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Microbiome at sites of gingival recession in children with Hutchinson–Gilford progeria syndrome

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Abstract

Background—Hutchinson–Gilford progeria syndrome (HGPS) is a rare premature aging disorder with significant oral and dental abnormalities. Clinical symptoms include various features of accelerated aging such as alopecia, loss of subcutaneous fat, bone abnormalities, and premature cardiovascular disease. In addition, children with HGPS have been observed to suffer from generalized gingival recession. Whether periodontal manifestations associated with this syndrome are the results of changes in the oral flora is unknown. The present study aimed to identify the microbial composition of subgingival sites with gingival recession in children with HGPS.

Methods—Nine children with HGPS were enrolled in this study. Plaque samples were collected from teeth with gingival recession. DNA samples were analyzed using the Human Oral Microbe Identification Microarray (HOMIM). Microbial profiles from HGPS children were compared with microbial profiles of controls from healthy individuals (n=9) and subjects with periodontal disease (n=9).

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First three authors had equal contribution to the study.

Disclosures

All authors have no conflict of interest.

Results—Comparison of microbial compositions of HGPS samples with periodontal health samples demonstrated significant differences for 2 bacterial taxa; *Porphyromonas catoniae* and *Prevotella oulori* were present in children with HGPS, but not normal controls. There were statistically significant differences of 20 bacterial taxa between HGPS and periodontal disease groups.

Conclusion—Typical periodontal pathogeneses were not present at sites with gingival recession in HGPS children. The microbial compositions of sites of gingival recession and attachment loss in HGPS were generally more similar to those of periodontal health than periodontal disease. Species other than typical periodontal pathogens may be involved in this recession.

Keywords

Gingival recession; Hutchinson–Gilford progeria syndrome; Microbiota; Periodontal diseases; Progeria

Introduction

Hutchinson–Gilford progeria syndrome (HGPS) is a rare premature aging disorder with an estimated incident of 1 in 4–8 million births,¹ and a prevalence of 1 in 20 million living individuals.² This syndrome is a sporadic, autosomal dominant genetic disorder that is caused by a point mutation in the lamin A (*LMNA*) gene.³ The point mutation leads to activation of a cryptic donor splice site resulting in production of abnormal lamin A protein, which is called progerin.³ Increased accumulation of progerin disrupts the integrity of the nuclear membrane and alters transcription, which result in alternations in cellular morphology, cellular functions, and premature cell death.¹ Clinical symptoms include various features of accelerated aging such as sclerotic skin, loss of subcutaneous fat, alopecia, joint contractures, bone abnormalities, and premature cardiovascular disease.^{4, 5}

In addition to pre-mature aging-related symptoms, children with HGPS display growth impairment and a variety of oral, dental, and craniofacial abnormalities.^{4, 6, 7} Craniofacial phenotypes comprise craniofacial disproportion, deficiency in sagittal and vertical growth of the jaws, micrognathia, and retrognathic appearance due to mandibular hypoplasia.^{6, 7} Typical oral and dental manifestations include hypodontia, delayed tooth eruption, dysmorphic teeth, severe dental crowding, ogival palatal arch, ankyloglossia, and presence of median sagittal palatal fissure.^{4, 6, 7}

While considerable attention has been given to HGPS over the past decade and several research groups have focused on the complex pathogenesis of this syndrome and physiological processes affected by this disorder,^{5, 8, 9} the report of periodontal manifestations have been limited to a single patient case report.⁶ In this report, the authors described periodontal manifestations of this syndrome in a 9-year old female with HGPS, and analyzed subgingival plaque from sites demonstrating periodontal breakdown for the presence of a limited panel of putative periodontal pathogens.⁶ *Treponema denticola*, *Porphyromonas gingivalis*, and *Tannerella forsythia* were detected, whereas *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* were not detected.⁶ It is not known whether periodontal manifestations associated with this syndrome are due to changes in the

