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Aggressive periodontitis in a 16-year-old Ghanaian adolescent, the original source of *Actinobacillus actinomycetemcomitans* strain HK1651 – a 10-year follow up

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Summary. The highly leukotoxic JP2 clone of *Actinobacillus actinomycetemcomitans* is strongly associated with periodontitis in adolescents. Availability of the DNA sequence of the complete genome of *A. actinomycetemcomitans* strain HK1651, a representative strain of the JP2 clone (www.genome.ou.edu/act.html), has provided new possibilities in basic research regarding the understanding of the pathogenesis of *A. actinomycetemcomitans* in periodontitis. This case report describes the periodontal treatment of the original source of *A. actinomycetemcomitans* HK1651, a 16-year-old Ghanaian adolescent girl with aggressive periodontitis. The bacterial examination involved polymerase chain reaction analysis for presence of JP2 and non-JP2 types of *A. actinomycetemcomitans*. The treatment, including periodontal surgery supplemented by antibiotics, arrested the progression of periodontitis for more than 10 years. Initially, infection by *A. actinomycetemcomitans*, including the JP2 clone, was detected at various locations in the oral cavity and was not limited to the periodontal pockets. Post-therapy, the JP2 clone of *A. actinomycetemcomitans* disappeared, while the non-JP2 types of *A. actinomycetemcomitans* remained a part of the oral microflora.

Introduction

In 1994, Brogan and co-workers showed that *Actinobacillus actinomycetemcomitans* strains characterized by a 530-bp deletion in the promoter region of the leukotoxin gene operon, called JP2 strains after the first isolate of this type [1], had an increased production of leukotoxin compared to *A. actinomycetemcomitans* strains without the 530-bp deletion (non-JP2 types) [2]. Later, it was demonstrated that the JP2 strains constitute a clone of *A. actinomycetemcomitans* serotype b, the highly leukotoxic JP2 clone [3,4]. Leukotoxin is able to kill important host immune cells as polymorphonuclear leucocytes [5–8].

Several studies have demonstrated an association between the presence of the JP2 clone of *A. actinomycetemcomitans* and periodontitis in adolescents [3,4,9–14]. Further, the manifestation and progression of clinical attachment loss in individuals positive for the JP2 clone of *A. actinomycetemcomitans* were more severe than in periodontally diseased individuals without this genotype of the species [10,15]. High prevalence of the JP2 clone of *A. actinomycetemcomitans* has been demonstrated in geographically widespread populations with African descent [3,4,13,14], and recent studies performed in North Africa have shown endemic occurrence of this type of *A. actinomycetemcomitans* in Morocco [9,10,15].

Aggressive periodontitis in adolescents is a rare disease in adolescents of northern European origin [16–19]. In recent years, however, the JP2 clone of *A. actinomycetemcomitans* has been isolated from African immigrants in several European countries [3,4,20,21] which has created renewed interest in the treatment of these relatively severe cases of periodontitis among adolescents. Here we report on clinical, radiographical, and microbiological findings, and the periodontal treatment performed in the original source of HK1651 *A. actinomycetemcomitans*, the strain from which the whole genomic DNA sequence is available at www.genome.ou.edu/act.html.

