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Microbiological Characterization in Children with Aggressive Periodontitis

ABSTRACT

The objective of this study was to characterize the subgingival microbiota of African-American children with Localized Aggressive Periodontitis (LAP). Fifty-one children were included. Subgingival plaque samples were taken from diseased (DD) and healthy sites (DH) in LAP and from healthy sites in HS and HC and analyzed by 16S rRNA-based microarrays. *Aggregatibacter actinomycetemcomitans* (Aa) was the only species found to be both more prevalent (OR = 8.3, p = 0.0025) and abundant (p < 0.01) in DD. *Filifactor alocis* (*Fa*) was also found to be more prevalent in DD (OR 2.31, CI 1.06-5.01, p = 0.03). Most prevalent species in healthy sites were *Selenomonas* spp, *Veillonella* spp, *Streptococcus* spp, *Bergeyella* sp, and *Kingella oralis*. Overall, *Streptococcus* spp, *Campylobacter gracilis, Capnocytophaga granulosa, Haemophilus parainfluenzae*, and *Lautropia mirabilis* were most abundant in healthy children, while *Aa, Fa, Tannerella* sp, *Solobacterium moorei, Parvimonas micra*, and *Capnocytophaga* sp were most abundant in LAP. Based on a comprehensive analysis with 16S rRNA-based microarrays, *Aa* was strongly associated and site-specific in LAP. In contrast, other species were found to be associated with healthy sites and individuals (ClinicalTrials.gov number CT01330719).

Abbreviations: healthy site in healthy sibling (HS); healthy site in healthy control child (HC).

KEY WORDS: localized aggressive periodontitis, diagnosis, microbiology, *Aggregatibacter actinomycetemcomitans*, HOMIM, subgingival microbiota.

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INTRODUCTION

Aggressive periodontitis (AgP) is comprised of a group of less frequent, often severe, rapidly progressive forms of periodontitis. This disease is characterized by an early age of onset, with molar and incisor teeth to be the first ones affected in localized forms (LAP), and a propensity for familial involvement. Albandar and Tinoco (Ickenstein *et al.*, 1999) reported a prevalence of AgP in children/adolescents from 0.1% (Europe) to 7.6% (Africa). Given its rare occurrence and difficulties in gathering large populations for study, our knowledge on AgP is still limited.

Numerous reports have aimed to characterize the microbiota associated with AgP. Some studies have associated the species *Aggregatibacter* (previously *Actinobacillus*) *actinomycetemcomitans* (*Aa*) with AgP in different populations (Zambon *et al.*, 1983; Kaldahl *et al.*, 1988; Haffajee *et al.*, 2003; Leung *et al.*, 2005; Yang *et al*., 2005; Kaner *et al*., 2007). Recent studies have shown a specific clone of *Aa* (JP2) to be associated with AgP in adolescents living in or originating from north and west Africa (Haubek *et al.*, 1996, 1997). This species has also been reported to be associated with the initiation of attachment loss in adolescents (Spinozzi and Langer, 1999; Haubek *et al*., 2008).

Other studies have found other species, such as *P. gingivalis (Pg)*, to be more prevalent in AgP (Gajardo *et al.*, 2005; Mayorga-Fayad *et al*., 2007). Thiha *et al.* (2007) reported that a population of Japanese individuals with either chronic or AgP was found to harbor no different amounts of the main periodontopathogenic species, *Aa, Pg*, and *T. forsythia*, although *Aa* was found to be more prevalent in LAP (Thiha *et al*., 2007).