oral microflora or pre-mature aging related changes in the connective tissue of subjects with HGPS. During the course of evaluations for several single-center clinical treatment trials that we have conducted at Boston Children's hospital,^{10–12} we universally observed presence of periodontal disease characterized by generalized gingival recession (Figure 1). Since bacterial biofilm plays a key role in the initiation and progression of periodontal diseases,¹³ determining the oral microbial profile of HGPS patients is crucial as the first step in investigating the possible etiology of progressive attachment loss in these patients. Therefore, we prospectively designed a study to identify the composition of the microbial flora inhabiting the sites with gingival recession in children with HGPS and to compare it with microbial profiles of patients with periodontal health and periodontal disease. We used a 16S rDNA-based microarray method, the Human Oral Microbe Identification Microarray (HOMIM),¹⁴ which is a culture-independent molecular method able to detect approximately 300 of the most prevalent, cultivated and not-yet-cultivated oral bacterial species.¹⁵

Materials and Methods

Study Population

The present study was conducted from June 2013 to April 2014. Participants were 2 years of age and older (age 4–12) with clinically and genetically confirmed c.1824 C>T, p. Gly608Gly classic HGPS, and ability to travel for regular study visits. Nine children were enrolled in this study. The demographic, dental status, and list of medications of patients are presented in Table 1. None of the patients had a history of antibiotic treatment within 6 months of data collection. The study protocol was reviewed and approved by Boston Children's Hospital Committee on Clinical Investigation. Informed consents were obtained from legal guardians after being fully informed about the study protocol. Interpreters were provided when required during the consenting process. Age-appropriate assent was also obtained.

Plaque samples

Collection of plaque samples was performed before any periodontal treatment for the subjects. In each subject, four to six sites (with the exception of one patient who only had 3 erupted teeth at the time of the study) in different teeth with gingival recession were sampled. Plaque samples were collected by rubbing a sterile periodontal probe on the tooth at and slightly below the gingival margin. Supragingival plaque was not removed prior to sampling. Plaque samples for each individual were pooled into a single tube containing 300 µl Tris Buffer^{**}. The samples were immediately frozen at –80 °C and delivered to the Forsyth Institute (Cambridge, MA) on dry ice to prevent thawing for microbiological analysis. All samples were collected by the same trained investigator.

DNA Isolation and HOMIM protocol

DNA was isolated from plaque samples using a lysozyme solution^{††} for overnight incubation before using a DNA purification kit^{††} according to the manufacturer's protocol.

^{**}Epicentre Biotechnologies, Madison, WI

^{††}Ready-LyseTM Lysozyme Solution, Epicentre Biotechnologies

Purified DNA samples were analyzed using HOMIM, which is a culture-independent 16S rRNA gene-based oligonucleotide, reverse capture microarray able to simultaneously detect approximately 300 of the most predominant oral bacterial species.^{14, 16} Briefly, 16S rRNA-based, reverse-capture oligonucleotide probes were printed on aldehyde-coated glass slides. Fluorescently-labeled 16S rRNA gene amplicons were hybridized at 55°C for 16 hours to probes on the slides. The microarray slides were scanned ^{§§}, and raw data were extracted using a software^{||} for microarray image analysis.

