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Prevalence of *fimA* genotypes of *Porphyromonas gingivalis* and periodontal health status in Chinese adults

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Background and Objective: *Porphyromonas gingivalis* fimbriae play a key role in colonization of the oral cavity. The *fimA* gene, which encodes fimbrillin (*FimA*), can be classified into six types (I–V and Ib) according to nucleotide sequence. In the present study, we investigated the relationship between the prevalence of *P. gingivalis*-specific *fimA* genotypes and periodontal health status in Chinese adults.

Material and Methods: One-hundred and fifteen patients with chronic periodontitis and 136 periodontally healthy adults were selected. *P. gingivalis* detection, determination of *fimA* genotypes, *and* the co-existence of *Actinobacillus actinomycetemcomitans* and *Tannerella forsythia* with various *fimA* types, were assessed by the polymerase chain reaction. Odds ratios and 95% confidence intervals were calculated for associating the *fimA*-specific genes with periodontitis.

Results: *P. gingivalis* was detected in 22.1% of healthy subjects and in 81.7% of the patients. A single *fimA* genotype was detected in most samples. In healthy adults, the most prevalent *fimA* genotype was type I (66.7%). However, type II was detected most frequently (43.6%) in the patient group, followed by type IV (30.9%). The frequency of co-existing *A. actinomycetemcomitans* and *T. forsythia* was highest in type II *fimA*-positive sites. Statistical analysis revealed that periodontitis was associated with occurrences of type I (odds ratio 0.97), Ib (odds ratio 13.26), II (odds ratio 36.62), III (odds ratio 4.57), IV (odds ratio 22.86) and V (odds ratio 1.19).

Conclusion: *P. gingivalis* type II followed by type IV were considered as disease-associated strains that account for the pathogenesis of chronic periodontitis in Chinese adults.

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Chronic periodontitis is a destructive inflammatory disease of periodontal tissues, which causes the absorption of alveolar bone and the damage of periodontal tissues (1). *Porphyromonas gingivalis*, a gram-negative, black-pigmented anaerobe, possessing various virulence factors, such as fimbriae (2), can colonize the oral surfaces, invade periodontal tissues and activate the host immunoreaction. It is widely considered that *P. gingivalis* is one of the most important pathogens causing chronic periodontitis (3,4).

P. gingivalis is reportedly detected not only at a high frequency in patients with periodontitis, but also at a low frequency in periodontally healthy individuals without marked gingival inflammation (5–7). Accumulated evidence has revealed a clonal heterogeneity in virulence among various *P. gingivalis* strains. According to the diversity of pathogenicity, *P. gingivalis* stains can be separated into two groups – disease-associated and nondisease-associated – and the variance of genetic heterogeneity was the foundation of the variance of pathogenicity in bacteria (8–12).

Major fimbriae, the main component parts of the *P. gingivalis* cell surface, play a key role in adhesion to host cells and co-adhesion with other plaque-forming bacteria (13–18). The *P. gingivalis fimA* gene, which encodes fimbrillin (the subunit of major fimbriae), is present as a single copy on the bacterial chromosome and no homologous sequences have been found in other black-pigmented *Porphyromonas* species (19). On the basis of the nucleotide sequence of its opening reading frame, Fujiwara *et al.* (20) classified *fimA* into four genotypes (I–IV). Subsequently, Nakagawa *et al.* (21,22) found new genotypes of *fimA* (V and Ib). Several epidemiological investigations have reported the distribution of *fimA* genotypes in subjects of different race and with different periodontal conditions. A majority of Japanese periodontitis patients were found to carry genotypes II and IV *fimA*, whereas in healthy Japanese adults, type I was the most prevalent *fimA* genotype (22–24). The predominant *fimA* genotypes in Caucasian and Brazilian periodontitis patients were II and I, and II and Ib, respectively (25,26). These results suggest that the variation in prevalence of the *P. gingivalis fimA* genotype may be associated with ethnicity, geographical location and periodontal status of the studied population.

