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Detection of a highly toxic clone of *Actinobacillus actinomycetemcomitans* (JP2) in a Moroccan immigrant family with multiple cases of localized aggressive periodontitis

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Summary. The JP2 clone of *Actinobacillus actinomycetemcomitans*, a high-leukotoxin-producing strain, characterized by a 530-basepair (bp) deletion in the promoter region of the leukotoxin gene operon and mainly found among individuals with African origin, is associated with localized aggressive periodontitis. The objective of the study was to examine the occurrence of periodontal disease in a Moroccan immigrant family living in Denmark in which the oldest son (14 year) was referred and treated for localized aggressive periodontitis. Further, the potential occurrence of the JP2 clone of *A. actinomycetemcomitans* in the family was examined. Here we present the clinical, radiographic, and microbiological findings from the family. Clinical and radiographic examination of the other family members revealed that 3 of 5 younger siblings had localized aggressive periodontitis, one had gingivitis and the mother had chronic periodontitis. Despite scaling followed by intensive maintenance therapy several family members, including the sibling with gingivitis, had further attachment loss at the 1-year examination. The JP2 clone of *A. actinomycetemcomitans* was isolated from subgingival plaque samples from 4 children with periodontitis. In contrast, it was not detected in plaque from the oldest boy, who had been treated for localized aggressive periodontitis by surgery combined with antibiotic therapy. The 4 children with periodontitis and colonized with the JP2 clone were treated by scaling and antibiotic administration. One month later the JP2 clone could still be detected in plaque samples. In conclusion, it is confirmed that members of immigrant families with African origin are potential carriers of the JP2 clone and that those families often have multiple family members with localized aggressive periodontitis. It is proposed that those families are given periodontal examination frequently to benefit from early diagnosis and treatment of the disease.

Introduction

Several population studies have indicated that occurrence of localized aggressive periodontitis (LAP) varies significantly between different parts of the world, with a tendency to generally higher prevalence of LAP in Africa and among individuals of African descent compared to Caucasians [1].

The occurrence and role of the JP2 clone of *Actinobacillus actinomycetemcomitans* in the aetiology of LAP is a matter of interest and debate. The JP2 clone of *A. actinomycetemcomitans* is characterized by a 530-basepair (bp) deletion in the promoter

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region of the leukotoxin gene operon and by a relatively high expression of leukotoxin [2]. There is a strong association between occurrence of the JP2 clone and LAP [3]. Further, LAP-patients with the JP2 clone have more advanced stages of LAP than LAP-patients without the JP2 clone in dental plaque [4].

Studies have revealed that Moroccan, Algerian and Ghanese immigrants living in northern Europe and with LAP may be colonized by the JP2 clone of *A. actinomycetemcomitans* [5,6]. Also Afro-Americans and individuals from Brazil were colonized by the JP2 clone [6]. In contrast, the JP2 clone was not detected in a collection of *A. actinomycetemcomitans* strains isolated from Caucasians in northern Europe [7]. More recently, it was confirmed, in a study performed in the USA, that *A. actinomycetemcomitans* strains with the 530-bp deletion was mainly found in individuals of African descent [8]. Recently, in a study performed in Morocco we showed that the JP2 clone of *A. actinomycetemcomitans* was present in 9% of a group of adolescents (14–19-year-old) [3].

Members of the species *A. actinomycetemcomitans* have been isolated from between 5 and 48% of 0–18-year-old individuals [9–13]. The occurrence of specific clonal types of *A. actinomycetemcomitans* as the JP2-type has until now mainly been studied in adolescents and adults [3,5,6,14–16]. Few studies describe cases of LAP among children being culture-positive for the JP2 clone [16–18]. In these studies an age predilection for being carrier of the JP2 clone has been suggested as no subjects over 21 years harboured the JP2 clone.

Early diagnosis of LAP is important for successful treatment. It is known that a substantial proportion (52%) of individuals with LAP have signs of periodontal disease (one or more sites with bone loss) in the primary dentition [19]. Knowing that the JP2 clone of *A. actinomycetemcomitans* is likely to be found in the Moroccan population and is strongly associated with LAP we initiated this family study. The aim was to examine whether younger siblings in a Moroccan immigrant family in Denmark, where the oldest son had been diagnosed and treated for LAP, also had LAP and/or were colonized by the JP2 clone of *A. actinomycetemcomitans*.

