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# Distribution of 8 periodontal microorganisms in family members of Chinese patients with aggressive periodontitis

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ABSTRACT

*Objective:* To date, no information on the distribution of periodontal microorganisms among family members of Chinese patients with aggressive peridontitis (AgP) is available. The aim of the present study was to investigate the probability of transmission of eight periodontal microorganisms between patients with aggressive periodontitis and their family members.

*Design:* Saliva and pooled subgingival plaque samples were collected from 103 participants from 41 nuclear families (including 41 AgP probands, 19 mothers, 22 fathers, 21 siblings). Eight periodontal microorganisms, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Campylobacter rectus*, *Prevotella intermedia*, *Prevotella nigrescens* and *Fusobacterium nucleatum* were detected in these samples by the polymerase chain reaction (PCR). In addition, the distribution of *fimA* genotypes was assessed in *P. gingivalis*-positive individuals by PCR.

*Results:* *P. gingivalis*, *T. forsythia*, *T. denticola*, *C. rectus* and *F. nucleatum* were the most frequently detected species both in AgP probands and in their relatives. Kappa statistical analysis revealed that the detection of *A. actinomycetemcomitans* (Kappa = 0.503) and *F. nucleatum* (Kappa = 0.565) in probands was highly consistent with that in their relatives. Most probands shared the identical *fimA* genotype of *P. gingivalis* with their relatives.

*Conclusions:* Our results suggested that the intrafamilial transmission of periodontal microorganisms may occur between Chinese patients with aggressive periodontitis and their relatives.



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*Abbreviations:* AgP, aggressive periodontitis; CP, chronic periodontitis; PD, probing depth; BI, bleeding index; AL, attachment loss.

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## 1. Introduction

Aggressive periodontitis (AgP) is a group of infrequent types of periodontal diseases with rapid attachment loss and bone destruction initiated at a young age. Many studies[1,2] have indicated that the prevalence of AgP is disproportionately high among certain families. In many families, the percentage of affected siblings may reach 40–50%,[3,4] or even higher.[5] The notable familial aggregation of AgP cases indicates that genetic factors might be important in susceptibility to AgP[6–8]. In addition, familial aggregation of periodontal disease may also reflect exposure to common environmental factors. Nasidze's observations suggest that similar lifestyles and diet lead to more similar oral microbiomes.[9] Certain infectious agents may cluster in families. The intrafamilial transmission of periodontal microorganisms may in part explain the familial aggregation of AgP and may have important prophylactic and treatment implications.

It has been shown that periodontal microorganisms are not restricted to subgingival areas, also being found in the saliva, supragingival plaque and various mucous membranes in patients with periodontitis.[10–12] Saliva and direct mucosal contact might be the main transmission routes of periodontal microorganisms. In a study[13] on the transmission of *Porphyromonas gingivalis* within families, it was noted that a *P. gingivalis*-colonized mother became a risk for colonization of their child and the risk of colonization was highest for a child when both parents were colonized with *P. gingivalis*. Umeda observed that *Tannerella forsythia*, *Prevotella intermedia* and *Prevotella nigrescens* were detected more frequently in children whose parents were positive for these microorganisms than in children whose parents were negative.[14] Similar results were obtained from other studies on children and their mothers.[15,16] Taken these observations together, the intrafamilial transmission of periodontal microorganisms may be suggested. However, detection of the same bacterial species in family members does not prove transmission. For further evidence, typing of the bacterial isolates is necessary. Methods of bacteria typing include molecular methods and phenotypic methods. *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* are detected frequently in patients with periodontitis, which makes these species prime candidates for studying person to person transmission.

To the best of our knowledge, no information on the distribution of periodontal pathogens among family members of Chinese patients with AgP is available. The aim of the present study was to investigate the probability of transmitting eight periodontal pathogens between patients with AgP and their family members.