With the latest advances in molecular biology techniques, recent studies have shown that recognized periodontal pathogens can also be recovered from healthy adults and children (Alaluusua and Asikainen, 1988; Yang *et al.*, 2002), and new uncultivated species have been associated with periodontitis (Kumar *et al.*, 2003; Riep *et al.*, 2009). Thus, with different reports in the literature, different techniques being used for the detection of periodontopathogens, and different populations studied, the role of specific periodontopathogens in the destructive process of AgP is still not clear. We have identified a cohort of African-American children diagnosed with LAP within one clinical setting in Florida. Since this is a homogeneous group in terms of race, age, and patterns of disease, and this is a rare type of disease, the objective of this study was to use 16S rRNA-based microarrays to run a comprehensive characterization of the subgingival microbial flora present in this disease when compared with healthy siblings and unrelated control children.

MATERIALS & METHODS

Demographics of the Study Population

This study aimed to characterize, clinically and microbiologically, a population of children with LAP in Florida. Children included in the study were recruited from the Leon County Health Department, Tallahassee, Florida, from February 2007 to August 2010. All children and their families were informed about the study protocol and signed an informed consent previously approved by the Institutional Review Board at University of Florida. Complete medical and dental histories were taken from all participants. Children with LAP were included as they were diagnosed with the disease and met inclusion criteria. Siblings of children with disease (healthy or diseased) were examined and included as well. Healthy unrelated control children were randomly selected from the same clinic and included as they met inclusion criteria. Attempts were made to match control children for age and gender. Inclusion criteria for all individuals were that they were between 5 and 21 years old, and were African-Americans. Localized aggressive disease was defined by the presence of less than 30% of sites but at least 2 teeth [incisor and/or first molar, but no more than 2 teeth other than first molar and incisors (Armitage, 1999)] with PD ≥ 5 mm in the presence of interproximal attachment loss and ≥ 2 mm bone loss detected on radiographic examination. Healthy participants were defined by the absence of pocket depth > 4 mm in the presence of attachment/bone loss (pseudo/gingival pockets with no attachment/bone loss were allowed). Patients in any category were excluded if they: had been diagnosed with any systemic diseases or conditions that could influence the progression and/or clinical characteristics of periodontal disease (*e.g.*, diabetes or blood disorders); had taken antibiotics within the preceding 3 mos or any medications that could influence the characteristics of the disease (*e.g.*, phenytoin, cyclosporine); were smokers; and/or were pregnant/lactating women.

Clinical Measurements

The following periodontal clinical parameters were taken by one calibrated examiner at the initial visit for all patients: pocket depth (PD), bleeding on probing (BoP); gingival margin position (GM); clinical attachment level (CAL: distance from the CEJ to the bottom of the pocket); Plaque Index (PI): and presence or absence of visible plaque. Measurements were performed with a UNC-15 periodontal probe at 6 sites *per* tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual) and were recorded with computer software (Florida Probe, Gainesville, FL, USA). Periapical and bite-wing x-rays were taken of the compromised teeth to confirm LAP diagnosis. Intra-examiner calibration was obtained when 80% of duplicate measures of probing depth and CAL were within 1 mm.

Collection of Bacterial Subgingival Biofilm

Bacterial subgingival biofilm was collected from 1 diseased site (first molar or incisor) with PD ≥ 5 mm and BoP and from 1 healthy site (PD ≤ 3 mm, no BoP) from children with LAP (non-affected molar or incisor). Samples were also collected from healthy sites from healthy siblings and healthy unrelated control children (molar or incisor). The area of collection was isolated with cotton rolls, and supragingival plaque was carefully removed. Collection was done with a sterile endodontic paperpoint. Following sampling, the paperpoint was stored at -70ºC until processed.

