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Subgingival microbiota in adult Down syndrome periodontitis

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Background and Objective: The subgingival microbiota in Down syndrome and non-Down syndrome adults receiving periodic dental care was examined for 40 bacterial species using checkerboard DNA–DNA hybridization and the results were related to clinical periodontal attachment loss.

Material and Methods: A total of 44 Down syndrome, 66 non-Down syndrome mentally retarded and 83 mentally normal adults were clinically evaluated. This involved, for each subject, the removal of subgingival specimens from three interproximal sites on different teeth; all subgingival samples per subject were then pooled and assessed for the presence and levels of 40 bacterial species using species-specific whole-genomic DNA probes and checkerboard DNA–DNA hybridization. Significant group differences in species proportions averaged across subjects were evaluated using the Kruskal–Wallis test, and associations between subgingival species and mean subject attachment loss within Down syndrome and non-Down syndrome subject groups were quantified using Pearson correlation and multiple linear regression analysis.

Results: Down syndrome subjects exhibited greater attachment loss than non-Down syndrome subjects ($p = 0.05$). Most microbial species were present in Down syndrome subjects at levels similar to non-Down syndrome subjects, except for higher proportions of *Selenomonas noxia*, *Propionibacterium acnes*, *Streptococcus gordonii*, *Streptococcus mitis* and *Streptococcus oralis* in Down syndrome subjects compared with non-Down syndrome study subjects, higher proportions of *Treponema socranskii* in Down syndrome subjects compared with non-Down syndrome mentally retarded subjects, and higher proportions of *Streptococcus constellatus* in Down syndrome subjects compared with mentally normal subjects. Down syndrome adults classified with periodontitis revealed higher subgingival levels of *T. socranskii* than Down syndrome subjects with no periodontitis ($p = 0.02$). Higher subgingival proportions of *S. constellatus*, *Fusobacterium nucleatum* ssp. *nucleatum*, *S. noxia* and *Prevotella nigrescens* showed significant positive correlations ($r = 0.35$–0.42) and higher proportions of *Actinomyces naeslundii* II and *Actinomyces odontolyticus* showed negative correlations ($r = -0.36$ to -0.40), with increasing mean subject attachment loss in Down syndrome adults.

Conclusion: Individuals with Down syndrome show higher levels of some subgingival bacterial species and specific associations between certain subgingival bacterial species and loss of periodontal attachment. These findings are consistent with the notion that certain subgingival bacteria may contribute to the increased level of periodontal disease seen in Down syndrome individuals and raise the question as to the reason for increased colonization in Down syndrome.

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Down syndrome is the most common chromosomal abnormality in live-born infants and is estimated to occur in approximately one in 732 infants in the USA (1). The syndrome is caused by trisomy of chromosome 21 (2). The most common manifestations of Down syndrome include a characteristic physical appearance and a variety of medical disorders, including mental retardation, congenital heart disease, thyroid dysfunction, Alzheimer's disease and abnormalities of the immune system (2).

It is well documented that individuals with Down syndrome are also at increased risk for developing destructive forms of periodontal disease (3,4), with the majority affected early in life with extensive gingival tissue inflammation, bleeding on probing, deepened probing depths, loss of periodontal attachment and crestal alveolar bone loss (3,4). As dentogingival microbial biofilm infections are considered essential for the initiation and progression of human periodontal diseases (5), the relationship between subgingival microorganisms and periodontal disease in individuals with Down syndrome has been the focus of several investigations (6–9). However, only mixed and inconclusive findings on this association are presently available, probably because of the limited number of study subjects examined, a primary focus on children and younger-age adults and the relatively small range of periodontopathic taxa evaluated.

The main objectives of the present study were: (i) to determine the presence and levels of 40 subgingival bacterial species in adult individuals with Down syndrome using species-specific whole-genomic DNA probes and checkerboard DNA–DNA hybridization; (ii) to investigate the subgingival microbial profile of Down syndrome adults matched with non-Down syndrome mentally retarded subjects and mentally normal subjects; and (iii) to assess the association between 40 subgingival bacterial species and loss of clinical periodontal attachment in individuals with and without Down syndrome.