## 2. Material and methods

### 2.1. Study population and clinical examination

From 2000 to 2011, a total of 103 participants from 41 nuclear families (including 41 AgP probands, 19 mothers, 22 fathers, 21 siblings) were recruited from the Department of Periodontology, Peking University School and Hospital of Stomatology. All the participants were members of the Chinese Han race. AgP patients who encouraged their relatives to participate in the study are termed probands. All the AgP patients were of the generalized type (GAgP). According to the 1999 international classification of periodontal diseases,[17] all participants were diagnosed as healthy, or having gingivitis, chronic periodontitis (CP), or AgP, based on full-mouth periodontal chartings (including assessments of probing depth (PD), attachment loss, bleeding index (BI)[18] at six sites per tooth) and full-mouth periapical radiographs. All the clinical parameters were recorded by three skilled, calibrated practitioners (HM, LX and LZ) as described by Shi.[19] Calibration was performed on 10 patients with severe periodontitis. The consistency of the replicated measurements of PD and AL for each examiner and paired measurements between two examiners were recorded. The clinical criteria used to define GAgP were as follows: (1) patients were under 35 years old; (2) they had rapid attachment loss and bone destruction; (3) at least eight teeth, three of them not being first molars and incisors, had PD $>$ 5 mm, AL $>$ 3 mm; (4) clinical diagnosis was confirmed by evidence of interproximal bone loss on full-mouth periapical radiographs. Participants were excluded if they (1) had a chronic medical disease or condition such as diabetes, cardiovascular disease, chronic kidney disease, hereditary disease and so on; (2) were pregnant or lactating; (3) had received periodontal treatment within the previous 6 months or antibiotic medication during the previous 3 months. Further classification of CP was based on the extent and severity of the clinically evident periodontal destruction. All the CP patients were divided into mild, moderate and severe types as described by Armitage.[20] According to the medical history, one relative was edentulous because he had a history of severe periodontitis. The study protocol was reviewed and approved by the Ethics Committee of the Peking University Health Science Center. The probands and all family members who agreed to attend the study provided written informed consent.

### 2.2. Sample collection and processing

Whole saliva and subgingival plaque samples were obtained 1 week after completing the full-mouth periodontal examination. Whole saliva samples were collected before subgingival plaque samples. Approximately 0.5 ml of unstimulated whole saliva from each individual was collected in a sterile Eppendorf tube. Subgingival plaque samples were obtained from the mesiobuccal sites of four first molars per participant (excluding sites with caries, interproximal restoration and crown, or if missing, premolars or second molars instead), and the four samples were pooled into a single sample tube. After isolating the sampled area with cotton rolls and gentle air drying, supragingival plaque was removed carefully with curettes, subgingival samples were obtained by placing a sterile Gracey curette at the apical extent of the pocket or gingival crevice and drawing it coronally with slight pressure. The sampled sites of periodontitis patients were characterized by PD $\geq$ 4 mm and AL $\geq$ 2 mm (if the mesiobuccal sites of the first molars had no pocket, other first or second molar sites were used).

Saliva and subgingival plaque samples were processed within 6 hours after collection (samples were temporarily stored at 4 °C immediately after collection). Saliva samples were centrifuged at 10,000 × *g* for 10 min, then the pellet was washed five times with 500 μL TE buffer (10 mmol/L Tris–HCl, pH 7.6, 1 mmol/L EDTA). Subgingival plaque samples were immersed in 500 μL TE buffer and mixed in a vortex mixer. The suspension was then centrifuged at 10,000 × *g* for 5 min, and the resulting pellet was washed twice with 500 μL TE buffer. The samples were stored immediately at −20 °C for DNA extraction.

### 2.3. DNA extraction and polymerase chain reaction (PCR) measurements

Genomic DNA was isolated from saliva and subgingival plaque sample with a commercial bacteria DNA mini kit (Watson Biotechnologies, Shanghai, PR China) following the manufacturer's instructions. DNA integrity was checked using 1.0% agarose gel electrophoresis. Bacterial 16S rDNA of the eight periodontal microorganisms (*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *Treponema denticola*, *Campylobacter rectus*, *P. intermedia*, *P. nigrescens* and *Fusobacterium nucleatum*) was amplified from the extracted DNA by PCR. The specific primer sequences and PCR protocol were based on reports by Ashimoto[21] and Baumgartner.[22] PCR amplification was performed by the GeneAmp PCR system 2700 (ABI, South San Francisco, CA, USA). PCR products were analysed by gel electrophoresis in 1.5% agarose.