DNA Isolation and Microarray Analysis

DNA was isolated from plaque samples with the use of a DNA Purification kit according to the manufacturer's instructions (MasterPure, EPICENTRE Biotechnologies, Madison, WI, USA). After purification, DNA concentration was tested with the Nanodrop (ND-1000 Spectrophotometer, Nanodrop Technologies Inc., Wilmington, DE, USA). A 200-ng quantity of each sample at a minimum concentration of 20 ng/μL was submitted to The Forsyth Institute for HOMIM (Human Oral Microbe Identification Microarray) analysis. [The method has been described in detail elsewhere (Colombo *et al*., 2009).] Briefly, 16S rRNA genes were PCR-amplified from DNA extracts with 16S rRNA universal forward and reverse primers and labeled *via* incorporation of Cy3-dCTP in a second nested PCR. HOMIM uses 16S rRNA-based, reverse-capture oligonucleotide probes (typically 18 to 20 bases), which are printed on aldehyde-coated glass slides and probed with labeled PCR products (described above) which are hybridized in duplicate. The microarray slides are scanned in an Axon 4000B scanner, and crude data are extracted with GenePix Pro software (Molecular Devices, Sunnyvale, CA, USA). Microbial profiles were generated from image files of scanned arrays with a HOMIM online analysis tool (http://bioinformatics.forsyth.org/homim/). Detection of a particular taxon was determined by the presence of a fluorescent spot for that unique probe. A mean intensity for each taxon was calculated from hybridization spots of the same probe, and signals were normalized by comparison of individual signal intensities with the average of signals from universal probes and calculated as described previously (Colombo *et al.*, 2009). Any original signal that was less than two times the background value was re-set to 1 and was assigned to the signal level 0. Signals greater than 1 were categorized into scores from 1 to 5, corresponding to ranked signal levels.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) with Tukey's multiple comparisons was applied among groups for clinical and demographic parameters. A paired *t* test was applied for differences in PD between DD and DH sites in LAP. ANOVA models were applied for the microbiological results among groups (diseased and healthy sites from LAP, healthy sites from siblings and unrelated control children). Least-squared means of each group were estimated, and a *post hoc t* test was performed to determine differences in the amount of each species. Logistic regression was performed, generating an odds ratio estimation, to compare the frequency of each probe among groups. Rank products analysis was performed to compare abundance of species between healthy individuals (sites pooled from healthy individuals) and those with disease (sites pooled in LAP individuals). SAS software (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Table 1. Clinical and Demographic Parameters for Different Groups Evaluated

	Age (yrs)	M/F	%PD > 4 mm	%BoP	%Plaque	Mean PD Sites/ All	Mean CAL	PD Site
LAP	14.29 ± 3.52	14m/17f	14.42 ± 9.51†	24.52 ± 32.84	45.16 ± 25.68	4.97 ± 0.69† 2.35 ± 0.42†	3.74 ± 1.51	6.00 ± 1.37 DD†‡ 2.18 ± 0.67 DH
Siblings	12.38 ± 3.89	9m/2f	3.25 ± 3.15	9.13 ± 6.58	31.75 ± 31.92	2.06 ± 2.21 1.91 ± 0.31	0	2.67 ± 0.7
Controls	13.44 ± 5.05	4m/5f	1.11 ± 1.45	7.00 ± 3.32	28.22 ± 25.81	1.37 ± 2.06 1.97 ± 0.07	0	2.50 ± 0.76
p Value	0.3939	0.0984	< 0.0001	0.0911	0.2518	< 0.0001 0.0017	< 0.0001	< 0.0001

Values are given by means ± Standard Deviation. M = male; F = female; Mean PD sites = Mean pocket depth of sites with PD > 4 mm; Mean PD all = mean PD of all sites; CAL = clinical attachment level of affected sites; BoP = bleeding on probing; PD site = Pocket depth from sampled site: DD = diseased site in LAP; DH = healthy site in LAP. †Statistically different from siblings and control children by ANOVA. ‡ Statistically different from DH site by paired *t* test.

RESULTS

Thus far, over 60 African-American children with LAP have been diagnosed in the Leon County Health Department. The present study addresses the periodontal conditions and microbial profiles of 31 LAP children, 11 healthy siblings, and 9 healthy unrelated control children. All children included were African-Americans. Mean age of children and gender distribution along with clinical parameters for all children are shown in Table 1.

