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ORIGINAL ARTICLE

Gastro-intestinal and oral microbiome signatures associated with healthy aging

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Abstract The human oral and gut microbiomes influence health via competition for a distinct niche in the body with pathogens, via metabolic capabilities that increase host digestive capacity and generate compounds engaged in signaling pathways and modulation of immune system functions. Old age alters our metabolic and regenerative capacity. Following recruitment of 65 human subjects in the age range of 70 to 82, we discerned healthy aging (HA) and non-healthy aging (NHA) cohorts discordant in the occurrence of one or more major diseases: (1) cancer, (2) acute or chronic cardiovascular diseases, (3) acute or chronic pulmonary diseases, (4) diabetes, and (5) stroke or neurodegenerative disorders. We analyzed these cohorts' oral microbiomes (saliva) and gut microbiomes (stool) to assess diversity and identify microbial biomarkers for HA. In contrast to the gut microbiome where no change was observed, we found that the saliva microbiome had higher α-diversity in the HA compared with the NHA group. We observed the genus *Akkermansia* to be significantly more abundant in the gut microbiota of the HA group. *Akkermansia muciniphila* is a colonic mucin-degrading bacterium believed to have beneficial effects on gastrointestinal health, particularly in the context of diabetes and obesity. Erysipelotrichaceae UCG-003 was a taxon increased in abundance in the HA cohort. *Streptococcus* was the only genus observed to be significantly decreased in abundance in both the gut and oral microbiomes of the HA cohort compared with the NHA cohort. Our data support the notion that these microbes are potential probiotics to decrease the risks of non-healthy aging.

Keywords Oral microbiome · Gut microbiome · 16S rRNA · Metagenomic analysis · Healthy aging · Chronic disease · Longevity · *Akkermansia* · *Streptococcus*

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Introduction

Healthy aging is influenced by human genetics and environmental factors, including nutrition and lifestyle. The aging process is generally characterized by a decline of physiological and immunological functions which, in turn, triggers acute and chronic diseases including organ failure. Chronic low-grade inflammation has long been thought to contribute to increased disease risks in the context of aging (Woods et al. 2012). Mitochondria and lysosomes are cellular organelles with

important roles in the degradation of cellular waste products and the recycling of their molecular building blocks for anabolic processes. Reduced function of these organelles and the consequential accumulation of cellular waste results in inflammation and, therefore, is linked to aging (Bratic and Larsson 2013; Carmona-Gutierrez et al. 2016; Harman 1972). One commonly studied model system for research on aging in multicellular organisms with a digestive tract is the nematode *Caenorhabditis elegans*. Associations between mitochondrial function and aging were demonstrating using *C. elegans* (Shen et al. 2014). Human genetic traits associated with healthy aging, even in cases of exceptional longevity (centenarians), are complex (Sebastiani et al. 2012, 2010). A *C. elegans* model was used to identify genes of potential importance to the aging process. Among those are *sir2* and *daf16*. Other genes encode proteins with apparent roles in longevity (Lin et al. 1997; Tissenbaum and Guarente 2001). A human Sir2 ortholog, SIRT3, has a potential mitochondrial NAD-dependent deacetylase function. Genotype variability in SIRT3 was statistically linked to longevity (Rose et al. 2003) and later described as a protein involved in reactive oxygen species (ROS) suppression and mitochondrial biogenesis (Kong et al. 2010) as well as tumor suppressor functions (Chen et al. 2014). Additionally, the insulin/IGF-1 signaling (IIS) pathway was identified as an important evolutionarily conserved pathway with a role in NF-kB signaling and the aging process. SIRT1 (a paralog of SIRT3) and class O Forkhead box (FoxO) transcription factors (human orthologs of the *C. elegans* protein Daf16) are NF-kB signaling inhibitors (Salminen and Kaarniranta 2010). In yet another study, genetic variability in the gene-encoding FOXO3A was strongly associated with longevity (Willcox et al. 2008).

