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GENETIC APPROACH TO THE STUDY OF EPIDEMIOLOGY AND PATHOGENESIS OF ACTINOBACILLUS ACTINOMYCETEMCOA4ITANS IN LOCALIZED JUVENILE PERIODONTITIS

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Abstract

Actinobacillus acrinomycetemcomirans isolates from periodontal pockets were examined for restriction fragment-length polymorphism using a characterized 4.7-kb DNA probe. A total of 6 patterns of RFLP was found in 133 isolates originating from 12 subjects. No relatedness was found between RFLP types and serotypes. Different periodontal sites within the same subject and different individuals within the same family sometimes showed only one type of *A. actinomycetemcomitans* RFLP. When members among the same family showed 2 RFLP types, children were always infected with the *A. acfinomycefemcomitans* strains found in at least one of the parents. These findings support the concept of familial spread of *A. actinomycetemcomitans. A. actinomycetemcomitans* RFLP type B, corresponding to reference strain JP2, seems to be particularly virulent, as indicated from the presence of RFLP type B in 3 subjects who converted from a healthy periodontal state to localized juvenile periodontitis. RFLP type B was not detected in any of the 21 *A. acrinomycetemcomitans*-infected patients with adult periodontitis. The RFLP method seems to be useful in determining the epidemiology and possibly the potential virulence of periodontal strains of *A. actinomycetemcomitans*.

Keywords

juvenile periodontitis, epidemiology, virulence, restriction fragment-length polymorphism, DNA probes, Actinobaccillus actinomycetemcomitans

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Summary

Actinobacillus acrinomycetemcomirans isolates from periodontal pockets were examined for restriction fragment-length polymorphism using a characterized 4.7-kb DNA probe. A total of 6 patterns of RFLP was found in 133 isolates originating from 12 subjects. No relatedness was found between RFLP types and serotypes. Different periodontal sites within the same subject and different individuals within the same family sometimes showed only one type of *A. actinomycetemcomitans* RFLP. When members among the same family showed 2 RFLP types, children were always infected with the *A. acfinomycefemcomitans* strains found in at least one of the parents. These findings support the concept of familial spread of *A. actinomycetemcomitans. A. actinomycetemcomitans* RFLP type B, corresponding to reference strain JP2, seems to be particularly virulent, as indicated from the presence of RFLP type B in 3 subjects who converted from a healthy periodontal state to localized juvenile periodontitis. RFLP type B was not detected in any of the 21 *A. acrinomycetemcomitans*-infected patients with adult periodontitis. The RFLP method seems to be useful in determining the epidemiology and possibly the potential virulence of periodontal strains of *A. actinomycetemcomitans*.

Keywords

juvenile periodontitis; epidemiology; virulence; restriction fragment-length polymorphism; DNA probes; *Actinobaccillus actinomycetemcomitans*

INTRODUCTION

Localized juvenile periodontitis is an infectious disease that results in rapid loss of alveolar bone support around permanent incisors and first molars (Lindhe and Slots, 1989). The disease has a familial nature (Baer, 1971) and *Actinobacillus actinomycetemcomitans* has been implicated as the aetiological agent (Slots and Taichman, 1988; Lindhe and Slots, 1989). However, some young individuals harbouring *A. actinomycetemcomitans* do not acquire the disease. Likewise, some sites in patients with localized juvenile periodontitis contain the organism without experiencing periodontal breakdown. While host-related factors most likely account for some of these observations (Genco and Slots, 1984), another consideration is the possible existence of virulent and avirulent strains of the bacterium. That virulence may differ among *A. actinomycetemcomitans* strains was suggested by Zambon, Slots and Genco (1983b), who detected serotype *b* strains twice as frequently in lesions of juvenile periodontitis as in those of adult periodontitis, by Tsai and Taichman (1986) who found leucotoxin-producing strains to be strongly associated with young patients

with localized periodontitis, and by Preus, Olsen and Namork (1987), who related bacteriophage-infected strains to disease-active periodontitis.

In order to assess further the epidemiology and relative virulence of *A. actinomycetemcomitans* strains, we have used probe-specific DNA fingerprinting to characterize clinical isolates from periodontal sites. We now report the analysis of *A. actinomycetemcomituns* strains from members of families with localized juvenile periodontitis and adults with advanced periodontitis who were followed in longitudinal studies.