Statistical analysis

Microbial community profiles were generated from image files of scanned HOMIM arrays using a HOMIM online analysis tool. The detection of a specific taxon was determined by the presence of a fluorescently-labeled spot for the targeted probe. A mean intensity for each taxon was calculated from the hybridization spots of the same probe and the signals were normalized and calculated as previously described.¹⁴ Signal levels ranges were obtained by raising normalized signal intensities to the power of 0.3. Two times the background value was assigned to the signal level 0; indicating absence of a particular taxon in a sample. Signals were categorized into scores from 1 to 5; corresponding to different signal levels. Pairwise sample dissimilarities were calculated based on the Pearson correlation coefficient and the dissimilarity matrices were subject to the principal coordinate analysis (PCoA) using the QIIME pipelines.¹⁷ PCoA reduces data dimensions and allows for visualization of microbial profiles comparisons at the sample and community levels. Data was compared with microbial profiles of historical controls from healthy individuals (n=9) and profiles from adults with chronic periodontitis (n=9). HOMIM data for periodontally healthy subjects and chronic periodontitis subjects was previously published.¹⁶ Briefly, all control subjects were 18 years of age and had 14 teeth. Periodontally healthy subjects presented 10% of sites with Bleeding on Probing (BOP) and <3 mm pocket depth (PD). Chronic periodontitis subjects presented > 10% of teeth with PD ≥ 5 mm with BOP. Exclusion criteria included periodontal treatment or use of antibiotic within the last 6 months, use of long-term anti-inflammatory medication, pregnancy, and nursing.¹⁶

Although not aged-matched, the periodontally healthy and periodontitis control groups allowed us to assess the microbial differences in HGPS. However, these control groups were historical, may introduce a selection bias into the study. Yet, it should be emphasized that the main objective of this study was to identify microbial profile of sites with gingival recession in children with HGPS, and the two historical control groups were added only to facilitate the interpretation of microbiologic data of HGPS group. In comparisons of microbiomes between each two groups, the Wilcoxon Rank Sum test was used (alpha threshold p-value < 0.05). To adjust for multiple comparisons, Benjamini-Hochberg corrections were used. For cluster analyses, Pearson correlation was used as a distance metric selection. The program MeV was used calculation for hierarchal clustering and generation of heatmap figures.

^{††} MasterPure DNA Purification Kit, Epicentre Biotechnologies

^{§§} Axon 4000B scanner, Axon GenePix 4000B, MDS Analytical Technologies, Sunnyvale, CA

^{||} GenePix Pro software, MDS Analytical Technologies

Results

Sites of gingival recession were clearly evident in all study participants (Figure 1). Comparison of the entire microbial profile of samples between HGPS and periodontal disease demonstrated that microbial profiles of sites with gingival recession in individuals with HGPS were clearly distinct from microbial profiles of those with periodontal disease (Figure 2A). Cluster analysis showed that microbial profile of samples from the same group clustered closely together, and a distinct clustering was evident between HGPS and periodontal disease samples (Figure 2B). The comparisons of microbiomes between the two groups demonstrated that 20 taxa were statistically different between the two groups (Table 2). The taxa that were detected significantly more often in HGPS group were:

Campylobacter concisus HOT-575 ($p = 0.031$), *Capnocytophaga granulosa* HOT-325 ($p = 0.014$), *Porphyromonas catoniae* HOT-283 / *Porphyromonas sp.* HOT-279 ($p = 0.045$), *Prevotella* Cluster II ($p = 0.045$), *Prevotella histicola* HOT-298 / *Prevotella melaninogenica* HOT-469 ($p = 0.045$), and *Prevotella oulori* HOT-288 ($p = 0.027$). *Porphyromonas gingivalis* and *Tannerella forsythia* were detected in HGPS group, but were not as predominant as in periodontal disease. *Treponema denticola* was detected in two HGPS subjects and *Prevotella intermedia* was found in one HGPS subject, but *Aggregatibacter actinomycetemcomitans* was not detected in HGPS or periodontally healthy subjects (data not shown). In contrast, the following typical periodontal pathogens were found significantly more often in periodontal disease samples: *Desulfobulbus sp.* HOT-041 ($p = 0.013$), *Filifactor alocis* ($p = 0.013$), *Parvimonas micra* ($p = 0.031$), *Tannerella forsythia* ($p = 0.014$), *Porphyromonas endodontalis* ($p = 0.029$), *Porphyromonas gingivalis* ($p = 0.027$). These species were not typically detected in HGPS samples (Figure 2B).

