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Presence of *Aggregatibacter actinomycetemcomitans* in young individuals: a 16-year clinical and microbiological follow-up study

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Abstract

Aim: To look for clinical signs of periodontal disease in young adults who exhibited radiographic bone loss and detectable numbers of *Aggregatibacter actinomycetemcomitans* in their primary dentition.

Material and methods: Periodontal status and radiographic bone loss were examined in each of the subjects 16 years after the baseline observations. Techniques for anaerobic and selective culture, and checkerboard, were used to detect periodontitis-associated bacterial species. The isolated *A. actinomycetemcomitans* strains were characterized by polymerase chain reaction.

Results: Signs of localized attachment loss were found in three out of the 13 examined subjects. *A. actinomycetemcomitans* was recovered from six of these subjects and two of these samples were from sites with deepened probing depths and attachment loss. Among the isolated *A. actinomycetemcomitans* strains, serotypes a–c and e, but not d or f, were found. None of the isolated strains belonged to the highly leucotoxic JP2 clone, and one strain lacked genes for the cytolethal distending toxin.

Conclusions: This study indicates that the presence of *A. actinomycetemcomitans* and early bone loss in the primary dentition does not necessarily predispose the individual to periodontal attachment loss in the permanent dentition.

Key words: adolescents; *Aggregatibacter actinomycetemcomitans*; periodontitis

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Periodontitis is an infectious disease that is initiated by the biofilm present on the teeth. The complexity of the microflora in the biofilm makes it difficult to determine the exact aetiology of the periodontal disease. The aggressive form of the disease, which preferentially affects younger individuals, is characterized by a rapid periodontal destruction (Albandar et al. 1991a, b, Lang et al. 1999). Periodontal disease in children and adolescents is a rare condition in individuals of northern European origin (Saxen 1980, Sjödin et al. 1989, 1993, Källestål et al. 1990). A wide variation in the reported prevalence may be related to differences in criteria, sampling methods and/or differences in the surveyed populations. The disease shows some racial predispositions, being more common in people of African and Asian origin (Van der Velden et al. 1989, Haubek et al. 1996).

The aggressive form of periodontal disease that affects younger individuals is considered to involve both a susceptible host and certain bacterial virulence factors. *Aggregatibacter actinomycetemcomitans* is a bacterial species associated with aggressive periodontitis predominantly in younger individuals, but may also be involved in rapidly destructive adult periodontitis (Zambon 1985, Fine et al. 2006). This Gram-negative, facultatively anaerobic, non-motile rod, which is grouped into six serotypes, a–f (DiRenzo et al. 1994, Asikainen et al. 1995) and various genotypes (Asikainen et al. 1995), pos-

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sesses two exotoxins: the leucotoxin and cytolethal distending toxin, Cdt (Fine et al. 2006). There is a considerable intra-species diversity of *A. actinomycetemcomitans*, and its association with disease is rather moderate (Van der Weijden et al. 1994, Fine et al. 2006, 2007). A specific clone of *A. actinomycetemcomitans*, JP2, is strongly associated with aggressive periodontitis in individuals of African descent (Haubek et al. 1996, 1997). The JP2 clone differs from other clones of the species by several genetic peculiarities, including a 530-bp deletion in the promoter gene of the leucotoxin operon that results in increased expression of the toxin (Brogan et al. 1994). This clone has been shown to have a significantly increased virulence potential as compared with other members of the species (Haubek et al. 2008). The more recently discovered Cdt affects various cell types by inhibiting proliferation and inducing cell death (Lara-Tejero & Galán 2000). The *cdt* genes, *a*, *b* and *c*, are present in about 80% of the *A. actinomycetemcomitans* strains, and their association with disease remains to be determined (Fine et al. 2006).

There is also an indication that periodontal attachment loss in the permanent dentition of adolescents is often preceded by bone loss in the primary dentition (Sjödin et al. 1989, 1993, Albandar et al. 1991a, Philstrom et al. 2005). There is an indication that adolescents with bone loss and/or attachment loss around their permanent teeth are at risk of developing additional periodontal disease as adults (Jenkins & Papapanou 2001, Albandar & Tinoco 2002, Philstrom et al. 2005).

