Oral Microbiology Immunology 2004: 19: 224–229
Printed in Denmark. All rights reserved

Oral Microbiology and Immunology

Distribution of *fimA* genotypes of *Porphyromonas gingivalis* in subjects with various periodontal conditions

C. G. Missailidis, J. E. Umeda, C. Ota-Tsuzuki, D. Anzai, M. P. A. Mayer
Department of Microbiology, Institute of Biomedical Sciences, Universidade de São Paulo, São Paulo, Brazil

Missailidis CG, Umeda JE, Ota-Tsuzuki C, Anzai D, Mayer MPA. Distribution of fimA *genotypes of* Porphyromonas gingivalis *in subjects with various periodontal conditions. Oral Microbiol Immunol 2004: 19: 224–229.* © Blackwell Munksgaard, 2004.

Fimbria encoded by the gene *fimA* is considered one of the main factors in the colonization of the oral cavity by *Porphyromonas gingivalis*. Allelic variation in *fimA* led to the classification of strains of *P. gingivalis* into six genotypes. The occurrence of *P. gingivalis* was determined by polymerase chain reaction using 16S rRNA primers in 302 subgingival samples obtained from 102 Brazilian subjects exhibiting different periodontal conditions. Distribution of *fimA* genotypes was assessed in 146 *P. gingivalis* positive samples by polymerase chain reaction using primers pairs homologous to the different *fimA* genes. *P. gingivalis* was detected in 51 of 57 (89.4%) patients with periodontal attachment loss, in six of 20 gingivitis patients (30.0%) and in two of 25 (8.0%) subjects with a healthy periodontium. Variant type II was the only type detected in 53 sites (39.3%), distributed among 19 periodontitis patients (37.3%) and in one patient with no periodontal destruction. Type Ib was the second most prevalent genotype in periodontitis patients (19.6%). Genotype V was not detected in the studied population. Type IV was the most commonly type found among gingivitis patients, either alone or in combination with other genotypes. Multiple genotypes were detected in nine sites (6.1%). A *fimA* genotype was not identified in 26 sites (17.8%) of 146 sites positive for *P. gingivalis,* suggesting that other alleles of *fimA* not yet sequenced may be prevalent in this population. These data demonstrated that *P. gingivalis* type II strains followed by type Ib are more prevalent in periodontitis patients from a multiracial population in Brazil, suggesting an increased pathogenic potential of these types.

Key words: fimbria; genotyping; *Porphyromonas gingivalis*; periodontitis

Marcia P. A. Mayer, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Avenida Lineu Prestes 1374, São Paulo, SP, 05508–900, Brazil
E-mail: mpamayer@icb.usp.br
Accepted for publication January 29, 2004

Porphyromonas gingivalis is a gram-negative strict anaerobic coccobacillus that expresses several virulence factors related to the colonization of oral sites, damage of the periodontal tissues, and evasion of the host response (15). The microorganism is strongly associated with the etiology of periodontitis in cross-sectional (28) and longitudinal studies (25).

P. gingivalis exhibits genotypic and phenotypic diversity, which may result in differences in the ability of individual clones to induce periodontal destruction (3, 11, 25). Most subjects are colonized by only one clonal type of *P. gingivalis* (6, 15), although the colonization by multiple clones has been reported (1, 2, 10, 11, 32).

P. gingivalis colonizes the oral cavity by adhering to host cells, such as epithelial cells and fibroblasts, matrix compounds as collagen and fibronectin, SHA (saliva-coated hydroxyapatite) and other organisms such as *Streptococcus sanguis*, *Actinomyces viscosus* and *Fusobacterium nucleatum* (29, 30) mediated by adhesins and proteases (14). Salivary receptors such as proline-rich proteins, glycoproteins, and stratherin interact with *P. gingivalis* fimbria, allowing the adhesion to solid surfaces (4).

P. gingivalis presents a main fimbria formed by subunits of 41 kDa, encoded by the chromosomal gene *fimA* (9). Another fimbria, formed by subunits of 67 kDa, morphologically, immunologically, and genetically distinct from the former, may

also be present (12). Mutations in *fimA* resulted in decreased adhesion to SHA, epithelial cells, and fibroblasts (13), and the mutant exhibited a reduced ability to induce periodontal bone loss in gnotobiotic rats (19).

Allelic variation in *fimA* among strains of *P. gingivalis* resulted in diversity in fimbrillin related to size and amino acid sequence at the N-terminus of the protein (9). Based on the differences in *fimA*, five genotypes of *P. gingivalis* had been described (I–V) (2, 21). A sixth variant, highly homologous to variant type I, was recently described and designated as type Ib (23).