Case report

A Moroccan immigrant family consisting of 7 members, a 38-year-old mother and 6 sons, 7–14-year-old, were clinically, radiographically and microbiologically examined. The father was not included in the study as he no longer lived with the family. The oldest son was referred from the Municipal Dental Service in Copenhagen, Denmark to the Dental School in Copenhagen for dental treatment of LAP (Fig. 1). At the referral, bitewing radiographs taken three years earlier showed manifest marginal bone loss in the primary molar regions documenting the presence of periodontitis. As the adolescent was of Moroccan origin the whole family was invited to an examination at the Dental School, while the regular dental care of the children was continued in the Municipal Dental Service. The children of the family as well as the mother were otherwise healthy and no systemic abnormality was noted in the medical history.

All family members were clinically examined, including measuring of periodontal pocket depth and probing attachment level, on six sites of all fully erupted teeth. Presence of periodontal pockets of 5 mm or more and clinical attachment loss of 2 mm or more is summarized in Table 1. Periodontal disease was diagnosed according to the classification of the 1999 International Workshop for classification of periodontal diseases and conditions [20].

The examination included an orthopantomograph of the mother and bitewing radiographs of the children. In addition, a set of full-mouth intraoral radiographs was obtained from the oldest brother before initiation of treatment. The proximal marginal bone level was registered at the radiographs as the distance between the cementum-enamel junction and the marginal bone level to the nearest 1 mm using a ruler. Pathological bone loss was suggested on sites with a distance > 2 mm [21].

Clinical and radiographic examination revealed that 3 of 5 younger siblings had LAP, one had gingivitis, one was healthy, and the mother had chronic periodontitis (Table 1). It can be seen that all family members except the 12-year-old child (patient no. 4) presented periodontal pockets of 5 mm or more. Furthermore, all except the 12- and 13-year-old boys had clinical attachment loss of 2 mm or more on two or more teeth. The 7-year-old dizygote twins showed clinical attachment loss on 3 and 4 primary molars, respectively (Table 1) (Fig. 2).

Despite a course of scaling, repeated oral hygiene instructions, and full mouth cleaning whenever needed, the 13-year-old boy with gingivitis showed bleeding on probing and radiographic loss of the