MATERIALS AND METHODS

Subjects and study design

A longitudinal study of young siblings of patients with localized juvenile periodontitis was begun; its purpose was to characterize the clinical, microbiological and immunological features associated with initiation of this periodontitis. A cross-sectional study of 21 patients with adult periodontitis and detectable *A. actinomycetemcomitans* was also made. The study of localized juvenile periodontitis included 5 families with at least one individual with the disease and at least one child, of 13 yr or less, serving as a subject susceptible to this periodontitis. At quarterly intervals, each subject at risk was examined clinically and microbiologically. The clinical examination included measurement of probing attachment level, probing pocket depth, radiographic alveolar bone height (yearly), degree of gingival inflammation and dental plaque. Periodontal breakdown was defined as an increase of 2 mm or more in the distance from the cement-enamel junction to the bottom of the periodontal pocket, or an increase of at least 2 mm in pocket depth. Subjects were assigned a 6-digit number, the first 2 of which specified the family and the last 2 identified the subject.

Clinical microbial procedures

Subgingival microbial samples were taken from 4 target (mesial surfaces of the 4 first molars) and 2 control sites (mesial surfaces of the upper left and right premolars). In addition, subgingival samples were obtained from any other periodontal site that had experienced breakdown as defined above. Bacterial samples were assigned a 6-digit number: the first 2 digits identified the subject, the next 2 digits the visit number, and the last 2 digits the site. Non-targeted breakdown sites were identified by tooth number and surface designation.

Sampling with paper points, transport in VMGA III (Möller, 1966), and primary cultivation on TSBV medium selective for *A. actinomycetemcomitans* (Slots, 1982a) was carried out as described by Slots, Feik and Rams (1990). *A. actinomycetemcomitans* isolates were identified according to small adherent colonies on TSBV, positive catalase reaction, and negative 4-methylum-belliferylfl-β-$_{D}$-galactoside reaction (Slots, 1982b; Alcoforado, McKay and Slots, 1987). At least two *A. actinomycetemcomitans* colonies were subcultured from each sample. Purity of culture was established by conventional microbiological methods.

Genetic microbial methods

The RFLP of clinical *A. actinomycetemcomitans* isolates was determined as described by DiRienzo *et al.* (1990). In brief, chromosomal DNA was extracted from each isolate and digested to completion with either *Eco*RI or *Hin*dIII. The restriction fragments were separated on 0.7% agarose gels for Southern blot analysis (Southern, 1975). The *A. actinomycetemcomitans* DNA blots were then hybridized with a radioactive probe, and the RFLP was determined.

The hybridization probe was made as follows. Chromosomal DNA from *A. actinomycetemcomitans* strain Y4 was digested to completion with *Eco*RI. The DNA fragments were ligated to the vector pBR328, previously digested with the same enzyme. The ligated sample was used to transform *Escherichia coli* DHI (*gyr*A, *rec*A, *rel*A, *end*A, *thi*, *hsd*R, *sup*E), and the transformants were screened for insertional inactivation of the chloramphenicol-resistant gene. Plasmid DNA was extracted from several clones and restricted with *Eco*RI. From a clone containing a 4.7-kb insert fragment, the insert DNA fragment was isolated and labelled with ^{32}P, using nick translation. This labelled DNA fragment failed to hybridize to chromosomal DNA from other bacterial species tested. The hybrid plasmid was restriction mapped using several enzymes.

RESULTS

The ability of the 4.7-kb probe to detect RFLP in *A. actinomycetemcomitans* is shown in Fig. 1. In some instances, *A. actinomycetemcomitans* from several sites in the same subject (lanes D, H and I) or from several subjects within the same family (lanes E, G and J) displayed the same RFLP bands. However, 2 different strains or variants of *A. actinomycetemcomitans* could also be detected in one subject (lanes D and F) or among members of the same family (lanes C and F). Analyses based on the distribution of *Hin*dIII sites within the same fragment revealed other RFLP profiles, but all isolates fell into the same groupings as those obtained with *Eco*RI (no data using *Hin*dIII restriction digestions are shown in this paper).

