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Clonal stability of *Porphyromonas gingivalis* in untreated periodontitis

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Abstract

Objectives: The objective of the present investigation was to study the clonal stability of *Porphyromonas gingivalis* in a population of Indonesian subjects, deprived of dental care and with varying degrees of periodontitis over a period of 8 years.

Material and Methods: In 1994, 105 subjects and in 2002, 103 subjects were *P. gingivalis* culture positive. Multiple isolates from each of these subjects were used for amplified fragment length polymorphism (AFLP) typing.

Results: Sixty-six individuals were *P. gingivalis* culture positive at both time points. In 31 subjects (47%) an exact identical *P. gingivalis* genotype distribution was found in 1994 and in 2002. In 26 of these subjects one genotype, in eight subjects two identical genotypes were found at both time points. In 70% of the subjects at least one *P. gingivalis* genotype was found in 1994 and 2002, whereas other genotypes were either newly detected or were no longer detectable. Identical genotypes were found in 26% of the sibships. Clonal stability in siblings was 39%. Horizontal transmission of *P. gingivalis* was only found in 2002 and was low (11%). In total, 56 *P. gingivalis* genotypes were identified in 1994 and 61 in 2002. Twenty-four appeared unique, whereas other genotypes were found in multiple subjects within as well as without families. One genotype occurred in 11 different subjects.

Conclusions: The clonal stability of *P. gingivalis* under natural conditions is high. Complete different genotype distribution was found in only 27% of the subjects. Transmission of *P. gingivalis* occurred frequently among siblings but not among spouses.

Key words: clonal stability; *P. gingivalis*; periodontitis; transmission

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Porphyromonas gingivalis is a gram-negative strict anaerobic coccobacillus that occurs in the human oral cavity. It is a major pathogen in destructive periodontal diseases in mainly adult patients (Slots & Ting 1999). The organism displays a wide array of virulence factors including capsule formation, endotoxin, fimbriae and a strong proteolytic activity (Holt et al. 1999). Several studies have shown that the prevalence of *P. gingivalis* in periodontally healthy individuals is low and the species may not be commensal to the oral cavity (Griffen et al. 1998, van Winkelhoff et al. 2002, Boutaga et al. 2003). The genetic diversity of the species in populations is high and an association between clonal type and disease type or disease severity has not been found (Loos et al. 1993, Menard & Mouton 1995). Caucasian subjects usually harbour one *P. gingivalis* clonal type (Loos et al. 1992, Saarela et al. 1993a, 1996, van Steenbergen et al. 1993) but multiple clones per subject have also been reported (Enersen et al. 2006). Recently, van Winkelhoff et al. (2007) reported on multiple clonal types in 34.5% of the subjects in an Indonesian population with varying degrees of periodontal disease. Identical clonal types were observed between relatives indicating that person-to-person transmission within family units occurs (for review see van Winkelhoff & Boutaga 2005). Intra-family distribution of *P. gingivalis* has been studied by Tuite-McDonnel et al. (1997) and they estimated that the relative risk of colonization by a spouse is 4 (CI 95% 2.3–7.0) if a husband or wife is colonized by *P. gingivalis*. The relative risk for children to obtain *P. gingivalis* was

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estimated 4.7 (CI 95% 3.1–7.0) when the mother was *P. gingivalis* positive and 3.0 (CI 95% 2.0–4.5) when the father was *P. gingivalis* positive. In children, *P. gingivalis* may colonize only transiently with increasing colonization stability at older age (Lamell et al. 2000). Also in adults a considerable volatility in acquisition and loss of *P. gingivalis* has been described (Cullinan et al. 2003). No information on clonal stability (colonization by one clonal type over a considerable length of time) of *P. gingivalis* is available.

In our ongoing longitudinal study ''Java Project on Periodontal Diseases'', we studied the natural development and progression of periodontal disease in a population that is deprived of dental care (van der Velden et al. 2006). Microbiological analysis of the subgingival plaque has been performed in 1994 and 2002 and has provided *P. gingivalis* isolates from different time points spanning a period of 8 years.

