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Original

Intra-familial distribution of nine putative periodontopathogens in dental plaque samples analyzed by PCR

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Abstract: It is of great importance to understand the distribution of periodontopathogens within family members when considering the risk of periodontitis in children. The purpose of this study was to investigate the distribution of periodontopathogens among family members. We used the polymerase chain reaction method to test 4, 8, and 7 probands with healthy gingiva, gingivitis, and periodontitis, respectively, and their 60 immediate family members. Plaque samples were collected from all erupted teeth sites using a sterile toothbrush. In 161 of the 165 positive cases, if a child harbored one of the periodontopathogens then at least one of the parents was also positive for the same bacterium. The prevalence of parent-child co-infection was 42.9% for *Actinobacillus actinomycetemcomitans*, 21.4% for *Porphyromonas gingivalis*, 29.2% for *Treponema denticola*, 59.5% for *Tannerella forsythensis (Bacteroides forsythus)* and 16.7% for *Prevotella intermedia*. Our results indicate that parents could be an important source of periodontopathogens for the colonization that occurs in their children. (J. Oral Sci. 46, 149-156, 2004)

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Introduction

Periodontal diseases are mainly associated with gram-negative bacteria that initiate a series of events leading to the loss of periodontal attachment and alveolar bone surrounding the tooth (1). Early childhood years are the critical period for the acquisition of certain bacteria, and close household contacts may be the source of acquisition for infants and children. Evidence of periodontopathogens as etiologic agents in periodontitis has primarily been collected from cross-sectional investigations or studies of patients sampled only at times when the diseases were detectable. Therefore, in order to prevent periodontal diseases and be able to evaluate the risk factors, it is necessary to investigate the transmission and distribution of periodontopathogens between children and their family members. Transmission of putative bacteria has been suggested to occur between spouses as well as between parents and children (2-4).

It is of particular interest to investigate the early colonization of children with periodontopathogens following establishment of periodontal diseases, as the number of organisms in a specific site may be low, and their presence could identify a patient as a carrier or being at risk of developing periodontitis in adolescence (5). However, to date, the most convincing data only implicate a few microorganisms, such as *Actinobacillus*

actinomycetemcomitans, *Porphyromonas gingivalis*, and *Prevotella intermedia*, as the etiologic agents in periodontitis.

It is now widely accepted that polymerase chain reaction (PCR) methods provide a more sensitive means of detection of putative bacteria species, as compared to conventional culture techniques (6-8). A PCR assay could also be suitable for the detection of periodontopathogens, especially in cases of subgingival plaque in children where there are a limited number of pathogens present.

The purpose of this study was to investigate the distribution of periodontopathogens among family members by using the PCR method.

Materials and Methods

Study Subjects

Test results for the 79 subjects (19 probands and 60 of their family members) in the present report, came from studies on the prevalence of periodontal diseases and periodontal bacteria (9). Consent for participation was obtained prior to the present study. Subjects with clinically healthy gingiva, along with those with gingivitis and periodontitis, were classified as follows (10). Those with an absence of inflammation, no bleeding on brushing, and no radiographic bone loss at all sites were regarded as the clinically healthy gingiva group. Subjects with mild to moderate inflammation at more than one site, as determined by the gingival index (11), and who had no radiographic bone loss were classified as the gingivitis group. Children and parents with attachment loss of greater than 3 and 5 mm, respectively, from at least one site among four teeth were defined as the periodontitis group. The age range for the children and parents was 1 - 18 years and 32 - 65 years, respectively. There were 4 probands with healthy gingiva, 8 with gingivitis, and 7 with periodontitis. Subjects who had taken antibiotics within the past 3 months were excluded.

Plaque Sampling

Dental plaque was collected with a sterile toothbrush for 1 minute from erupted teeth, as has been previously described (10). Plaque adhering to the toothbrush was removed by washing several times in a tube of sterile distilled water. Thereafter, the plaque samples were immediately transported to our research laboratory and stored at -20°C before the extraction of genomic DNA.