Several studies analyzing the diversity of *P. gingivalis* reported that most isolates exhibited a unique profile, suggesting that this organism is essentially an opportunistic pathogen (15, 24).

On the other hand, the analysis of specific genes suggested that certain clones might exhibit a higher pathogenic potential than others (8). Studies concerning the pathogenic potential of distinct *fimA* genotypes suggested that *fimA* genotype II strains could be more virulent than others. The association between *fimA* genotype II strains with severe periodontitis was observed in a very homogeneous population in Japan (1, 2) and among 15 periodontitis patients in Germany (10). Type I was the second most prevalent genotype among German patients and type IV the second most prevalent type among Japanese, suggesting differences in frequency of *fimA* alleles according to the geographic location or ethnicity origin. Type Ib was also associated with periodontitis in Japanese patients, mainly among mentally disabled subjects (23).

This study aimed to detect *P. gingivalis* in subgingival samples obtained from subjects with different periodontal conditions from a multiracial population from Brazil, and to determine the genotype based on *fimA* allelic variation. The detection of *P. gingivalis* was performed by polymerase chain reaction (PCR) using species-specific 16S rRNA primers in 302 subgingival samples obtained from 102 subjects. The *P. gingivalis* positive samples were then submitted to genotyping using primers pairs specific to each *fimA* type (2, 21).

Material and methods

Subgingival samples were obtained from 102 subjects, 41 men and 61 women, aged 14–75 years old, receiving dental treatment at the Dental School, University of São Paulo and University Camilo Castelo Branco, both situated in São Paulo, SP, Brazil. Prior to sample collection, the patients were requested to sign an informed consent to participate in the study and to answer questions concerning smoking habits, antibiotics usage, and previous periodontal treatment. The Ethical Committee of the Biomedical Sciences Institute approved this study. All patients were clinically examined to register pocket probing depth and bleeding upon probing. Subjects reporting usage of antibiotics, periodontal treatment in the previous 6 months or diseases associated with periodontitis such as diabetes or AIDS were excluded from the analysis.

The patients were divided into two groups according to clinical and radiographic characteristics. Group I comprised 45 subjects with no loss of attachment, 25 exhibiting a healthy periodontium (IA), and 20 diagnosed as gingivitis patients, presenting clinical evidence of gingival inflammation and bleeding on probing in more than 30.0% of the sites (IB). Group II comprised 57 patients diagnosed as having periodontitis, exhibiting periodontal sites with attachment loss and alveolar bone destruction. Among the periodontitis patients, 15 were classified as having aggressive and 42 as having chronic periodontitis. From each of 102 patients, three subgingival sites were sampled, for a total of 302 sites. Group IA sites exhibited no bleeding on probing. All sampled sites in group IB exhibited bleeding on probing. Group II sites exhibited bleeding on probing, bone destruction, loss of attachment and pocket depth of at least 4 mm, and the three deepest pocket depth sites were selected for sampling. Four samples were excluded from the analysis, two from a gingivitis patient (group IB) and two from a periodontitis patient (group II) due to loss during manipulation.

After removal of supragingival plaque with a sterile curette, the subgingival plaque was collected by inserting three paper points into the deepest portion of the pocket/sulcus for 10 s. The paper points were immediately transferred to the transport media VMGA III (20) and sent to the Oral Microbiology Laboratory, Biomedical Science Institute, University of São Paulo. The samples were kept at 37°C for 15 min, homogenized and aliquots of 100 μl were transferred to tubes containing 300 μl of sterile MilliQ water. Cells were lysed and DNA was extracted by immersing the tubes in boiling water for 10 min (31).

Detection of *P. gingivalis* was performed by PCR using primers homologous to *16S rRNA* gene, described in Table 1. DNA from the strain *P. gingivalis* ATCC 33277 was used as positive control. A negative control with no added template DNA was performed in every group of reactions. Amplification by PCR was performed in the Gene Amp PCR system 2400 (Perkin Elmer Corporation, Foster, CA). The 25 μl PCR reaction consisted of 1X buffer, 0.5 U of Tet DNA polymerase (Ultratools DNA Polymerase, Biotools B & M Laboratories, Madrid, Spain), 200 μM of dNTP (Invitrogen, São Paulo, Brazil), 50 pmol of each oligonucleotide primer, and 5 μl of template DNA. $MgCl_2$ concentration was adjusted to 3 mM. The PCR conditions included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, with a final step of 72°C for 7 min (2). The products obtained from each reaction were resolved by electrophoresis in 1.5% agarose gels in Tris-acetate-EDTA buffer (TAE). Digital images of the ethidium bromide stained gels were obtained with the Photo PC 3100Z (Epson, Hemel-Hempstead, UK).