The aim of the present study was to investigate clonal stability of *P. gingivalis* genotypes over a period of 8 years under natural conditions i.e. in subjects without dental interventions. We also studied distribution of *P. gingivalis* genotypes within family units and between couples.

Material and Methods

Microbiological samples were obtained from subjects participating in a longitudinal, prospective study and living in a village with approximately 2,000 inhabitants at the Malabar/Poerbasari tea estate on Western Java, Indonesia. This population was selected because it had not received regular dental care and had not been exposed to preventive dental care programs. If necessary, tooth extraction was performed by a general physician. Therefore, this population was suitable for study of the natural development and progression of periodontitis. The population consisted mostly of tea labourers that received basic medical care and were employed by a government-owned tea estate, PTP XIII. The study population of this ongoing investigation has been described in detail by van der Velden et al. (2006). In 1994, 158 (69 males, 89 females) and in 2002, 123 subjects of the initial population of 255 subjects in 1987 were available for microbiological evaluation. In these subjects, the deepest bleeding site with the greatest amount of attachment loss in each quadrant of the dentition was selected for sampling with two sterile paper points. Pooled samples were cultured and analysed for the presence and levels of *P. gingivalis*.

Microbiological procedures

The pooled subgingival plaque samples were vortexed for 30 s and 10-fold serial dilutions were prepared in sterile saline. Aliquots of 100 μL were plated on 5% sheep blood agar plates (Oxoid no. 2, Oxoid Ltd., Basingstoke, UK), supplemented with haemin (5 mg/l) and menadione (1 mg/l) and anaerobically incubated (BBL Gaspak Anaerobic System, Beckton, Dickinson and Company, Sparks, MD, USA) at 37°C for up to 14 days. The total colony forming units were determined and dark-pigmented colonies were streaked to purity and identified using standard techniques (van Winkelhoff et al. 2002). One to four *P. gingivalis* isolates per subject were kept on beads at -80°C until use.

Amplified fragment length polymorphism typing

Bacterial strains were anaerobically grown as described above. Cells were suspended to a density of 0.5–1.0 McFarland in 2.5 ml TE 1-buffer [1 M Tris, 0.1 M EDTA (pH 8.0)]. Hundred microlitres of this bacterial suspension was used for automated DNA extraction and purification with the MagNA Pure DNA Isolation kit III (Bacteria, Fungi, Roche Molecular Diagnostics, Almere, The Netherlands). The protocol included 1 h of pre-treatment with proteinase K (20 mg/ml) and lysis buffer (Bacteria, Fungi, Roche Molecular Diagnostics) at 56°C. After isolation, the DNA was eluted in 100 μL elution buffer (Bacteria, Fungi, Roche Molecular Diagnostics) and stored at -20°C till use (Boutaga et al. 2003).

AFLP typing is based on the procedure as described earlier (Enersen et al. 2006, Tuite-McDonnel et al. 1997). Five microlitres of DNA was added to 5 μl of restriction-ligation reaction mixture containing 1 × T4 DNA ligase buffer (New England Biolabs, Beverly Hills, MA, USA), 0.05 M NaCl, 1 mg/ml of bovine serum albumin (New England Biolabs), 5 pmol of the *Pst*I adapter (Eurogentec, Ovree, Belgium), 20 pmol of the *Mse*I adapter (Eurogentec), 80 U of T_4 DNA ligase (New England Biolabs), 0.5 U of *Pst*I (New England Biolabs), 2 U of *Mse*I (New England Biolabs). This mixture was incubated for 3 h at 37°C. After incubation, the restriction-ligation reaction was diluted 1:20 with TE 0.1-buffer pH 8.0.