Genomic DNA Preparation

Actinobacillus actinomycetemcomitans ATCC29522^T, *Porphyromonas gingivalis* ATCC33277^T, *Treponema denticola* ATCC33521^T, *Tannerella forsythensis* (*Bacteroides forsythus*) ATCC43037^T, *Prevotella intermedia* ATCC25611^T, *P. nigrescens* ATCC33563^T, *Capnocytophaga gingivalis* ATCC33624^T, *C. sputigena* ATCC33612^T, and *C. ochracea* ATCC27872^T were used as controls. Cells from broth cultures (1.5 to 3 ml), colonies picked up in the logarithmic phase or plaque samples from the subjects were harvested by centrifugation at 1600 $\times$ *g* for 20 minutes. The supernatants were discarded, and individual cell pellets were stored at -20°C without additional preparation until DNA isolation. The genomic DNA preparation from each subject's plaque sample was obtained by a standard miniprep procedure, as previously described (12), to which we added an RNase treatment (13). Concentrations of DNA in the samples from the culture and dental plaque were calculated by measuring the A_{260}, and the quality was estimated by the A_{260}/A_{280} ratio (14). The genomic DNA samples were stored at -20°C before use.

PCR Primers

The bacterial species investigated in this study were selected based on a list of putative periodontopathogens (7,15). The species-specific PCR primers used are shown in Table 1. The primers for eubacteria 16S ribosomal DNA sequence (GenBank accession number M75035) were used to confirm the presence of bacteria in plaque samples as a positive control (16). All primers were purchased from Amersham Pharmacia Biotech, AB, Uppsala, Sweden.

PCR Protocol

For PCR, bacterial DNA from the plaque samples was amplified in a 50 µl reaction mixture containing 50 ng of the DNA sample, 1 $\times$ PCR buffer, 0.2 mM deoxyribonucleotide mixture (Amersham Pharmacia Biotech, AB, Uppsala, Sweden), 50 pmol of each primer, and 2 units of *Taq* DNA polymerase (Amersham Pharmacia Biotech, AB, Uppsala, Sweden) in a thermal cycler (program temp control system PC-700, ASTEC, Fukuoka, Japan).

Each set of PCR analyses included a negative control (water blank) in addition to the positive control. Before the actual PCR cycles, the reaction components without the enzyme were kept at 95°C for 5 minutes to prevent false hybridization and subsequent extension of the primers in the suboptimal temperatures used during the initial heating period (hot-start). The conditions used for PCR amplification of each species and eubacterial 16S rDNA have been previously described (7,15,16). After amplification, 15 µl of the PCR products was analyzed by electrophoresis on a 1.2 - 1.5% agarose gel. After staining with ethidium bromide, the newly synthesized DNA

fragments were visualized under a 302 nm ultraviolet light. The size of the PCR products was estimated from the electrophoretic migration of products relative to a 100 base-ladder marker (Amersham Pharmacia Biotech, AB, Uppsala, Sweden).

Statistical Analysis

The significance of association between occurrence of the studied species in parents and their children was determined by a chi-square test (17).

Results

Clinical and microbiological descriptions of the healthy proband families are shown in Table 2. Two fathers had healthy gingiva, 1 had gingivitis, and 1 had periodontitis, while 2 mothers had healthy gingiva and 2 had gingivitis, and 5 children had healthy gingiva and 3 had gingivitis. Among these 16 individuals that were from 4 families, all were negative for *A. actinomycetemcomitans*. We detected *P. gingivalis*, *T. denticola*, and *P. intermedia* in 5 parents, 2 fathers, and 3 parents, respectively, although these were not found in any children aged 3 to 13 years. Of the healthy proband families, 7, 6, 8, and 1 of the parents, and 2, 5, 7, and 1 of the children were positive for *T. forsythensis* (*B. forsythus*), *P. nigrescens*, *C. gingivalis*, and *C. sputigena*, respectively, while all members were positive for *C. ochracea.* No child was found to be positive for a tested species that was negative in his/her parents.