The *fimA* genotype-specific primers of *P. gingivalis*, designed by Amano et al.[23] and Nakagawa et al.[24] were used for *fimA* typing. Briefly, 25 μl of the PCR reaction mixture contained 2 μl of sample, 2.5 μl of 10 × PCR buffer, 1 unit Taq DNA polymerase, 0.2 mM of each dNTP, 0.4 μM of each primer, and 2.5 mM $MgCl_2$ for types I, II, and V, and 1.5 mM $MgCl_2$ for types III, IV and Ib. PCR amplification was carried out under previously described cycle conditions.[25] Amplification of six genotypes (I–V and Ib) resulted in products of 392 bp, 257 bp, 247 bp, 251 bp, 462 bp and 271 bp, respectively. In samples wherein both type I and II *fimA* were detected simultaneously, another PCR amplification was performed using the type Ib primer pair, after which the 271 bp amplified fragments were digested with RsaI (TaKaRa Biotechnology, Dalian, PR China) for distinguishing type Ib fimA as described by Nakagawa.[24] The samples in which the restricted amplifications resulted in two fragments of 162 bp and 109 bp were considered to be type Ib. PCR products were analysed by 2–3% agarose gel electrophoresis.

The gel was stained with 50 μl/L GoldView™ (SBS Genetechnology, Beijing, PR China) and photographed under 300 nm ultraviolet illumination. DL 2000 and 100 bp ladder (TaKaRa Biotechnology, Dalian, PR China) served as the molecular weight marker.

### 2.4. Statistical analysis

All data were analysed using the statistical program SPSS 13.0. Quantitative values (such as age, PLI, PD, BI and AL) were analysed by the Student *t* test (normal distribution) and Mann–Whitney test (non-normal distribution). If the eight microorganisms were detected in any of the two kinds of sample per participant, the subject was regarded as positive for these microorganisms. The difference in the prevalence of the eight microorganisms and frequencies of the different genotypes of *P. gingivalis* between AgP probands and their relatives were tested by chi-square statistics. The kappa statistic was used to determine the degree of agreement in detecting eight periodontal microorganisms between probands and their relatives. Correlations between the numbers of bacterial species present in probands and their relatives were analysed using Spearman's correlation test. A *P* value $< 0.05$ was considered statistically significant.

## 3. Results

A total of 103 participants were recruited for this study. Table 1 summarizes the demographic and clinical characteristics of the AgP probands and their relatives. One relative had no periodontal chart or X-ray because he was edentulous. The mean age of AgP probands was significantly lower than that of their relatives. The percentage of males in AgP probands was also significantly lower than that of their relatives. The frequency of current smokers in probands was significantly lower than that in relatives. More severe clinical indices, including PD, AL, BI and percentage of sites with PD ≥ 4 mm and PD ≥ 6 mm were observed in AgP probands than in relatives. The number of lost teeth was not different between the two groups.

The family structure is shown in Table 2, 28 out of 41 probands provided fathers and/or mothers and 18 probands provided 21 siblings. Clinical periodontal diagnoses of the relatives are presented in Table 3. 8.1% of the relatives recruited in this study were diagnosed with AgP, 40.3% had severe CP, and 32.3% had moderate CP.

Table 4 shows the detection frequencies of eight microorganisms from probands and their relatives. The prevalence of the eight microorganisms was not significantly different between the AgP probands and their relatives. *P. gingivalis*,

**Table 1 – The demographic and clinical characteristics of AgP probands and their relatives.**

| Variables | Probands N = 41 | Relatives N = 62[c] |
|---|---|---|
| Age (years)[a] | | |
| Mean ± SD | 24.4 ± 5.6 | 41.8 ± 12.5 |
| Range | 11–35 | 8–62 |
| Males (%)[a] | 9 (22%) | 31 (50%) |
| Current smokers (%)[a] | 2 (4.9%) | 21 (33.9%) |
| Probing depth (mm)[a] | 4.9 ± 1.0 | 3.7 ± 0.9 |
| Attachment Loss (mm)[a] | 4.5 ± 1.4 | 3.0 ± 1.2 |
| Bleeding Index (BI)[a,b] | 3.1 (2.5, 3.5) | 3.7 (3.4, 4.0) |
| % of sites with PD ≥ 4 mm[a] | 70.7 ± 16.2 | 45.4 ± 22.0 |
| % of sites with PD ≥ 6 mm[a,b] | 31.4 (19.5, 51.6) | 2.3 (0, 11.5) |
| Number of teeth lost[b] | 0 (0, 1.8) | 0.5 (0, 3.3) |

Values are given as mean ± SD unless otherwise noted.
[a] $P < 0.01$, probands versus relatives.
[b] Median value (interquartile range).
[c] One relative had no periodontal chart and X-ray because he was edentulous.