In total, 422 bacterial species were analyzed in the subgingival plaque samples of all individuals. All samples collected were successfully analyzed. Of these, 52 bacterial species showed significant differences among groups for abundance (Tables 2 and 3) and 21 for prevalence (Fig.).

Two species of *Aa* were more frequently detected in diseased sites in LAP individuals: *Aa_ot531_AA84* (OR = 8.33, CI = 2.11 - 32.90, p = 0.0025) and *Aa_ot531_P02* (OR = 5.82, CI = 1.79 - 18.97, p = 0.0034), along with *Filifactor alocis*_ot539_AB95 (OR = 2.31, CI = 1.06 - 5.01), when compared with healthy sites in LAP (*Aa* species) and HS (*Aa* and *F. alocis*) (Fig.). Interestingly, *Aa* was also found in 2/9 HC and 4/11 HS and a few healthy sites children with LAP, albeit in less abundance (Table 2).

The two probes of *Aa* mentioned above were also more abundant in diseased sites in LAP when compared with healthy sites in both LAP and HC (Table 2, $p < 0.001$). Some of the most abundant species in HC were *Bergeyella* sp, *Campylobacter concisus, Haemophilus parainfluenzae, Rothia dentocariosa* and *mucilaginosa, Neisseria flavescens, Streptococcus* sp, and *Treponema lecithinolyticum*, whereas the most abundant in HS were *Actinomyces, Campylobacter, Capnocytophaga, Cardiobacterium valvulum, Porphyromonas endodontalis, Leptotrichia hofstadii, Sphaerocytophaga*, and *Treponema* sp. (Table 2).

In comparisons of healthy and diseased individuals as a group, species such as *Lautropia mirabilis, Haemophilus parainfluenzae, Streptococcus Cluster II, Campylobacter gracilis*, and *Capnocytophaga granulosa* were most abundant in healthy individuals ($p < 0.01$), whereas *Tannerella* sp, *Filifactor alocis*, the two probes for *Aa, Parvimonas micra, Capnocytophaga* sp, and *Solobacterium moorei* were more abundant in children with LAP ($p < 0.01$, Table 3).

DISCUSSION

The prevalence of aggressive periodontal disease in children and young adults in the US is around 1 to 2% and is estimated to be up to 3 times more prevalent in Blacks (Ickenstein *et al.*, 1999). Although the prevalence of this disease is relatively low and is apparently confined to specific susceptible groups, early stages of this disease could easily go underdiagnosed until clearer signs of alveolar bone loss are detected in radiographic examination. Therefore, the detection of specific bacteria associated with this disease in children at a young age, before disease breakdown occurs, would be desirable for the successful prevention and/or treatment of this disease.

In the present investigation, we evaluated diseased and healthy sites of 31 children with LAP, 11 healthy siblings, and 9 healthy unrelated control children. We have identified several bacterial species that could be playing a destructive or even a possible protective role in this disease.

Aa was very strongly associated with LAP in this population, in both prevalence and abundance. This species has also been associated with AgP/LAP in previous studies (Kaldahl *et al.*, 1988; Haffajee *et al.*, 2003; Kaner *et al.*, 2007). We also observed *Aa* to be very site-specific, since it was more prevalent and abundant in diseased *vs*. healthy sites in LAP. Similarly, Lopez *et al*. found 6% of healthy sites *vs*. 44% diseased sites harboring *Aa* in localized juvenile periodontitis in Chile (Lopez *et al*., 1995). This is an interesting finding, since this disease is localized to specific sites in the mouth.