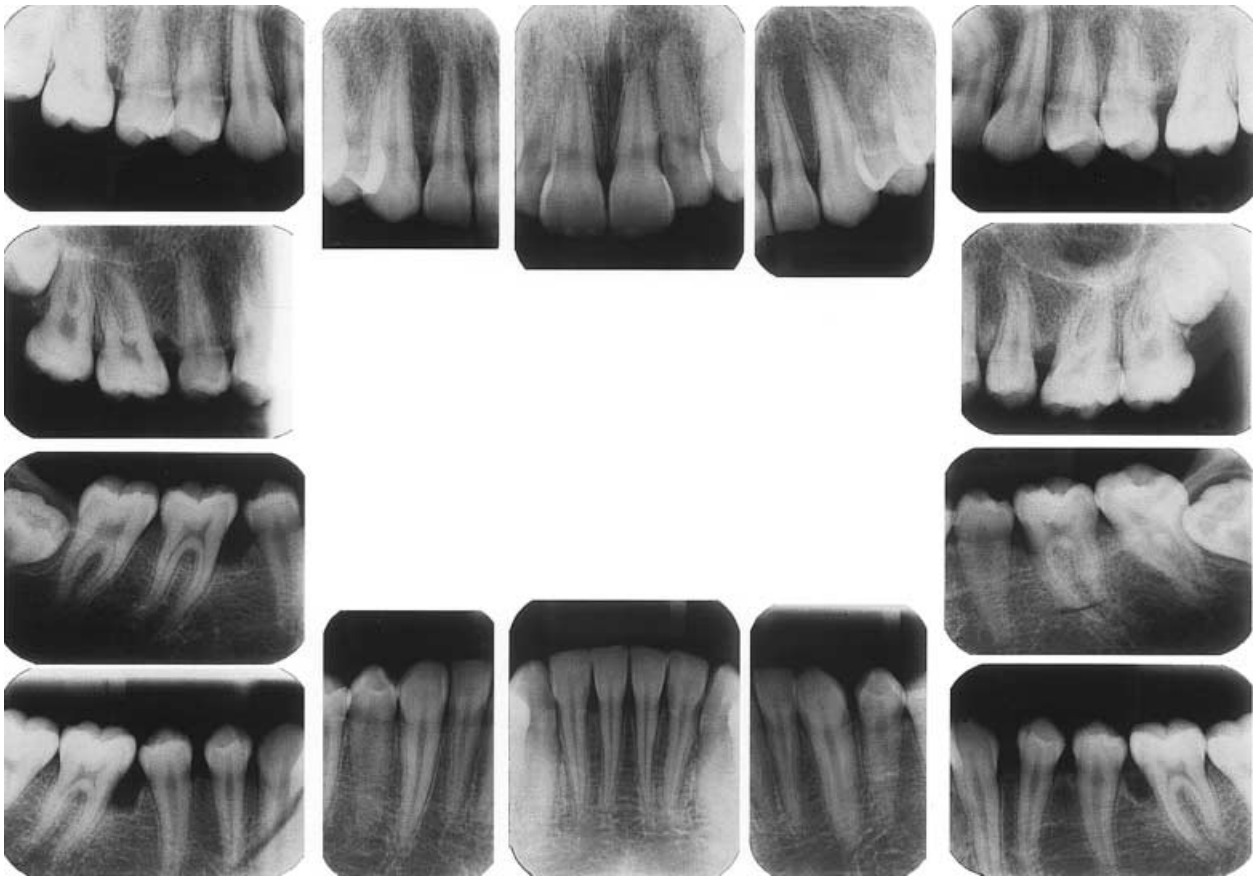

Fig. 1. A 14-year-old Moroccan boy with localized aggressive periodontitis. On full-mouth intraoral radiographs bone loss was visible at all permanent first molars and the upper left central and lateral incisors.

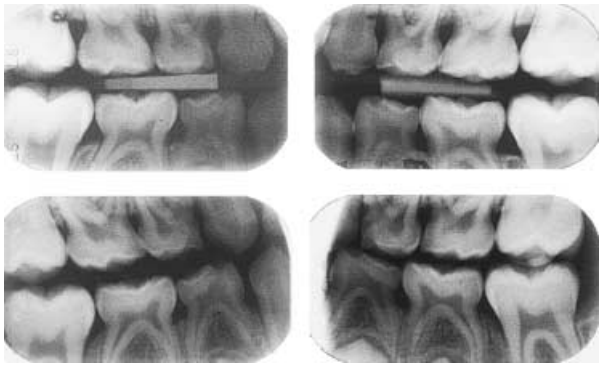

Fig. 2. Bitewing radiographs of the dizygote twins (7-year-old) with localized aggressive periodontitis. Marginal bone loss was visible in one of the twins (upper two radiographs) at the teeth 54, 63 and 64, and on the other twin (lower two radiographs) at the teeth 64 and 65, where the distance between the cementum-enamel junction and the marginal bone level was 3 mm on one of the proximal surfaces.

lamina dura of the alveolar crest at the first left lower molar and between the first and second right lower incisors at the one-year follow-up examination indicating initial periodontitis. The three boys already diagnosed with LAP also showed further marginal bone loss on at least one of the primary molars, after 1 year.

A subgingival plaque sample was then collected from between 2 and 4 pockets with depth between 3 and 7 mm (pooled samples) from each family member and placed in tubes with saline (0·9%) (Table 2). Occurrence of *A. actinomycetemcomitans* with and without the 530-bp deletion in the promoter region of the leukotoxin gene was detected by polymerase chain reaction (PCR) using the primers ltx3 and ltx4 directly on plaque samples as described by Poulsen and co-workers [22]. The PCR method used is highly specific in the detection of *A. actinomycetemcomitans* and more sensitive than cultivation of plaque samples followed by identification of *A. actinomycetemcomitans* [22]. The 3 children with

Table 1. Clinical periodontal findings in a Moroccan immigrant family consisting of a mother and her 6 sons.