Table 1. PCR primers

Analysis	Primers	Sequence	Product (bp)
P. gingivalis 16S rDNA	Pg16SrDNA F	5′TGT AGA TGA CTG ATG TGT AAA ACC 3′	197
	Pg16SrDNA R	5′ACG TCA TCC CCA CCT TCC TC 3′	
Type I *fimA*	*FimA* I F	5′CTG TGT GTT TAT GGC AAA CTT C 3′	
	FimA R	5′AAC CCC GCT CCC TGT ATT CCG A 3′	392
Type Ib *fimA*	*FimA* Ib F	5′CAG CAG AGC CAA AAA CAA TCG 3′	271
	FimA Ib R	5′TGT CAG ATA ATT AGC GTC TGC 3′	
Type II *fimA*	*FimA* II F	5′ACA ACT ATA CTT ATG ACA ATG G 3′	
	FimA R	5′AAC CCC GCT CCC TGT ATT CCG A 3′	257
Type III *fimA*	*FimA* III F	5′ATT ACA CCT ACA CAG GTG AGG C 3′	
	FimA R	5′AAC CCC GCT CCC TGT ATT CCG A 3′	247
Type IV *fimA*	*FimA* IV F	5′CTA TTC AGG TGC TAT TAC CCA A 3′	
	FimA R	5′AAC CCC GCT CCC TGT ATT CCG A 3′	251
Type V *fimA*	*FimA* V F	5′ AAC AAC AGT CTC CTT GAC AGT G 3′	
	FimAV R	5′TAT TGG GGG TCG AAC GTT ACT GTC 3′	462

PCR primers homologous to each *fimA* variant (Table 1) were used for genotyping (1, 2). DNA from *P. gingivalis* strains ATCC 33277, HW24D-1, 6/26, HG564, and HNA99, corresponding to *fimA* types I, II, III, IV, and V, respectively, generously provided by Dr. Atsuo Amano (Faculty of Dentistry, Osaka University, Japan), were used as positive controls. The composition and conditions for DNA amplification were the same as described above.

Periodontitis samples (n = 34) in which types I and II were detected simultaneously and one sample in which types I, II, and III were detected simultaneously were submitted to another amplification reaction using type Ib primer pairs. After amplification, the 271 bp amplicons were digested with *Rsa*I and electrophoresed. The samples in which the restricted amplicons resulted in two fragments of 162 bp and 109 bp were considered type Ib (23). One own clinical isolate of *P. gingivalis* type Ib [strain 315B (a)] was used as positive control.

To avoid contamination, the establishment of the reactions and product analysis were performed in separate rooms. Negative and positive controls were included in every experiment. The sensitivity of detection of *P. gingivalis* was estimated by performing the amplification reactions using template DNA extracted from VMGA III transport medium containing decreasing amounts of cells of *P. gingivalis*.

The Chi-squared test was used to compare the frequencies of occurrence of *P. gingivalis* in patients in each disease category. The detection of the bacteria was also compared according to type of periodontitis, race, gender, smoking habits and pocket depth. *P*-values < 0.05 were considered significant. The frequencies of *fimA* alleles according to group allocation of sites and patients were also compared by Chi-squared test.

Results

The prevalence of *P. gingivalis* was analyzed in 302 subgingival samples obtained from 102 subjects, 41 males and 61 females, with different periodontal conditions. PCR for detection of *P. gingivalis* was able to produce an amplicon using template DNA obtained from as little as 3.7 *P. gingivalis* cells. Due to the dilution steps, only samples presenting values equal to or greater than 9×10^3 colony-forming units (CFU) of *P. gingivalis* were considered positive for the presence of the bacterium.

Table 2. Detection of *P. gingivalis* by PCR in 302 sites according to periodontal condition

Periodontal condition	*P. gingivalis* negative n (%)	*P. gingivalis* positive n (%)	No. of sites n (%)
Healthy	72 (96.0)	3 (4.0)	75 (24.8)
Gingivitis	50 (86.2)	8 (13.8)	58 (19.2)
Periodontitis	34 (20.1)	135 (79.9)	169 (56.0)
Total	156 (51.7)	146 (48.3)	302 (100.0)

$\chi^2 = 154.16$. $P = 0.000$.