Material and Methods

Study sites

This study was performed in cooperation with the Georgia Department of Human Resources/Georgia Regional Hospitals (GRH). The study included three subject groups: Down syndrome; non-Down syndrome mentally retarded; and mentally normal. Down syndrome and non-Down syndrome mentally retarded study subjects were recruited from GRH Hospital Centers in Atlanta, Savannah and Augusta, Georgia. All Down syndrome and non-Down syndrome mentally retarded subjects were patients of record and receiving periodic dental care at one of the three hospital locations. Some of these individuals were institutionalized, while others were outpatients living in group-homes or with their families.

Subject inclusion criteria

Study subject inclusion criteria and recruitment were as previously described. In brief, Down syndrome study subjects had a confirmed diagnosis of trisomy 21, a minimum of 10 teeth present, no other medical conditions known to affect periodontal status (i.e. diabetes mellitus), no systemic antibiotic therapy in the 3 mo prior to entry into the study, no history of cigarette smoking, were adults ≥ 18 years of age and were able to cooperate with the study examiners. The same inclusion criteria were used to select the other study group subjects, except for the need of a confirmed diagnosis of mental retardation without trisomy 21 for non-Down syndrome mentally retarded subjects and an absence of mental retardation for mentally normal subjects. All study subjects needed to be able to communicate with and understand spoken English.

Subject recruitment

The attending dentist-in-charge of the dental clinic at each of the three GRH Hospital Centers reviewed available records and identified dentate Down syndrome patients who met the study criteria and would be able to participate in a dental examination. Non-Down syndrome mentally retarded subjects, where their mental retardation was secondary to head trauma at birth, were matched to the Down syndrome patients on age, race and gender using the same hospital records and were selected on the basis of their ability to cooperate and sit for a dental examination without the need for sedation. Mentally normal subjects, matched for gender, race and age with the Down syndrome subjects, were recruited from the general population living in the vicinity of the three GRH Hospital Centers and were under the care of private practice dentists.

A total of 289 subjects were initially screened for the study: 26 were disqualified for medical reasons; 46 completed only portions of the study evaluations, but were not able to return to complete the remaining portions; and 217 completed most of the study evaluations. Demographic and clinical data for the entire subject populations are reported elsewhere. For the present study, clinical and microbiological data were obtained for 193 subjects (44 Down syndrome, 66 non-Down syndrome mentally retarded and 83 mentally normal controls).

Informed consent

The study protocol and informed consent process were approved by the Georgia Regional Hospital Institutional Review Board. Prior to commencing the study, informed consent was obtained and documented for all subjects. The GRH Hospital Center dentist-in-charge contacted the family or legal guardian/caretaker of each potential Down syndrome or non-Down syndrome mentally retarded subject, explained the study protocol and obtained their consent to enroll the subject in the study. In addition to obtaining family or legal guardian/caretaker written informed consent, the study protocol was also explained directly to each Down syndrome or non-Down syndrome mentally retarded subject prior to commencing the study examination procedures, and their personal consent was obtained

and witnessed. Mentally normal study subjects consented on their own behalf.

Clinical evaluations

As previously described (10), two experienced dental hygienists blinded to the objectives of the study performed all examinations. The examiners were calibrated and standardized in the use of the clinical evaluation measures employed in the study (10). The examiners enumerated missing teeth and teeth with carious lesions, then recorded the Loe and Silness gingival index (11), the Quigley Hein plaque index (12), probing depth and clinical periodontal attachment levels on six sites per tooth. Third molars were excluded. Periodontitis was defined as present when ≥ 5% of the teeth scored exhibited attachment loss of ≥ 5 mm (13).