Table 3 shows clinical and microbiological descriptions of the 8 families (16 parents and 19 children total) with a proband with gingivitis. Among them, the parents and proband of a single family were found positive for *A. actinomycetemcomitans*. Further, 8 fathers, 2 mothers, and 1 child were positive for *P. gingivalis*, 4 fathers, 3

Table 1 PCR primer sets used in this study

Primer sets (5'-3')	Size* (bp)	Reference
Actinobacillus actinomycetemcomitans AAA CCC ATC TCT GAG TTC TTC TTC ATG CCA ACT TGA CGT TAA AT	557	Ashimoto, *et al.*, 1996
Porphyromonas gingivalis AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	404	Ashimoto, *et al.*, 1996
Treponema denticola TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	311	Ashimoto, *et al.*, 1996
Tannerella forsythensis GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	641	Ashimoto, *et al.*, 1996
Prevotella intermedia TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	575	Ashimoto, *et al.*, 1996
Prevotella nigrescens ATG AAA CAA AGG TTT TCC GGT AAG CCC ACG TCT CTG TGG GCT GCG A	804	Ashimoto, *et al.*, 1996
Capnocytophaga gingivalis AGA GTT TGA TCC TGG CTC AG GGA CGC ATG CCC ATC TTT CAC CAC CGC	227	Conrads, *et al.*,1996
Capnocytophaga sputigena AGA GTT TGA TCC TGG CTC AG GAT GCC GCT CCT ATA TAC CAT TAG G	185	Conrads, *et al.*,1996
Capnocytophaga ochracea AGA GTT TGA TCC TGG CTC AG GAT GCC GTC CCT ATA TAC TAT GGG G	185	Conrads, *et al.*,1996
Universal primers for positive control CAG GAT TAG ATA CCC TGG TAG TCC ACG C GAC GGG CGG TGT GTA CAA GGC CCG GGA ACG	625	Goncharoff, *et al.*, 1993

* Expected size of PCR products.

Table 2 Clinical and microbiological descriptions of the healthy proband families

Family	Member	Sex	Age (years)	Dentition	Periodontal condition	Positive or negative								
						Aa	Pg	Td	Tf	Pi	Pn	Cg	Cs	Co
1	Proband	M	5	Mixed	Healthy	-	-	-	-	-	-	+	-	+
	Father	M	41	Permanent	Periodontitis	-	+	+	+	-	-	+	-	+
	Mother	F	46	Permanent	Gingivitis	-	+	-	-	-	-	+	-	+
2	Proband	M	5	Primary	Healthy	-	-	-	-	-	+	-	-	+
	Father	M	43	Permanent	Healthy	-	-	-	+	-	+	+	+	+
	Mother	F	41	Permanent	Healthy	-	+	-	+	-	+	+	-	+
	Triplets	M	5	Primary	Gingivitis	-	-	-	-	-	+	+	-	+
	Triplets	M	5	Primary	Healthy	-	-	-	-	-	+	+	-	+
3	Proband	F	3	Primary	Healthy	-	-	-	-	-	-	+	-	+
	Father	M	42	Permanent	Gingivitis	-	+	-	+	+	+	+	+	+
	Mother	F	41	Permanent	Gingivitis	-	+	-	+	+	+	+	+	+
	Sister	F	12	Permanent	Gingivitis	-	-	-	+	-	+	+	-	+
4	Proband	M	13	Permanent	Healthy	-	-	-	+	-	+	+	-	+
	Father	M	41	Permanent	Healthy	-	-	+	+	+	+	+	+	+
	Mother	F	40	Permanent	Healthy	-	-	-	+	-	+	+	-	+
	Brother	M	8	Mixed	Gingivitis	-	-	-	-	-	-	+	+	+

+: positive; -: negative

mothers, and 4 children were positive for *T. denticola*, 15 parents and 14 children were positive for *T. forsythensis*, 6 parents and 3 children were positive for *P. intermedia*, 13 parents and 17 children were positive for *P. nigrescens*, 16 parents and 18 children were positive for *C. gingivalis*, and 9 parents and 7 children were positive for *C. sputigena*, while all were found positive for *C. ochracea*. Three children were positive for *C. sputigena* and one child was positive for *P. intermedia*, even though those bacteria were not found in their parents.

Table 4 shows clinical and microbiological descriptions of the 7 periodontitis proband families (14 parents and 14 children total). Among them, 1 father, 1 mother, and 2 children were found positive for *A. actinomycetemcomitans*, 5 fathers, 5 mothers, and 5 children were positive for *P. gingivalis*, 7 parents and 3 children were positive for *T. denticola*, 13 parents and 8 children were positive for *T. forsythensis*, 7 parents and 2 children were positive for *P. intermedia*, 12 parents and 11 children were positive for *P. nigrescens*, 13 parents and 11 children were positive for *C. gingivalis*, 8 parents and 4 children were positive for *C. sputigena*, and 14 parents and 12 children were positive for *C. ochracea*. No child was found positive for a tested species that was negative in his/her parents. In 161 of the 165 positive cases, if a child harbored one of the periodontopathogens, then at least one of the parents was also positive for that bacterium.