This present study investigated the prevalence of *P. gingivalis fimA* genotypes in Chinese patients with chronic periodontitis compared with periodontally healthy adults, and studied the relationships between *P. gingivalis fimA* genotypes and clinical parameters. The detection frequency of *Actinobacillus actinomycetemcomitans* and *Tannerella forsythia* in the sites harboring each *P. gingivalis fimA* type were also examined to investigate the relationship between *fimA* genotypes of *P. gingivalis and* the co-existence of these pathogens. The odds ratios and 95% confidence intervals were calculated for finding a specific *fimA* genotype associated with the development of periodontitis in Chinese subjects.

Material and methods

Subjects

Based on the Third National Epidemiological Survey on Oral Health in China, a total of 136 periodontally healthy adults were selected randomly from Chengdu, Sichuan, China, for participation in this study. One-hundred and fifteen patients with chronic periodontitis, referred to the Department of Periodontology at the Dental Hospital, West China School of Stomatology (Sichuan University, Chengdu, China), were selected for this study. The periodontally healthy controls (44 men aged 25–75 years, mean age, 44.3 ± 10.6; and 92 women aged 25–74 years, mean age, 42.4 ± 11.3) possessed ≥20 teeth and had healthy gingiva, no radiographic evidence of bone loss, and the probing pocket depth and probing attachment loss were < 3 mm and < 1 mm, respectively, for all sites. All patients with chronic periodontitis (67 men aged 25–74 years, mean age, 47.9 ± 11.8; 48 women aged 26–75 years, mean age, 44.5 ± 12.4) were systemically healthy, and had periodontitis associated with periodontal pockets deeper than 4 mm and possessed ≥16 teeth. No subjects had any significant medical history, systemic diseases, or disorders that might affect the outcome of the periodontal therapy, and those that had received either periodontal therapy or antibiotic medication during the 3 mo prior to the study were excluded.

Clinical examinations and microbiological sample collections

All the subjects were requested to sign an informed consent to participate in the study. The Sichuan University Health Guideline for Studies Involving Human Subjects approved this study.

Prior to taking subgingival plaque samples, probing depth and bleeding on probing were determined. The pocket probing depth was measured at six points around the circumference of Ramfjord teeth (27) (mesio-, mid- and disto-buccal; disto-, mid- and mesio-lingual) with a round-ended probe tip 0.4 mm in diameter. Probing depth was measured from the gingival margin to the deepest probing point; bleeding on probing was scored as follows: (+) immediate bleeding on probing or (–) no bleeding.

Subgingival plaque samples were taken from the deepest molar site of Ramfjord teeth in patients and from the mesio-buccal molar site of Ramfjord teeth in healthy subjects. After removal of supergingival plaque with a sterile curette, three sterile paper-points were inserted into the site, held static for 10 s, and then taken out and immersed at once in a sterile plastic tube. The paper points were immediately placed on ice and then mixed with Chelex®-100 (Bio-Rad Laboratories, Hercules, CA, USA) and incubated at 56°C for 30 min, after which they were boiled at 100°C for 10 min and centrifuged for 20 min. The resulting supernatant was subjected to further analysis, and then stored at −20°C.

Polymerase chain reaction (PCR)

Detection of *P. gingivalis*, *A. actinomycetemcomitans* and *T. forsythia* were performed by the PCR using primers homologous to the *16S rRNA* gene, as described by Tran *et al.* (28) and Ashimoto *et al.* (6). The *fimA* type-specific primers, designed by Amano *et al.* and Nakagawa *et al.* (22,24), were used for *fimA* typing and detection (Fig. 1). The PCR amplification consisted of 2 μL of template DNA, 100 pmol of each oligonucleotide primer (TaKaRa Biotech Co, Ltd, Dalian, China), 2.5 μL of ×10 buffer, 3 mM $MgCl_2$, 200 μM dNTPs, 0.2 μL of *Taq*DNA polymerase (Fermentas Life Science, Vilnius, Lithuania) and sterile Tris-distilled water, to a total