Mitochondrial functions are influenced by environmental factors such as toxicants and steroid hormones that a large human population is exposed to (Meyer et al. 2013; Velarde 2014). The metabolism of nutrients, with a final stage in mitochondria, generates oxidants that may contribute to degenerative aging diseases (Ames et al. 1993). Low intake of antioxidant-rich foods is thought to be a factor in aging diseases (Ames et al. 1993). Given the role of human microbiota colonizing the digestive tract in the uptake and metabolism of foods, orally ingested toxicants and steroid hormones, relationships between the composition of human microbiota and aging likely exist (Heintz and Mair 2014; Kumar et al. 2016). The rapid progress in next-generation sequencing (NGS) technologies and associated bioinformatics analysis tools have permitted the taxonomic characterization of human oral cavity and gut microbiota using metagenomics. Links between the highly complex microbiota and disease states can be identified (Eckburg et al. 2005; Gill et al. 2006; Round and Mazmanian 2009). To name a few studies and perspectives, associations of gut microbial changes with dysregulated adaptive immune cells (Round and Mazmanian 2009) and nutrient metabolism (Kau et al. 2011) were established. Caries has been linked to a distinct composition of the oral microbiome, and cariogenic microbes were identified (Gomez et al. 2017). Claesson et al. (2012) reported that fecal microbiota of older individuals had greater interindividual variations compared with younger adults. These investigators also found gut microbiota of people in long-term care facilities to be less diverse than those of community-dwelling older adults. Data on the abundance of the cytokines IL-6 and IL-8, diastolic blood pressure, and weight suggested that quantitative changes of *Ruminococcus*, *Prevotella*, *Oscillobacter* CAG, and *Bacteroides* taxa in the gut microbiome played a role in healthy aging (Claesson et al. 2012). Many bacteria residing in the digestive tract produce metabolites absorbed by the host's intestinal mucosa where they induce physiological changes. For example, the type 2 diabetes drug metformin was found to increase the life span of *C. elegans* when co-cultured with *Escherichia coli* by altering folate and methionine metabolism (Cabreiro et al. 2013). Such gut microbiota-mediated metabolic adaptations may also occur in humans who take this therapeutic drug. In a study on Parkinson's disease, a progressive neurological disorder that mostly elderly people suffer from, the concentrations of short-chain fatty acids (SCFAs) were decreased compared with an age-matched control group (Unger et al. 2016). Produced by many GI commensal organisms, SCFAs modulate signaling pathways including the inhibition of histone deacetylases and activation of G protein–coupled receptors. SCFAs are absorbed by enterocytes and enter the blood stream (Tan et al. 2014). Examples of SCFA producers are Lachnospiraceae family members that convert lactate to propionate and *Faecalibacterium*, *Eubacterium*, and *Roseburia,* species that produce butyrate from butyryl-CoA. There is evidence of cross-feeding of such SCFAs among gut commensals (Rios-Covian et al. 2016). Changes in the oral microbiota were also linked to increased risks of cardiovascular disease, a set of morbidities afflicting many older adults (Leishman et al. 2010).

To our knowledge, no data have been published on the correlation of gut and oral microbial profiles with elderly cohorts discordant with respect to incidence of at least one major disease in their lifetimes. We enrolled a cohort of 65 human subjects, half of whom had evidence of healthy aging (HA) and half of whom self-reported having suffered from at least one major disease (defined here as non-healthy aging (NHA)). The enrolled older adults provided stool and saliva samples collected at two timepoints 4 to 6 months apart. The NHA group had a medical history of cancer, stroke or other neurological disease, heart attack or other cardiovascular disease, chronic pulmonary disease, or diabetes/diabetic complications. Gut and oral cavity microbiomes (from stool and saliva samples, respectively) were phylogenetically profiled to determine diversity and taxonomic differences between the two groups. REAP surveys (Gans et al. 2003) were used to correlate data on nutritional intake and disease status with the microbiomes.

Materials and methods

Study population, recruitment, and specimen collections

A case-control, prospective study was conducted. The internal review boards (IRB) of Danbury Hospital, Danbury, CT, and the J. Craig Venter Institute, Rockville, MD, approved a human-subject consent form and a protocol outlining the risks and benefits of participation, as well as a questionnaire that requested a detailed medical history and nutrition via a Rapid Eating and Activity Assessment for Patients (REAP) (Gans et al. 2003). IRB approvals for the study were received in November 2013 (name: The Rudy Ruggles Systems Biology Study to Identify and Elucidate Health Aging; protocol number: 13-01) and continued for the time period of data generation and analysis. Participants who enrolled in the study visited a physician at Danbury Hospital in the context of routine or disease follow-up health care. Provided that their age was in the 70–82 range, they were asked about their interest in volunteering for the study. Sixty-five human subjects of both genders were enrolled in 2014 and agreed to provide saliva, stool, blood, and urine specimens twice during the active enrollment period. By early 2015, recruitment and specimen collections were completed. Medical history data allowing grouping subjects into the HA vs. NHA cohorts were available for all 65 participants. The NHA group had a medical history linked to one or more of the following major disease categories: (1) cancer, (2) acute or chronic cardiovascular disease, (3) acute or chronic pulmonary disease, (4) chronic liver disease, (5) diabetes and diabetic complications, (6) stroke or neurodegenerative disorder. Human subjects who were in the HA group did not report being diagnosed with any of these diseases in their lifetimes. A standard stool collection protocol developed by the Human Microbiome Project initiative (Human Microbiome Project 2012) was used for this study. Stool and saliva specimens were shipped to the main study site (JCVI), cooled at 5–10 °C overnight, and then frozen at − 80 °C until used for 16S rDNA sequencing experiments.