Patient no.	Age (years)	Gender	Number of teeth present		Periodontal diagnosis*	Teeth with periodontal pockets ≥ 5 mm	Teeth with clinical attachment loss ≥ 2 mm
			Primary	Permanent			
1	38	f	0	27	CP	1 | 1 5 (31, 35, 41)	5 1 | 1 4 5 (31, 34, 35, 41, 45)
2	14	m	0	28	LAP	6 | 1 2 6 6 1 | 1 6 (16, 21, 22, 26, 31, 36, 41, 46)	6 | 1 2 6 6 | 6 (16, 21, 22, 26, 36, 46)
3	13	m	0	26	Gingivitis	6 | 6 (36, 46)	0
4	12	m	2	23	Healthy	0	0
5	10	m	12	12	LAP	E | 2 D E (55, 22, 64, 65)	E D | D E (54, 55, 64, 65)
6	7**	m	14	10	LAP	D | D (54, 64)	D | D E D (54, 64, 65, 74)
7	7**	m	12	10	LAP	| D E (64, 65)	D | D E (54, 64, 65)

*CP, Chronic periodontitis; LAP, Localized aggressive periodontitis; **Dizygote twins.

Table 2. Occurrence of *A. actinomycetemcomitans* strains with and without the 530-bp deletion in the promoter region of the leukotoxin gene in subgingival plaque collected from a Moroccan immigrant family consisting of a mother and her 6 sons.

Patients no.	Teeth from which subgingival plaque samples were obtained	Detection of *A. actinomycetemcomitans* with the 530-bp deletion (JP2 clone)	Detection of the *A. actinomycetemcomitans* without the 530-bp deletion
		One year after baseline examination	
1	41, 35	–	+
2	26, 46	–*	–
3	36, 46	+**	–
4	46, 75	–	–
5	54, 84	+**	–
6	54, 64, 74, 84	+**	–
7	64, 74, 84	+**	–

*has been treated (scaling, surgery and antibiotic therapy); **has been treated (scaling only).

LAP and the 13-year-old boy with initial periodontitis were colonized by the JP2 clone. The JP2 clone was not detected in plaque from the oldest boy, who prior to the plaque sampling had received periodontal treatment for LAP including flap surgery with scaling of the root surfaces followed by twice-daily rinses with 0·1% Chlorhexidine digluconate solution for 3 weeks. Systemic amoxicillin (3×375 mg/day) was prescribed for 14 days during the surgical periodontal therapy. At his examination 1 year later treatment arrest of the periodontal destruction was documented both clinically and radiographic (Fig. 3). The mother was the only one colonized by *A. actinomycetemcomitans* strains without the characteristic 530-bp deletion (Table 2).

Treatment, including scaling combined with systemic amoxicillin 3×375 mg/day prescribed for 10 days for the 4 younger siblings positive for the JP2 clone and with periodontitis did not arrest the disease. The JP2 clone of *A. actinomycetemcomitans* could still be detected one month after the initiation of the local treatment and systemic antibiotic therapy.

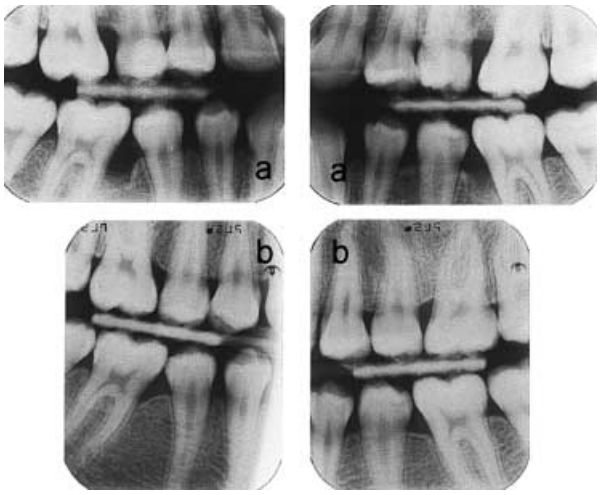

Fig. 3. Bitewing radiographs of the oldest son before treatment was initiated (a) and one year after treatment (b). Healing of the marginal crest at the first molars is evident.