Five microlitres of the diluted restriction-ligation mixture was added to 5 μL of the AFLP amplification mixture [1 × GeneAmp PCR buffer (Applied Biosystems, IJssel, The Netherlands), 2 mM dNTP's (Promega, Leiden, The Netherlands), 15 mM $MgCl_2$, (Applied Biosystems, IJssel, The Netherlands) 20 ng *PST*-0-FAM primer (Eurogentec), 60 ng *MSE*-C primer (Eurogentec), 1 U AmpliTaq DNA polymerase (Applied Biosystems, IJssel, The Netherlands)]. The mixture was amplified in a GeneAmp PCR System 9700 machine (Perkin Elmer, Groningen, The Netherlands). The amplification conditions were 2 min. at 72°C followed by 12 cycles consisting of 30 s at 94°C, 30 s at 65°C (with this temperature decreasing 0.7°C with each succeeding cycle) and 60 s at 72°C. This sequence was followed by 23 cycles consisting of 30 s at 94°C, 30 s at 56°C and 60 s at 72 °C and a final incubation of 10 min. at 72°C (Cullinan et al. 2003). The product was stored at 4°C.

The samples were prepared for capillary electrophoresis by adding 2.5 μl of the PCR product to 22 μl deionized formamide and 0.5 μl of ROX-labelled GeneScan-500 (Applied Biosystems, Foster City, CA, USA) as an internal standard. The samples were run on an ABI 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA), AFLP patterns were analysed with BioNumerics software version 3.0 (Applied Maths, Sint-Martens-Latum, Belgium). The similarities between normalized AFLP patterns (range 50–500 bp) were calculated with the Pearson correlation and dendrograms were obtained by the unweight pair group method using arithmetic averages (UPGMA) clustering.

Reproducibility

The reproducibility of the AFLP typing method was established by triple testing of five *P. gingivalis* strains. On the basis of the calculated SD a cutoff level of 85% similarity was used to designate isolates as one genotype (Rijnsburger et al. 2007).

Results

The number of subjects, culture positive for *P. gingivalis* in 1994 and in 2002 is summarized in Table 1. In 1994, 105

Table 1. Number of subjects with detectable *P. gingivalis* (Pg+) in 1994 and 2002

1994	2002	Number (%)
$N = 105$ Pg+	$N = 103$ Pg+	
+	–	39 (27.5%)
–	+	37 (26.1%)
+	+	66 (46.5%)

Table 2. Number (%) of subjects with 1, 2 or 3 genotypes of *P. gingivalis* in 1994 and 2002

N gentotypes	1994	2002
1	74 (70.5%)	75 (72.8%)
2	25 (23.8%)	22 (21.4%)
3	6 (5.7%)	5 (4.8%)
Total	105	103

and in 2002, 103 subjects were culture positive for *P. gingivalis*. A considerable volatility of acquisition and loss of *P. gingivalis* in the population was found; 66 subjects were positive at both time points.

The total number of *P. gingivalis* isolates available for AFLP genotyping amounted to 805, of which 387 were obtained in 1994 and 418 in 2002. The average number of tested isolates per subject was 3.7 in 1994 and 4.0 in 2002. The number of *P. gingivalis* genotypes per subject in 1994 and in 2002 is shown in Table 2 and amounted to a total of 56 different *P. gingivalis* genotypes in 1994 and 61 in 2002. In 1994, 74 of the 105 subjects (70.5%) of the subjects had one genotype, two and three genotypes were found in 25 (23.8%) and six subjects (5.7%), respectively. A similar distribution was observed in 2002. The results of the AFLP genotyping of the *P. gingivalis* isolates of the 66 subjects that were *P. gingivalis* culture positive in 1994 and in 2002 are shown in Table 3. In 31 of the 66 (47%) of the subjects 1 or 2 identical *P. gingivalis* genotypes were detected in 1994 and 2002 (A → A, A+B → A+B). In 46 of the 66 subjects (69.7%) of the subjects at least one identical *P. gingivalis* genotype was detected in 1994 and in 2002. (Table 3: A → A, A → A+B, A+B → A, A+B → A+B, A+B → A+C, A+B+C → A, A+B+C → A+D, A+B+C → A+B+D). In 20 of the 66 subjects (30.3%) a complete different genotype distribution was observed in 2002.