A total of 6 RFLP types were identified among the 133 *A. actinomycetemcomitans* isolates examined (Table 1). No correlation was found between *A. actinomycetemcomitans* RFLP types and serotypes (Table 1, footnote). The Table also shows that the RFLP method may be useful for tracing the transmission of strains or variants of *A. actinomycetemcomitans.* In family 1, an *A. actinomycetemcomitans* isolate from the mother had a hybridization profile similar to that of *A. actinomycetemcomitans* ATCC 29524 (type E; see footnote of Table 1 for designation of RFLP types), and an isolate from the father revealed another RFLP (type a). The daughter (010003) had one isolate with type E RFLP and 7 isolates with type a. She had probably acquired strains from both the mother and father. The son (010004) had 2 isolates which matched the RFLP of the father (type a), and 2 isolates (type b) that did not match the RFLP of the father, the mother, or the reference strains. In families 6 and 10, 4 children were infected with *A. actinomycetemcomitans* strains similar to those found in at least one of the parents (Table 1).

Table 2 suggests that the RFLP method can be used to detect possible differences in virulence among subgingival *A. actinomycetemcomitans* isolates. Over a period of 2 yr, subject 100040 provided 87 *A. actinomycetemcomitans* isolates, which revealed 2 hybridization patterns. One was RFLP type B, which was similar to that of *A. actinomycetemcomitans* strain JP2; the other was a previously undetected RFLP type, designated c. An interesting observation was the identification of RFLP type B in 6 of the 9 sites that converted from health to periodontitis (sites 11D, 12D, 12M, 13M, 24D, 36L, 42B, 42D). In contrast, 6 stable subject sites consistently yielded RFLP type c, except for site 36M, which at one sampling time showed type B. It is of interest that 4 of the stable sites constituted tooth surfaces at high risk for the development of localized juvenile periodontitis (16M, 26M, 36M, 46M). The mother (100039) of subject 100040 was a patient with localized periodontitis who also harboured *A. actinomycetemcomitans* RFLP type B.

The implication of a particularly high virulence potential of *A. actinomycetemcomitans* RFLP type B is supported from findings in 2 other subjects. Subject 020007, who had active lesions of localized juvenile periodontitis upon entering the study yielded strains of RFLP

type B. Likewise, subject 070031, who converted to localized juvenile periodontitis shortly after initial examination, had all *A. actinomycetemcomitans* isolates belonging to RFLP type B. Thus, the 3 subjects with active disease listed in Table 1 all had *A. actinomycetemcomitans* strains of RFLP type B.

The RFLP profile was also studied for strains isolated from 21 patients with adult periodontitis. The RFLP analysis produced a variety of profiles that have yet to be categorized. However, none of these isolates had the RFLP type B profile.

DISCUSSION

Although specific DNA sequences are conserved in strains of *A. actinomycetemcomitans,* the RFLP of the organism varies sufficiently to use specific DNA sequences for typing clinical isolates on Southern blots (DiRienzo *et al.,* 1990). The basis for DNA fingerprinting in our study was the variable occurrence and location of *Eco*RI or *Hin*dIII restriction sites within a 4.7-kb segment of *A. actinomycetemcomitans* chromosomal DNA.

Our findings confirm and extend previous data on oral transmission of *A. actinomycetemcomitans.* In accordance with the biochemical and serological study of Zambon, Christersson and Slots (1983a), we found that each infected child harboured at least one *A. actinomycetemcomitans* genotype which concomitantly was present in one of the parents. However, Zambon *et al.* (1983a) reported that all infected persons within each of 5 families harboured the same *A. actinomycetemcomitans* biotype and serotype, whereas our work revealed one family with at least 3 genotypes of *A. actinomycetemcomitans.* Furthermore, we showed that *A. actinomycetemcomitans* serotype *b* includes several genotypes. In a limited study, Preus and Olsen (1988) used restriction endonuclease fingerprinting to suggest that an infection with *A. actinomycetemcomitans* in a patient with the Papillon-Lefevre syndrome originated from the family dog. The available data indicate that DNA typing is more sensitive than conventional microbiological techniques for tracing *A. actinomycetemcomitans* infections. DNA probes may also delineate differences in virulence among *A. actinomycetemcomitans* isolates. The case in point was subject 100040 who showed periodontal breakdown in all but one site (36M at visit 40) infected with *A. actinomycetemcomitans* RFLP type B. The representative strain of *A. actinomycetemcomitans* RFLP type B is JP2, originally isolated from a 11-yr-old patient with localized juvenile periodontitis (N. S. Taichman, unpublished). In subject 100040, the classical sites of lesions of localized periodontitis remained stable and the predominant *A. actinomycetemcomitans* RFLP was of type c. Patient 100040 most likely became infected by 2 strains of *A. actinomycetemcomitans,* and the more pathogenic RFLP type B strain predominated over the RFLP type c strain in disease-active sites. The possibility also exists that one infecting strain (RFLP type B obtained from the mother) mutated during oral infection, and the mutant variant (RFLP type c) had a lower pathogenic potential than the parent strain. That genetic alterations in *A. actinomycetemcomitans* may result in altered virulence was suggested by Preus and Olsen (1987), who associated the triggering of a lytic bacteriophage in *A. actinomycetemcomitans* with the development of disease. In any event, the establishment of a differing virulence potential among various *A. actinomycetemcomitans* RFLP types may form the basis for delineating gene products of critical importance for disease development. Also, the use of DNA probes to indicate particularly pathogenic *A. actinomycetemcomitans* strains may be helpful in diagnosing subjects at high risk for periodontitis (Slots *et al.,* 1988).