Additionally, *Aa* was present in some healthy control children and siblings, as observed in other studies (Alaluusua and Asikainen, 1988; Yang *et al.*, 2002; Riep *et al.*, 2009). However, it was less prevalent and less abundant in these individuals when compared with LAP disease sites and slightly more prevalent and abundant in siblings when compared with control children, although that difference was not significant. Recent longitudinal evaluations of *Aa* in children and adolescents indicated that the

Table 2. Significant Levels of Species Abundance in the Different Sites/Groups

Most Abundant Species in LAP Disease Sites (DD)	DD	DH	HC	HS	p Value
Aggregatibacter actinomycetemcomitans_ot531_AA84	1.8235[a]	0.2143[b]	0.4444[b]	1.2308[ab]	<.0001
Aggregatibacter actinomycetemcomitans_ot531_P02	2.1765[a]	0.4286[b]	0.4444[b]	1.5385[ab]	0.0002
Most Abundant Species in LAP Healthy Sites (DH)	**DD**	**DH**	**HC**	**HS**	**p Value**
Capnocytophaga sp clones *BM058* and *BU084_ot329_O08*	0.0588[a]	0.4286[b]	0.2222[ab]	0.3077[ab]	0.0364
Capnocytophaga sputigena_ot775_AC15	0.0000[a]	0.1786[b]	0.0000[ab]	0.0000[ab]	0.0308
Catonella morbi and sp clone *BR063_ot164_165_O56*	0.7941[a]	1.3214[b]	0.6667[ab]	1.0000[ab]	0.0441
Eubacterium sp clone *7 69_ot846_AB31*	0.0000[a]	0.1786[b]	0.0000[ab]	0.0000ab	0.0028
Eubacterium[11][G-7] yurii_ot377_W84	1.3235[a]	2.1071[b]	1.2222[ab]	1.4615[ab]	0.0344
Fusobacterium periodontium_ot201_R20	0.2941[a]	1.0714[b]	0.5556[ab]	0.6154[ab]	0.0086
Kingella denitrificans_ot582_X41	0.0294[a]	0.2500[b]	0.0000[ab]	0.0000[ab]	0.0436
Prevotella melaninogenica and sp clone *BE073_ot298_469_T81*	0.0588[a]	0.4286[b]	0.0000[ab]	0.0000[ab]	0.0142
Selenomonas sp clones *DS071* and *EW084_ot138_146_Q52*	0.2647[a]	0.7857[b]	0.1111[ab]	0.7692[ab]	0.0214
Selenomonas sp clones *DS071* and *EW084_ot138_146_X69*	0.1176[a]	0.5357[b]	0.0000[ab]	0.4615[ab]	0.0088
Streptococcus anginosus and *intermedius_ot543_644_AB82*	0.5294[a]	1.3214[b]	1.2222[ab]	0.5385[ab]	0.0048
Streptococcus anginosus and *intermedius_ot543_644_Q62*	1.7647[a]	2.8929[b]	2.6667[ab]	2.2308[ab]	0.0021
Streptococcus constellatus and *intermedius_ot576_644_F48*	0.9412[a]	1.8214[b]	1.7778[ab]	1.1539[ab]	0.0056
Streptococcus mitis bv2 and sp clone *FP064_ot069_398_Q64*	0.1471[a]	0.5357[b]	0.4444[ab]	0.2308[ab]	0.0146
Streptococcus sanguinis and sp clone *C3MLM097_ot058_758_AB75*	0.0882[a]	0.7143[b]	0.5556[ab]	0.5385[ab]	0.0026
Streptococcus sanguinis and sp clone *C3MLM097_ot058_758_AB78*	0.1176[a]	1.0714[b]	0.6667[ab]	0.6154[ab]	0.0002