Table 5 shows the percent distribution of periodontopathogens tested in families with healthy gingiva, as well as those with a proband with gingivitis or periodontitis. *A. actinomycetemcomitans* was not found in any individual from a healthy proband family. Further, *P. gingivalis*, *T. denticola*, and *P. intermedia* were not found in any of the children tested. The prevalence of children in gingivitis and

Table 3 Clinical and microbiological descriptions of the gingivitis proband families

Family	Member	Sex	Age (years)	Dentition	Periodontal condition	Positive or negative								
						Aa	Pg	Td	Tf	Pi	Pn	Cg	Cs	Co
5	Proband	F	5	Primary	Gingivitis	-	-	-	-	-	-	+	+	+
	Father	M	41	Permanent	Healthy	-	-	-	-	-	-	+	-	+
	Mother	F	39	Permanent	Gingivitis	-	-	-	+	-	+	+	-	+
	Brother	M	9	Mixed	Gingivitis	-	-	-	+	-	-	+	+	+
6	Proband	M	9	Mixed	Gingivitis	-	+	+	+	+	+	+	-	+
	Father	M	41	Permanent	Gingivitis	-	+	+	+	-	+	+	+	+
	Mother	F	36	Permanent	Periodontitis	-	-	-	+	-	+	+	-	+
	Brother	M	8	Mixed	Gingivitis	-	-	-	+	-	+	+	-	+
7	Proband	M	3	Primary	Gingivitis	-	-	-	-	-	+	+	+	+
	Father	M	44	Permanent	Healthy	-	+	-	+	+	+	+	-	+
	Mother	F	42	Permanent	Periodontitis	-	+	-	+	+	+	+	-	+
	Brother	M	15	Permanent	Gingivitis	-	-	-	+	+	+	+	-	+
	Sister	F	12	Permanent	Gingivitis	-	-	-	+	-	+	+	-	+
	Brother	M	8	Mixed	Gingivitis	-	-	-	+	-	+	+	-	+
	Brother	M	1	Primary	Gingivitis	-	-	-	-	-	+	+	-	+
8	Proband	M	10	Mixed	Gingivitis	+	-	-	-	-	+	+	+	+
	Father	M	53	Permanent	Gingivitis	+	+	+	+	-	+	+	+	+
	Mother	F	38	Permanent	Periodontitis	+	+	+	+	-	-	+	+	+
	Brother	M	18	Permanent	Gingivitis	-	-	-	+	-	+	+	-	+
	Brother	M	16	Permanent	Gingivitis	-	-	+	+	-	+	+	-	+
9	Proband	F	10	Mixed	Gingivitis	-	-	+	-	-	+	+	+	+
	Father	M	39	Permanent	Healthy	-	-	+	+	+	-	+	+	+
	Mother	F	40	Permanent	Healthy	-	-	+	+	+	+	+	+	+
	Brother	M	8	Mixed	Gingivitis	-	-	+	+	-	+	+	-	+
10	Proband	F	10	Mixed	Gingivitis	-	-	-	+	-	+	-	-	+
	Father	M	40	Permanent	Healthy	-	-	+	+	-	+	+	-	+
	Mother	F	36	Permanent	Periodontitis	-	-	+	+	+	+	+	+	+
	Sister	F	7	Mixed	Gingivitis	-	-	-	+	-	+	+	-	+
11	Proband	F	8	Mixed	Gingivitis	-	-	-	+	-	+	+	+	+
	Father	M	38	Permanent	Gingivitis	-	-	-	+	-	+	+	+	+
	Mother	F	38	Permanent	Gingivitis	-	-	-	+	-	+	+	-	+
	Sister	F	5	Primary	Gingivitis	-	-	-	+	-	+	+	-	+
12	Proband	F	7	Mixed	Gingivitis	-	-	-	+	+	+	+	+	+
	Father	M	44	Permanent	Healthy	-	-	-	+	-	+	+	+	+
	Mother	F	37	Permanent	Gingivitis	-	-	-	+	+	+	+	+	+