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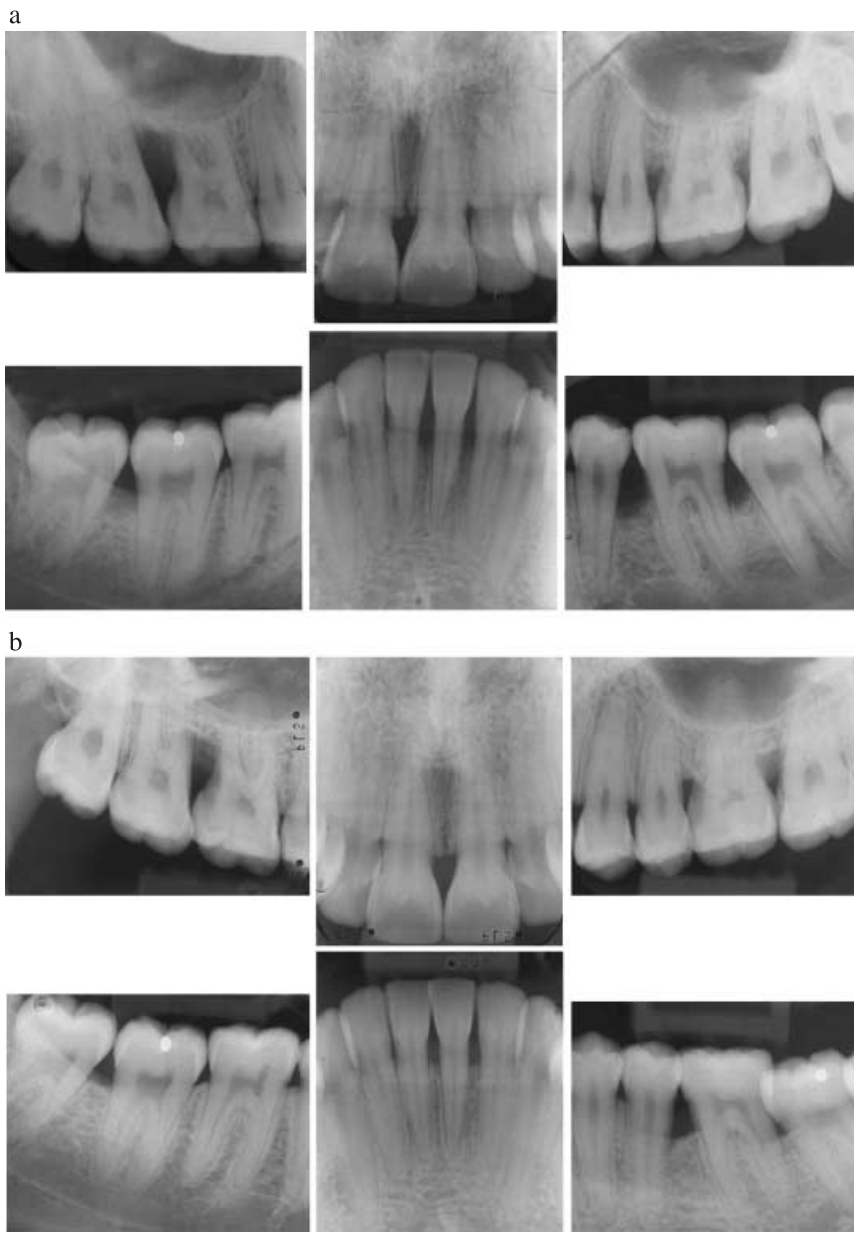

Fig. 1. (a) Intraoral radiographs of a 16-year-old Ghanaian female with localized aggressive periodontitis before surgical treatment supplemented by antibiotics. Alveolar bone loss was visible at all permanent first and second molars, except right lower first molar. Further, several incisors in both upper and lower region had bone loss. (b) Intraoral radiographs 1 year after surgical treatment supplemented by antibiotics. The radiographs show arrest of the disease and more distinct lamina dura on the top of the alveolar bone crest in the majority of the proximal areas.

Case report

A Ghanaian adolescent, 16 years of age, was referred to the Department of Periodontology, School of Dentistry, University of Copenhagen for treatment of aggressive periodontitis (Fig. 1a). The girl was otherwise healthy, and no systemic abnormality was noted in the medical history. The girl was born and lived in Ghana until the age of 12 years where after she moved to Denmark. The patient was followed for 11 years. In two periods, however, a dentist was not visited, a 2-year period 6 months after referral and a 5-year period 6 years after baseline (Table 1).