Microbiological evaluations

Three sites per subject were sampled for microbial analysis after completion of the clinical evaluations. In periodontitis subjects, three interproximal sites with probing depth > 5 mm were randomly chosen from three different teeth in separate quadrants. In non-periodontitis subjects, three interproximal sites with probing depth < 4 mm were randomly chosen from three different teeth in separate quadrants. After removal of supragingival plaque and isolation to prevent saliva contamination, subgingival plaque samples were collected using a sterile Gracey curette from the selected sites. All subgingival samples per subject were pooled into a single Eppendorf tube containing 450 μL of 10 mM Tris–HCl/1 mM EDTA, pH 7.6 (TE buffer) and then 300 μL of 0.5 M NaOH was added. The samples were immediately frozen at −70°C and delivered within a 3-mo period to the Forsyth Institute (Boston, MA, USA) for microbiological analysis.

Upon arrival at the Forsyth Institute, the presence and levels of 40 bacterial species were determined in each pooled subgingival plaque sample using species-specific whole-genomic DNA probes and a modification (14) of the checkerboard DNA–DNA hybridization technique (15). In brief, each sample was lysed by boiling for 10 min, then neutralized with 800 μL of ammonium acetate; the denatured DNA was placed in lanes on a nylon membrane using a Minislot device (Immunetics, Cambridge, MA, USA). After fixation of DNA to the membrane, the membrane was placed in a Miniblotter 45 (Immunetics), with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole-genomic DNA probes constructed to each of the 40 bacterial test species, as previously described (16), were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes were detected using antibody to digoxigenin conjugated with alkaline phosphatase. Chemifluorescence detection of signals was performed using AttoPhos substrate (Amersham Life Science, Arlington Heights, IL, USA) and a computer-linked Storm Fluorimager (Molecular Dynamics, Sunnyvale, CA, USA) to read the intensity of fluorescence signals resulting from DNA probe–target hybridization. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each of the test bacterial species. The concentration of each DNA probe was adjusted to permit detection of 10^4 cells of a given test bacterial species. The sensitivity and specificity of the species-specific DNA probes was as previously reported (16). Storm Fluorimager signals were converted to absolute counts by comparison with the standards on the same membrane. Failure to detect a signal was recorded as zero. A total of 193 pooled subgingival plaque samples were evaluated.

Data analysis

Three-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test, were used on mean values, and chi-square tests were used on proportional data, to examine for statistically significant between-group differences in demographic and clinical parameters. The percentage of DNA probe counts for each of the tested bacterial species in pooled subgingival patient samples was determined for each study subject, with species proportions then averaged across subjects in each of the three study subject groups. Significant differences in test species proportions among study subject groups were evaluated using the Kruskal–Wallis test, adjusted for 40 comparisons (17). The association between subgingival species and the mean subject attachment level within Down syndrome and non-Down syndrome subject groups was quantified using Pearson correlation coefficients adjusted for subject age, race, gender and supragingival plaque levels. In instances where a significant association ($p \leq 0.05$) was found in either group, differences in partial associations between groups were tested with multiple linear regression analysis, with interaction terms (group, species and group by species) modeled in addition to previous adjustment variables. Significance testing of between-group, species-specific interaction terms on mean subject attachment level were used to identify microbial species, at a critical p-value of ≤ 0.01, that were unique correlates of mean subject attachment level in individuals with Down syndrome.

Results

Clinical findings

Table 1 summarizes demographic and clinical data of the 193 study subjects. All three subject groups exhibited similar race and gender distributions. ANOVA showed differences between the three subject groups on age ($p = 0.0001$) and institutional living ($p = 0.001$). Follow-up analysis with Tukey's HSD test showed that the non-Down syndrome mentally retarded subjects were older than the Down syndrome and the mentally normal subjects ($p = 0.05$), and the number of institutionalized subjects in the non-Down syndrome mentally retarded group was higher than in the Down syndrome group ($p = 0.05$). Clinically, the percentage of subjects with periodontitis (as defined above) was similarly distributed among the three subject groups. ANOVA showed