The study population harboured 23 families with two or more siblings with a total of 54 subjects (Table 4). In 1994, in 13 families two or more siblings were culture positive for *P. gingivalis*. In 6/23 sibships (26%) an identical *P. gingivalis* genotype was found in two or more siblings within a family unit, but different families had different *P. gingivalis* genotypes. In 2002, out of the same 23 families also in six sibships (26%) an identical *P. gingivalis* genotype was found among the siblings. In the study population a total of 27 married couples were present. In 1994, in none and in 2002, in 3/27 (11%) of the couples an identical *P. gingivalis* genotype was detected in both spouses (Table 5).

In total, 56 AFLP genotypes were identified in 1994. Twenty-four genotypes of *P. gingivalis* were unique, i.e. occurred in only one study subject. All other 32 genotypes were found in more than one subject. We identified one *P. gingivalis* genotype (#46) that was found in 11 subjects, only two of which were family members. Figure 1 shows the number of subjects sharing a given *P. gingivalis* genotype in the study population. In Fig. 2, 3 examples of transmission within family units, i.e. between siblings and between families, i.e. spouses are shown. In example A, *P. gingivalis* genotype # 41 is spread within family #70 and may be transmitted to family #42 through marriage between subjects #100 and #55, or vice versa. Likewise, in example B, it is shown that *P. gingivalis* genotype #6 is spread with family unit # 87 and may be transmitted to family #63 through marriage between subjects #125 and #128. In example C, *P. gingivalis* genotype # 20 was found both in 1994 and 2002 in subject #154 and probably transmitted by marriage to subjects #155.

Table 3. Distribution of *P. gingivalis* (Pg) genotypes in 1994 and 2002

1994	2002		Change in genotype distribution		
	N		N	1994	2002
1 genotype $N = 74$	27*	Pg not detected			
	37	1 gt†	26	A	= A
			11	A	= B
	8	2 gt	6	A	= A+B
			2	A	= B+C
	2	3 gt	2	A	= B+C +D
2 genotypes $N = 25$	10	Pg not detected			
	7	gt	4	A+B	= A
			3	A+B	= C
	8	2 gt	5	A+B	= A+B
			2	A+B	= A+ C
			1	A+B	= C+D
3 genotypes $N = 6$	2	Pg not detected			
	1	1 gt	1	A+B+C	= A
	1	2 gt	1	A+B+C	= A+D
	1	3 gt	1	A+B+C	= A+B+D
	1	1 gt	1	A+B+C	= D

*Number of subjects.
†Genotype.
The number of *P. gingivalis* positive subjects in 1994 was 105 of which 66 were retrieved in 2002.

Table 4. Number of sibships with 0, 1 or ⩾2 subjects culture positive for *P. gingivalis* in 1994 and 2002 (54 siblings in 23 sibships) and the number (%) of sibships with an identical *P. gingivalis* genotype

	Number of Pg+ subjects in sibships			# (%) sibships with identical Pg genotype
	0	1	⩾2	
1994	1	9	13	6/23 (26%)
2002	1	7	15	6/23 (26%)

Table 5. Number of couples with 0, 1 or 2 subjects culture positive for *P. gingivalis* in 1994 and 2002. (54 subjects in 27 couples) and the number (%) of couples with an identical *P. gingivalis* genotype

	Number of Pg+ subjects in couple			# (%) couples with identical Pg genotype
	0	1	2	
1994	3	11	13	0
2002	3	6	18	3 (11%)

Discussion

The digestive tract is in principle an open system and can be colonized by microorganisms from the environment