A previous population-based study examined signs of periodontal disease in children living in Sweden (Sjödin & Mattson 1994).The study showed that 0.8% of the examined 7–9-year-old subjects exhibited radiographic periodontal bone loss at ≥ 2 proximal surfaces of their teeth. Twenty-six of the identified children were available for further examination (Sjödin et al. 1995). In 14 of these 26 cases (54%), *A. actinomycetemcomitans* was found in samples obtained from pockets with the deepest probing depths. In addition, the prevalence of this bacterium was correlated to leucotoxin-neutralizing capacity in the serum. In a matched reference group, neither the microorganism nor any leucotoxin-neutralizing capacity was found in any of the subjects. These results indicate that *A. actinomycetemcomitans* may be an important aetiologic factor in this age group.

The aim of the present study was to look for clinical signs of periodontal disease and the presence of *A. actinomycetemcomitans* among these subjects 16 years after the baseline observations. The stability of the infection and the disease progression were evaluated. The *A. actinomycetemcomitans* strain isolates from baseline and from the follow-up were characterized.

Material and Methods

Study population

The study population consisted of 13 subjects who all exhibited bone loss and were colonized by *A. actinomycetemcomitans* 16 years ago. The healthy controls were not available for this follow-up study. At baseline, in the child population, the initial case group consisted of twenty-six 7–9-year-old children. In each child, a subgingival plaque sample was taken from the primary tooth sites with the deepest probing depths (Sjödin & Mattson 1994). Fourteen of these subjects fulfilled the criteria for the present follow-up study performed 16 years after the baseline examinations. The criteria were radiographic signs of proximal attachment loss at their primary teeth and the presence of *A. actinomycetemcomitans* in their deep pockets (Sjödin & Mattson 1994, Sjödin et al. 1995). These 14 individuals (23–25 years) included eight females and five males of different ethnic origins (seven Asian, five Caucasian, and two African). We were not able to locate one male of African origin for the follow-up examination. Three subjects were both smokers and snuff users. One subject only smoked (≥ 10 cigarettes/day) and one subject only used snuff. All subjects had completed high school and two subjects were now studying at a university. Nine of the subjects regularly went to dental checkups, while four subjects only went for emergency treatment. Most of them brushed their teeth twice daily, while inter-dental cleaning was more occasional. None of the subjects had received antibiotics during the 6 months before the follow-up examination.

Clinical examination

Clinical examination and radiography of all subjects were carried out by a single examiner in Public Dental Clinics in the counties of Örebro, Stockholm, Gothenburg and Halland in Sweden. The follow-up examination included a systemic health questionnaire. The participants were also interviewed about their oral hygiene habits, earlier dental treatment, the use of tobacco or snuff, family situation, educational and employment status.

Full-mouth plaque index and dental status (caries, fillings, and missing teeth) were examined. Full-mouth probing pocket depth (PPD) was recorded from the gingival margin to the bottom of the pocket to the nearest millimetre, using a manual periodontal probe (Hu-Friedy PCP 11, Chicago, IL, USA). Bleeding on probing (BOP) was recorded after 30 s. PPD and BOP were measured at six sites per tooth of all teeth present. Bitewing X-rays and periapical frontal radiographs were performed in each subject.

All participants gave their conformed consent and the study was approved by the Ethics Committee of Umeå University, Sweden.

Microbiological examination

Compared with the baseline study, the microbiological examination at the follow-up study was more extensive. First, the DNA/DNA checkerboard hybridization method was used to acquire an overall picture of a possible infection of the subjects by periodontitis-associated bacterial species. For this purpose, subgingival plaque samples were collected distally from the second molars in both jaws from all the individuals and sent to the oral microbiology laboratory at the University of Gothenburg. The analyses were performed according to Dahlen & Leonhardt (2006).