+: positive; -: negative

Table 4 Clinical and microbiological descriptions of the periodontitis proband families

Family	Member	Sex	Age (years)	Dentition	Periodontal condition	Positive or negative								
						Aa	Pg	Td	Tf	Pi	Pn	Cg	Cs	Co
13	Proband	F	6	Primary	Periodontitis	-	+	+	+	+	+	-	-	+
	Father	M	32	Permanent	Periodontitis	-	+	+	+	+	+	+	-	+
	Mother	F	34	Permanent	Gingivitis	-	-	-	-	-	-	+	-	+
	Brother	M	9	Mixed	Gingivitis	-	+	+	+	-	+	+	-	+
	Brother	M	8	Mixed	Gingivitis	-	+	+	-	-	+	-	-	+
14	Proband	M	13	Permanent	Periodontitis	-	+	-	+	-	+	+	-	+
	Father	M	50	Permanent	Gingivitis	-	+	-	+	-	+	+	+	+
	Mother	F	45	Permanent	Gingivitis	-	+	+	+	-	+	+	-	+
	Brother	M	11	Permanent	Healthy	-	-	-	+	-	+	+	-	+
15	Proband	F	11	Mixed	Periodontitis	-	-	-	+	-	+	+	-	-
	Father	M	43	Permanent	Gingivitis	-	+	+	+	+	+	-	+	+
	Mother	F	37	Permanent	Periodontitis	-	+	-	+	+	+	+	-	+
	Sister	F	15	Permanent	Gingivitis	-	-	-	-	-	-	+	-	+
16	Proband	F	9	Mixed	Periodontitis	-	-	-	+	-	+	+	-	+
	Father	M	36	Permanent	Gingivitis	-	-	+	+	+	+	+	-	+
	Mother	F	36	Permanent	Periodontitis	-	+	-	+	-	+	+	+	+
	Brother	M	7	Mixed	Gingivitis	-	-	-	+	-	+	+	-	+
17	Proband	M	6	Mixed	Periodontitis	-	-	-	-	-	-	+	+	+
	Father	M	43	Permanent	Gingivitis	-	-	-	+	-	+	+	-	+
	Mother	F	42	Permanent	Periodontitis	+	-	-	+	-	+	+	+	+
	Sister	F	5	Primary	Periodontitis	-	-	-	-	-	-	-	-	-
18	Proband	M	6	Mixed	Periodontitis	-	-	-	+	-	+	+	+	+
	Father	F	65	Permanent	Gingivitis	-	+	+	+	-	+	+	+	+
	Mother	F	35	Permanent	Gingivitis	-	+	-	+	+	+	+	+	+
19	Proband	M	7	Mixed	Periodontitis	+	+	-	-	+	+	+	+	+
	Father	M	53	Permanent	Gingivitis	+	+	+	+	+	-	+	+	+
	Mother	F	50	Permanent	Periodontitis	-	+	+	+	+	+	+	+	+
	Brother	M	5	Mixed	Gingivitis	+	-	-	-	-	+	+	+	+

+: positive; -: negative

periodontitis proband families, respectively, was 5.3% and 14.3% for *A. actinomycetemcomitans*, 5.3% and 35.7% for *P. gingivalis*, 21.1% and 21.4% for *T. denticola*, and 15.8% and 14.3% for *P. intermedia*. The prevalence of children for *T. forsythensis* and *C. sputigena* in the diseased proband families was higher than that in healthy proband families, while *P. nigrescens*, *C. gingivalis*, and *C. ochracea* were commonly present in both parents and children.

Table 6 shows the occurrence of *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythensis*, and *P. intermedia* among family members. The percentage of positive children in which either the father or mother was positive was 42.9% for *A. actinomycetemcomitans*, 21.4% for *P. gingivalis*, 29.2% for *T. denticola*, 59.5% for *T. forsythensis*, and 16.7% for *P. intermedia*. There was a significant association between the occurrence of *A. actinomycetemcomitans* ($P < 0.0001$) and *T. denticola* ($P = 0.014$) among parents and their children.