Table 1. Summary of demographic and clinical features of 193 subjects with microbiological data

Variable	Down syndrome (n = 44)	Non-Down syndrome mentally retarded (n = 66)	Mentally normal (n = 83)	p-Value
Demographics				
Age	35.81 (1.83)	46.15 (1.40)	40.93 (1.33)	0.0001[a]
Age range (years)	18–56	22–84	18–73	
Percentage Caucasian	81.82	80.30	68.67	NS
Percentage male gender	47.73	57.58	44.58	NS
Percentage institutionalized	40.91	84.85	NA	0.001[b]
Clinical				
Number of missing teeth	4.38 (0.58)	4.60 (0.48)	1.80 (0.42)	0.0001[c]
Plaque index	1.71 (0.11)	1.85 (0.09)	1.28 (0.08)	0.001[c]
Gingival index	0.94 (0.04)	1.01 (0.04)	0.71 (0.03)	0.0001[c]
Probing depth (mm)	2.58 (0.08)	2.34 (0.06)	2.41 (0.06)	0.07
Attachment level (mm)	2.67 (0.11)	2.20 (0.09)	2.24 (0.08)	0.0006[d]
Percentage of sites with attachment level ≥5 mm	11.30 (1.78)	3.84 (1.45)	4.46 (1.29)	0.002[d]
Percentage of subjects classified with periodontitis	72.7	71.1	78.8	0.55

Data are presented as mean (standard error) or percentage. p-Value gives the probability that the groups differ in either an analysis of variance (ANOVA) test or chi-square test for means or proportions, respectively.
Follow-up analysis with Tukey's honestly significant difference (HSD) test showed: [a]Mental retardation > Down syndrome and mentally normal (p = 0.05); [b]Mental retardation > Down syndrome (p = 0.05); [c]Mentally healthy < mental retardation and Down syndrome (p = 0.05); [d]Down syndrome > mental retardation and mentally normal (p = 0.05).

significant differences between the three subject groups on gingival index, plaque index, attachment level (both mean whole-mouth and percentage of sites with attachment level ≥ 5 mm) and the number of missing teeth ($p < 0.05$). Follow-up analysis with Tukey's HSD showed that subjects in both the Down syndrome and non-Down syndrome mentally retarded groups had higher values for gingival index (p = 0.05), plaque index (p = 0.05) and number of missing teeth (p = 0.05) than mentally normal subjects. Even though similar proportions of subjects in each of the three study groups were classified with periodontitis, Down syndrome subjects exhibited greater loss of clinical periodontal attachment (both mean whole-mouth and percentage of sites with attachment level ≥ 5 mm) than non-Down syndrome subjects (p = 0.05).

Microbiological findings

Figure 1 displays the mean proportions of DNA probe counts of the 40 test bacterial species among the three subject groups, irrespective of the clinical status of subjects within each group. Species were ordered and grouped according to the microbial complexes described by Socransky *et al.* (18). All of the bacterial species were detected in the subgingival plaque samples of each group, and most species were present at similar levels in each group. No between-group differences in subgingival proportions were found among the evaluated species (n = 9) in the red, purple and *Actinomyces* microbial complexes, as well as for 19 (61.3%) of the other 31 evaluated organisms.

However, after adjusting for 40 comparisons, Down syndrome subjects yielded significantly higher subgingival levels of *Selenomonas noxia*, *Propionibacterium acnes*, *Streptococcus gordonii*, *Streptococcus mitis* and *Streptococcus oralis* relative to non-Down syndrome study subjects; higher levels of *Treponema socranskii* than non-Down syndrome mentally retarded subjects; and higher levels of *Streptococcus constellatus* compared with mentally normal subjects. Non-Down syndrome mentally retarded subjects had significantly higher subgingival proportions of *Prevotella intermedia*, *Prevotella melaninogenica* and *Eubacterium nodatum* relative to the other two study groups and had higher levels of *S. constellatus* compared with mentally normal subjects. Mentally normal subjects revealed significantly higher subgingival levels of *T. socranskii* than non-Down syndrome mentally retarded subjects, higher levels of *Capnocytophaga ochracea* than Down syndrome subjects and higher levels of *Capnocytophaga sputigena* than the other two subject groups.