Second, the culture method used included the detection not of only *A. actinomycetemcomitans* but also the periodontitis-associated bacterial species mentioned below. Single-site subgingival plaque samples as well as mucosa samples were collected from all 13 subjects. The plaque samples were taken from inflamed periodontal pockets with PPD ≥ 5 mm or when these pockets were absent, from randomly selected pseudopockets. After

removal of plaque, the site was dried by placing cotton rolls. An absorbent endodontic paper point was inserted into the bottom of each pocket for 30 s. For the mucosa samples, the tongue, tonsils and cheeks were swabbed with cotton buds. Both the paper points and the cotton buds were transferred to an anaerobic transport medium (VMGA III) and culturing was performed within 24 h.

The samples were processed within 24 h according to the following procedure: serially diluted aliquots were spread on Columbia-base agar supplemented with hemin (0.05 mg/ml), K-vitamin (0.01 mg/ml) and lysed horse blood (5%) for detection of *Porphyromonas gingivalis*, *Prevotella intermedia/Prevotella nigrescens*, *Tannerella forsythia* and *Parvimonas micra*. *A. actinomycetemcomitans* was detected on the TBV medium, the selective medium described by Slots (1982), but without serum. The bacterial species were identified according to established methods (Lakio et al. 2002). Proportional recovery of the species was achieved by comparing the numbers of their colonies with the total number of bacterial colonies on the blood agar medium.

Characterization of *A. actinomycetemcomitans* strains by polymerase chain reaction (PCR)

Among all 86 strains, 36 strains were isolated at baseline and 50 at the follow-up examination. While the criterion for selection of strains at baseline was one colony per site (one to six per subject), when possible, it was three colonies of each morphology type per site at the follow-up examination (one to 14 per subject). Characterization of the strains included serotyping, genotyping, leucotoxin promoter gene typing and screening for *cdt* genes. For the PCR-based characterization, DNA was isolated from the strains after growth for 3 days on the blood agar medium mentioned above. The cells were harvested and suspended into sterile water to yield an optical density of 2.0 at 600 nm. The suspension was boiled for 8 min. and then centrifuged for 5 min. at 20,000 × *g*. The supernatant was frozen in aliquots and used as a template in the PCR-based characterization of the isolates. The PCR mixtures (2.5 μl) contained 1 μM of each primer, 0.2 mM deoxyribonucleotides (Amersham Biosciences, Freiburg, Germany), 0.17 mg/ml bovine serum albumin (Roche, Mannheim, Germany), 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 2.5 AmpliTaq polymerase (Roche) and 2.5 μl template. The primers used in this study and the temperature profiles for amplification of the various genes have been described elsewhere, for serotyping a–e (Suzuki et al. 2001), for serotype f (Kaplan et al. 2002), for leucotoxin promoter genetyping (Brogan et al. 1994) and for the CDT-ABC gene-typing (Ahmed et al. 2001). The PCR products were analysed by agarose (1.2%) gel electrophoresis in a Tris-acetate (40 mM; pH 8.3) buffer containing 1 mM EDTA. The gel was stained with ethidium bromide and photographed under ultraviolet light.

The AP-PCR genotyping of the *A. actinomycetemcomitans* isolates was performed as described previously (Saarela et al. 1992, Asikainen et al. 1995, Doğan et al. 1999).

Table 1. Clinical characteristics of the 13 subjects at the follow-up examination

Study population	1	2	3	4	5	6	8	9	10	11	12	13	14
Number of teeth	32	28	29	30	29	30	32	31	32	28	31	29	32
Number of pockets (PPD) (mm)													
4	23	27	60	15		2	21	23	26	17	20	13	9
≥ 5	4	6	5	4					9		1		3
≥ 6		5							1		1		2
≥ 7		2									1		
BOP (%)	21	51	57	27	4	28	37	34	45	37	59	30	17
ABL (*number of sites*)													
Radiographic measurements [CEJ-AC (m,d)] (mm)													
≥ 2									4				
3		6							5		4		
4		4							2		2		
PI (%)	47	50	42	49	31	24	54	57	55	41	68	20	40
Deposits													
Supragingival calculus	x	x	x	x			x	x	x	x	x	x	x
Subgingival calculus	x	x	x				x		x		x		