Discussion

The toothbrushing sampling method used in this study collected supragingival plaque and subgingival plaque, though it may not be appropriate for detection of periodontal pathogens. The majority of recent studies have focused on the composition of subgingival plaque (18-20), however, the role of supragingival plaque in oral microbial ecology and the initiation of periodontal diseases are less clear. Recently, some researchers have found that putative periodontal pathogens can be harbored in supragingival plaque, suggesting a possible role for this environment as a reservoir for the pathogens that can lead to their spread into or reinfection of subgingival sites (21-23). Therefore, it is of great importance to be able to detect the presence of putative periodontal pathogens in both supragingival and subgingival plaque samples from subjects so that the risk of periodontal disease development can be determined. Moreover, our analyses using 16S rDNA primers confirmed the presence of bacteria in all of the plaque samples (data

Table 5 Distribution of 9 putative periodontopathogens in the families with a healthy gingiva, gingivitis, or periodontitis proband

		Number of subjects	Percent of positive subjects								
			Aa	Pg	Td	Pi	Cs	Tf	Pn	Cg	Co
Healthy proband family	Father	(N=4)	0.0	50.0	50.0	50.0	75.0	100.0	75.0	100.0	100.0
	Mother	(N=4)	0.0	75.0	0.0	25.0	25.0	75.0	75.0	100.0	100.0
	Children	(N=8)	0.0	0.0	0.0	0.0	12.5	25.0	62.5	87.5	100.0
Gingivitis proband family	Father	(N=8)	12.5	37.5	50.0	25.0	62.5	87.5	75.0	100.0	100.0
	Mother	(N=8)	12.5	25.0	37.5	50.0	50.0	100.0	87.5	100.0	100.0
	Children	(N=19)	5.3	5.3	21.1	15.8	36.8	73.7	89.5	94.7	100.0
Periodontitis proband family	Father	(N=7)	14.3	71.4	71.4	57.1	57.1	100.0	85.7	85.7	100.0
	Mother	(N=7)	14.3	71.4	28.6	42.9	57.1	85.7	85.7	100.0	100.0
	Children	(N=14)	14.3	35.7	21.4	14.3	28.6	57.1	78.6	78.6	85.7

not shown).

The concordance of colonization within families was examined to assess the frequency of transmission of 9 putative periodontopathogens. In all except 4 cases (97.6%), when a child harbored 1 of the 9 putative periodontopathogens, at least 1 of the parents was also positive for that bacterium. We found some positive children with negative parents for a few bacteria and this might have been due to infection from an extra-familial source. The high detection sensitivity of the PCR method employed allowed us to evaluate the probability of bacterial transmission from parents to children, even though the bacterial strain genotypes were not identical in this study.

Overall in the present study, parent-child concordance for *A. actinomycetemcomitans*, *T. denticola*, and *P. nigrescens* was more evident within the families than that seen for *P. gingivalis* and *P. intermedia*, which were not frequently found to be transmitted from parent to child. In previous arbitrarily primed PCR (AP-PCR) analyses, children most often carried an amplitype for *A. actinomycetemcomitans* identical to one of their parents (24). Similar results from restriction endonuclease analyses were also reported for adult periodontitis parents and their children with regard to *A. actinomycetemcomitans* and *P. gingivalis* (2). In contrast, AP-PCR results indicate there was parent-child transmission of *A. actinomycetemcomitans* in 6/19 (32%) families while *P. gingivalis* was not found to have been transmitted from parent to child in any of the study subjects (25). Furthermore, *A. actinomycetemcomitans* and *P. gingivalis* were rarely present in the oral cavities of healthy children aged 2 to 12 years (10). In another report, *P. intermedia* was found to be more associated with periodontal diseases (9). Using PCR ribotyping, *P. intermedia* and *P. nigrescens* of the same subtype were shown to be able to colonize spouses or

Table 6 Occurrence of *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythensis* and *P. intermedia* in family members