Comparison of microbial profiles between groups by periodontal status (periodontitis and nonperiodontitis) confirmed the lack of major differences between the three subject groups (data not shown). However, within the Down syndrome group, periodontitis subjects exhibited higher levels of *T. socranskii* than nonperiodontitis subjects (p = 0.02). Within non-Down syndrome groups, periodontitis subjects showed higher proportions of *Aggregatibacter actinomycetemcomitans* (p = 0.017) and *Treponema denticola* (p = 0.005) than nonperiodontitis subjects.

Table 2 summarizes the significant correlations ($p \leq 0.1$) noted between mean subject attachment level and subgingival levels of each test bacterial species, for Down syndrome and non-Down syndrome groups, with correlations adjusted for age, race, gender and supragingival plaque levels. Because the non-Down syndrome mentally retarded and mentally normal groups showed similar levels of association (data not shown), they were combined into a joint non-Down syndrome comparison group. The association between microbial species and mean subject attachment level differed markedly between Down syndrome and non-Down syndrome subjects. In Down syndrome subjects, increasing subgingival proportions of *S. constellatus*, *Fusobacterium nucleatum* ssp. *nucleatum*, *S. noxia* and *Prevotella nigrescens* were positively associated, and increasing proportions of *Actinomyces naeslundii* II and *Actinomyces odontolyticus* were

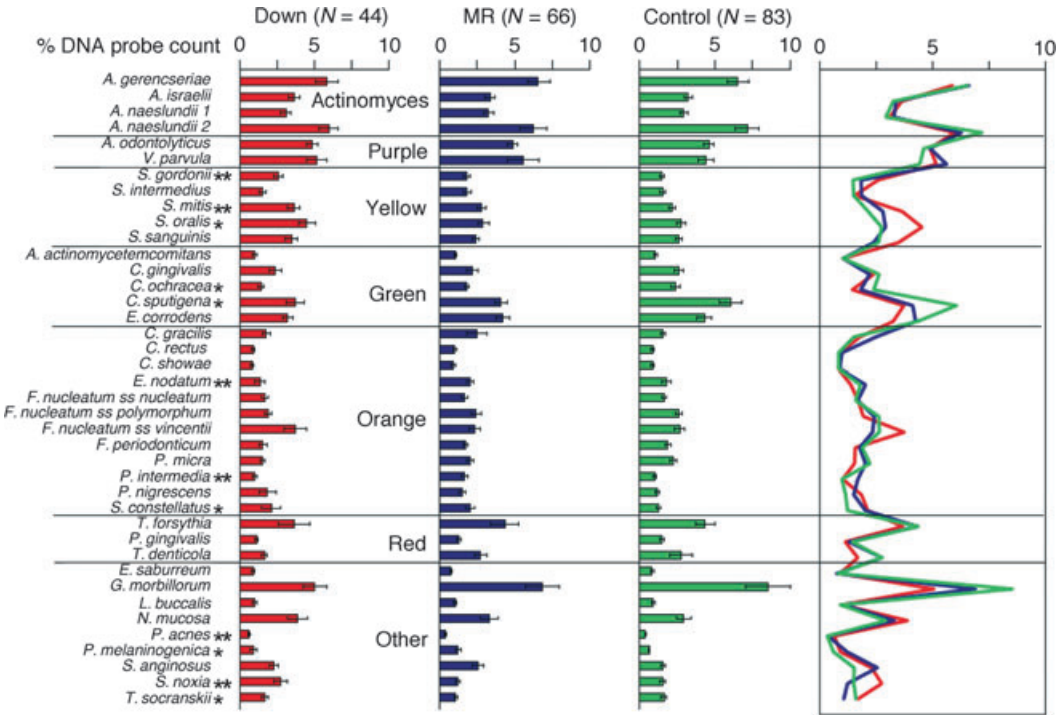

Fig. 1. Bar chart of the mean percentage of the DNA probe count of individual species in subgingival plaque samples taken from Down syndrome subjects, non-Down syndrome mentally retarded subjects and mentally normal (control) subjects. The bars represent the mean percentages and the whiskers the standard error of the mean. The percentage of the DNA probe count for each species was computed per subject and then averaged across subjects in each group. $*p < 0.05$, $**p < 0.01$.