A clinical examination, including measurements of probing pocket depth (PPD) on six sites of 28 teeth (excluding the third molars), was performed at baseline and after 2·5, 3·5, 6, and 11 years. Furthermore, the examinations included full-mouth intraoral radiographs, except at the 6-year examination (Table 1). The proximal marginal bone level was determined at the radiographs as the distance from the cemento-enamel junction (CEJ) to the alveolar crest (AC), and alveolar

Table 1. Periodontal characteristics of a Ghanaian adolescent during a 10-year follow-up period.

	At referral	Before surgery	After surgery	At discharge	At 10-year recall
	At baseline	2·5 years after baseline	3·5 years after baseline	6 years after baseline	11 years after baseline
Number of teeth with PPD[a] ≥ 5 mm	19	9	5	6	6
Number of sites with PPD ≥ 5 mm	54 (168)[e]	21 (168)	5 (168)	8 (168)	9 (168)
Mean PPD (in mm) of sites ≥ 5 mm	7·0	7·9	5·0	5·4	5·0
Number of teeth with ABL[b]	15	15	15	–	14
Number of sites with ABL	18 (56)[f]	21 (56)	18 (56)	–	21 (52)
Mean distance from CEJ[c]-AC[d] (in mm) of sites with ABL	3·3	4·2	3·3	–	3·2

[a]PPD, probing pocket depth; [b]ABL: alveolar bone loss was defined on sites with a distance from CEJ – AC ≥ 2 mm [22].
[c]CEJ, cemento-enamel junction.
[d]AC, alveolar crest; [e]PPD was measured on six sites of 28 teeth (number of measurable periodontal sites in parentheses).
[f]The distance in mm from the cemento-enamel junction to the alveolar crest was measured on mesial and distal surfaces of 28 teeth (number of measurable proximal surfaces on radiographs in parentheses).

Table 2. Occurrence of JP2 and non-JP2 types of *Actinobacillus actinomycetemcomitans* at four sampling occasions.

Sampling occasions				Detection of *A. actinomycetemcomitans* with the 530-bp deletion (JP2 type)	Detection of *A. actinomycetemcomitans* without the 530-bp deletion (non-JP2 types)
No.	Relative to treatment	Sites microbiologically sampled			
First	Before surgery	Periodontal sites with ABL*	16, 26, 35, 31 (pooled)	+	–
Second	Before surgery	Periodontal sites with ABL*	16	+	–
			35	+	–
		Healthy sites	15	–	–
			34	–	–
		Tongue	Right side	+	–
			Left side	+	–
		Cheek	Right side	+	–
			Left side	+	+
		Tonsils	Right side	–	–
			Left side	+	+
Third	After surgery	Periodontal sites with ABL*	16	–	+
			35	–	–
Fourth	At the 10-year recall	Deepest periodontal sites	17, 16 26, 27 (pooled)	–	+

*ABL, alveolar bone loss.

bone loss (ABL) was defined as sites with a distance from CEJ – AC ≥ 2 mm [22].

After the baseline examination, a 6-month period of treatment including motivation, oral hygiene instruction, and subgingival scaling was performed, followed by a 2-year period without dental care due to drop out. Two and a half year after baseline the patient attended the dental clinic regularly again. Another short hygiene phase was initiated. This phase was followed by performance of periodontal flap surgery in all premolar/molar regions and in the lower incisor region of the mouth supplemented by systemic amoxycillin (3 × 375 mg/day) and metronidazole (3 × 250 mg/day) for 12 days. After another 2·5 year of regular supportive therapy (from 3·5 to 6 years after baseline) the patient was discharged from the clinic. Eleven years after baseline (10-year recall) the patient was recalled for a follow-up examination.