When comparing the microbial profile of samples from HGPS children with those from periodontally healthy subjects, PCoA demonstrated some distinctions in the microbial profiles between the two groups (Figure 3A). However, the comparisons of microbiomes found significant differences between the two groups for two *Prevotella* species: *Prevotella sp.* HOT 317 ($p = 0.009$) and *Prevotella sp.* HOT 310 ($p = 0.033$), which were noticeable absent from HGPS samples, but were detected in most periodontally-healthy subjects (Figure 3B and Table 2).

Clear distinction and clustering was also found in the entire microbial profile of samples between periodontal health and periodontal disease groups (Figure 4A and 4B). Statistically significant differences were found between the two groups for 46 taxa, which are listed in Table 2.

PCoA showed that the microbial profiles of HGPS samples were more similar to the microbial profile of samples of periodontal health sample than samples of periodontal disease (Figure 4C).

Discussion

This investigation represents the first prospectively designed, comprehensive cohort-based analysis of microbial profiles of sites with gingival recession in individuals with HGPS. The

results demonstrated that microbial profiles of dental plaques from individuals with HGPS were more similar to the microbial compositions of periodontal health than of periodontal disease.

Similar to previously published studies,^{14, 16} HOMIM analysis of samples from periodontal disease sites demonstrated a high prevalence of periodontal pathogens, including classical periodontal pathogens,^{18, 19} such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Porphyromonas endodontalis*, and *Parvimonas micra*, as well as newly-associated bacterial species,¹⁶ such as *Desulfobulbus sp.*, *Filifactor alocis*, *Fretibacterium sp.*, and *Peptostreptococcus sp.* These putative periodontal pathogens were at much lower levels or in some cases totally absent from samples of children with HGPS. Species that were predominant in HGPS, namely *P. catoniae* and *P. oulora*, are not commonly detected in periodontal disease. However, *P. catoniae* has been associated with shallow periodontitis sites,²⁰ and *P. oulora* may be associated with refractory periodontitis in adults.²¹ These data suggest that species other than the typical periodontal pathogens may be involved the gingival recession in children with HGPS.

Increased production and accumulation of progerin in cells are responsible for premature aging phenotypes of HGPS, which are manifested in the connective tissue.^{22, 23} Altered extracellular matrix in HGPS patients is believed to contribute to clinical features such as scleroderma-like skin phenotype and development of vascular lesions.²⁴ It has been also shown that HGPS fibroblasts have impaired DNA repair, reduced telomere length, retarded turnover, and decreased viability compared to normal fibroblasts.^{23, 25, 26} This would result in impaired growth and maturation of connective tissue as well as its reduced ability to remodel.²³ HGPS fibroblasts also are more susceptible to oxidative stress, and their nuclear lamina has a reduced capacity to rearrange following mechanical strain, which impairs cell viability under mechanical stress.^{27, 28} In addition, the level of nucleotide pyrophosphatase transcription, which has a significant role in soft tissue maintenance, is dramatically reduced in HGPS fibroblasts.^{22, 29, 30} Furthermore, similar to normal aging, the function and hemostasis of stem cells are compromised in Hutchinson–Gilford progeria syndrome.^{31–34} Moreover, it has been demonstrated that accumulation of progerin in cells results in a proinflammatory response characterized by sustained expression of leukocyte adhesion molecules and proinflammatory cytokines such as IL-8 and MCP-1.³⁵ Therefore, these changes in the connective tissue of HGPS children may affect periodontal hemostasis and be responsible for the gingival recession phenotype in these children. However, understanding the exact pathophysiology of gingival recession in patients with HGPS and its relevance to the etiology of gingival recession in the aging population requires further investigation.