Table 3. Detection of *P. gingivalis* in 102 patients with different periodontal conditions

	Frequency of occurrence (%)			
	Healthy subjects	Gingivitis subjects	Periodontitis subjects	
			Chronic	Aggressive
P. gingivalis negative	23 (92.0)	14 (70.0)	4 (9.5)	2 (13.3)
P. gingivalis positive	2 (8.0)	6 (30.0)	38 (90.5)	13 (86.7)
Total	25	20	42	15

$\chi^2 = 55.32$. $P = 0.000$.

P. gingivalis was detected in 146 (48.3%) sites. The distribution of these sites is shown in Table 2. *P. gingivalis* was detected in only 11 of 133 sites with no periodontal breakdown (8.3%), and in 135 of 169 sites with periodontitis (79.9%), 75.6% (59 of 78 sites) sites with shallow pockets (4–6 mm depth), 86.6% sites exhibiting pocket depths 7–9 mm (58 of 67 sites) and 75% of deep pockets (> 10 mm) (18 of 24 sites) harbored *P. gingivalis*. No statistically significant differences in *P. gingivalis* detection were observed according to periodontal pocket depth among sites with periodontitis.

P. gingivalis was detected in 59 (57.8%) among 102 subjects with different periodontal conditions (Table 3). Chi-squared test revealed significant differences in *P. gingivalis* detection among the groups ($P < 0.05$). However, no statistically significant differences in *P. gingivalis* detection were observed between chronic and aggressive periodontitis patients. Among 31 women with periodontitis, *P. gingivalis* was detected in 28 (90.3%), whereas the organism was detected in 23 of 26 (88.4%) men with periodontitis. *P. gingivalis* was detected in 3 of 15 men (20.0%) and in 5 of 30 women (16.6%) with no periodontal destruction. No differences in *P. gingivalis* occurrence according to gender were detected. The bacteria were detected in 17 of 21 subjects reporting smoking habits. However, smoking could be a confounding factor, since 15 of these *P. gingivalis* positive subjects were diagnosed as periodontitis patients. Five of 16 Caucasian gingivitis patients (31.2%) and one of four gingivitis patients of African origin (25.0%) presented the bacterium. Among periodontitis patients, *P. gingivalis* was detected in 35 out of 40 Caucasian (87.5%), and in 16 out of 17 subjects with African backgrounds (94.1%). No differences between *P. gingivalis* detection between Caucasians and African-Brazilian subjects were found.

The sites that were positive for *P. gingivalis* were screened to determinate the genotype based on *fimA* allelic variations. A single band with the expected size was obtained in every reaction, as shown in Fig. 1 for the positive control reactions. Genotypic analysis was performed by PCR

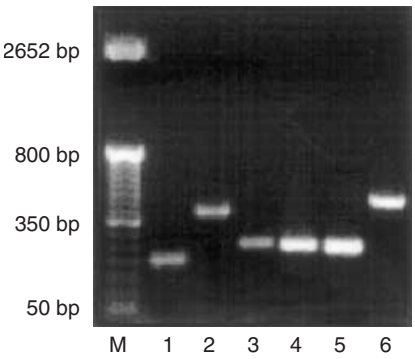

Fig. 1. Amplicons after electrophoresis on a 1.5% agarose gel. **1**: PCR product using *16SrRNA P. gingivalis* primers (197 bp) and DNA from strain *P. gingivalis* ATCC 33277. **2–6**: PCR products using primers homologous to gene *fimA* I, II. III, IV and V and DNA from strains ATCC 33277 (392 bp), HW24D-1 (257 bp), 6/26 (247 bp), HG564 (251 bp), HNA99 (462 bp), respectively. M, molecular weight marker (50 bp DNA Ladder, Gibco/Life Technologies, Carlsbad, CA).

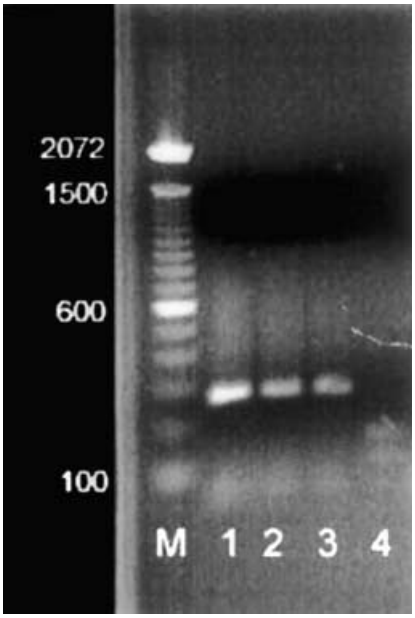

Fig. 2. Detection of type Ib *fimA* by PCR amplification and *Rsa*I digestion. The *fimA* gene fragments were successfully amplified both from *P. gingivalis* ATCC 33277 (type I *fimA*) in lane 1 and 315B (a) (type Ib *fimA*), in lane 2, using *fimA* type Ib primers. The PCR products of type I and type Ib *fimA* (271 bp) were subjected to *Rsa*I digestion, as show in lanes 3 and 4, respectively. The type Ib *fimA* gene fragment was digested with *Rsa*I, resulting in 162 bp and 109 bp fragments. M, molecular weight marker (100 bp DNA Ladder, Gibco/ Life Technologies, Carlsbad, CA).

