, two, three and four distinct STs were detected, respectively, from infected sites, and in a Finnish patient two distinct STs were found. However, the sampled sites in these studies were unknown.

The fact that *P. gingivalis* can be detected in diseased periodontal sites and in healthy gingival sulci (33, 64, 86, 87) suggests that it is a species of variable pathogenic potential, and single sites have been hypothesized to be colonized by more virulent clones (49, 51, 87).

The diversity of *P. gingivalis* in single sites was investigated by MLST in patients with ‘refractory’ periodontitis (84) (Fig. 4). The diagnosis represents cases of treatment-resistant disease (91, 92) where a variable number of sites may harbor clones of higher virulence. Furthermore, the knowledge of the complexity of the subgingival microbiota in periodontal disease could (87, 88) allow for the possibility of DNA exchange between different strains in the same periodontal pocket. With increasing number of cells over time in one infected site, such events could represent fitness selection of surviving clones with regard to local growth conditions as well as the hosts immune response, which may contribute to further progression of the disease (88).

The clonal diversity in single sites (84) demonstrated colonization by multiple STs. In each of 15 ‘refractory’ periodontal pockets, it was detected from 8–1 STs (Fig. 4), and these results raise the question of a much larger clonal diversity within a patient than earlier reported. The study (84), in regard to virulence and diagnosis, also shows the presence of two STs from two different subjects that were previously recorded in the MLST isolate database and represented strains sampled from cases of aggressive periodontitis (92) in Germany (ST14 and ST78) (www.pubmlst.org/pgingivalis). These may well correspond to more virulent clones, as is the case with other human pathogens (87).

Genetic variation accumulates in many bacterial species at housekeeping loci as frequently by homologous recombination (replacement of corresponding small chromosomal segments from related strains) as by point mutations (3, 88, 93, 94). Based on many MLST data sets from several species, the eBURST program (www.mlst.net) was developed to explore the diversification of closely related bacterial strains on a short time scale (3, 9, 95).

The analysis by eBURST of the allelic profiles in single periodontal pockets where more than three clones were sampled (84) showed 10 clonal complexes defined by single locus variants (9, 95) (Fig. 4). With the exception
Fig. 2. Genetic relationships among 138 isolates of *P. gingivalis* in the MLST database (www.pubmlst.org/pgingivalis). A distance matrix was calculated by the unweighted pair group method with arithmetic averages (UPGMA) on the basis of the differences in the allelic profiles. Clusters at a genetic distance of 0.1 were designated 1 to 30 at a cutoff level of about 50%. Columns to the right of the tree designate ST number, *fimA* genotyping results (see below), and continents of origin (EU, Europe; NA, North America; AF, Africa; AS, Asia). Multiple colored squares mark isolates with multiple positive *fimA* genotyping reactions. *fimA* genotyping was not performed for the German isolates (see later).
of one subject (7) (see later) (Fig. 4), the variation in each periodontal site was only related to pepO and recA genes. The alleles of these genes at each site showed multiple identical polymorphic sites in non-adjacent nucleotides with only one variable nucleotide between the alleles (unpublished alignments) (84). Because recombination generally occurs and involves larger segments of chromosomal DNA than the fragments analyzed by MLST, it is most likely that the same polymorphic sites in different alleles of the genes actually reflected recombination rather than point mutations occurring within the investigated site. Furthermore, it is unlikely that multiple point mutations would arise in the same polymorphic sites in the different alleles. These findings are in accordance with other studies concluding that recombination seems to occur to a larger extent among bacterial species than previously expected (93–95). By calculation of the average values of \( dN/dS \) which were <1 for both genes (average \( dN/dS \) recA = 0.000, \( dN/dS \) pepO = 0.2924; unpublished data), the results of the variation indicated synonymous substitutions with no change in gene products.