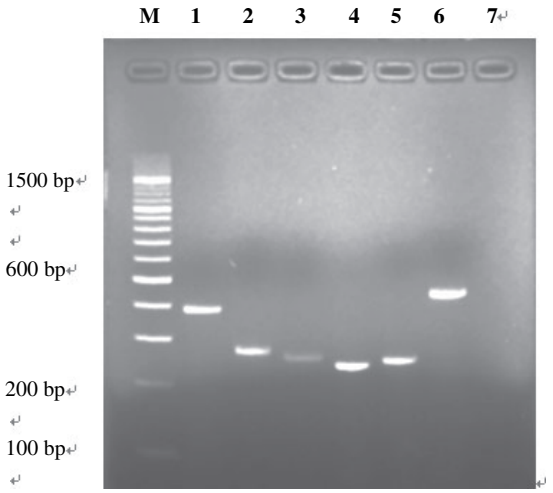

Fig. 1. Electrophoresis of amplification products on a 1.5% agarose gel. Lanes 1–7, polymerase chain reaction products using primers homologous to gene *fimA* I (392 bp), Ib (271 bp), II (257 bp), III (247 bp), IV (251 bp), V (462 bp) and the negative control, respectively. M, molecular weight marker (100 bp DNA ladder; Fermentas Life Science, Vilnius, Lithuania).

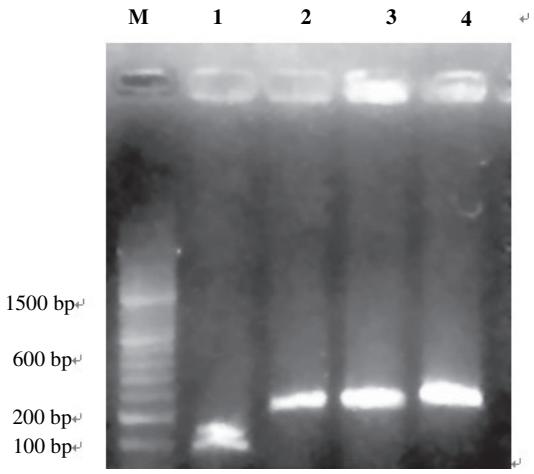

Fig. 2. Detection of type Ib *fimA* by polymerase chain reaction amplification and *Rsa*I digestion. Lanes 3 and 4, *fimA* amplicons from *Porphyromonas gingivalis* 551 (type Ib *fimA* clinical isolate) and *P. gingivalis* ATCC33277 (type I *fimA*) using *fimA* Ib primers. Lanes 1 and 2 show amplicons of lanes 3 and 4 digested with *Rsa*I. M, molecular weight marker (100 bp DNA ladder; Fermentas Life Science, Vilnius, Lithuania).

volume of 25 μL. PCR amplification was carried out in a thermal cycler with previously described cycle conditions (6,22,24,28). In samples where both type I and II *fimA* were simultaneously detected, another PCR amplication was performed using the type Ib primer pair, after which the amplified fragments were digested with *Rsa*I (Fermentas Life Science) for distinguishing type Ib *fimA*, as described previously (22) (Fig. 2). *P. gingivalis* ATCC33277, *P. gingivalis* W83, *A. actinomycetemcomitans* ATCC29533 and *T. forsythia* ATCC43037 (provided by the Key Laboratory for Oral Biomedical Engineering of Ministry of Education, Sichuan University) were used as positive controls. To test the reliability of the results, 10% of samples were selected at random and re-analyzed by the PCR.

Analysis of PCR products

The products obtained from each reaction were resolved by electrophoresis in 1.5% agarose gels in Tris acetate-boric acid buffer. Digital images of the ethidium bromide-stained gels were obtained with the Gel Doc 2000 (Bio-Rad, Hercules, CA, USA).

Statistical analysis

A chi-square test was used to analyze statistically the comparative frequencies of bacterial occurrence in periodontally healthy and periodontitis groups, the frequency of each *fimA* genotype with clinical periodontal indices, and the effect of the co-existence of *A. actinomycetemcomitans* and *T. forsythia* on each *fimA* genotype (p-values of < 0.05 were considered significant). Odds ratios and 95% confidence intervals were calculated for a significant association of *fimA*-type occurrences with periodontal health status. Computations were carried out by means of statistical analysis software SPSS®11.0 (SPSS Inc., Chicago, IL, USA).