significantly negatively correlated, with increasing mean attachment level in subjects. In comparison, non-Down syndrome subjects showed increasing levels of *A. actinomycetemcomitans, Eikenella corrodens, S. mitis, S. oralis* and *Neisseria mucosa* to be positively associated, and increasing levels of *Actinomyces gerencseriae* to be negatively associated, with increasing mean subject attachment level.

Further significance testing of between-group, species-specific interaction terms on mean subject attachment level showed *S. constellatus*, *F. nucleatum* ssp. *nucleatum*, *A. naeslundii* II, *A. odontolyticus* and *Streptococcus sanguinis* to each demonstrate a nonzero association with mean subject attachment level within the Down syndrome group that was statistically different from the association found in non-Down syndrome subjects, indicating that these bacterial species were related to attachment level in Down syndrome subjects but not in non-Down syndrome subjects. Interestingly, none of the nonzero microbial species associations with mean subject attachment level obtained in the non-Down syndrome group were significantly different from those found in Down syndrome subjects.

Discussion

The present study findings demonstrated that adult Down syndrome periodontitis subjects, while exhibiting greater periodontal pathology than non-Down syndrome subjects (10), yielded subgingival microbiological profiles similar to those found in patients with chronic periodontitis. Most of the 40 subgingival bacterial species evaluated using checkerboard DNA–DNA hybridization were present at similar levels in adult Down syndrome subjects compared with matched non-Down syndrome mentally retarded and mentally normal subjects, including the major periodontal pathogens *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *P. intermedia*, *Parvimonas micra*, *F. nucleatum* and *Campylobacter rectus*, consistent with previous culture-based studies (19).

Regarding the few bacterial species found to exhibit significant between-group differences, Down syndrome adults showed higher subgingival proportions of *S. noxia*, *P. acnes*, *S. gordonii*, *S. mitis* and *S. oralis* compared with non-Down syndrome study subjects, higher *T. socranskii* levels than non-Down syndrome mentally retarded subjects and higher *S. constellatus* levels relative to mentally normal subjects. *S. gordonii*, *S. mitis* and *S. oralis* are pioneer organisms that initiate early microbial colonization and assist in the further development of microbial plaque by creating a foothold for other bacteria to attach to. Furthermore, *S. gordonii* contains genes that facilitate the attachment of free-floating *P. gingivalis* to adherent plaque biofilm (20). *P. acnes* is an opportunistic pathogen normally found on skin and has been associated with persistent apical periodontal infections (21). The presence of *P. acnes* at elevated levels in the

Table 2. Correlations relating periodontal attachment loss with levels of each microbial species within non-Down syndrome and Down syndrome groups, after adjusting for age, gender, race and plaque index