**Table 2 – Family structure of the study.**

| Parents | Number of sibships | | | |
|---|---|---|---|---|
| | 0 | 1 | ≥2 | Total |
| With mother | 6 | – | – | 6 |
| With father | 8 | 1 | – | 9 |
| With parents | 9 | 2 | 2 | 13 |
| Without parents | – | 12 | 1 | 13 |
| Total | 23 | 15 | 3 | 41 |

**Table 3 – Periodontal clinical diagnosis of the relatives.**

| Diagnosis | Frequency *N* | Percentage |
|---|---|---|
| AgP | 5 | 8.1% |
| Mild CP | 9 | 14.5% |
| Moderate CP | 20 | 32.3% |
| Severe CP | 25 | 40.3% |
| Gingivitis | 1 | 1.6% |
| Healthy | 1 | 1.6% |
| Edentulous | 1 | 1.6% |
| Total | 62 | 100% |

*T. forsythia*, *T. denticola*, *C. rectus* and *F. nucleatum* were the most frequently detected species both in AgP probands and their relatives. Among the 41 families, relatives of 15 AgP probands were positive for *A. actinomycetemcomitans* (at least one relative from one family was positive for this pathogen), and relatives of 26 AgP probands were negative for it. *A. actinomycetemcomitans* was detected more frequently in probands whose relatives were positive for *A. actinomycetemcomitans* than in probands whose relatives were negative for it (66.7% versus 19.2%, $P < 0.01$). In addition, the number of bacterial species in probands was positively correlated to the number in their relatives ($R = 0.395$, $P = 0.001$). Kappa statistical analysis revealed that the detection of *A. actinomycetemcomitans* and *F. nucleatum* in probands was highly consistent with that in their relatives (Table 5).

Since *P. gingivalis* was one of the most frequently detected species in AgP probands and their relatives, the *fimA* genotype of *P. gingivalis* was further analysed. The distribution of six *fimA* genotypes in AgP probands and their relatives carrying

**Table 5 – Kappa statistic of detection of 8 periodontal microorganisms in 41 probands and 62 relatives.**

| Microorganisms | Kappa value | *P* value |
|---|---|---|
| *A. actinomycetemcomitans* | 0.503 | 0.001 |
| *P. gingivalis* | −0.059 | 0.641 |
| *T. forsythia* | −0.048 | 0.67 |
| *T. denticola* | 0.109 | 0.37 |
| *C. rectus* | −0.025 | 0.82 |
| *P. intermedia* | 0.11 | 0.386 |
| *P. nigrescens* | 0.183 | 0.149 |
| *F. nucleatum* | 0.565 | 0.001 |

*P. gingivalis* is summarized in Table 6. Most individuals harboured one genotype of *fimA*. The prevalence of *fimA* I in probands was significantly lower than that in their relatives. The distribution of other genotypes of *fimA* was not significantly different between the two groups. Type II was the most prevalent *fimA* genotype both in AgP probands and their relatives. Among the 34 families who were *P. gingivalis*-positive in subgingival plaque from both probands and all their relatives, 20 probands (58.8%) shared the identical *fimA* genotype with their relatives. Among the 30 families who were positive for *P. gingivalis* in saliva from both probands and all their relatives, 20 probands (60.6%) shared the identical *fimA* genotype with their relatives. Among the 10 parents who were positive for *P. gingivalis* in their subgingival plaque, 7 couples (70%) shared the identical *fimA* genotype. Among the 11 parents who were positive for *P. gingivalis* in their saliva, 10 couples (90.9%) shared the identical *fimA* genotype. Fig. 1 shows the distribution of *fimA* genotype of *P. gingivalis* in 12 typical families. In 80 individuals who were colonized by *P. gingivalis* both in subgingival plaque and saliva samples, 74 (92.5%) subjects harboured the identical genotype in both samples.