Results

Distribution of *P. gingivalis* among periodontally healthy adults and patients with chronic periodontitis

P. gingivalis was detected in 30 out of 136 (22.1%) subgingival plaque samples from periodontally healthy adults (Table 1). Although the frequency of *P. gingivalis* detection was higher among the 55–64 years age-group, there was no distinctive statistical difference among various age groups. In patients, 94 of 115 (81.7%) samples were positive for *P. gingivalis*, with the highest, but nonsignificant, frequency in the 35–44 years age group. In each age group, a significant difference was found between periodontally healthy subjects and periodontitis patients in the prevalence of *P. gingivalis* ($p < 0.05$).

Table 1. Distribution of Porphyromonas gingivalis in periodontally healthy adults and in patients with chronic periodontitis

Age (years)	Periodontally healthy adults		Chronic periodontitis patients	
	Examined	*P. gingivalis* detected (%)	Examined	*P. gingivalis* detected (%)
25–34	18	3 (16.7)	14	10 (71.4)
35–44	63	13 (20.6)	28	27 (96.4)
45–54	18	4 (22.3)	32	25 (78.1)
55–64	14	4 (28.6)	33	25 (75.8)
65–75	23	6 (26.1)	8	7 (87.5)
Total	136	30 (22.1)	115	94 (81.7)

Significant differences ($p < 0.05$) were found between periodontally healthy adults and patients with chronic periodontitis in all age groups.

Distribution of *fimA* genotypes among the periodontally healthy subjects and the periodontitis patients

The distribution of *fimA* genotypes among *P. gingivalis*-positive samples was subsequently analyzed (Table 2). Among the healthy subjects, only a single *P. gingivalis fimA* type was detected in most of the samples (24/30). Two different *fimA* genotypes were detected in five samples, all of which comprised type I and another *fimA* genotype. Type I *fimA* was detected most frequently (66.7%, $p < 0.05$), followed by type V (16.7%) and type III (10%). Moreover, one subgingival plaque sample showed a positive *P. gingivalis* 16*S rRNA* PCR result, but the PCR assay of *fimA*-specific primers was negative. The distribution of *fimA* genotypes was also analyzed in periodontitis patients carrying *P. gingivalis*. A single *fimA* gene was detected in 73.4% of all samples, 22.3% of samples had two different *fimA* genotypes and 4.3% had three. The combined detection rate of type II with other *fimA* genotypes was the highest. A majority of the patients were found to carry type II *fimA* organisms (43.6%, $p < 0.01$), followed by type IV (30.9%, $p < 0.05$) and type Ib (20.2%).

Table 2. Distribution of six fimA genotypes in periodontally healthy adults and chronic periodontitis patients carrying Porphyromonas gingivalis

fimA type	Frequency of occurrence (%)			
	Periodontally healthy adults		Chronic periodontitis patients	
	n	(%)	*n*	(%)
I	15	(50.0)	8	(8.5)
Ib	1	(3.3)	9	(9.6)
II	0	(0)	23	(24.7)
III	3	(10.0)	6	(6.4)
IV	2	(6.7)	20	(21.3)
V	3	(10.0)	3	(3.2)
Subtotal	24	(80.0)	69	(73.4)
I and Ib	1	(3.3)	2	(2.1)
I and II	2	(6.7)	2	(2.1)
I and V	2	(6.7)	1	(1.1)
Ib and II	0	(0)	2	(2.1)
Ib and III	0	(0)	3	(3.2)
Ib and IV	0	(0)	1	(1.1)
II and III	0	(0)	4	(4.3)
II and IV	0	(0)	6	(6.4)
Subtotal	5	(16.7)	21	(22.3)
I, Ib, and II	0	(0)	1	(1.1)
I, II, and III	0	(0)	1	(1.1)
I, II, and IV	0	(0)	1	(1.1)
Ib, II, and IV	0	(0)	1	(1.1)
Subtotal	0	(0)	4	(4.3)
Untypeable	1	(3.3)	0	(0)

Significant differences were found for the following comparisons: type I vs. types Ib, II, III, IV, V and untypeable in all periodontal subjects ($p < 0.05$).
Significant differences among the *fimA* genotypes in chronic periodontitis patients were found for the following comparisons: type II vs. types I, Ib, III and V ($p < 0.01$); type IV vs. types I, Ib, III and V ($p < 0.05$); and type II/IV vs. types I/Vand Ib/IV ($p < 0.05$).