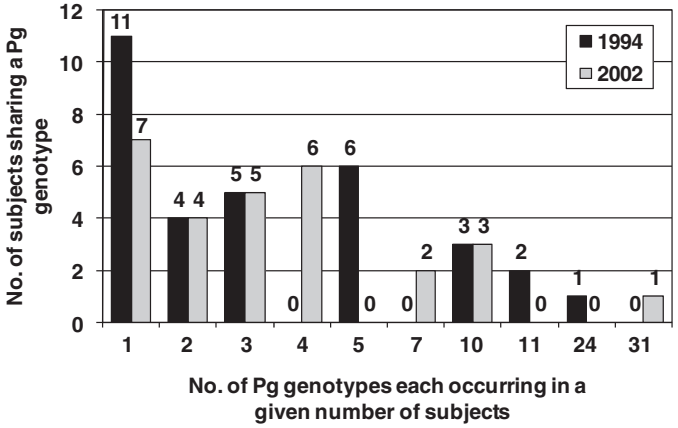

Fig. 1. Distribution of *P. gingivalis* genotypes among subjects in the study population in 1994 and 2002. Note that each of 24 genotypes in 1994 occurred in one single subject whereas one specific genotype was detected in 11 different individuals.

A

2002 Pg genotype#	41		41	16	-		
1994 Pg genotype#	-		-	-	41		
Subject #	**55**	x	**100**	107	117		
Family #	*42*		*70*				

B

2002 Pg genotype#	6	6	-			6		-	
1994 Pg genotype #	-	6	6			-		29	
Subject#	124	163	**125**	x		**128**		157	
Family #		*87*				*63*			

C

2002 Pg genotype#	20		20+28	59	
1994 Pg genotype#	20		-	-	
Subject #	**154**	x	**155**	69	
Family #	*108*		*51*		

Fig. 2. Three examples of transmission of *P. gingivalis* within families units (siblings) and between spouses. Genotype numbers represent distinct *P. gingivalis* genotypes. Subjects are represented by a number. Numbers in italic represent family units, numbers in bold represent spouses that link two families.

including other subjects. The colonization dynamics of the gut has been investigated and it has been found that the *Escherichia coli* population consists of a mixture of transient and persistent clones (Caugant et al. 1981). Population dynamics of oral bacterial species over a significant length of time has poorly been studied. To our knowledge, this is the first study that has focused on clonal stability of *P. gingivalis* under natural conditions, i.e. in subjects without periodontal treatment. We were able to study this phenomenon in 66 individuals representing 46.5% of the study population; 53.5% the study subjects had detectable levels of *P. gingivalis* on one occasion only. This observation indicates a rather high volatility of colonization in this population which is in agreement with previous observations (Lamell et al. 2000, Cullinan et al. 2003). Establishment of the colonization rate of a pathogen in a study population is determined by the detection limit of the technique used. In the present study, we used the culture technique for isolation, growth and AFLP typing of *P. gingivalis*. For the detection of the species approximately 10^4 bacterial cells are required. This means that culture negative subjects may have been colonized by numbers of *P. gingivalis* $< 10^4$. The number of isolates per subject that were tested averaged between 3.7 and 4 which also limit the possibility to detect different genotypes present in low numbers. Loos et al. (1992) suggested, on the basis of a statistical model, that at least 29 isolates from a single pocket must be tested for the lowest true proportion of a one observed clonal type to reach 90% probability that only one clonal type is present (at 95% confidence level). Also, 29 sites must be sampled from an oral cavity if only one clonal type was observed and if one wants to conclude exclusivity of infection. We used a pooled sample of four pockets and genotyped four isolates per subject. Therefore, the conclusions of the present study must be interpreted with caution.

Hohwy et al. (2001) studied the population dynamics of *Streptococcus mitis* on buccal mucosa in young and adult subjects over a period of 9–10 months. In young subjects, a given genotype was found only once whereas adult subjects showed genotype stability more often. This phenomenon was also reported by Lamell et al. (2000) who found a positive relationship between increasing age and persistent colonization with *P. gingivalis*.