using five primers pairs specific for each allelic variant of *fimA*. In order to detect type Ib, samples giving products after amplification with primers specific to both types I and II were screened for the detection of type Ib by amplification and digestion of the amplicons. Those amplicons exhibiting an *Rsa*I site as shown in Fig. 2 were considered as type Ib.

The distribution of *fimA* genotypes of *P. gingivalis* in 146 subgingival samples positive for *P. gingivalis* is shown in Table 4. All *fimA* allelic variants, except for type V were detected in the studied population. A single *fimA* variant was detected in 111 sites (76.1%), whereas two or more *fimA* variants of *P. gingivalis* were detected in 9 sites (6.1%). A specific *fimA* genotype could not be determined in 26 sites positive for *P. gingivalis* (17.8%).

The distribution of *fimA* genotypes in 59 subjects carrying *P. gingivalis* is shown in Table 5. A specific genotype could not be found in 17.0% of subjects, whereas a single genotype could be detected in 66.0% of the subjects. The occurrence rate of each *fimA* genotype was calculated as follows:

Table 4. Detection of types of *P. gingivalis* based on allelic variation of *fimA* gene in 146 sites with different periodontal conditions which *P. gingivalis* was detected

Allelic variant	No. of sites (%)	Periodontal condition of sampled site		
		Healthy (%)	Gingivitis (%)	Periodontitis* (%)
FimA I	6 (4.1)	0	0	6 (4.4)
FimA II	54 (37.0)	1 (33.3)	0	53 (39.3)
FimA Ib	33 (22.6)	0	0	33 (24.4)
FimA III	9 (6.2)	0	0	9 (6.7)
FimA IV	9 (6.2)	0	2 (25.0)	7 (5.2)
FimA V	0	0	0	0
subtotal	111 (76.1),	1 (33.3)	2 (25.0)	108 (80)
FimA I/II	1 (0.7)	0	0	1 (0.7)
FimA II/III	3 (2.0)	0	0	3 (2.3)
FimA I/II/III	1 (0.7)	0	0	1 (0.7)
FimA I/IV	3 (2.0)	0	3 (37.5)	0
FimA II/IV	1 (0.7)	0	1 (12.5)	0
subtotal	9 (6.1)	0	4 (50)	5 (3.7)
Untypable	26 (17.8)	2 (66.7)	2 (25.0)	22 (16.3)
Total	146 (100.0)	3 (100.0)	8 (100.0)	135 (100.0)

*$\chi^2 = 260.84$. $P = 0.000$.

- patients with no attachment loss (n = 8): type I in 25.0%, type Ib in 0%, type II in 25.0%, type III in 0%, type IV in 50.0%.
- periodontitis patients (n = 51): type I in 11.7%, type Ib in 27.4%, type II in 47.0%, type III in 11.7%, type IV in 5.8%.

Since multiple genotypes were found in some of the patients, the total percentage number exceeds 100%. Statistically significant differences in the prevalence of *fimA* allelic variants were found among sites and patients exhibiting periodontitis ($P < 0.05$). This analysis was not possible in subjects without periodontal breakdown due to the low percentage of subjects harboring *P. gingivalis* in this group.

Discussion

The present data confirm the relation between *P. gingivalis* and periodontal breakdown in the Brazilian population. The detection of *P. gingivalis* was performed by amplification of the *16S rRNA* gene because this method has a higher sensitivity and specificity than other techniques such as culture (26) and use of DNA probes (27). It could be speculated that the culture negative/PCR positive sites detected in several studies (5, 26) would harbor levels of the bacteria too low to induce periodontal destruction. Thus, the sensitivity of the present method was estimated. The PCR was able to detect only 3.7 cells, and due to the dilution of

Table 5. Detection of types of *P. gingivalis* based on allelic variation of *fimA* gene in 59 subjects with different periodontal conditions carrying *P. gingivalis*