Microbiological samples were collected by the paper point method in periodontal sites and by swaps with cotton buds from cheeks, tonsils, and tongue (Table 2). At the first of two sampling occasions before surgical treatment, a pooled plaque sample was collected from four of the deepest periodontal pockets to determine the presence of the JP2 and non-JP2 types of *A. actinomycetemcomitans* (Table 2). The second sampling occasion before surgical treatment included 10 bacterial samples from various locations in the oral

cavity (from 2 sites with periodontitis, 2 healthy sites, 2 samples from the tongue, the cheeks, and the tonsils) (Table 2). After surgical treatment, subgingival plaque (the third sampling occasion) was collected from the same two periodontitis sites (JP2 positive sites) as sampled before surgical treatment. Finally, a pooled sample from four sites of the deepest periodontal pockets at that point of time was collected 11 years after baseline. At the first, second and the third sampling occasions, conventional cultivation technique for isolation of *A. actinomycetemcomitans* strains was used followed by subtyping of leukotoxin promoter type by polymerase chain reaction (PCR) as previously described [4]. Three to six isolates from each of the *A. actinomycetemcomitans*-positive samples were subtyped by PCR. At the 10-year recall (the fourth sampling occasion) the occurrence of JP2 and non-JP2 types of *A. actinomycetemcomitans* was detected by PCR using plaque sample suspended in 0·9% w/v saline as previously described [23].

The clinical and radiographical findings for the study period are shown in Table 1. At baseline, the majority of teeth (19 out of 28) had PPD ≥ 5 mm and more than half of the teeth had ABL (Table 1). After an initial 6-month period with treatment and a 2-year period without dental care, another examination showed that the number of teeth and number of sites with PPD ≥ 5 mm had decreased, but mean PPD for sites ≥ 5 mm had increased from 7·0 to 7·9 mm (Table 1). In addition, an increase in alveolar bone loss was recorded compared to baseline (Table 1). At the examination of the patient 1 year after surgery, arrest of the periodontal destruction was documented both clinically and radiographically (Fig. 1b, Table 1). At the discharge the clinical examination showed that the periodontal condition was stabilized (Table 1). At the 10-year recall only minor changes of the dental status were found compared to the findings at the examination 5 years earlier (Table 1).

Initially, a pooled plaque sample from four periodontal sites revealed that the patient was infected by the JP2 clone of *A. actinomycetemcomitans* (Table 2). At the second sampling occasion 10 bacterial samples were positive for the JP2 clone of *A. actinomycetemcomitans*, except the two shallow pockets and the right tonsil (Table 2). In addition, the cheek and the tonsil in the left side were positive for non-JP2 types of *A. actinomycetemcomitans*. After surgery, plaque sampling of the same two periodontal pockets (teeth 16 and 35) as sampled before surgical treatment revealed that these pockets were no longer positive for the JP2 clone. However, one of the periodontal pockets (tooth 16) was positive for the non-JP2 types of *A. actinomycetemcomitans*. The JP2 clone of *A. actinomycetemcomitans* was not detected at the 10-year recall, although the non-JP2 type of *A. actinomycetemcomitans* was found (Table 2).

Discussion

The present study demonstrates a successful treatment of localized aggressive periodontitis in a 16-year-old Ghanaian adolescent culture-positive for the JP2 clone of *A. actinomycetemcomitans* prior to therapy. Treatment successfully arrested ABL and eradicated or at least reduced levels of highly leukotoxic JP2 clone of *A. actinomycetemcomitans* below the level of detection. In contrast, non-JP2 types of *A. actinomycetemcomitans* remained a part of the oral microflora after treatment.