Veillonella atypica and *parvula_ot161_524_Q67*	1.0000[a]	2.0714[b]	2.0000[ab]	1.5385[ab]	0.0066
Veillonella EF509966 Crohns_not_oral_AD63-C	0.8529[a]	1.6786[b]	1.5556[ab]	1.2308[ab]	0.0074
Veillonella parvula_ot161_D96	0.0294[a]	0.2143[b]	0.1111[ab]	0.0769[ab]	0.0386
Veillonella parvula_ot161_M04	0.3529[a]	0.8571[b]	0.6667[ab]	0.5384[ab]	0.0169
Most Abundant Species in Healthy Control Sites (HC)	**DD**	**DH**	**HC**	**HS**	**p Value**
Bergeyella sp clone *AK152_ot322_AD84*	0.1176[a]	0.1200[ab]	0.6600[b]	0.1500[ab]	0.0210
Campylobacter concisus_ot575_X33	0.0000[a]	0.0000[a]	0.1111[b]	0.0000[b]	0.0062
Haemophilus parainfluenzae_ot718_W79	0.7941[a]	1.1429[a]	1.8889[b]	1.7692[b]	0.0412
Neisseria flavescens_ot610_AA76	0.1176[a]	0.2500[ab]	0.5556[b]	0.0769[ab]	0.0357
Rothia dentocariosa and *mucilaginosa_ot587_681_E52*	0.0000[a]	0.1786[ab]	0.3333[b]	0.0769[ab]	0.0304
Streptococcus Cluster II_ot071_755_758_Q59	0.7353[a]	1.7500[a]	2.3333[b]	1.4616[b]	0.0007
Streptococcus Cluster III_ot755_758_767_768_Q65	0.9706[a]	1.7857[b]	2.0000[b]	1.2308[ab]	0.0085
Streptococcus constellatus and *intermedius_ot576_644_AB77*	0.7353[a]	1.7857[b]	2.0000[b]	0.8462[ab]	0.0019
Streptococcus parasanguis I and *II_ot411_721_AB05*	0.1176[a]	0.4286[b]	0.6667[b]	0.2308[ab]	0.0101
Treponema lecithinolyticum_ot653_D52	0.0000[a]	0.0000[a]	0.1111[b]	0.0769[b]	0.0524
Most Abundant Species in Healthy Sibling Sites (HS)	**DD**	**DH**	**HC**	**HS**	**p Value**
Actinomyces Cluster II_ot180_181_671_701_AC99	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
Actinomyces meyeri and *odontolyticus_ot671_701_AA88*	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
Actinomyces sp strain *B27SC_ot178_W02*	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
Cardiobacterium valvulum_ot540_AD79	0.0000[a]	0.0714[a]	0.0000[b]	0.4615[b]	0.0003
Cardiobacterium valvulum_ot540_AD80	0.0000[a]	0.0357[a]	0.0000[b]	0.2308[b]	0.0008
Leptotrichia hofstadii_ot224_AA58	0.0000[a]	0.0000[ab]	0.0000[ab]	0.1539[b]	0.0310
Porphyromonas endodontalis_ot273_N99	0.000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
Sphaerocytophaga S3_ot337_X23	0.0882[a]	0.2857[ab]	0.4444[ab]	0.5385[b]	0.0370
SR1[G-1] sp _X112_ot345_AC72	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
SR1[G-1] sp _X112_ot345_AC73	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
Treponema denticola_ot584_O40	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310
Treponema sp clone *_T021_ot231_AC45*	0.0000[a]	0.0000[ab]	0.0000[ab]	0.0769[b]	0.0310