## 4. Discussion

This study recruited 41 AgP probands and their 62 first degree blood relatives.

Table 3 demonstrated that the prevalence of AgP and severe CP was high in these families. Our previous studies

**Table 4 – Detection frequencies of eight microorganisms from probands and their relatives (*N* (%)).**

| Microorganism | Probands | | | Relatives | | |
|---|---|---|---|---|---|---|
| | Plaque N = 41 | Saliva N = 34[b] | Subject[a] N = 41 | Plaque N = 59 | Saliva N = 59[b] | Subject[a] N = 62 |
| *A. actinomycet-emcomitans* | 8 (19.5) | 11 (32.4) | 14 (34.1) | 14 (23.7) | 12 (20.3) | 20 (32.3) |
| *P. gingivalis* | 39 (95.1) | 34 (100) | 39 (95.1) | 51 (86.4) | 52 (88.1) | 58 (93.5) |
| *T. forsythia* | 39 (95.1) | 29 (85.3) | 40 (97.6) | 50 (84.7) | 54 (91.5) | 57 (91.9) |
| *T. denticola* | 36 (87.8) | 29 (85.3) | 38 (92.7) | 52(88.1) | 43 (72.9) | 55 (88.7) |
| *C. rectus* | 39 (95.1) | 32 (94.1) | 39 (95.1) | 54 (91.5) | 58 (98.3) | 61 (98.4) |
| *P. intermedia* | 22 (53.7) | 24 (70.6) | 29 (70.7) | 40 (67.8) | 33 (55.9) | 46 (74.2) |
| *P. nigrescens* | 23 (56.1) | 21 (61.8) | 29 (70.7) | 34 (57.6) | 32 (54.2) | 47 (75.8) |
| *F. nucleatum* | 37 (90.2) | 31 (91.2) | 37 (90.2) | 51 (86.4) | 46 (78) | 57 (91.9) |

The values in the parentheses represent %.

[a] Results of the subject level is a combination of results of subgingival plaque and saliva. If the microorganism was detected in any one of the two kinds of sample per subject, the subject was regarded as positive for this microorganism.

[b] Saliva samples of 10 subjects (including 7 probands and 3 relatives) were not collected because of personal reasons of these subjects.

**Table 6 – Distribution of six *fimA* genotypes in AgP probands and their relatives carrying *Porphyromonas gingivalis* (N (%)).**

| *fimA* genotype | Probands | | Relatives | |
|---|---|---|---|---|
| | Plaque N = 39 | Saliva N = 34 | Plaque N = 48 | Saliva N = 52 |
| *fimA* I | 4 (10.3)[a] | 2 (5.9)[a] | 14 (29.2) | 13 (25) |
| *fimA* II | 20 (51.3) | 15 (44.1) | 18 (37.5) | 22(42.3) |
| *fimA* III | 2 (5.1) | 3 (8.8) | 2 (4.2) | 2 (3.8) |
| *fimA* IV | 1 (2.6) | 1 (2.9) | 3 (6.3) | 2 (3.8) |
| *fimA* V | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| *fimA* Ib | 9 (23.1) | 9 (26.5) | 11 (22.9) | 11 (21.2) |
| Subtotal | 36 (92.3) | 30 (88.2) | 48 (100) | 50 (96.2) |
| *fimA* I/III[b] | 1 (2.6) | 1 (2.9) | 0 (0) | 1 (1.9) |
| *fimA* I/IV[b] | 0 (0) | 1 (2.9) | 0 (0) | 0 (0) |
| *fimA* II/IV[b] | 0 (0) | 1 (2.9) | 0 (0) | 0 (0) |
| *fimA* II/V[b] | 1 (2.6) | 1 (2.9) | 0 (0) | 0 (0) |
| Subtotal | 2 (5.1) | 4 (11.8) | 0 (0) | 1 (1.9) |
| Untypable | 1 (2.6) | 0 (0) | 0 (0) | 1 (1.9) |
| Total | 39 (100) | 34 (100) | 48 (100) | 52 (100) |