Fuijse et al. (2004) studied clonal diversity and stability of *Eikenella corrodens* in non-diseased subjects and in subjects with aggressive periodontitis. They found a positive relationship between disease status and the number of genotypes of the species. Diseased subjects had on average 3.0 and periodontally healthy subjects had on average 1.5 clonal types. They observed a complete change of subgingival *E. corrodens* genotypes types between baseline and at 9 months follow-up examination in 58% of the subjects. This contrasts the observation in the present study; we found 69.7% of the subjects, culture positive in 1994 and 2002, still colonized by a specific *P. gingivalis* genotype eight years after the first examination. This shows a rather stable colonization pattern of *P. gingivalis*. Moreover, in 47% of the subjects that were culture positive at both time points, we found an identical genotype distribution over the 8 years period. This clonal stability may be related to the lack of dental intervention other than extractions in case of severe caries or odontogenic abscesses. We hypothesize that the lack of periodontal intervention explains the high clonal stability by the mechanism of colonization resistance, which is a mechanism of prevention of colonization by exogenous organisms. Also, the subgingival plaque may not be affected by exogenous microorganisms to the same extent as the microflora on oral mucosal surfaces.

Besides clonal stability, transmission was also observed in this study. We found identical *P. gingivalis* genotypes among siblings in 26% of the sibships in 1994 and 2002. This may indicate either transmission from sibling to sibling (horizontal transmission) or vertical transmission from one or both of the parents to their children. The possibility of vertical transmission could unfortunately not be studied because parents of the study subjects were not involved in this investigation.

Transmission between spouses was not observed in 1994. In 2002, however, in three couples identical genotypes were identified which is evidence for horizontal transmission. This seems a low transmission rate in comparison to other studies (Petit et al. 1993, Saarela et al. 1993b). Asikainen et al. (1996) found transmission of *P. gingivalis* in 20% of the couples. Van Steenbergen et al. (1993) isolated *P. gingivalis* from 10 out of eighteen spouses married for 10–27 years with patients that suffered from a *P. gingivalis*-associated periodontitis. Eight couples, both culture positive for *P. gingivalis*, participated in a study on clonal distribution using restriction enzyme analysis technique (REA). An identical *P. gingivalis* was found in six out of the eight couples tested (75%). This observation shows that horizontal transmission of *P. gingivalis* can be as high as 33% (six of 18 couples). The difference between these observations and the results of the present study cannot be explained by difference in typing technique since recently it was shown that the REA and the AFLP technique result in identical conclusions on clonality for *P. gingivalis* (Rijnsburger et al. 2007). A possible explanation for the low transmission rate between spouses in the present study involves the colonization resistance, the lack of dental treatment and possibly different behaviour. On the basis of the present observations, it can be speculated that periodontal treatment may increase the change for horizontal transmission of *P. gingivalis* between spouses. Reducing the sub- and supragingival bacterial load may reduce the colonization resistance of the periodontal microflora, which may increase the change of exogenous microorganisms to colonize and invade the subgingival area.

In the present study, we were able to study the spread of *P.* gingivalis genotypes into the community. We found 24 *P. gingivalis* genotypes that were each detected in one subject only. In contrast, we also found identical *P. gingivalis* clones in more than one subject. The penetration of clones into the study population varied between genotypes. The most obvious example of a relativity high penetration was genotype #46 which was detected in 11 subjects, only two of which were family members. This genotype had obviously spread into the community and had colonized different families. This is to our knowledge the first observation of clonal penetration of *P. gingivalis* into a community outside family units.