A. actinomycetemcomitans	Child			Co-infection ratio (%)	χ^2
	+	-	Total		*P* value
Father$^+$ or Mother$^+$	3	4	7	42.9	15.72
Father$^-$ and Mother$^-$	0	34	34	-	*P*<0.0001
P. gingivalis	Child			Co-infection ratio (%)	χ^2
	+	-	Total		*P* value
Father$^+$ or Mother$^+$	6	22	28	21.4	3.26
Father$^-$ and Mother$^-$	0	13	13	-	*P*=0.071
T. denticola	Child			Co-infection ratio (%)	χ^2
	+	-	Total		*P* value
Father$^+$ or Mother$^+$	7	17	24	29.2	5.98
Father$^-$ and Mother$^-$	0	17	17	-	*P*=0.014
T. forsythensis	Child			Co-infection ratio (%)	χ^2
	+	-	Total		*P* value
Father$^+$ or Mother$^+$	24	17	41	59.5	NA
Father$^-$ and Mother$^-$	0	0	0	-	NA
P. intermedia	Child			Co-infection ratio (%)	χ^2
	+	-	Total		*P* value
Father$^+$ or Mother$^+$	4	20	24	16.7	1.08
Father$^-$ and Mother$^-$	1	16	17	-	*P*=0.299

+: positive, -: negative, NA: not available

parents and children with gingivitis (26). Moreover, there are still other reports that are compatible with these previous studies (3,4).

It has been suggested that when trying to determine the risk of periodontal disease in children, parents could be an important source of the periodontopathogens. In the present study, regardless of the periodontal condition of the subjects, *P. nigrescens*, *C. gingivalis*, and *C. ochracea* were found to have been established earlier in the oral cavity of children due to their high prevalence in their respective parent. Also, *T. forsythensis* was moderately transmittable within families, whereas *P. gingivalis*, *T. denticola*, and *P. intermedia* were rarely transmittable from parents to periodontal-healthy children. In the healthy proband families, the prevalence of *P. gingivalis* was relatively

high in fathers (60.0%) and mothers (75.0%), although no child was found to be positive for this bacterium or *T. denticola* and *P. intermedia*. It was also noted that as periodontal conditions worsened, periodontopathogens were more frequently transmitted. In the periodontitis proband families, the prevalence of *P. gingivalis* was 71.4% in both fathers and mothers, and 35.7% in children. It has been shown that there are disease-associated as well as non-disease-associated strains of *P. gingivalis*, and that their infectious traits that influence periodontal health status could perhaps be differentiated based on the clonal variation of *fimA* genes (27). In addition, individuals who harbor putative pathogens frequently do not manifest signs of periodontal disease, which might be attributed to host defenses, bacterial antagonism, and a possible lack of pathogenicity by the infectious organisms (28).

Children with healthy gingiva are likely to have parents with healthy gingiva, as compared to those with periodontal disease. A family history may be informative and help clinicians to select the appropriate therapy for children with periodontitis. As a result, clinicians who treat children should be aware of the familial periodontal condition, associated microbiota, host response, and risk factors, in order to facilitate an early diagnosis (29). Finally, identification of the route of transmission could be helpful, since the risk of developing periodontal diseases may be increased.

Although the number of subjects in the present study was limited, we believe that our results confirm that periodontal bacteria are likely to be transmitted from parents to children. To avoid oral colonization by periodontopathogens in children, parents should be examined for the presence of the organisms, and, if detected, undertake an appropriate preventive program to eliminate them (25). However, a future longitudinal study is required in order to be able to fully comprehend and establish the chronology of the infection and the probable direction of pathogen transfer.

In conclusion, the present study results indicate that parents could be an important source of the periodontopathogens that are found to orally colonize in their children.