Relationships of *P. gingivalis fimA* genotypes to clinical parameters

In patients with chronic periodontitis, the relationships of probing depth and bleeding on probing with the prevalence of *fimA* types were analyzed. As shown in Table 3, in two groups, the prevalence of type II and type IV *fimA* was higher than the prevalence of the other *fimA* types ($p < 0.05$), and the prevalence of type V *fimA* was lower than the others ($p < 0.05$). A significant difference was found for the incidence of type IV *fimA* between the two groups ($p < 0.05$). Bleeding on probing was found in 83 patients (88.3%) (Table 4). In the bleeding on probing (+) group, the prevalence of type II and type IV *fimA* was higher than that of the other *fimA* types ($p < 0.05$), and the prevalence of type V was lower than that of the other *fimA* types ($p < 0.05$). Significant differences were obtained on the incidence of I–IV and Ib *fimA* types between the two groups ($p < 0.05$). There was no significant difference between the distribution of various *fimA* genotypes and age.

Combined detection frequency of *T. forsythia*, *A. actinomycetem comitans* and *P. gingivalis fimA* genotypes

Among the 30 *P. gingivalis*-positive samples of the healthy subjects, six were detected together with *T. forsythia* and one was detected together with *A. actinomycetemcomitans*. Of the

Table 3. Relationship of fimA genotype distribution with probing depth n (%) in periodontitis patients

PD	*n* (%)	Genotype					
		I *fimA*	Ib *fimA*	II *fimA*	III *fimA*	IV *fimA*	V *fimA*
4–6 mm	56 (59.6)	11 (19.6)	13 (23.2)	19 (33.9)[a]	9 (16.1)	20 (35.7)[a,c]	3 (5.3)
≥ 7 mm	38 (40.4)	5 (13.2)	6 (15.8)	21 (55.3)[b]	5 (13.2)	9 (23.7)[b,c]	1 (2.9)
Total	94 (100)	16 (17.0)	19 (20.2)	41 (43.6)	14 (14.9)	29 (30.9)	4 (4.3)

[a]II *fimA* and IV *fimA* vs. other *fimA* genotypes ($p < 0.05$).
[b]II *fimA* and IV *fimA* vs. other *fimA* genotypes ($p < 0.05$).
[c]A significant difference was obtained on the incidence of genotype IV *fimA* between the two groups ($p < 0.05$).
PD, probing depth.

Table 4. Relationship of fimA genotype distribution with bleeding on probing n (%) in patients with periodontitis

BOP	*n* (%)	Genotype					
		I *fimA*	Ib *fimA*	II *fimA*	III *fimA*	IV *fimA*	V *fimA*
+	83 (88.3)	12 (14.5)	15 (18.1)	38 (45.8)[a]	11 (13.3)	27 (32.5)[a]	3 (3.6)
–	11 (11.7)	4 (36.3)	4 (36.3)	3 (27.3)	3 (27.3)	2 (18.2)	1 (9.1)
Total	94 (100)	16 (17.0)	19 (20.2)	41 (43.6)	14 (14.9)	29 (30.9)	4 (4.3)

[a]Genotype II *fimA* and IV *fimA* vs. other *fimA* genotypes ($p < 0.05$).
Significant differences were obtained on the incidence of genotypes I–IV and Ib *fimA* between the two groups ($p < 0.05$).
BOP, bleeding on probing.

94 samples containing *P. gingivalis* from patients with chronic periodontitis, 60 also contained *T. forsythia* and 34 also contained *A. actinomycetemcomitans*. Among the periodontitis patients, the co-existence frequency of *T. forsythia* with type II *fimA* strains was 53.3%. In the combined detection sites of *P. gingivalis* and *A. actinomycetemcomitans*, the colonization of type II *fimA* strains reached 44.1%. The detection frequency of these combinations in type II *fimA*-positive sites differed significantly from the sites with other types of *fimA* ($p < 0.05$) (Table 5).

Analysis on the relevance of *P. gingivalis fimA* genotypes with the periodontal health status of Chinese adults

Significant associations of specific *fimA* genotypes with periodontal health status were demonstrated, as shown in Table 6.