In summary, over a period of 8 years, a rather high volatility of colonization by *P. gingivalis* was observed. Clonal stability of *P. gingivalis* in subjects culture positive for this bacterium, was found in the majority of subjects. In addition, transmission of the pathogen between family members was also frequently observed. Clones were identified that were able to spread not only among spouses and siblings penetrate but also to other members of the community. Further studies on the spread of *P. gingivalis* and more information of the route of transmission seem necessary in order to develop future preventive measures for transmission of this periodontal pathogen.

References

Asikainen, S., Chand, C. & Slots, J. (1996) Likelihood of transmitting *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in families with periodontitis. *Oral Microbiology and Immunology* **11**, 387–394.

Boutaga, K., van Winkelhoff, A. J., Vandenbroucke-Grauls, C. M. J. E. & Savelkoul, P. H. M. (2003) Comparison of real-time PCR and culture for detection of *Porphyromonas gingivalis* in subgingival plaque samples. *Journal of Clinical Microbiology* **41**, 4950–4954.

Caugant, D., Levin, A. B. R & Selander, R. K. (1981) Genetic diversity and temporal variation in the *E. coli* population of a human host. *Genetics* **98**, 467–490.

Cullinan, M. P., Hamlet, S. M., Westerman, B., Palmer, M. E., Faddy, M. J. & Seymour, G. J. (2003) Acquisition and loss of *Porphyromonas gingivalis, Actinobacillus actinomytcetemcomitans* and *Prevotella intermedia* over a 5-year period: effect of a triclosan/copolymer dentrifice. *Journal of Clinical Periodontology* **30**, 532–541.

Enersen, M., Olson, I., van Winkelhoff, A. J. & Caugant, D. A. (2006) Multilocus sequence typing of *Porphyromonas gingivalis* strains from different geographic origins. *Journal of Clinical Microbiology* **44**, 35–41.

Fuijse, O., Chen, W., Rich, S. & Chen, C. (2004) Clonal diversity and stability of subgingival *Eikenella corrodens*. *Journal of Clinical Microbiology* **42**, 2036–2042.

Griffen, A. L., Becker, M. R., Lyons, S. R., Moeschberger, M. L. & Leys, E. J. (1998) Prevaland ce of *Porphyromonas gingivalis* and periodontal health status. *Journal of Clinical Microbiology* **36**, 3239–3242.

Hohwy, J., Reinholdt, J. & Kilian, M. (2001) Population dynamics of *Streptococcus mitis* in its natural habitat. *Infection & Immunity* **69**, 6055–6063.

Holt, S. C., Kesavalu, L., Walker, S. & Genco, C. A. (1999) Virulence factors of *Porphyromonas gingivalis*. *Periodontol 2000* **20**, 168–238.

Lamell, C. W., Griffen, A. L., McClellan, D. L. & Leys, E. J. (2000) Acquisition and colonization stability of *Actinobacillus actinomytcetemcomitans* and *Porphyromonas gingivalis* in children. *Journal of Clinical Microbiology* **38**, 1196–1199.

Loos, B. G., Dyer, D. W., Whittam, T. S. & Selander, R. K. (1993) Genetic structure of populations of *Porphyromonas* (*Bacteroides*) *gingivalis* associated with periodontitis and other infections. *Infection & Immunity* **61**, 204–212.

Loos, B. G., van Winkelhoff, A. J., Dunford, R. G., Genco, R. J., de Graaff, J., Dickinson, D. P. & Dyer, D. W. (1992) A statistical approach to the ecology of *Porphyromonas gingivalis*. *Journal of Dental Research* **71**, 353–358.

Menard, C. & Mouton, C. (1995) Clonal diversity of the taxon *Porphyromonas gingivalis* assessed by random amplified polymorphis DNA fingerprinting. *Infection & Immunity* **63**, 2522–2531.

Petit, M. D., van Steenbergen, T. J. M., Scholte, L. M., van der Velden, U. & de Graaff, J. (1993) Epidemiology and transmission of *Porhyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* among children and their family members. A report of 4 surveys. *Journal of Clinical Periodontology* **20**, 641–650.