The actual number of bacterial samples collected in this case, followed by the use of conventional cultivation techniques may include a risk of getting false-negative results [23–25]. However, another method, PCR directly on dental plaque suspended in saline, became available to us during the follow-up period and was used at the 10 years follow-up [23]. This method is highly specific in the detection of *A. actinomycetemcomitans* and more sensitive than cultivation followed by PCR subtyping [23,26]. Most patients are colonized by one type of *A. actinomycetemcomitans*, whereas few are colonized by two or more clonal types [27–29]. In this case, the patient was initially only positive for the JP2 clone of *A. actinomycetemcomitans* based on analysis of a pooled plaque sample (4 periodontal sites) by conventional culturing techniques. However, it is tempting to suggest that the patient at this point of time as well might have been positive for the non-JP2 types of *A. actinomycetemcomitans* if a higher number of sites in the oral cavity had been sampled, and if a higher number of colonies from each sample had been subtyped. This is supported by the finding that the patient was culture-positive for both JP2 and non-JP2 types of *A. actinomycetemcomitans* when the patient was resampled. In contrast, it appears unlikely that the patient was positive for the JP2 type of *A. actinomycetemcomitans* at the end of the follow-up period as identical results were obtained 6 years apart. Further, the method used for the detection at the end of the follow-up period was demonstrated to be more sensitive than the method used previously [23].

Only periodontal sites were resampled after surgery because of these sites being considered focus sites for the *A. actinomycetemcomitans* infection and the relevant sites in relation to the manifestation of the disease.

One may wonder why the JP2 type of *A. actinomycetemcomitans* disappeared when the non-JP2 type remained a part of the flora after treatment. One reason may be that *A. actinomycetemcomitans* was eradicated during the treatment, but the patient was reinfected, e.g. from family members, by the non-JP2 types of *A. actinomycetemcomitans*. It is also possible that the JP2 and non-JP2 types of *A. actinomycetemcomitans* were not eradicated completely by the treatment, and that a subsequent increase in antibody levels against the leukotoxin because of the increasing age of the patient may have been detrimental to the highly leukotoxic JP2 clone [30,31], or that the periodontal therapy may have changed the ecology so that only the non-JP2 types of *A. actinomycetemcomitans*, which is considered a part of the normal flora (an opportunistic pathogen), survived the altered ecological conditions of the periodontium.

Despite the fact that the patient did not consult a dentist at all during the last 5 years of the follow-up period, no further progression of the periodontitis was seen after the complete 11-year period. A strong host response against the oral microflora combined with a lowering of the level (below detection level) or eradication of the JP2 clone of *A. actinomycetemcomitans* after intensive treatment may be important factors in the arrest of the initially very active periodontitis in this patient. There is evidence that infection with the JP2 clone of *A. actinomycetemcomitans* is important to consider in the progression of periodontitis in adolescents, particularly in individuals of African descent [10,12]. The JP2 clone of *A. actinomycetemcomitans*, however, may not be the direct or the only causal factor related to the disease. The occurrence of the JP2 clone of *A. actinomycetemcomitans* may be linked to other important factors, e.g. other coexisting pathogenic bacteria or a specific periodontitis-susceptible genetic constitution of the host, in the pathogenesis of aggressive periodontitis.

As demonstrated, *A. actinomycetemcomitans* infection was not limited to the periodontal pockets. It could be speculated that if antibiotics are to be used as part of the periodontal therapy in patients with aggressive periodontitis, antibiotics administered systemically rather than locally are likely to be more effective.

What this paper adds

- *Actinobacillus actinomycetemcomitans* strain HK1651, belonging to the JP2 clone strongly associated with aggressive periodontitis, is an important reference strain as the DNA sequence of the complete genome is available. This paper describes clinical, radiological, and microbiological findings, and the periodontal therapy performed in an aggressive periodontitis patient, the original donor of *A. actinomycetemcomitans* strain HK1651.

Why this paper is important for paediatric dentists

- The JP2 clone of *A. actinomycetemcomitans* is most frequently found in individuals of African origin. This report confirms the importance of being aware that individuals of African descent, living in widespread areas of the world, may be infected by the JP2 clone.
- If use of antibiotics is considered as supplement to the periodontal treatment of aggressive periodontitis in children and adolescents, the paediatric dentist has to be aware that not only periodontal pockets, but also other locations in the oral cavity, may be infected by *A. actinomycetemcomitans*. Therefore, systemic rather than local agents may be relevant.

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