Table 5. Combined detection frequency of Tannerella forsythia, Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis fimA genotypes

fimA genotype	Periodontally healthy adults *n* (%)		Chronic periodontitis patients *n* (%)	
	T. forsythia	*A. actinomycetemcomitans*	*T. forsythia*	*A. actinomycetemcomitans*
I	4 (66.7)	0 (0)	11 (18.3)	9 (26.5)
Ib	0 (0)	0 (0)	13 (21.7)	5 (14.7)
II	2 (33.4)	0 (0)	32 (53.3)[a]	15 (44.1)[b]
III	1 (16.7)	1 (100)	9 (15.0)	5 (14.7)
IV	0 (0)	0 (0)	9 (15.0)	12 (35.3)
V	1 (16.7)	0 (0)	0 (0)	0 (0)

[a]The detection frequency of the co-existence between type II *fimA* and *T. forsythia* differed significantly from that of other *fimA* genotypes ($p < 0.05$).
[b]The detection frequency of the co-existence between type II *fimA* and *A. actinomycetemcomitans* differed significantly from that of other *fimA* genotypes ($p < 0.05$).

Table 6. Relationship of Porphyromonas gingivalis fimA genotypes with periodontitis

Factors	Odds ratio	95% Confidence interval
P. gingivalis (all *fimA* types)	16.36	8.744–30.618
fimA type		
I	0.97	0.461–1.907
Ib	13.26	3.018–58.29
II	36.62	8.608–155.6
III	4.57	2.556–8.173
IV	22.86	5.315–98.24
V	1.19	0.291–4.864

Discussion

Epidemiological research has shown that the frequency of *P. gingivalis* in the subgingival plaque samples of patients with chronic periodontitis is high, ranging from 50.3 to 85.7% (23–26). Further studies discovered that *P. gingivalis* exists not only in patients with periodontitis, but also in the subgingival plaque samples of healthy people. Griffen *et al*. (29) investigated the distribution of *P. gingivalis* in the subgingival plaque samples of 181 periodontally healthy people and reported an occurrence of 25%; Yang *et al*. (30) reported that *P. gingivalis* occurred in 23.1% of 91 periodontally healthy Taiwanese subjects. *P. gingivalis* was detected in 36.8% of Japanese periodontally healthy adults (24). The present study investigated the prevalence of *P. gingivalis* in the subgingival plaque specimens of 115 chronical periodontitis patients and 136 periodontally healthy Chinese adults. *P. gingivalis* was detected in 81.7% of patients and in 22.1% of healthy subjects, similar, but not identical to, data obtained from other groups. The discrepancy may result from differences in ethnic groups, customs, geographical locations, etc.

In research on the prevalence of *fimA* genotypes of *P. gingivalis* with various periodontal conditions, a majority of Japanese patients were found to carry type II organisms, followed by type IV and type Ib; type I was widely distributed among the healthy subjects (22–24). The prevalence of *fimA* genotypes among Brazilan adults of different periodontal health status showed that

type II *fimA* was highest among the periodontitis patients, followed by type Ib (25). The present study investigated the distribution of *P. gingivalis fimA* genotypes in the subgingival plaque samples of Chinese periodontally healthy subjects and chronic periodontitis patients, and proved that *P. gingivalis* with type I *fimA* was widely distributed among the periodontally healthy people, whereas type II and type IV *fimA* organisms were widely distributed among the patients with chronic periodontitis. This result is similar to those reported by Amano *et al.* (24) and Nakagawa *et al.* (22), but different from that of Missailidis *et al.* (25). This difference is possibly a result of differences in factors such as geographical location and ethnic origin.

Our study showed that *P. gingivalis* with type II and type IV *fimA* were more frequently detected in the 4–6 mm and ≥7 mm probing depth groups, which indicated that type II and type IV *fimA* genotypes were associated with the initiation and progress of periodontitis in Chinese subjects. We also demonstrated that *P. gingivalis* with type II and IV *fimA* were more frequently detected in the bleeding on probing (+) group, which is different from the data of Fujise *et al.* (31). Fujise *et al.* reported that *fimA* I-positive sites at baseline were followed by a significantly higher frequency of persistent bleeding on probing after treatment than *fimA* I-negative sites. Miura *et al.* (32) revealed that *P. gingivalis* clinical isolates with type I *fimA* had higher Arg-gingipain and Lys-gingipain activities in culture supernatants, suggesting that *P. gingivalis* possessing type I *fimA* might facilitate the action of both fimbriae and proteinase. Therefore, further investigations are necessary to clarify the relationship between the destruction of periodontal tissue and the interaction of fimbriae with other *P. gingivalis* virulence factors.