It should be emphasized that pathogenicity is a multifactorial property influenced by the habitat of the bacterium and a number of host determinants (Slots and Genco, 1984). Identification of *A. actinomycetemcomitans* strains of high and low virulence is only a first

step to understanding pathogenesis. Future research has to delineate the determinant that is responsible for virulence, prove that it is relevant to infection *in vivo,* relate its chemical structure to its biological action, and determine possible protective and destructive host responses to it.

In conclusion, this study illustrates the potential value of molecular techniques in studying the epidemiology and virulence of *A. actinomycetemcomitans* in patients with localized juvenile periodontitis. Efforts to find the molecular basis for *A. actinomycetemcomitans*-mediated periodontal tissue destruction will undoubtedly continue.

Acknowledgments

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Abbreviation

RFLP restriction fragment-length polymorphism.

REFERENCES

Alcoforado GAP, McKay TL, Slots J. Rapid method for detection of lactose fermenting oral microorganisms. Oral Microbia. Immun. 1987; 2:35–38.

Baer PN. The case for periodontosis as a clinical entity. J. Periodont. 1971; 42:516–520. [PubMed: 5284178]

DiRienzo JM, Cornell S, Kazoroski L, Slots J. Probe-specific DNA fingerprinting applied to the epidemiology of localized juvenile periodontitis. Oral Microbial. Immun. 1990; 5:49–56.

Genco RJ, Slots J. Host responses in periodontal diseases. J. dent. Res. 1984; 63:441–451. [PubMed: 6366001]

Lindhe, J.; Slots, J. Periodontal disease in children and young adults. In: Lindhe, J., editor. Textbook of Clinical Periodontology. 2nd edn. Munksgaard: Copenhagen; 1989. p. 193-220.

Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Odont. Tidskr. 1966; 74:1–380.

Preus HR, Olsen I. Possible transmittance of *A. actinomycetemcomitans* from a dog to a child with rapidly destructive periodontitis. J. periodont. Res. 1988; 23:68–71. [PubMed: 2963906]

Preus HR, Olsen I, Namork E. The presence of phage-infected *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis patients. J. clin. Periodont. 1987; 14:605–609.

Slots J. Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J. clin. Microbial. 1982a; 15:606–609.

Slots J. Salient biochemical characters of *Actinobacillus actinomycetemcomitans*. Archs Microbial. 1982b; 131:60–67.

Slots J, Genco RJ. Black-pigmented *Bacteroides* species, *Capnocytophaga* species, and *ActinobaciIlus actinomycetemcomitans* in human periodontal disease: virulence factors in colonization, survival, and tissue destruction. J. dent. Res. 1984; 63:412–421. [PubMed: 6583243]

Slots, J.; Taichman, NS. Actinobaciiius actinomycetemcomitans. In: Newman, MG.; Nisengard, R., editors. Oral Microbiology and Immunology. Philadelphia: Saunders; 1988. p. 251-255.

Slots, J.; Taichman, NS.; Oler, J.; Listearten, MA. Does the analysis of the subgingival Dora have vahie in predicting periodontal breakdown?. In: Guggenheim, B., editor. Periodontology Today. Basel: Karger; 1988. p. 132-140.