Subjects with signs of periodontitis are indicated with filled columns.
Subject 7 did not participate in the follow-up examination.
x signifies presence of calculus.
PPD, probing pocket depth; BOP, bleeding on probing; ABL, alveolar bone loss was defined on sites with a distance from CEJ–AC ≥ 2 mm; CEJ, cemento-enamel junction; AC, alveolar crest; CEJ–AC, the distance in millimetre from the cemento-enamel junction to the alveolar crest was measured in radiographs on the mesial and distal surfaces on 28 teeth; PI, Plaque index.

Results

Clinical characteristics

The presence of gingivitis was a common finding among the young adult subjects (23–25 year old) at the follow-up examination. Loss of attachment and early signs of periodontal disease were detected clinically and radiographically in three (23%) (subjects 2, 10, 12) out of the 13 examined subjects (Table 1). Further clinical characteristics are summarized in Table 1. All 14 subjects had radiographic attachment loss at proximal surfaces of their deciduous teeth at the baseline examination when the subjects were 7–9 years of age (Sjödin & Mattson 1994, Sjödin et al. 1995).

Microbiological findings

The screening for periodontal pathogens by the checkerboard method revealed rather low scores (Table 2). Scores >4 were detected only in six subjects. *A. actinomycetemcomitans* was a low-score (<2) species among the subjects (Table 2). However, when the cultivation procedure was used for analysing mucosa and subgingival samples, the presence of *A. actinomycetemcomitans* was detected in six (46%) of the subjects (Table 3). *A. actinomycetemcomitans* was recovered in subgingival plaque samples from six of the young adults at the follow-up examination and from three of these six subjects in pooled samples from buccal mucosa, tongue and tonsils (Table 3). In two of these subjects (10, 12), the presence of *A. actinomycetemcomitans* correlated with sites of deepened probing depths and attachment loss. In one of the subjects (2), attachment loss and several

Table 2. Distribution of bacterial species in plaque samples according to checkerboard analysis (number of bacteria; 5 ≥ 10^6, 4 = 10^6, 3 ≥ 10^5, 2 = 10^5, 1 ≤ 10^5, 0 = no reaction)