It should be noted that the objective of the present study primarily was identification of microbial profile of sites with gingival recession in children with HGPS. Previously published data set from the two historical control groups of periodontal health and periodontal disease were only used to facilitate the interpretation of microbial profile of HGPS group. Direct comparison of microbial profile of HGPS group with historical control groups introduces several issues such as possibility of selection bias, differences in the method of the plaque collection between the HGPS groups and historical control groups, and lack of age-matching comparisons. Hence, these limitations should be considered when

interpreting the results. Additional studies are necessary to determine whether the microbiomes of HGPS are directly involved in gingival recession, or if anatomical abnormalities and premature aging related changes in the periodontium predisposed these patients to disease, or both.

Investigating rare syndromes with extreme phenotypes may lead to a better global understanding of the disease process. Hence, Hutchinson–Gilford progeria syndrome may represent a unique opportunity to study the etiology and disease process of generalized gingival recession and clinical attachment loss. Furthermore, investigating periodontal status of patients with HGPS will contribute to the HGPS phenotype database and advance our understanding of complex pathogenesis of this syndrome, which eventually may lead to implementation of treatment strategies to improve the quality of life of these patients.

Conclusion

The present study presents a comprehensive characterization of the microbial communities at sites with gingival recession in patients with HGPS. Primarily, it was found that typical periodontal pathogens were not present at sites with gingival recession in HGPS children. Secondly, the microbial profiles of sites of gingival recession and attachment loss in HGPS were more similar to the microbial profile of samples with periodontal health than periodontal disease. These data suggest that additional bacterial taxa and not the typical periodontal pathogens might be associated with this gingival recession in HGPS. Future studies are needed to understand the pathophysiology of gingival recession in these children.

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Key findings

Although the oral phenotype includes gingival recession, the microbial compositions of sites with gingival recession of these children are more similar to those of periodontal health than periodontal disease.

Figure 1.

(A) Intra-oral photograph of a six-year old child with HGPS illustrating gingival recession at teeth D (2mm), E (4mm), F (3mm), O (3mm), and P (3mm); (B) Intra-oral photograph of a nine-year old child with HGPS demonstrating gingival recession at teeth M (2mm), P (1mm), R (3mm), and S (5mm). Root caries was also evident on teeth M and R; (C and D) Figures C and D demonstrate the progression of gingival recession in a child with HGPS. At age of four years, minimal recession was observed at tooth S (C), but 5 mm recession was evident six years later (D). Black arrows demonstrates sites with gingival recession.

Figure 2.

(A) PCoA demonstrating a clear distinction between microbial profiles present in HGPS (blue) and periodontal disease (red). (B) Hierarchical clustering of statistically significant bacterial taxa that differentiate the microbiomes of subjects with HGPS and periodontal disease. Hierarchical cluster analysis applied to the normalized fluorescence intensity signal from the microarray data, on a 0 to 5 scale where 0 is not present (black) and 5 is present in high levels (red). Twenty significantly different bacterial taxa between the groups are shown. *Parvimonas micra* and *Porphyromonas gingivalis* are each shown twice since there were 2 different probes for each species.

Figure 3.

(A) PCoA demonstrating a relative distinction between microbial profiles present in HGPS (blue) and periodontal health (green). (B) Hierarchical clustering of statistically significant

bacterial taxa that differentiate the microbiomes of subjects with HGPS and periodontal health.

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Figure 4.

(A) PCoA demonstrating a clear distinction between microbial profiles present in periodontal health (green) and periodontal disease (red) in non-HGPS subjects. (B) Hierarchical clustering of bacterial taxa that were significantly different between periodontal

health and periodontal disease groups. Significant differences were found for 46 bacterial taxa between the groups. *Parvimonas micra* and *Porphyromonas gingivalis* are each included two times as there were 2 different probes for each species. (C) PCoA demonstrating distinctions between microbial profiles present in HGPS (blue), periodontal health (green) and periodontal disease (red). Microbial profiles of HGPS were more similar to periodontal health than to periodontal disease in non-HGPS subjects.