The values in the parentheses represent %.
[a] Versus saliva samples of relatives, $P < 0.05$.
[b] Concomitant presence of listed *fimA* genotype in the same sample.

indicated that genetic factors were important in susceptibility to AgP.[6–8]

The present study aimed to determine the likehood of intrafamilial transmission of eight periodontal microorganisms between Chinese patients with AgP and their first-degree blood relatives. The present results showed that the eight periodontal microorganisms were widely distributed in the saliva of AgP probands and their relatives, which is consistent with the findings of other studies.[10,26] Chinese families are accustomed to plunge their chopsticks into shared dishes and some parents even chew food for their infants. These habits may contribute to the transmission of periodontal microorganisms among family members. Most AgP probands in this study shared the same household with their relatives. Although some of the families did not share the same household when their saliva and subgingival plaque samples were collected, the length of cohabitation was much longer than the time they were apart according to their questionnaire. It has been reported that the clonal stability of *P. gingivalis* from most subjects was very high over a period of 8 years under natural conditions (the subjects did not receive periodontal treatment).[27] Another study reported that, irrespective of periodontal treatment, colonization by the same *A. actinomycetemcomitans* strain was remarkably stable within the individuals.[28] Rasiah also found that, using denaturing gradient gel electrophoresis (DGGE) method over a period of 7 years within one individual, the bacterial composition of saliva was relatively stable.[29] All these results make our study reasonable, the participants recruited in this study likewise did not receive any periodontal therapy within the previous 6 months.

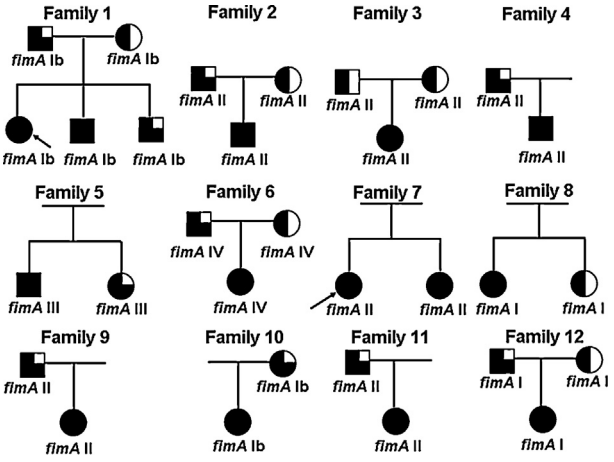


**Fig. 1 – The distribution of *fimA* genotype of *P. gingivalis* in 12 typical families. *FimA* genotype was marked for each individual. Squares designate males and circles designate females. The proband is indicated with an arrow in families which have more than one AgP affected. ● ■, AgP affected; ◔ ◪, relatives affected by severe periodontitis; ◧ ◐, relatives affected by moderate periodontitis.**

*A. actinomycetemcomitans* has long been considered a major etiologic agent of AgP patients.[30,31] Although the prevalence of *A. actinomycetemcomitans* in this population was relatively low compared with that of other microorganisms, *A. actinomycetemcomitans* was detected more frequently in probands whose relatives were positive for *A. actinomycetemcomitans* than in probands whose relatives were not, and Kappa statistical analysis revealed that the detection of *A. actinomycetemcomitans* in AgP probands was highly consistent with that in their relatives. These results suggested the intra-familial transmission of *A. actinomycetemcomitans* between Chinese AgP patients and their family members. However, a limitation of our study is that we did not type the bacterial isolates of *A. actinomycetemcomitans*. Using arbitrarily primed-PCR to genotype *A. actinomycetemcomitans* isolates from family members, Asikainen found that 11 of 12 families had identical genotypes among family members.[32] Dogan found that the AgP probands in a Turkish population shared *A. actinomycetemcomitans* clonal types with their parents in five of six (83%) families and with their siblings in three of six (50%) families.[33] Similar conclusions were drawn from other studies.[34–36] In contrast to this finding, a comparison of the PCR-generated amplitypes of *A. actinomycetemcomitans* has shown that there is a wide distribution of amplitypes among probands and their immediate relatives.[37] However, identical genotypes in family members are not 100% proof of transmission, as finding identical genotypes might occur by chance. So Dogan et al.[33] additionally exploited the *A. actinomycetemcomitans* serotype–genotype distribution in a group of unrelated Turkish individuals to evaluate the probability of finding identical clonal types of *A. actinomycetemcomitans* in family members by chance alone, and the results indicated that the probability was very low.