Rijnsburger, M. C., Delwel, S., Boutaga, K., van Steenbergen, T. J. M., Savelkoul, P. H. M. & van Winkelhoff, A. J. (2007) Comparison of REA and AFLP typing of *Porphyromonas gingivalis* isolated from spouses. *Oral Microbiology & Immunology* **22**, 369–373.

Saarela, M., Mattö, J., Asikainen, S., Jousimies-Somer, H., Torkko, H., Pyhälä, L., Stucki, A. M., Hannula, J. & Hölltä, P. (1996) Clonal diversity of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella nigrescence* in two families. *Anaerobe* **2**, 19–27.

Saarela, M., Stucki, A. M., von Troil-Linden, B., Alaluusua, S., Jousimies-Somer, H. & Asikainen, S. (1993a) Intra-and inter-individual comparison of *Porphyromonas gingivalis* genotypes. *FEMS Immunology and Medical Microbiology* **6**, 99–102.

Saarela, M., von Troil-Linden, B., Torrko, H., Stucki, A. M., Alaluusua, S., Jousimies-Somer, H. & Asikainen, S. (1993b) Transmission

between spouses. *Oral Microbiology and Immunology* **8**, 349–354.

Slots, J. & Ting, M. (1999) *Actinobaccillus actinomycetemcomitans* and *Porhyromonas gingivalis* in human periodontal disease: occurrence and treatment. *Periodontolology 2000* **20**, 82–121.

Tuite-McDonnel, M., Griffen, A. L., Moeschberger, M. L., Dalton, R. E., Fuerst, P. A. & Leys, E. J. (1997) Concordance of *Porphyromonas gingivalis* colonization in families. *Journal of Clinical Microbiology* **35**, 455–461.

van der Velden, U., Abbas, F., Armand, S., Loos, B. G., Timmerman, M. F., van der Weijden, G. A., van Winkelhoff, A. J. & Winkel, E. G. (2006) Java project on periodontal diseases. The natural development of periodontitis: risk factors, risk predictors and risk determinants. *Journal of Clinical Periodontology* **33**, 540–548.

van Steenbergen, T. J. M., Petit, M. D., Scholte, L. H., van der Velden, U. & de Graaff, J. (1993) Transmission of *Porphyromonas gingivalis* between spouses. *Journal of Clinical Periodontology* **20**, 340–345.

van Winkelhoff, A. J. & Boutaga, K. (2005) Transmission of periodontal pathogens and models of infection. *Journal of Clinical Periodontology:* **32** (Suppl. 6), 16–27.

van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. A. & van der Velden, U. (2002) *Porphyromonas gingivalis, Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* **29**, 1023–1028.

Van Winkelhoff, A. J., Rijnsburger, M. C., Abbas, F., Timmernman, M. F., van der Weijden, G. A., Winkel, E. G. & van der Velden, U. (2007) Java project on periodontal diseases. A study on transmission of *Porphyrmonas gingivalis* in a remote Indonesian population. *Journal of Clinical Periodontology* **34**, 480–484.

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Clinical Relevance

Scientific rationale for the study: No information is available on the clonal stability of *Porphyromonas gingivalis* in patients with periodontitis. In a longitudinal study (Java Project on Periodontal Diseases) we have been able to study the clonal stability of *P. gingivalis* in a cohort of subjects with untreated periodontitis over a period of eight years (1994–2002).
Principle findings: A considerable volatility of acquisition and loss of *P. gingivalis* was found; 47% of the subjects were culture positive in 1994 and 2002. In this group clonal stability of at least one *P. gingivalis* genotype was found in 70% of the subjects. In 47% an identical genotype distribution was found. In 26% of the sibships transmission of *P. gingivalis* among siblings was found. Transmission between spouses occurred in 11% of the couples.
Practical implications: Prevention of transmission of *P. gingivalis* among family members may be an effective tool to reduce the odds to develop destructive periodontal diseases.