Slots J, Feik D, Rams TE. *Actinobacillus actinomycetemcomitans* and *Bacteroides intermedius* in human periodontitis: age relationship and mutual association. J. clin. Periodont. 1990 (In press).

Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. molec. Biol. 1975; 98:503–517. [PubMed: 1195397]

Tsai C-C, Taichman NS. Dynamics of infection by leucotoxic strains of *Actinobaciilus actinomycetemcomitans* in juvenile periodontitis. J. clin. Periodont. 1986; 11:330–331.

Zambon JJ, Christersson LA, Slots J. *Actinobacillus actinomycetemcomitans* in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. J. Periodont. 1983a; 54:707–711. [PubMed: 6358452]

Zambon JJ, Slots J, Genco RJ. Serology of oral Actinobaciilus actinomycetemcomitans and serotype distribution in human periodontal disease. Infect. Immun. 1983b; 41:19–27. [PubMed: 6407997]

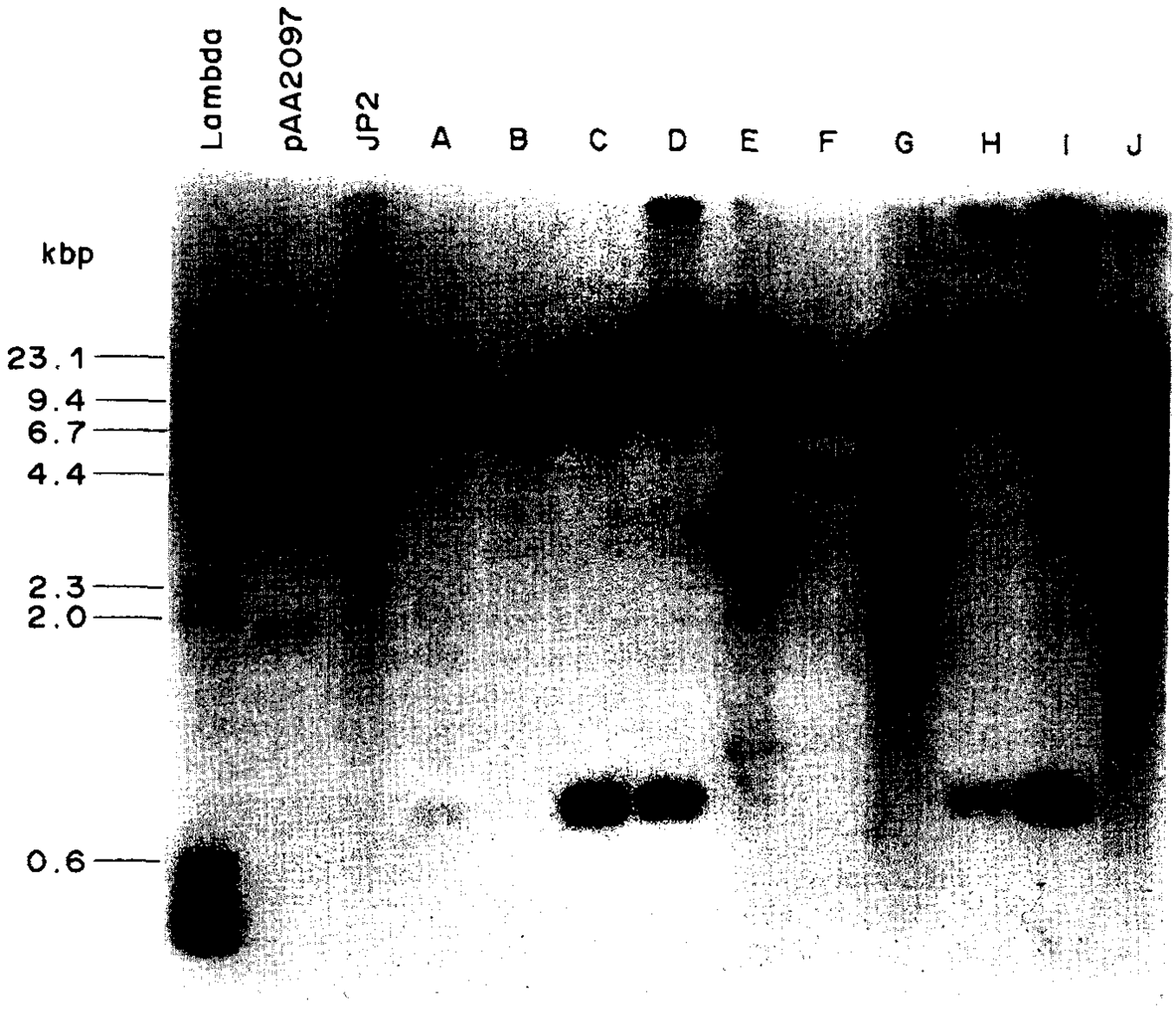

Plate 1

Fig. 1.

Example of RFLP analysis of selected subgingival isolates of *A. actinomycetemcomitans.* Chromosomal DNA was extracted from *A. acrinomycetemcomifans* recovered from subjects participating in a longitudinal study of localized juvenile periodontitis. Restriction fragments were Southern blotted and hybridized with a 4.7-kb DNA probe as described in Materials and Methods. Lambda, nick-translated lambda DNA markers; pAA2097, probe DNA; JP2, chromosomal DNA fragments from strain JP2. DNA samples are listed according to subject number. Lane A, 010004; lane B, 020007; lane C, 010004; lane D, 010003; lane E, 060026; lane F, 010003; lane G, 060026; lane H, 010003; lane I, 010003; lane J, 060028. The autoradiogram is shown. Reprinted with permission from DiRienzo *et al.* (1990).

Table 1

Subject description and A. *actinomycetemcomitans* isolates studied

Family	Subject	Sex	Family relationship	Number of isolates examined	RFLP designation*	Conversions
1	010001	Male	Father	1	a	
	010002	Female	Mother	1	E	
	010003	Female	Child	8	a, E	
	010004	Male	Child	4	a, b	
2	020007	Male	Child	1	B	(+)†