Microbial species (percentage DNA)	Strain	Non-Down syndrome (n = 149)	Down syndrome (n = 44)	Interaction significance
Actinomyces gerencseriae	ATCC 23840	−0.24 (0.005)		> 0.15
Actinomyces israelii	ATCC 12102			
Actinomyces naeslundii 1	ATCC 12104	−0.16 (0.06)		
Actinomyces naeslundii 2	ATCC 43146		−0.40 (0.01)	0.001
Actinomyces odontolyticus	ATCC 17929		−0.36 (0.03)	0.001
Veillonella parvula	ATCC 10790			
Streptococcus gordonii	ATCC 10558			
Streptococcus. intermedius	ATCC 27335			
Streptococcus mitis	ATCC 49456	0.27 (0.001)		0.03
Streptococcus oralis	ATCC 35037	0.20 (0.02)		0.09
Streptococcus sanguinis	ATCC 10556	0.15 (0.07)	−0.27 (0.10)	0.006
Aggregatibacter actinomycetemcomitans	ATCC 43718 and 29523	0.29 (< 0.001)		> 0.15
Capnocytophaga gingivalis	ATCC 33624			
Capnocytophaga ochracea	ATCC 33596			
Capnocytophaga sputigena	ATCC 33612			
Eikenella corrodens	ATCC 23834	0.24 (0.004)		0.06
Campylobacter gracilis	ATCC 33236	−0.14 (0.09)		
Campylobacter rectus	ATCC 33238			
Campylobacter showae	ATCC 51146			
Eubacterium nodatum	ATCC 33099			
Fusobacterium nucleatum ssp. *nucleatum*	ATCC 25586		0.42 (0.009)	0.002
Fusobacterium nucleatum ssp. *polymorphum*	ATCC 10953			
Fusobacterium nucleatum ssp. *vincentii*	ATCC 49256			
Fusobacterium periodonticum	ATCC 33693			
Parvimonas micra	ATCC 33270			
Prevotella intermedia	ATCC 25611			
Prevotella nigrescens	ATCC 33563		0.35 (0.04)	0.10
Streptococcus constellatus	ATCC 27823		0.42 (0.008)	0.01
Tannerella forsythia	ATCC 43037			
Porphyromonas gingivalis	ATCC 33277	0.15 (0.07)		
Treponema denticola	FI B1			
Eubacterium saburreum	ATCC 33271			
Gemella morbillorum	ATCC 27824		−0.30 (0.07)	
Leptotrichia buccalis	ATCC 14201			
Neisseria mucosa	ATCC 19696	0.24 (0.003)		0.11
Propionibacterium acnes	ATCC 11827 and 11828			
Prevotella melaninogenica	ATCC 25845			
Streptococcus anginosus	ATCC 33397			
Selenomonas noxia	ATCC 43541		0.37 (0.02)	0.06
Treponema socranskii	FI S1		0.31 (0.06)	

ATCC, American Type Culture Collection (Manassas, VA, USA); FI, Forsyth Institute (Boston, MA, USA).
Data presented as correlation coefficient (p-value). Values with $p > 0.10$ are not shown.

subgingival microbiota of Down syndrome subjects may be related to a thumb-sucking and/or a finger-biting habit (22). *S. noxia* is a periodontal pathogen associated with periodontal disease activity in interproximal sites (23). *T. socranskii* has been associated with severity of periodontal tissue destruction (24,25). *S. constellatus* is an important periodontal pathogen associated with refractory forms of periodontitis (26–28). The presence of these organisms at elevated levels in Down syndrome individuals may contribute to their increased susceptibility to periodontitis.

It is important to note that all subjects in this study received dental care on a regular basis, including periodic periodontal dental prophylaxis/scaling. These repeated periodontal treatment procedures probably affected the subgingival microbial colonization patterns detected in the study subjects, particularly in regard to the suppression of red-complex *P. gingivalis*, *T. denticola* and *T. forsythia* species (29), and potentially minimized the harmful effects of elevated supragingival plaque levels in Down syndrome and non-Down syndrome mentally retarded subjects (30). The positive associations found between various streptococci and mean subject attachment level in non-Down syndrome study subjects may indeed reflect more an outcome of successful periodontal therapy rendered than any potential etiologic association (31).