From the investigation on the distribution of *fimA*-type *P. gingivalis* in young Japanese subjects (aged 3–18 years), Tamura *et al.* (33) discovered that none of the *P. gingivalis*-positive samples showed a positive reaction to the type II *fimA*-specific primer, and that *P. gingivalis* with type IV *fimA* existed only among the older age groups. *P. gingivalis* with type II and IV *fimA* are widely distributed among adult periodontitis patients (22–26). These results suggested that the distribution of *fimA* genotypes might have a certain type of relationship with age. By studying the distribution of *fimA* types among different age groups of healthy subjects and patients, it was found that although there was no significant difference in the distribution of *fimA* genotypes in various age groups, the prevalence of type II and type IV *fimA* strains increased with increasing age in the patient group, peaking, respectively, in the 35–44- and 55–64-year age-groups; the detection rates of type I and type III, on the contrary, decreased with increasing age in patients over 35–44 years of age (data not shown). Previous research showed that with increasing age and change of oral hygiene conditions, bacteria predominating in the subgingival biofilm would be changed accordingly (33–35). Whether there is a similar type of predominant competitive interaction in the *fimA*-type disease-associated and non-disease-associated strains is still unresolved and required further research with larger collection of samples.

It has been recognized that bacterial species exist in complex in subgingival plaque (36–40). Gumr *et al.* (40) investigated the distribution of *P. gingivalis* and *T. forsythia* in the subgingival plaque samples from pockets of different probing depths, and found a strong association between them. In research on the distribution of *A. actinomycetemcomitans*, the detection of *A. actinomycetemcomitans* in the subgingival plaque samples of patients with chronic periodontitis was 31–74% (37–39), suggesting that *A. actinomycetemcomitans* not only acts as the main periodontal pathogen for aggressive periodontitis, but also combines, in chronic periodontitis, with other periodontal pathogens to cause periodontal destruction and to aggravate the inflammation of periodontal tissues. Besides facilitating the initial interaction between the bacteria and the host cells, fimbriae also participate in the co-adhesion of *P. gingivalis* with the primary colonizing organisms and other gram-negative secondary colonizing organisms in the plaque biofilm, which contributes to formation of the subgingival biofilm (15–18). Are there any differences in the co-adhesion of various *fimA P. gingivalis* with *T. forsythia* and *A. actinomycetemcomitans*? By analyzing the *P. gingivalis*-positive samples of the periodontally healthy subjects and the patients with periodontitis, the research of the present study detected the co-existence of various *fimA P. gingivalis* together with *T. forsythia and A. actinomycetemcomitans*. In the *P. gingivalis*-positive samples of the patients, the detection frequency of *T. forsythia* and *A. actinomycetemcomitans*, combined with type II *fimA* strains, differed significantly from other types ($p < 0.05$). The result is similar to that of Mirua *et al.* (32), suggesting that *P. gingivalis* with type II *fimA* has a stronger tendency to co-adhere to other periodontal pathogens, promoting the development of subgingival biofilm.

By analyzing the relationship between various *fimA* genotypes of *P. gingivalis* and the periodontal health status of Chinese adults, we calculated the odds ratio and the 95% confidence interval for a significant association of *fimA*-type occurrences with periodontal health status. The results demonstrated that the relationship of type II *fimA* with periodontitis was remarkable, being significantly stronger than any other genotype, followed by type IV and type Ib; type I and type V were demonstrated to be weakly related to periodontitis and had no statistical value. This research established that type II and type IV *P. gingivalis* are conspicuously related to the initiation and progression of chronic periodontitis in Chinese adults. Therefore, disease-associated *fimA*-specific *P. gingivalis* exists in Chinese adults, and further study is necessary to investigate the difference of *P. gingivalis fimA* genotypic pathogenic mechanisms.

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