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References

1. Zambon JJ (1994) Familial transmission of periodontal pathogens as a risk factor for periodontal disease progression. Compendium 15, 996-1000
2. Petit MDA, van Steenbergen TJM, Timmerman MF, de Graaff J, van der Velden U (1994) Prevalence of periodontitis and suspected periodontal pathogens in families of adult periodontitis patients. J Clin Periodontol 21, 76-85
3. Mättö J, Saarela M, von Troil-Lindén B, Könönen E, Jousimies-Somer H, Torkko H, Alaluusua S, Asikainen S (1996) Distribution and genetic analysis of oral *Prevotella intermedia* and *Prevotella nigrescens*. Oral Microbiol Immunol 11, 96-102
4. van Steenbergen TJM, Bosch-Tijhof CJ, Petit MDA, Van der Velden U (1997) Intra-familial transmission and distribution of *Prevotella intermedia* and *Prevotella nigrescens*. J Periodontal Res 32, 345-350
5. Watanabe K, Frommel TO (1993) Detection of *Porphyromonas gingivalis* in oral plaque samples by use of the polymerase chain reaction. J Dent Res 72, 1040-1044
6. Tran SD, Rudney JD (1996) Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. J Clin Microbiol 34, 2674-2678
7. Ashimoto A, Chen C, Bakker I, Slots J (1996) Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 11, 266-273
8. Meurman JH, Wahlfors J, Korhonen A, Alakuijala P, Väisänen P, Torkko H, Jänne J (1997) Identification of *Bacteroides forsythus* in subgingival dental plaque with the aid of a rapid PCR method. J Dent Res 76, 1376-1380
9. Okada M, Hayashi F, Nagasaka N (2001) PCR detection of 5 putative periodontal pathogens in dental plaque samples from children 2 to 12 years of age. J Clin Periodontol 28, 576-582
10. Okada M, Hayashi F, Nagasaka N (2000) Detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in dental plaque samples from children 2 to 12 years of age. J Clin Periodontol 27, 763-768
11. Löe H, Silness J (1963) Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 21, 533-551
12. Wilson K (1994) Preparation of genomic DNA from bacteria. In Current protocols in molecular biology, Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, eds, Greene

publishing Associated and Wiley-Interscience, New York, 2.4.1-2.4.2
13. Smith GLF, Socransky SS, Smith CM (1989) Rapid method for the purification of DNA from subgingival microorganisms. Oral Microbiol Immunol 4, 47-51
14. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd ed, Cold Spring Harbor Laboratory, New York, E. 5
15. Conrads G, Mutters R, Fischer J, Brauner A, Lutticken R, Lampert F (1996) PCR reaction and dot-blot hybridization to monitor the distribution of oral pathogens within plaque samples of periodontally healthy individuals. J Periodontol 67, 994-1003
16. Goncharoff P, Figurski DH, Stevens RH, Fine DH (1993) Identification of *Actinobacillus actinomycetemcomitans*: polymerase chain reaction amplification of *lkt*A-specific sequences. Oral Microbiol Immunol 8, 105-110
17. Dixon WJ, Massey FJ Jr (1969) Introduction to statistical analysis. 3rd ed, McGraw-Hill, New York, 237-243
18. Moore WEC, Moore LVH (1994) The bacteria of periodontal diseases. Periodontol 2000 5, 66-77
19. Haffajee AD, Socransky SS (1994) Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 5, 78-111
20. Zambon JJ (1996) Periodontal diseases: microbial factors. Ann Periodontol 1, 879-925
21. Gmür R, Guggenheim B (1994) Interdental supragingival plaque - a natural habitat of *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter rectus*, and *Prevotella nigrescens*. J Dent Res 73, 1421-1428
22. Ximénez-Fyvie LA, Haffajee AD, Socransky SS (2000) Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol 27, 648-657
23. Ximénez-Fyvie LA, Haffajee AD, Socransky SS (2000) Microbial composition of supra- and subgingival plaque in subjects with adult periodontitis. J Clin Periodontol 27, 722-732
24. Preus HR, Zambon JJ, Dunford RG, Genco RJ (1994) The distribution and transmission of *Actinobacillus actinomycetemcomitans* in families with established adult periodontitis. J Periodontol 65, 2-7
25. Asikainen S, Chen C, Slots J (1996) Likelihood of transmitting *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in families with periodontitis. Oral Microbiol Immunol 11, 387-394
26. Fukui K, Kato N, Kato H, Watanabe K, Tatematsu N (1999) Incidence of *Prevotella intermedia* and *Prevotella nigrescens* carriage among family members with subclinical periodontal disease. J Clin Microbiol 37, 3141-3145
27. Amano A, Kuboniwa M, Nakagawa I, Akiyama S, Morisaki I, Hamada S (2000) Prevalence of specific genotypes of *Porphyromonas gingivalis fimA* and periodontal health status. J Dent Res 79, 1664-1668
28. Greenstein G, Lamster I (1997) Bacterial transmission in periodontal diseases: a critical review. J Periodontol 68, 421-431
29. Wara-aswapati N, Howell TH, Needleman HL, Karimbux N (1999) Periodontitis in the child and adolescent. ASDC J Dent Child 66, 167-174