Abundance values are represented here as the least-square mean intensity value (from HOMIM ranked signal scale 0-5) for each group. Means followed by different superscript letters represent statistical differences among groups by ANOVA. DD = diseased sites in children with disease; DH = healthy sites in children with disease; HS = healthy site in healthy sibling; HC = healthy site in healthy control child. Different letters show statistically significant differences among groups.

Table 3. Most Abundant Species in Healthy and Diseased Individuals

Most Abundant Species in Healthy Individuals				
Taxa	p Values	Fold-change	Mean	SD
Lautropia mirabilis_ot0.00122_X44	0.004672897	0.8911174	0.058425285	0.27918294
Haemophilus parainfluenzae_ot718_W79	0	0.5493658	1.1729078	1.4559939
Streptococcus Cluster II_ot0.00171_755_758_Q59	1.40E-04	0.6446244	1.3452182	1.3010002
Campylobacter gracilis_ot623_Q0.0014	0	0.6073211	1.6900116	1.4961808
Campylobacter gracilis_ot623_X34	0	0.58508325	2.1496668	1.5956601
Capnocytophaga granulosa and sp clone *BB167_ot325_326_AA89*	0	0.49718076	1.7015518	1.6428522
Most Abundant Species in LAP Individuals				
Taxa	p Values	Fold-change	Mean	SD
Solobacterium moorei_ot678_AC0.0012	0.00182243	1.3059351	0.33411482	0.74152595
Filifactor alocis_ot539_AB94	0.001214953	1.3419528	0.9087584	1.6108342
Aggregatibacter actinomycetemcomitans_ot531_AA84	0.001682243	1.2631507	1.1615744	1.7507974
Parvimonas micra_ot111_L97	4.67E-05	1.5799938	1.4027699	1.5877612
Aggregatibacter actinomycetemcomitans_ot531_P0.0012	4.67E-05	1.3636757	1.4258847	1.9681077
Parvimonas micra_ot111_V0.0015	0	1.7198296	1.4487239	1.6045148
Capnocytophaga sp clone *_X0.00166_ot335_AD22*	7.48E-04	1.285298	1.8162187	1.0944762
Tannerella sp clone *BU0.00163_ot286_T83*	0.004299066	1.1626025	0.2996665	0.6835908

The Table shows the most abundant bacterial taxa in diseased and healthy groups by Rank products. SD = standard deviation. 'Mean' indicates mean abundance of species in the group (top, healthy or bottom, LAP). Fold-change indicates change in mean abundance between healthy and LAP groups.

presence of this species in healthy children could represent a risk marker for LAP initiation (Spinozzi and Langer, 1999; Haubek *et al.*, 2008). A longitudinal analysis of these children will enable us to detect the possible initiation of disease in the healthy groups, especially the siblings, who are at greater risk for this disease.

Other species associated with diseased individuals included *Parvimonas micra* and *Filifactor alocis*, which is in agreement with previous studies (Kumar *et al*., 2003, 2006). *P. gingivalis* has been associated with AgP in other populations (Gajardo *et al.*, 2005; Mayorga-Fayad *et al*., 2007); however, this species was not frequently present in this population.

An interesting finding in the present study was regarding health-associated species. Most of those were present in larger quantities and proportions in healthy sites, including in an individual with disease, such as *Selenomonas* sp, *Veillonella*, and *Streptococcus.* In contrast, some species not typically detected, such as *Bergeyella* sp, *Kingella oralis*, and *Neisseria* sp, were more frequently found in HC and HS. This could indicate that some health-associated species could be playing a possible protective role in maintaining periodontal health. Stingu *et al.* have reported a higher prevalence of *S. sanguinis* in healthy individuals compared with those with AgP (Stingu *et al.*, 2008). These authors observed that the absence of *S. sanguinis* was associated with clinical periodontal breakdown. In addition, Colombo *et al.* reported that *Capnocytophaga, Cardiobacterium, Haemophilus, Kingella, Lautropia, Neisseria, Rothia, Streptococcus*, and *Veillonella* spp. were more associated with periodontal therapeutic success (Colombo *et al*., 2012). Thus, there is a possible protective role of healthy subgingival species in periodontal disease that needs to be further elucidated. Longitudinal analysis of treatment responses in this cohort and analysis of a greater number of individuals will enable us to associate some of these species with treatment response.

Finally, the pathogenesis of periodontitis does not seem to rely solely on bacterial composition. It also depends upon the immune and inflammatory responses to bacterial colonization of the gingival sulcus (Offenbacher, 1996; Page and Kornman, 1997). We have already reported a hyper-inflammatory response by an elevated release of inflammatory mediators both systemically (Shaddox *et al.*, 2010) and locally (Alfant *et al*., 2008; Shaddox *et al.*, 2011) in this LAP population. Therefore, there is a multitude of factors that could be associated with the rapid periodontal breakdown in LAP (for review, see Schenkein *et al.*, 2007). Thus, questions remain to be answered to better define this disease and to enable us to establish proper treatment and prevention.