Discussion

The present study shows occurrence of the JP2 clone of *A. actinomycetemcomitans* in 4 individuals in a family with multiple cases of LAP. The JP2 clone was isolated from subgingival plaque collected from pockets around primary first molars in 3 children with LAP affecting several teeth. This indicates that infection with the JP2 clone of *A. actinomycetemcomitans* may happen early in life and that sites around primary molars may serve as a reservoir and appropriate ecological niche for growth of the JP2 clone of *A. actinomycetemcomitans*. Several studies have suggested an early acquisition of *A. actinomycetemcomitans* [11]. Here it is shown that the JP2 clone of *A. actinomycetemcomitans* is one of the clonal types of *A. actinomycetemcomitans* that can colonize children early in life. In some studies, an age predilection of infection with the JP2 clone [16–18] has been suggested, as the clone was more prevalent among younger individuals (< 14 years of age) and absent or present in very low numbers only in the subgingival microbiota of older individuals. This hypothesis is supported by the findings in this study as the mother did not have the JP2 clone at detectable level. Transmission of *A. actinomycetemcomitans* strains from parents to children has been demonstrated (vertical transmission) [23,24]. However, if the JP2 clone of *A. actinomycetemcomitans* is below detection level in adults we must question how the JP2 clone of *A. actinomycetemcomitans* is transmitted. It is not clear whether the main route of transmission of the JP2 clone is vertical or horizontal or a combination of both.

It is well known that LAP often responds less favourably to scaling and surgery than other forms of periodontitis. Furthermore, scaling and surgery has been ineffective in eliminating *A. actinomycetemcomitans* in these patients [25]. It has therefore been suggested that conventional therapy is supplemented with systemic antibiotic administration. Both tetracycline and metronidazole have been recommended as favourable antibiotics in treatment of *A. actinomycetemcomitans*-associated aggressive periodontitis [25–27]. Finally, a combination of systemic amoxicillin and metronidazole as a supplement to surgical treatment has been suggested as the most effective treatment of *A. actinomycetemcomitans*-associated aggressive periodontitis [28]. In this study, treatment of the oldest son, including scaling and surgery supplemented with amoxicillin, was successfully carried out as healing of periodontal lesions and no further periodontal destruction was found one year after initiation of treatment (Fig. 3). In contrast to the successful treatment of the oldest son, for whom we have no information on the microbiological findings before initiation of treatment, the treatment regime consisting of oral hygiene instruction and scaling was not sufficient to stop progress of LAP in the affected younger children (patients 3, 5, 6, 7). Besides, oral hygiene instruction, scaling, and antibiotic therapy with amoxicillin were not sufficient to eliminate or reduce the JP2 clone of *A. actinomycetemcomitans* to below the level of detection. It appears that radical treatment including extraction of primary molars and/or prescription of the recommended combination of both amoxicillin and metronidazole, despite the difficulties in patient compliance and cooperation and possible general side-effects, may be needed to improve the periodontal status and to possibly prevent a potential transition of the disease from the primary to the permanent dentition. However, factors such as, e.g. tooth migration after early extraction of primary teeth, are undesirable and cooperation of children in periodontal treatment and in simultaneous intake of medication in a large family may be challenging for the children, the parents and the dentist. More studies on treatment of LAP and prevention of transition of the disease from the primary to the permanent dentition are needed.

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Résumé. Le clone JP2 de *Actinobacillus actinomycetemcomitans*, une souche hautement productrice de leucotoxine, caractérisé par une délétion de 530 paires de bases (bp) dans la région du promoteur de l'opéron du gène de la leucotoxine et essentiellement trouvé chez des individus d'origine africaine, est associé à des parodontites agressives localisées. L'objectif de l'étude a été d'évaluer la présence de maladies parodontales dans une famille immigrante vivant au Danemark dont le fils aîné (14 ans) a été adressé et traité pour parodontite agressive localisée. La présence potentielle du clone JP2 de *Actinobacillus actinomycetemcomitans* a également été recherchée dans la famille.
Nous présentons dans cet article les données cliniques, radiographiques et microbiologiques de cette famille. L'examen clinique et radiographique a révélé que 3 des 5 autres membres de la fratrie présentaient une parodontite agressive localisée, 1 une gingivite et la mère une parodontite chronique. Malgré le détartrage suivi par une maintenance intensive, plusieurs membres de la famille dont l'enfant avec gingivite montraient une perte ultérieure d'attachement lors de la visite à un an. Le clone JP2 d'*Actinobacillus actinomycetemcomitans* a été isolé d'échantillons de plaque sous-gingivale chez les 4 enfants avec parodontite. A l'opposé, il n'a pas été détecté dans la plaque du frère aîné qui avait été traité pour parodontite agressive localisée par chirurgie combinée à une antibiothérapie. Les 4 enfants avec parodontite et colonisés par le clone JP2 ont été traités par détartrage et traitement antibiotique. Un mois plus tard, le clone JP2 pouvait toujours être détecté dans les échantillons de plaque.
En conclusion, cette étude confirme que les membres de familles immigrantes issues d'Afrique sont potentiellement porteuses du clone JP2 et que ces familles ont souvent de nombreux membres de la famille avec parodontite agressive localisée. Il est proposé d'effectuer fréquemment un examen parodontal dans ces familles pour bénéficier d'un diagnostic et d'une prise en charge précoces.