Demographic characteristics, teeth present, and list of medications of the patient population

Table 1

Patient #	Age (years)	Gender	Ethnicity (country of origin)	Teeth present	Medication list
1	6	F	African American (US)	Incomplete primary dentition; only 3 primary teeth erupted; no teeth are mobile or exfoliating	Lonafarnib, Pravastatin, Vitamin D
2	6	M	White (US)	Full primary dentition; no teeth are mobile or exfoliating	Aspirin, * Lonafarnib, Pravastatin, Recombinant growth hormone, Vitamin D
3	6	M	White (US)	Incomplete primary dentition; no eruption of 2 nd primary molars; no teeth are mobile or exfoliating	Aspirin, * Levalbuterol, Lonafarnib, Montelukast sodium, Pravastatin, Propranolol, Vitamin D
4	6	M	White (Sweden)	Incomplete primary dentition; teeth J, K, and T are not erupted; no teeth are mobile or exfoliating	Aspirin, * Lonafarnib, Pravastatin, Vitamin D
5	11	F	Hispanic/Latina (Argentina)	Mixed dentition; sampled teeth are not mobile or exfoliating	Aspirin, * Lonafarnib, Pravastatin, Vitamin D
6	9	F	Middle Eastern/North African (Egypt)	Incomplete primary dentition; teeth J and K are not erupted; no teeth are mobile or exfoliating	Lonafarnib, Pravastatin, Vitamin D
7	12	F	White (US)	Mixed dentition; sampled teeth are not mobile or exfoliating	Aspirin, * Lonafarnib, Pravastatin, Vitamin D
8	7	F	White (South Africa)	Incomplete primary dentition; teeth B, K, and T are not erupted; no teeth are mobile or exfoliating	Aspirin, * Lonafarnib, Pravastatin, Vitamin D
9	4	F	White (US)	Full primary dentition; no teeth are mobile or exfoliating	Lonafarnib, Pravastatin, Recombinant growth hormone, Vitamin D

F= Female; M = Male

* Low dosage (2–3 mg/kg/day)

Table 2

List of bacterial taxa that were significantly different between the groups

	Target taxon	P value
Different between the HGPS and periodontal disease		
Detected more often in HGPS group	<i>Campylobacter concisus</i> HOT-575	0.031
	<i>Capnocytophaga granulosa</i> HOT-325	0.014
	<i>Porphyromonas catoniae</i> HOT-283 / <i>Porphyromonas</i> sp. HOT-279	0.045
	<i>Prevotella</i> Cluster II	0.045
	<i>Prevotella histicola</i> HOT-298 / <i>Prevotella melaninogenica</i> HOT-469	0.045
	<i>Prevotella oulori</i> HOT-288	0.027
Detected more often in periodontal disease group	<i>Peptostreptococcaceae</i> [11][G-4] spp. HOT-103,369	0.014
	<i>Porphyromonas endodontalis</i> HOT-273 / <i>Porphyromonas</i> spp. HOT-285,395	0.029
	<i>Veillonellaceae</i> [G-1] spp. HOT-129,135,148	0.045
	<i>Desulfobulbus</i> sp. HOT-041	0.013
	<i>Eubacterium</i> [11][G-5] saphenum HOT-759	0.045
	<i>Filifactor alocis</i> HOT-539	0.013
	<i>Fretibacterium</i> Cluster	0.014
	<i>Fretibacterium</i> sp. HOT-359 / <i>Fretibacterium</i> sp. HOT-362	0.014
	<i>Parvimonas micra</i> HOT-111	0.031
	<i>Parvimonas micra</i> HOT-111	0.045
	<i>Peptostreptococcaceae</i> [13][G-1] sp. HOT-113	0.027
	<i>Porphyromonas gingivalis</i> HOT-619	0.027
	<i>Porphyromonas gingivalis</i> HOT-619	0.018
	<i>Tannerella forsythia</i> HOT-613	0.014
Different between the HGPS and periodontal health		
Detected more often in periodontal health group	<i>Prevotella</i> sp. HOT 317	0.009
	<i>Prevotella</i> sp. HOT 310	0.033
Different between the periodontal health and periodontal disease		
Detected more often in periodontal health group	<i>Gemella haemolysans</i> HOT-626 / <i>Gemella sanguinis</i> HOT-757	0.022
	<i>Gemella haemolysans</i> HOT-626	0.025
	<i>Haemophilus parainfluenzae</i> HOT-718	0.005
	<i>Prevotella</i> sp. HOT-310	0.012
	<i>Prevotella</i> sp. HOT-317	0.017
	<i>Rothia dentocariosa</i> HOT-587 / <i>Rothia mucilaginosa</i> HOT-681	0.023
	<i>Streptococcus australis</i> HOT-073	0.005
	<i>Streptococcus oralis</i> HOT-707 / <i>Streptococcus</i> sp. HOT-064	0.005
Detected more often in periodontal disease group	<i>Fretibacterium</i> spp. HOT-360,362,453	0.046
	<i>Peptostreptococcaceae</i> [11][G-4] spp. HOT-103,369	0.005
	<i>Porphyromonas endodontalis</i> HOT-273 / <i>Porphyromonas</i> spp. HOT-285,395	0.005
	<i>Selenomonas infelix</i> HOT-639 / <i>Selenomonas</i> spp. HOT-126,479,481	0.023