In summary, we have encountered a very similar pattern of LAP in African-Americans in north Florida. Specific bacteria, such as *Aa*, seem to play a destructive role in this disease, whereas other species may be playing a more protective role against LAP. Longitudinal evaluation of this group could lead us to important understanding of the role of these bacteria in the initiation and progression of LAP and its treatment.

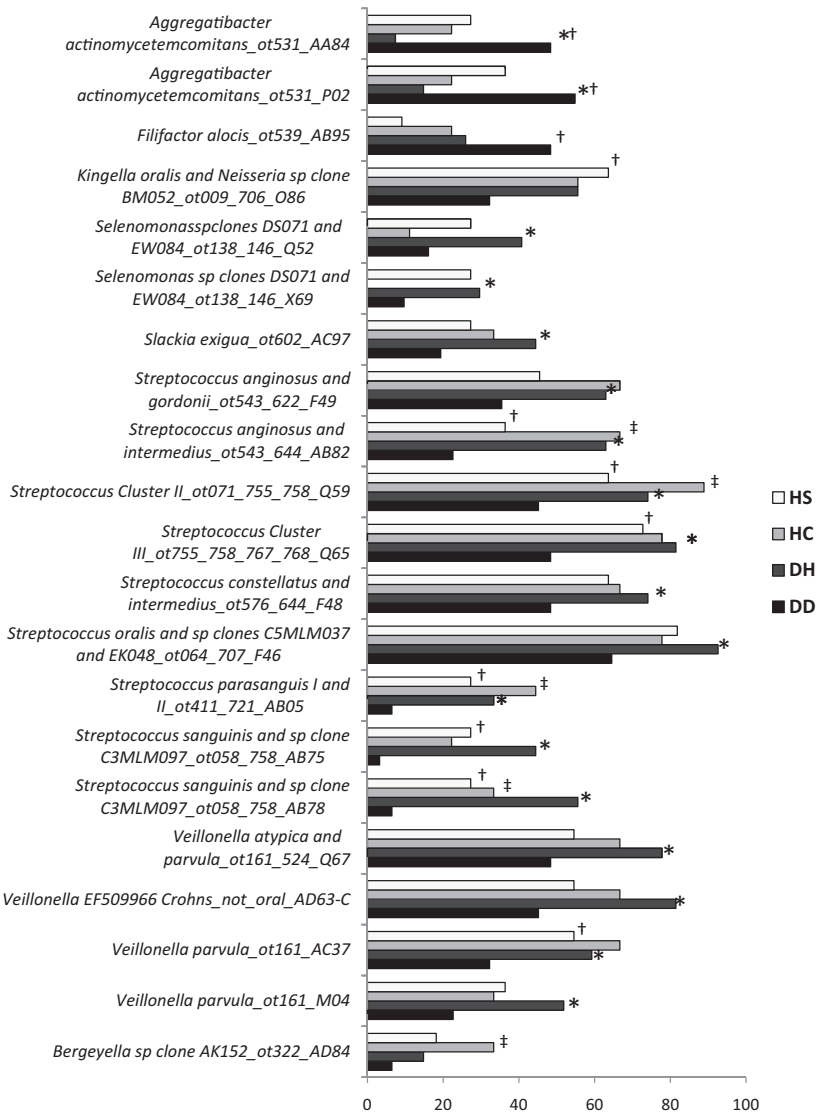

Figure. Bacterial species which showed significantly different prevalence among sites/groups. DD = diseased sites in children with disease; DH = healthy sites in children with disease; HS = healthy sites in healthy sibling; HC = healthy sites in healthy control children. Significant differences denoted by * DD *vs*. DH, † DD *vs*. HS, and ‡DD *vs*. HC.

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