Allelic variant	No. of subjects n (%)	Periodontal condition of the subject	
		Healthy and gingivitis n (%)	Periodontitis* n (%)
FimA I	1 (1.7)	0 (0.0)	1 (1.9)
FimA Ib	10 (17.0)	0 (0.0)	10 (19.6)
FimA II	20 (33.9)	1 (12.5)	19 (37.3)
FimA III	3 (5.0)	0 (0.0)	3 (5.9)
FimA IV	5 (8.4)	2 (25.0)	3 (5.9)
FimA V	0 (0.0)	0 (0.0)	0 (0.0)
Subtotal	39 (66.0)	3 (37.5)	36 (70.6)
FimA I/Ib	3 (5.0)	0 (0.0)	3 (6.0)
FimA I/II	1 (1.7)	0 (0.0)	1 (1.9)
FimA I/IV	1 (1.7)	1 (12.5)	0 (0.0)
FimA Ib/II	1 (1.7)	0 (0.0)	1 (1.9)
FimA II/III	2 (3.4)	0 (0.0)	2 (4.0)
FimA I/II/III	1 (1.7)	0 (0.0)	1 (1.9)
FimA I/II/IV	1 (1.7)	1 (12.5)	0 (0.0)
Subtotal	10 (17.0)	2 (25.0)	8 (15.7)
Untypable	10 (17.0)	3 (37.5)	7 (13.7)
Total	59 (100.0)	8 (100.0)	51 (100.0)

*$\chi^2 = 95.76$. $P = 0.000$.

the sample in transport media, the samples positive for *P. gingivalis* should have at least 9×10^3 CFU *P. gingivalis*, indicating that only those sites exhibiting high levels of the bacteria were considered positive.

In the present study, *P. gingivalis* was detected in 89.4% of patients with periodontitis, similar to data obtained in Japan (1) and Chile (18), but at higher prevalence than reported in Scotland (26). *P. gingivalis* was found in only 17.7% patients with no periodontal breakdown, similar to others who reported a prevalence of *P. gingivalis* detected by PCR methods ranging from 10% to 36.3% in patients with no loss of attachment (1, 5). The variability in prevalence may be due to methodologic variation, such as dilution of the samples and amplification of other regions besides *16S rRNA* (26), but could also be attributed to ethnicity or geographic location of the studied population (11, 18).

The diversity among *P. gingivalis* strains, based either on phenotypic characteristics or on genotyping, was shown by several studies (17, 32), and this diversity may reflect the virulence potential of different clones.

Two or more allelic variants of *P. gingivalis* in the same site were detected in only 6.1% of the samples positive for *P. gingivalis* reported here, and in 17% of subjects, indicating a monoclonal infection by *P. gingivalis* in the majority of sites and subjects, as shown by others (7, 17). The high percentage of sites infected by more than one clonal type shown in other studies analyzing *fimA* variants may be due to homology with more than one primer pair or probe by the same *fimA* type (32). Since *fimA* is found in a single copy in the chromosome of *P. gingivalis* (9). Analyzing clinical isolates from German patients, Eick et al. (10) reported that DNA from six of 15 strains of *P. gingivalis* exhibited more than one *fimA* genotype, most of them exhibiting amplicons for types I and II, indicating that these strains were probably type Ib.

We found that the allelic variant type II was more prevalent in sites from periodontitis patients, followed by the variant Ib, whereas type IV was only found in 5.2% of periodontitis sites. Among 11 sites with no loss of attachment in which *P. gingivalis* was detected, variant type IV was detected in six, either alone or associated with other types (Table 4).

Studies performed in Japan (2, 21) and Germany (10) also revealed that type II strains of *P. gingivalis* predominate in periodontitis patients. However, in the Japanese population, there was a similar frequency of types Ib and IV among periodontitis patients (1), whereas in the Brazilian patients the prevalence of type Ib was much higher than type IV. Genotype IV was also less prevalent than type I/II (probably type Ib) in the study reported by Eick et al. (10), analyzing 15 clinical isolates from German patients. The higher prevalence of genotype II presented in these studies, including ours, contrasts with the data obtained by Griffen et al. (11) analyzing strains of *P. gingivalis* isolated in Ohio, USA, by interspace ribosomal polymorphism. These authors demonstrated that type hW83, corresponding to *fimA* type IV strains, was more frequently associated with periodontitis than the others. However, distinct regions of the chromosome were analyzed by the two methods, and there are no studies determining the relation between ISR types and *fimA* variants.

In the studied Brazilian population, variant type IV was associated with sites exhibiting no loss of attachment in which *P. gingivalis* was detected. Type I was detected among healthy subjects only associated with type IV, differing from the high prevalence of *fimA* type I in *P. gingivalis* positive sites in these subjects reported in Japan (1).