Subjects	Site	PPD (mm)	*Pg*	*Pi*	*Pn*	*Tf*	*Aa*	*Fn*	*Td*	*Pm*	*Cr*
1	1	4	0	0	0	0	0	0	0	0	0
	2	4	5	1	1	3	1	0	3	0	3
	3	4	0	2	2	0	2	0	3	1	0
	4	4	1	0	0	1	0	0	0	0	0
2	1	4	0	0	1	1	0	1	1	0	1
	2	4	0	0	2	0	0	0	0	0	0
	3	6	1	1	2	3	1	1	3	1	1
	4	5	0	0	1	1	1	0	1	1	1
3	1	4	0	1	0	1	0	0	1	0	0
	2	4	0	1	1	0	1	1	0	1	0
	3	5	0	4	4	3	1	2	2	1	1
	4	5	0	2	1	3	1	2	3	1	1
4	1	4	0	1	0	0	0	0	1	1	0
	2	4	0	1	1	1	1	1	2	1	1
	3	5	0	1	2	1	1	1	3	2	1
	4	5	0	1	1	1	0	1	1	1	0
5	1	4	0	1	1	0	0	1	0	0	0
	2	4	0	1	1	0	0	1	0	1	1
	3	4	0	1	0	0	0	1	1	0	0
	4	4	0	1	1	1	1	2	3	2	0
6	1	4	0	1	0	0	0	1	0	0	0
	2	4	0	1	0	0	0	0	0	0	0
	3	5	0	2	2	3	0	1	0	1	0
	4	5	0	4	2	1	0	1	0	1	0
*7											
8	1	4	4	2	1	2	0	1	4	1	1
	2	5	4	2	1	2	0	0	3	1	1
	3	5	1	1	1	1	0	0	3	1	0
	4	4	1	2	3	3	1	1	3	1	1
9	1	4	0	2	4	2	1	1	2	1	0
	2	4	0	2	3	1	1	1	1	1	0
	3	4	0	0	0	0	0	0	0	0	0
	4	4	1	1	0	0	1	0	3	3	0
10	1	5	0	1	2	0	1	1	0	1	0
	2	5	0	1	1	1	1	0	1	1	1
	3	6	0	1	1	0	1	0	1	0	0
	4	5	0	3	2	1	1	0	3	1	0
11	1	4	1	1	1	0	1	1	1	1	0
	2	4	0	1	1	0	1	1	1	0	0
	3	4	0	1	3	3	1	1	3	1	1
	4	4	0	1	2	1	1	1	1	1	0
12	1	4	2	2	2	1	0	1	3	1	1
	2	4	2	1	1	1	0	0	3	1	1
	3	4	2	0	0	0	0	0	1	0	0
	4	7	2	0	0	1	0	0	1	1	1
13	1	4	0	1	1	0	1	1	0	1	0
	2	4	0	1	1	0	1	1	0	1	0
	3	5	1	2	1	1	1	1	2	1	1
	4	5	1	1	1	2	1	1	2	1	1
14	1	5	4	3	1	4	1	1	3	1	2
	2	4	3	2	2	3	1	1	3	1	1
	3	4	1	3	3	1	1	1	2	1	1
	4	6	1	2	3	1	0	1	1	1	0

*Subject 7 did not participate in the follow-up examination.

Pg, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Pn*, *Prevotella nigrescens*; *Tf*, *Tannerella forsythia*; *Aa*, *A. actinomycetemcomitans*; *Fn*, *Fusobacterium nucleatum*; *Td*, *Treponema denticola*; *Pm*, *Parvimonas micra*; *Cr*, *Campylobacter rectus*.

sites with deepened probing depths (> 5 mm) were detected, but no *A. actinomycetemcomitans* were found. The cultivation procedure supported the findings by the checkerboard method that periodontal pathogens could not be detected or were detected in low proportions (Table 3). In general, the proportions, as well as the total number of *A. actinomycetemcomitans*, in the subgingival plaque samples of these six subjects were low (Table 3). In all the examined subjects, other periodontal pathogens were also detected in relatively low numbers.

A. actinomycetemcomitans was detected in subgingival plaque samples of all these 14 subjects at the baseline examination, while none of the matched referents harboured this bacterium at baseline (Sjödin et al. 1995). The proportion of subjects with an ethnic background other than Caucasian was high in the selected study population (Table 3).

Leucotoxin promoter type and *cdt* genes among the *A. actinomycetemcomitans* strains

None of the subjects harboured *A. actinomycetemcomitans* strains with the JP2 leucotoxin promoter type, either in the isolates from childhood nor in the isolates from the 16-year follow-up examination. *cdt* genes (*a–c*) were detected in all characterized *A. actinomycetemcomitans* strains except one. This strain represented serotype c and was isolated from a subject of African origin (subject 7) (Table 4).

Distribution of serotypes and AP-PCR genotypes among the *A. actinomycetemcomitans* strains

Among the *A. actinomycetemcomitans* serotypes, serotypes a–c and e, but not d or f, were detected among the 36 strains isolated from the fourteen 7–9-year-old subjects at the baseline examination (Table 4). Among the seven Asian subjects, three harboured two different serotypes, a and b, a and c, a and e, respectively, while one African subject had three serotypes, a–c. Two serotypes, b and c, were detected in one of the Caucasian subjects. Among the strains isolated from the six *A. actinomycetemcomitans*-positive young adults, serotypes a–c, and f were identified. All of the isolates were serotypeable. Among two of the 13 examined young adults, two different serotypes were detected. In five of six *A. actinomycetemcomitans*-positive subjects at the follow-up examination, strains representing the same serotype could be detected in both the baseline and the follow-up samples (Table 4). None of the *A. actinomycetemcomitans* AP-PCR genotypes that were detected in a subject at baseline could be recovered in samples from the same subject at the follow-up examination (Table 4).