Among Down syndrome group subjects in the present study, subgingival proportions of *S. constellatus*,

F. nucleatum ssp. *nucleatum*, *S. noxia* and *P. nigrescens* showed statistically significant positive correlations, and subgingival proportions of *A. naeslundii* II and *A. odontolyticus* showed significant negative correlations, with increasing mean subject attachment level, although all of these correlations were relatively modest in magnitude ($r = 0.35–0.42$). Subgingival proportions of the suspected periodontal pathogen *T. socranskii* also tended to associate (albeit nonsignificantly) with mean subject attachment level in Down syndrome subjects. *S. constellatus*, *F. nucleatum* ssp. *nucleatum*, *S. noxia*, *P. nigrescens* and *T. socranskii* are all frequently associated with the subgingival microbiota of chronic periodontitis lesions in mentally normal adults, whereas *A. naeslundii* II and *A. odontolyticus* are strongly associated with stable healthy periodontal conditions (18,32). *S. constellatus* and *F. nucleatum* are difficult to eliminate with scaling and root-planing alone (29). The association of these two organisms with loss of periodontal attachment in Down syndrome individuals suggests that organisms resistant to conventional mechanical debridement may contribute to the severity of periodontitis in this vulnerable population.

In previous studies of Down syndrome in children, where little or no periodontal therapy was performed prior to microbiological evaluations, Meskin *et al.* (7) found no differences in the prevalence of *Bacteroides melaninogenicus* (now *Prevotella melaninogenica*) in subgingival plaque from institutionalized children with Down syndrome vs. normal or cerebral palsy-affected children. In contrast, increased levels of *A. actinomycetemcomitans* have been reported in Down syndrome children (6,33). Similarly, Amano *et al.* (34) demonstrated, by PCR, that 10 periodontal pathogens exhibited an increased PCR-positive prevalence in children with Down syndrome, and Sakellari *et al.* (8) found various periodontal pathogens to colonize children, adolescents and young adults with Down syndrome, earlier and at higher levels, than healthy individuals and subjects with cerebral palsy.

In Down syndrome adults most comparable to our study population, Reuland-Bosma *et al.* (19) compared 17 Down syndrome adults with moderate periodontitis and daily caretaker-performed oral hygiene with a well-matched control group and found no statistically significant differences in the subgingival prevalence and culturable proportions of seven periodontal bacterial pathogens. Our findings, with 40 subgingival bacterial species, confirm and extend their observations by examining a larger number of Down syndrome subjects with varied ethnicities and evaluating a wider range of disease and health-associated bacterial species. Additionally, this study better controlled for mental challenge and its associated behavioral sequelae as factors that may influence the association between attachment level and certain bacterial species in Down syndrome subjects. In this regard, we have previously reported that while Down syndrome adults have a higher prevalence of periodontal disease than non-Down syndrome mentally retarded adults, they have similar levels of mental challenge (10). The limitations of the present study include the limited number of sampled sites ($n = 3$) per subject, pooling of subgingival specimens within subjects before microbiological evaluation, no inclusion of children or adolescents in the study populations and no assessment of herpesviruses-implicated Down syndrome periodontitis (35).

Nevertheless, the occurrence of more severe periodontal pathology in Down syndrome adults, with the presence of subgingival bacterial species typical of patients with chronic periodontitis, suggests that altered host–microbe interactions are particularly important in the pathogenesis of Down syndrome periodontitis. Indeed, the presence of immune deficiencies, altered cell functions and oxidative stress alterations in individuals with Down syndrome support this notion (36–38)). Interestingly, we recently reported that the carriage of several interleukin-1 (IL-1) genetic polymorphisms (IL-1A +4845, IL-1B +3954 and IL-1RN +2018) was inversely related to periodontal attachment loss in Down syndrome subjects (39), in contrast to their positive associations in some patients with chronic periodontitis (40). Further research to delineate the nature of host immune responses to both bacterial and viral challenges in the subgingival environment of Down syndrome subjects is warranted.

In conclusion, despite the similarities between the subgingival microbial composition of individuals with and without Down syndrome, individuals with Down syndrome show higher levels of some bacterial species and specific associations between certain bacterial species and loss of periodontal attachment. These findings are consistent with the notion that certain subgingival bacteria may contribute to the increased level of periodontal disease seen in Down syndrome individuals and raise the question as to the reason for such increased colonization in Down syndrome.

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