No specific *fimA* genotype was detected in 17.8% sites positive for *P. gingivalis*, or in 17.0% subjects carrying *P. gingivalis*. This percentage is higher than the 5.1% reported in Japan (1), suggesting that other *fimA* types may be present in the studied population, a hypothesis that is currently under investigation.

Adherence is an essential step for microbial colonization (12) and allelic variation in *fimA* may reflect differences in function and immunogenicity of *P. gingivalis* fimbria (17). Other virulence-related factors might also correlate with the *fimA* genotype, such as increased BAPNA activity in type II strains, higher hemagglutination ability in type IV strains and higher elastolytic activity in type I (10). The virulence potential of type II strains was also revealed by an increased ability to adhere to Hep-2 cells than the other genotypes, although the ability to adhere to human gingival fibroblasts was similar (22). Despite the high prevalence of *fimA* genotype II strains among periodontitis patients, almost all characterization studies of *P. gingivalis* fimbriae and invasion assays have been performed with type I (14, 16). Furthermore, it was reported that only fimbrillin type II was internalized in epithelial cells and accumulated around the nucleus, differing from the other types (22).

Genotype II is the most prevalent among periodontitis patients either from a homogeneous population as in Japan or from a multiracial group in Brazil. Although studies characterizing virulence factors and population structure of *P. gingivalis* belonging to different *fimA* types are still needed, the close association between *P. gingivalis fimA* type II and periodontitis suggests that the pathogenic potential of this variant is higher than others, and may help explain differing clinical outcomes of periodontal *P. gingivalis* infections.

Acknowledgments

This study was supported by FAPESP Grant 00/10112–0. We also thank Dr. Francisco Pustiglione, and Dr. Elisa Russo, Dental School, University of São Paulo, and Dr. Eduardo Saba Chuffji, Dental School, University Camilo Castelo Branco. We also thank C. Rosana Prisco for statistical analysis.

References

1. Amano A, Kuboniwa M, Nakagawa I, Akiyama S, Morisaki I, Hamada S. Prevalence of specific genotypes of *Porphyromonas gingivalis fim*A and periodontal health status. J Dent Res 2000: **79**: 1664–1668.
2. Amano A, Nakagawa I, Kataoka K, Morisaki I, Hamada SI. Distribution of *Porphyromonas gingivalis* strains with *fimA* genotypes in periodontitis patients. J Clin Microbiol 1999: **37**: 1426–1430.
3. Amano A, Premaraj T, Kuboniwa M, et al. Altered antigenicity in periodontitis patients and decreased adhesion of *Porphyromonas gingivalis* by environmental temperature stress. Oral Microbiol Immunol 2001: **16**: 124–128.
4. Amano A, Sharma A, Lee J-Y, Sojar HT, Raj PA, Genco RJ. Structural domains of *Porphyromonas gingivalis* recombinant fimbrillin that mediate binding to salivary proline-rich protein and statherin. Infect Immun 1996: **64**: 1631–1637.
5. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 1996: **11**: 266–273.
6. Asikainen S, Chen C, Alaluusa S, Slots J. Can one acquire periodontal bacteria and periodontitis from a family member? J Am Dent Assoc 1997: **128**: 1263–1271.
7. Asikainen S, Chen C, Slots J. Likelihood of transmitting *Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis* in families with periodontitis. Oral Microbiol Immunol 1996: **11**: 387–394.
8. Curtis MA, Hanley SA, Aduse-Opoku J. The *rag* locus of *Porphyromonas gingivalis*: a novel pathogenicity island. J Periodontal Res 1999: **34**: 400–405.