6	060025	Male	Father	0	—	
	060026	Female	Mother	3	G	
	060027	Male	Child	2	G	
	060028	Male	Child	11	G	
	060029	Male	Child	1	G	
	060083	Female	Child	0	—	
7	070030	Female	Mother	0	—	
	070031	Female	Child	12	B	+
	070032	Female	Child	0	—	
10	100039	Female	Mother	2	B	
	100040	Male	Child	87	B, c	+
	100054	Male	Father	0	—	

*Upper case letters represent RFLP of reference strains: Y4 (serotype *b)* = A; JP2 (serotype *b)* = B; ATCC 29522 (serotype *b) = C;* ATCC 29523 (serotype *a*) = D; ATCC 29524 (serotype *b)* = E; NCTC 9710 (serotype *c*) = F; 627 (serotype *a) = G.* Lower case letters represent RFLP patterns other than those of the reference strains.

†Converted immediately before entering study.

Table 2

RFLP patterns of *A. acfinomycetemcomitans* isolates from subject 100040

Site*	Visit number†													
	0	**11**	**20**	**30**	**40**	**41**	**50**	**51**	**60**	**61**	**70**	**71**	**80**	**90**
16M		400211	400311	400411	400511	400611	400711	400811	400911	401011		401211	401311 (5 isolates)	
14M	400012		400312	400412	400512	400612	400712	400812	400912	401012		401212	401312	
24M	400013		400313	400413	400513	400613	400713			401013		401213		
26M			400314	400414	400514	400614		400814				401214		
36M		400215		400415	400515‡		400715							
46M	400016		400316		4430516	400616	400716	400816	400916	401016				
pool	400017												400017	
										11D	11D		11D (5 isolates)	
										12D			12D	
											12M		12M	12M
										13M			13M	
								24D	24D	24D	24D		24D	
											36L 36L		36L	
											42B 42B		42B (5 isolates)§	
											42D 42D		42D	
													25M	

*Breakdown occurred at sites IlD, 12D, 12M, 13M, 24D, 36L, 42B, 42D, and 25M (all non-target sites).

†Visit number 0 represents baseline. Visits were scheduled every 3 months (intervals of IO). Additional visits are designated by odd-numbered intervals.

‡Boxed samples represent RFLP type B, unboxed samples represent RFLP type C.

§Only I out of 5 isolates in sample 42B from visit 80 had an RFLP of type B.