In the present study, kappa statistical analysis also revealed that the prevalence of *F. nucleatum* in probands was highly consistent with that in their relatives (Table 5). This finding captured our attention because *F. nucleatum* in dental plaque plays a significant role in biofilm formation

and maturation, and it is the major co-aggregation bridging organism that links early colonizing commensals and late pathogenic colonizers in dental biofilms. Diaz found that *F. nucleatum* was able to support the growth of *P. gingivalis* in aerated and $CO_2$-depleted environments in which *P. gingivalis*, as a monoculture, was not able to survive.[38] *F. nucleatum* is a type of bacteria in the orange complex as described by Socransky, and orange complex bacteria are closely related to red complex bacteria (*P. gingivalis*, *T. forsythia*, *T. denticola*), and the members of the red complex are rarely found in the absence of members of the orange complex.[39] Consequently, if *F. nucleatum* is easily transmitted among family members, the family member will be susceptible to infection by red complex bacteria. It has also been reported that the average number of bacterial species in plaque samples harbouring *F. nucleatum/periodonticum* was significantly greater than in those without them in both children and their mothers,[16] which implies that *F. nucleatum* and *F. periodonticum* have important roles in biofilm formation. We found that the number of bacterial species in probands was positively correlated to the number in their relatives, which is consistent with a study of periodontitis-free Japanese children and their mothers.[15]

The present results showed that *P. gingivalis* was one of the most frequently detected species both in AgP probands and in their relatives. *P. gingivalis* is suspected of participating in AgP patients of certain populations.[25,40] A study based on an Indonesian population found identical *P. gingivalis* genotypes among siblings in 6 of the 13 families (46%), but none of the 13 couples shared an identical *P. gingivalis* genotype.[41] Van Winkelhoff's observations also showed that transmission of *P. gingivalis* occurred frequently among siblings but not among spouses.[27] However, other investigators, using different bacteria typing methods, have found that horizontal transmission (an individual infects unrelated individual) of *P. gingivalis* between spouses existed.[42,43]

Fimbriae was thought to play an important role in the colonization and invasion of periodontal tissues by *P. gingivalis*.[44] The *fimA* gene, encoding fimbrillin (a subunit protein of fimbriae), has been classified into six genotypes based on the nucleotide sequences (types I, Ib, II, III, IV and V).[45] It was found in our study that type II was the most prevalent *fimA* genotype both in AgP probands and their relatives. Type II of *fimA* binds to epithelial cells most efficiently through specific host receptors compared with other *fimA* genotypes.[24] In a study from Japan, it was suggested that type II of *fimA* may be an important factor in transmission of *P. gingivalis* between spouses because the proportion of type II from couples with probable intrafamilial transmission (the couples shared the same pulsed field gel electrophoresis patterns) was significantly higher than in couples without evidence of transmission.[42] It was found in the present study that most couples (parents of AgP probands) shared identical *fimA* genotype, which supported the transmission of *P. gingivalis* between spouses who has shared the same household for a long time (the minimum time of cohabitation was 12 years). Our observations also indicated that most probands (more than 55%) shared the identical *fimA* genotype with their relatives. These results suggest that *P. gingivalis* could be transmitted between AgP probands and their relatives. In this study, the prevalence of type I in probands was significantly lower than that in their relatives. This phenomenon could be attributed to deeper PD in AgP probands than in their relatives. Amano found that type I of *fimA* was detected more frequently in shallow pockets.[46]

In summary, the findings of this study indicated that intrafamilial transmission of periodontal microorganisms may occur between Chinese patients with AgP and their relatives. So the relatives of the AgP patients who lived in close contact with them were suggested to get involved in the treatment plan of the patients to achieve a long-term effect.

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## Competing interests

None declared.

## Ethical approval

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