An especially noteworthy observation was detected in one periodontal site. A very distinct clone (ST 109) was observed in a clonal complex together with variants of seven closely related others (Fig. 4). The observation could be explained as an example of a recombinational event in the allele of the affected recA gene of ST 109 giving rise to the other variants. This is supported by Tribble et al. (88) who demonstrated the ability of different \( P. \) gingivalis strains to transfer chromosomal DNA to each other by conjugation, representing an example of the underlying mechanism for allele swapping and the genetic variation of \( P. \) gingivalis (88). Furthermore, Frandsen et al. (76) showed signs of frequent recombination by identifying a random distribution of two virulence-associated mobile genetic elements among their investigated strains.

**fimA gene; genotyping and sequencing**

Because the results of the studies summarized here (79, 81) provided further evidence of a high genetic diversity and a clear indication of some degree of clonality of \( P. \) gingivalis, an eventual association of virulence genes with specific clones could be a valuable supplement to our knowledge of the characteristics of the species. Thus, all strains were investigated by \( fimA \) genotyping.

Eighty two \( P. \) gingivalis isolates were investigated by the \( fimA \) genotyping method, a method used in a number of studies with direct PCR technique on clinical material for detection of prevalence of \( fimA \) genotypes from sites with various periodontal conditions (56–62). The results from the cultured isolates confirmed the results from the clinical investigations that \( fimA \) II and IV genotypes seem to be predominant in chronic periodontitis-affected sites (56, 60, 79).

The association of these genotypes to cardiovascular disease has also been described earlier.

A relatively high prevalence of isolates positive in PCR for multiple \( fimA \) genotypes was observed in the two bacterial populations (25.6 and 30.1%, respectively) (79), similar to the reports from the clinical material in other investigations (56–61). Because the \( fimA \) gene occurs as a single copy in the chromosome (53), detection of multiple \( fimA \) genotypes in the clinical studies has been explained by (1) the presence of several different genotypes colonizing the same site (56, 61), (2) the possible existence of new unidentified genotypes (57, 58, 61), or (3) the existence of \( fimA \) non-typable strains (presence of new genotypes) (56–60). All the investigated isolates in our studies were typable. Because the investigation from cultured isolates resulted in similar findings as in clinical PCR studies, we anticipated that the reason for the multiple positive PCR reactions could be a previously not identified variation within the \( fimA \) gene.

In an attempt to explain these results (\( fimA \) genotyping), a set of universal primers were designed for the purpose of sequencing the whole gene in a selected number of isolates. These isolates were chosen based on the results from the genotyping method, focusing mainly on those with multiple positive PCR reactions to the specific primers for \( fimA \) genotype I, Ib, and II because of the high prevalence of these combinations. The reference strains for genotype \( fimA \) III and V were also selected for sequencing.
The analysis verified a conserved fimA gene with only minor variations among the selected isolates, mainly point mutations. Only one sign of recombination could be detected for an isolate, indicating that a fragment from fimA type III was inserted into the fimA type II gene of another isolate. However, our multiple sequence alignments of the total fimA gene sequences resembled the alignments published by Fujiwara et al. (55), also showing minor variations in single nucleotides of the respective isolates.

The fimA genotyping from the clinical study of single 'refractory' periodontitis sites also revealed some sites harboring isolates reacting to multiple specific PCR primer sets. It is likely that the observations of multiple reactions for a number of investigated strains in both studies can be explained partly by the primer design of the fimA genotyping method and partly by the small variation of the fimA gene between isolates. These results put a question mark to the fimA genotyping method, suggesting either redesign or reevaluation. Furthermore, results from alignment of the different fimA sequences revealed generally a conserved gene with only small variations in certain segments (79, 81).

**fimA genotyping vs. MLST**

The relationship between the distribution of fimA genotypes and MLST data of *P. gingivalis* has not been investigated earlier. The tendency of clustering of the fimA genotypes (79, 81) with isolates of the same or closely related STs harboring the same fimA genotype strengthens our conclusion of a weakly clonal population structure of *P. gingivalis*, but with no strong relationship between fimA genotypes and the STs identified (Fig. 2).

**Conflict of interest and funding**

There is no conflict of interest in this study for the author.

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