Table 3. Detection of selected bacterial species (% of total viable bacteria) in samples from subgingival plaque and mucosa by cultivation

Subjects	Ethnicity	Site	Total number/ Sample (million)	*Aa* (%)	*Pg* (%)	*Pi/Pn* (%)	*Tf* (%)	*Pm*%
1	Caucasian	38d	35	0.06	ND	ND	ND	ND
		mucosa	34	ND	ND	ND	ND	ND
2	Asian	27b	14	ND	ND	1.4	7.1	ND
		36m	12	ND	ND	ND	ND	14
		46m	0.69	ND	ND	ND	ND	17
		mucosa	59	ND	ND	ND	ND	ND
3	Caucasian	21m	11.6	0.7	ND	ND	8.6	ND
		mucosa	98.4	ND	ND	ND	ND	ND
4	Caucasian	25m	4.1	ND	ND	ND	ND	ND
		mucosa	76	ND	ND	ND	ND	ND
5	Caucasian	mucosa	27.8	ND	ND	ND	ND	ND
6	Caucasian	47d	2.5	ND	ND	ND	ND	ND
		mucosa	42.2	ND	ND	ND	ND	ND
7*	African							
8	Asian	38d	10.7	ND	ND	ND	ND	0.9
		46d	7.9	0.07	ND	ND	ND	3.3
		mucosa	110	0.02	ND	ND	ND	ND
9	Asian	26d	1.8	ND	ND	ND	1.1	ND
		mucosa	39.2	ND	ND	ND	ND	ND
10	African	37m	16	4.4	ND	ND	2.3	2.3
		45d	14.6	1.2	ND	1.1	0.5	ND
		46d	1.6	0.09	ND	2.4	ND	2.4
		mucosa	49	0.0002	ND	ND	ND	ND
11	Asian	47d	6.7	ND	ND	ND	ND	ND
		mucosa	51.2	ND	ND	ND	ND	ND
12	Asian	47d	5.7	0.43	ND	ND	ND	3.5
		38d	9	0.007	ND	ND	ND	2.2
		mucosa	21.6	ND	ND	ND	ND	2.8
13	Asian	17d	13.8	ND	ND	ND	ND	ND
		mucosa	118	ND	ND	ND	ND	ND
14	Asian	47d	16.3	5.4	ND	ND	ND	ND
		mucosa	87	0.009	2.3	ND	ND	ND

Subjects with detectable numbers of *A. actinomycetemcomitans* are indicated by filled rows.
*Subject 7 did not participate in the follow-up examination.
Level of detection $<0.0001\%$.
Aa, *A. actinomycetemcomitans*; *Pg*, *P. gingivalis*; *Pi/Pn*, *P. intermedia/P. nigrescens*; *Tf*, *T. forsythia*; *Pm*, *Parvimonas micra*; ND, not detected.

Table 4. Distribution of *A. actinomycetemcomitans* serotypes and genotypes of the isolates from baseline (child) and in the follow-up (adult) isolates

Subject	Ethnicity	Serotype		AP-PCR genotype	
		child	adult	child	adult
1	Caucasian	c	c	3	1
2	Asian	c		4	
3	Caucasian	a	a	7	1
4	Caucasian	c		15	
5	Caucasian	b		8	
6	Caucasian	b		8	
		c		3	
7	African	a	*	7	*
		b	*	13	*
		c	*	19	*
8	Asian	c	c	4	3
			f		3
9	Asian	a		1	
		c		15	
10	African	a	c	1	3
		b	b	2	9
		b		1	
11	Asian	a		1	
		e		20	
12	Asian	e	f	6	17
13	Asian	b		2	
14	Asian	b		2	
		a	a	10	1
			a		1

Subjects with detectable numbers of *A. actinomycetemcomitans* as adults are indicated by filled squares.
*Subject 7 did not participate in the follow-up examination.