9. Dickinson DP, Kubiniec MA, Yoshimura F, Genco RJ. Molecular cloning and sequencing of the gene encoding the fimbrial subunit protein of *Bacteroides gingivalis*. J Bacteriol 1988: **170**: 1658–1665.
10. Eick S, Rödel J, Einax JW, Pfister W. Interaction of *Porphyromonas gingivalis* with KB cells: comparison of different clinical isolates. Oral Microbiol Immunol 2002: **17**: 201–208.
11. Griffen AL, Lyons SR, Becker MR, Moeschberger ML, Leys EJ. *Porphyromonas gingivalis* strain variability and periodontitis. J Clin Microbiol 1999: **37**: 4028–4033.
12. Hamada N, Watanabe K, Arai M, Hiramine H, Umemoto T. Cytokine production induced by a 67-kDa fimbrial protein from *Porphyromonas gingivalis*. Oral Microbiol Immunol 2002: **17**: 197–200.
13. Hamada N, Watanabe K, Sasakawa C, Yoshikawa M, Yoshimura F, Umemoto T. Construction and characterization of a *fimA* mutant of *Porphyromonas gingivalis*. Infect Immun 1994: **62**: 1696–1704.
14. Hamada S, Amano A, Kimura S, Nagakawa I, Kawabata S, Morisaki I. The importance of fimbriae in the virulence and etiology of some oral bacteria. Oral Microbiol Immunol 1998: **13**: 129–138.
15. Lamont RJ, Jenkinson HF. Subgingival colonization by *Porphyromonas gingivalis*. Oral Microbiol Immunol 2000: **15**: 341–349.
16. Lamont RJ, Yilmaz Ö. In or out: the invasiveness of oral bacteria. Periodontology 2000 2003: **30**: 61–69.
17. Loos BG, Dyer DW. Restriction fragment length polymorphism analysis of the fimbrillin locus, *fimA* of *Porphyromonas gingivalis*. J Dent Res 1992: **71**: 1173–1181.
18. Lopez NJ. Occurrence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in progressive adult periodontitis. J Periodontol 2000: **71**: 948–954.
19. Malek R, Fisher JG, Caleca A, et al. Inactivation of the *Porphyromonas gingivalis fimA* gene blocks periodontal damage in gnotobiotic rats. J Bacteriol 1994: **176**: 1052–1059.
20. Möller AJR. Microbiological examination of root canals and periapical tissues of human teeth. Odontol Tidskr 1966: **74** (Suppl): 380.
21. Nakagawa I, Amano A, Kimura RK, Nakamura T, Kawabata S, Hamada S. Distribution and molecular characterization of *Porphyromonas gingivalis* carrying a new type of *fim*A gene. J Clin Microbiol 2000: **38**: 1909–1914.
22. Nakagawa I, Amano A, Kuboniwa M, Nakamura T, Kawabata S, Hamada S. Functional differences among *fimA* variants of *Porphyromonas gingivalis* and their effects on adhesion to and invasion of human epithelial cells. Infect Immun 2002: **70**: 277–285.
23. Nagakawa I, Amano A, Ohara-Nemoto Y, et al. Identification of a new variant of *fimA* gene of *Porphyromonas gingivalis* and its distribution in adults and disabled populations with periodontitis. J Periodontal Res 2002: **37**: 425–432.
24. Özmeriç N, Preus HR, Olsen I. Genetic diversity of *Porphyromonas gingivalis* and its possible importance to pathogenicity. Acta Odontol Scand 2000: **58**: 183–187.
25. Rams TE, Listgarten MA, Slots J. Utility of 5 major putative periodontal pathogens and selected clinical parameters to predict periodontal breakdown in patients on maintenance care. J Clin Periodontol 1996: **23**: 346–354.
26. Riggio MP, MacFarlane TW, MacKenzie D, Lennon A, Smith AJ, Kinane D. Comparison of polymerase chain reaction and culture methods for detection of *Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis* in subgingival plaques samples. J Periodontal Res 1996: **31**: 496–501.
27. Siqueira JF, Rôças IN, Uzeda M, Colombo AP, Santos KRN. Comparison of 16S rDNA-based PCR and checkerboard DNA-DNA hybridization for detection of selected endodontic pathogens. J Med Microbiol 2002: **51**: 1090–1096.
28. Slots J. Update on *Actinobacillus Actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease. J Int Acad Periodontol 1999: **1**: 121–126.
29. Stinson MW, Haraszthy GG, Zhang XL, Levine MJ. Inhibition of *Porphyromonas gingivalis* adhesion to *Streptococcus gordonii* by human submandibular-sublingual saliva. Infect Immun 1992: **60**: 2598–2604.
30. Stinson MW, Safulko K, Levine MJ. Adherence of *Porphyromonas (Bacteroides) gingivalis* to *Streptococcus sanguis in vitro*. Infect Immun 1991: **59**: 102–108.
31. Teanpaisan R, Douglas CWI. Molecular fingerprinting of *Porphyromonas gingivalis* by PCR of repetitive extragenic palindromic (REP) sequences and comparison with other fingerprinting methods. J Med Microbiol 1999: **48**: 741–749.
32. Zhang YJ, Yasui S, Yoshimura F, Ishikawa I. Multiple restriction fragment length polymorphism genotypes of *Porphyromonas gingivalis* in single periodontal pockets. Oral Microbiol Immunol 1995: **10**: 125–128.