	Target taxon	P value
	<i>Treponema</i> spp. HOT-237,242	0.046
	<i>Veillonellaceae</i> [G-1] spp. HOT-129,135,148	0.023
	<i>Bacteroidales</i> [G-2] sp. HOT-274	0.005
	<i>Bacteroidales</i> [G-2] sp. HOT-274	0.013
	<i>Campylobacter concisus</i> HOT-575 / <i>Campylobacter rectus</i> HOT-748	0.022
	<i>Campylobacter concisus</i> HOT-575 / <i>Campylobacter rectus</i> HOT-748	0.021
	<i>Catonella morbi</i> HOT-165 / <i>Catonella</i> sp. HOT-164	0.041
	<i>Desulfobulbus</i> sp. HOT-041	0.005
	<i>Dialister invisus</i> HOT-118	0.025
	<i>Eubacterium</i> [11][G-3] brachy HOT-557	0.046
	<i>Eubacterium</i> [11][G-5] saphenum HOT-759	0.023
	<i>Eubacterium</i> [11][G-6] nodatum HOT-694	0.023
	<i>Eubacterium</i> [11][G-7] yurii HOT-377 / <i>Peptostreptococcaceae</i> [11][G-7] sp. HOT-106	0.023
	<i>Filifactor alocis</i> HOT-539	0.005
	<i>Fretibacterium</i> Cluster_D70	0.005
	<i>Fretibacterium</i> sp. HOT-359 / <i>Fretibacterium</i> sp. HOT-362	0.005
	<i>Fusobacterium periodonticum</i> HOT-201	0.005
	<i>Parvimonas micra</i> HOT-111	0.005
	<i>Parvimonas micra</i> HOT-111	0.005
	<i>Peptostreptococcaceae</i> [13][G-1] sp. HOT-113	0.046
	<i>Peptostreptococcaceae</i> [13][G-1] sp. HOT-113	0.012
	<i>Peptostreptococcus stomatis</i> HOT-112	0.023
	<i>Porphyromonas gingivalis</i> HOT-619	0.012
	<i>Porphyromonas gingivalis</i> HOT-619	0.005
	<i>Prevotella intermedia</i> HOT-643	0.023
	<i>Stomatobaculum</i> sp. HOT-097	0.012
	<i>Streptococcus constellatus</i> HOT-576	0.046
	<i>Tannerella forsythia</i> HOT-613	0.005
	<i>Treponema maltophilum</i> HOT-664	0.025
	<i>Treponema parvum</i> HOT-724	0.046