Zusammenfassung. Der JP2 Klon von Actinobacillus actinomycetemcomitans (AA), ein in hohem Maße Leukotoxin-produzierender Stamm, charakterisiert durch eine 530-Basenpaare-Deletion in der Promotorregion des Leukotoxin Gen-Operons und überwiegend isoliert aus Individuen afrikanischer Abstammung, ist mit aggressiver Parodontitis assoziiert.
Ziel dieser Studie war es, das Vorkommen von parodontaler Erkrankung bei einer marokkanischen Einwandererfamilie zu untersuchen, die in Dänemark lebt und deren ältester Sohn überwiesen und behandelt wurde aufgrund lokalisierter aggressiver Parodontitis.
Außerdem wurde das mögliche Auftreten des JP2 Stamms von AA innerhalb der Familie untersucht. In diesem Fallbericht stellen wir die klinischen, röntgenologischen und mikrobiologischen Untersuchungsergebnisse der Familie vor. Die Untersuchung ergab, dass drei der fünf jüngeren Geschwister lokalisierte Parodontitis aufwiesen, eines hatte Gingivitis, die Mutter chronische Parodontitis. Trotz Scaling und intensiver Nachbetreuung zeigten mehrere Familienmitglieder weiteren Attachmentverlust nach einem Jahr. Der JP2 Stamm von AA wurde bei vier Familienmitgliedern mit Parodontitis nachgewiesen, nicht jedoch bei dem ältesten Jungen, welcher initial zusätzlich antibiotisch behandelt worden war. Die vier Kinder mit Parodontitis und JP2 wurden darauf ebenfalls kombiniert mechanisch-antibiotisch behandelt. Es bestätigt sich, dass Familien afrikanischer Herkunft potentiell träger von JP2 sein können und oft mehrere Familienmitglieder aggressive Parodontitis aufweisen. Solche Familien sollten regelmäßig parodontologisch untersucht werden um von der Frühdiagnose und Frühbehandlung zu profitieren.

Resumen. El clon JP2 del *Actinobacillus actinomycetemcomitans*, una cepa con gran producción de leucotoxinas, caracterizada por una delación en la base pareada.
530 (bp) en la región promotora del operon del gen de leucotoxina y generalmente localizada en individuos de origen africano, está asociada con la periodontitis agresiva localizada. El objetivo de este estudio fue examinar la ocurrencia de enfermedad periodontal en una familia inmigrante de Marruecos

que vive en Dinamarca en la cual el hijo mayor (14 años) fue referido y tratado por una periodontitis agresiva localizada. Además, fue examinado el potencial de ocurrencia del clon JP2 del *A. actinomycetemcomitans* en la familia. Aquí presentamos los hallazgos clínicos, radiográficos y microbiológicos en la familia. El examen clínico y radiográfico de los otros miembros de la familia reveló que 3 de los 5 hermanos menores tenían periodontitis agresiva localizada, uno tenía gingivitis y la madre tenía periodontitis crónica. A pesar del tratamiento de raspado y de la intensiva terapia de mantenimiento, varios miembros de la familia, incluyendo al hermano con gingivitis, tuvieron pérdida de la inserción gingival en el control anual.

El clon JP2 del *A. actinomycetemcomitans* fue aislado de las muestras de placa subgingival de los 4 niños con periodontitis. En contraste, no fue detectado en la placa del hijo mayor, que había sido tratado de periodontitis agresiva localizada con un tratamiento combinado de cirugía y antibióticos. Los 4 niños con periodontitis y colonizados con el clon JP2 fueron tratados con raspados y administración de antibióticos. Un mes después, el clon JP2 podía detectarse aún en las muestras de placa. En conclusión, se confirma que los miembros de familias inmigrantes de origen africano son portadores potenciales del clon JP2 y que esas familias con frecuencia tienen múltiples miembros de la familia con periodontitis agresiva localizada. Se propone que se les brinde un examen periodontal frecuente a esas familias para que se beneficien del diagnóstico y tratamiento precoz de la enfermedad.

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