Discussion

In this study, we examined periodontal status and the oral microflora of 13 selected young adults, based on the detection of bone loss and *A. actinomycetemcomitans* in their childhood, at baseline, and 16 years earlier (Sjödin & Mattson 1994, Sjödin et al. 1995). Albeit very small, the material of this study is exceptional. Firstly, bone resorption among children is very uncommon. Secondly, these subjects were infected with *A. actinomycetemcomitans*, a bacterial species closely associated with an aggressive form of periodontitis. Thirdly, we had a unique possibility to re-examine these subjects with early-onset bone resorption after 16 years.

The initial case group, identified in their childhood with bone loss at ≥ 2 tooth surfaces, with deepened probing depths, probing attachment loss, suppuration on probing and proximal calculus, exhibited multiple signs of ongoing periodontal disease (Sjödin & Mattson 1994, Sjödin et al. 1995). *A. actinomycetemcomitans* was detected in cultivable numbers from subgingival plaque samples in 14 of the 26 children (7–9 years old) with attachment loss, while none of the periodontally healthy referents harboured this bacterium (Sjödin et al. 1995). This strongly indicates an association between the prevalence of *A. actinomycetemcomitans* and attachment loss in this child population. There were several apparent questions. Could we detect a predisposition for the disease? Was the *A. actinomycetemcomitans* infection stable over time? Could we observe any correlation between the presence or the proportions of various clones of the bacterial species and bone resorption?

In the 16-year follow-up study, we identified signs of localized attachment loss in three (23%) out of 13 subjects examined. *A. actinomycetemcomitans* was recovered in cultures from six of these 13 subjects at generally low-to-intermediate levels. In two of the subjects, *A. actinomycetemcomitans* from was detected in sites with deepened probing depths and attachment loss. This is in line with previous findings (Mombelli et al. 1994), because the most likely site to isolate *A. actinomycetemcomitans* from is the one most affected. However, it is also possible to isolate *A. actinomycetemcomitans* from shallow pockets/pseudopockets or mucosal membranes (Eger et al. 1996). *A. actinomycetemcomitans* is found in both inactive and active periodontal sites in patients with periodontitis, but the bacterium occurs in significantly higher proportions in active sites with progression of the disease (Dzink et al. 1985, van Winkelhoff et al. 1994). Screening the subjects for periodontal pathogens by the checkerboard method revealed rather low scores for all the tested bacterial strains.

One of the examined subjects with attachment loss showed elevated proportions of anaerobic periodontitis-associated subgingival bacterial species, but no detectable *A. actinomycetemcomitans*. We also detected *A. actinomycetemcomitans* in samples from buccal mucosa, tongue and tonsillar crypts. Pseudopockets located in the second and third molars were sampled, and in some cases, these pockets proved to be reservoirs of *A. actinomycetemcomitans*. The samples were not collected from the same locations in childhood and in adulthood because the subjects were still in their primary dentition at the time of the baseline examination. The subgingival presence of *A. actinomycetemcomitans* in these children seemed to be linked to their periodontal condition at that time (Sjödin et al. 1995). In this restricted material of the young adults, the rather low isolation frequencies of *A. actinomycetemcomitans* connected to the clinical findings make it difficult to draw firm conclusions about the aetiological role of this bacterium.

The subjects in the present study had all received regular dental care during their childhood and teens, some of them occasionally after leaving high school. Most of the individuals lived close to the area where they had lived as children. Chronic gingivitis was a common finding in this age group. Attachment loss among otherwise healthy young adults is a rare finding in industrial countries (Albandar 1989, Källestål et al. 1990, Albandar et al. 1999, Albandar & Tinoco 2002). It seems reasonable to assume that the three individuals with attachment loss in early adulthood are more susceptible to periodontal disease and might be at risk of further disease progression.

Life style factors, such as smoking, seem to add a significant risk of more severe and prevalent disease, and smoking may increase the clinical expression of periodontal disease in already susceptible subjects (Grossi et al. 1994, Schenkein et al. 1995, Gunsolley et al. 1998). Two of the subjects with attachment loss had been smokers since their early teens. One subject, an African female with attachment loss, had complications with caries and extensive fillings in her molars. Of course, local factors, such as fillings, caries and open contacts, may have contributed to the bone loss in her case.

The distribution of *A. actinomycetemcomitans* varies among different ethnic populations. Studies from African and Asian countries have shown a high prevalence of *A. actinomycetemcomitans* in surveyed populations (van der Weijden et al. 1994, Papapanou et al. 2002). Seven of the 14 examined children with bone loss were of Asian origin (Sjödin et al. 1995). The contribution of race/ethnicity to further development of periodontitis can only be hypothesized in the presence of other variables, such as age, socioeconomic status, oral hygiene and dental care, in these individuals.

Most *A. actinomycetemcomitans*-infected subjects harbour a single serotype that remains stable for several years (Saarela et al. 1992). Some individuals in this study were colonized by strains representing two or three serotypes. Serotype b was found in six of the children, while in the adults, serotype b was recovered from only one individual. This person, a female of African origin, also showed clinical attachment loss. *A. actinomycetemcomitans* serotype b has been shown to be highly prevalent in young individuals with periodontitis (Zambon et al. 1983), while serotype c has been the most common serotype in healthy carriers (Asikainen et al. 1995). Several studies have shown a predominance of serotype c in periodontitis patients of Chinese, Vietnamese, Korean and Japanese origin (Rylev & Kilian 2008).

In five of the six *A. actinomycetemcomitans*-positive subjects at the follow-up examination, we detected isolates of the same serotype as they had had at the baseline study. However, none of the *A. actinomycetemcomitans* AP-PCR baseline genotypes were the same in the samples from the same subject at the follow-up examinations. Although the phenomenon has been previously reported to be rare (Saarela et al. 1999), our findings indicate a common genotype conversion during this 16-year follow-up period. On the other hand, the change in genotype may well be due to a later colonization by a different *A. actinomycetemcomitans* strain. A given AP-PCR genotype is almost always of a set of serotypes (Saarela et al. 1999). For instance, serotype c strain does not usually display genotype 1. Therefore, the change seen in subject #1 from genotype 3 in childhood to genotype 1 in adulthood may be especially interesting and worth further investigations.

In summary, in three (23%) out of 13 subjects infected with *A. actinomycetemcomitans*, early signs of periodontal attachment loss were observed after a period of approximately 16 years. In two of the three subjects, *A. actinomycetemcomitans* was detected at re-examination. These results indicate an increased proportion of diseased subjects compared with previous examinations of randomly selected Scandinavian adolescents (Källestål et al. 1990). Our findings indicate that periodontitis affecting the primary dentition does not necessarily indicate the presence of periodontal attachment loss in the permanent dentition. Unfortunately, this study is far too small to draw more specific conclusions. However, these data might be valuable, together with other similar studies, when evaluating the risk markers and environmental factors in the development of chronic periodontitis later in life (Machtei et al. 1999, Fine et al. 2007).

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Clinical Relevance

Scientific rationale for the study: The present study describes a restricted, but unique child population, with a prevalence of *Aggregatibacter actinomycetemcomitans* and attachment loss in their primary dentition. These individuals were re-examined as young adults after a 16-year period.
Principal findings: In some individuals, marginal bone loss in the primary dentition was associated with periodontal attachment loss later in adult life. Our findings also indicate that this condition does not necessarily lead to periodontitis in the permanent dentition according to this follow-up longitudinal study.
Practical implications: An additional follow-up study of these young adults will be needed to evaluate the further development of their periodontal conditions.