Stool DNA extraction

DNA for 16S rRNA PCR reactions was extracted from stool samples using a MoBIO powersoil purification kit. We aliquoted approximately 1 g of stool into a centrifuge tube and resuspended it in 800 μl of lysis buffer (1 M Tris-HCl, 2 mM EDTA, 1.2% Triton X-100). Stool samples were incubated at 75 °C for 10 min, allowed to cool to room temperature, and followed by the addition of 60 μl 200 mg/ml lysozyme and 5 μl RNase A. Stool lysates were incubated overnight at 37 °C, and then added to lysing tubes provided in the MoBIO powersoil purification kit. The DNA was extracted using the manufacturer's specifications and eluted in 100 μl of the solution (Rajagopala et al. 2016).

Saliva DNA extraction

Saliva specimens were thawed at 4 °C and vortexed thoroughly. A 300-μl aliquot was resuspended in 300 μl TES buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, and 1.2% Triton X-100). The sample was pulse-vortexed and incubated at 75 °C for 10 min followed by cooling to 20 °C. To this suspension, we added 60 μl chicken egg lysozyme (200 μg/ml), 5.5 μl mutanolysin (20 U/ml; Sigma–Aldrich), and 5 μl linker RNase A. Gently mixing, the suspension was incubated for 60 min at 37 °C. After addition of 100 μl 10% SDS and 42 μl proteinase K (20 mg/ml), the lysate was digested overnight at 55 °C. DNA was extracted by adding an equal volume of phenol:chloroform:isoamylalcohol (25:24:1; pH 6.6). The suspension was vortexed and centrifuged at 13,100×*g* for 20 min. The aqueous phase was

transferred to a clean sterile microcentrifuge tube. The residual sample was re-extracted repeating the previous step, followed by a final extraction using an equal volume of chloroform:isoamylalcohol (24:1) and centrifugation at 13,100×*g* for 15 min. To the aqueous phase, we added 3 M sodium acetate (pH 5.2) at one tenth of the volume. DNA was precipitated by adding an equal volume of ice-cold isopropanol. Incubations at − 80 °C for 30 min or at − 20 °C overnight followed. Precipitated DNA was centrifuged at 13,000×*g* for 10 min and was washed with 80% ethanol. The centrifugation step was repeated after renewed addition of 80% ethanol. The air-dried DNA pellet was resuspended in TE buffer (20 mM Tris-HCl, pH 8.0; 1 mM Na-EDTA) and stored at − 20 °C. PCR inhibitors were removed using the MOBio PowerClean DNA purification kit according to the manufacturer's specifications.

16S rDNA analysis by MiSeq sequencing

DNA extracted from stool samples was amplified using primers that targeted the V1-V3 regions of the 16S rRNA gene (Rajagopala et al. 2016). These primers included the i5 and i7 adaptor sequences for Illumina MiSeq pyrosequencing as well as unique 8-bp indices incorporated in both primers such that each sample received its unique barcode pair. This method of incorporating the adaptors and index sequences in the primers at the PCR stage provided minimal loss of sequence data when compared with previous methods that would ligate the adaptors to every amplicon after amplification. This method also allows generating sequence reads which are all in the same 5′-3′ orientation. Using approximately 100 ng of extracted DNA, the amplicons were generated with Platinum Taq polymerase (Life Technologies, CA) and by using the following cycling conditions: 95 °C for 5 min for an initial denaturing step followed by 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s for a total of 35 cycles followed by a final extension step of 72 °C for 7 min and then stored at 4 °C. Once the PCR for each sample was completed, the amplicons were purified using the QIAquick PCR purification kit (Qiagen Valencia, CA), quantified fluorometrically using SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific), normalized, and pooled in preparation for bridge amplification followed by Illumina MiSEQ sequencing using the dual index 2 × 300-bp format (Roche, Branford, CT) following the manufacturer's protocol.

Processing and filtering of sequence reads

Operational taxonomic units (OTUs) were generated de novo from raw Illumina sequence reads using the UPARSE pipeline (Edgar 2013). Paired-end reads were trimmed of adapter sequences, barcodes, and primers prior to assembly. Sequences of low quality and singletons were discarded. Sequences were subjected to a de-replication step, and abundances were determined. Chimera filtering of the sequences occurred during the clustering step. We used the Wang classifier (method = Wang) and bootstrapped using 100 iterations (iters = 100). We set mothur to report full taxonomies only for sequences where 80 or more of the 100 iterations are the same (cutoff = 80). Taxonomies were assigned to the OTUs with mothur (Schloss et al. 2009) using version 123 of the SILVA 16S ribosomal RNA database (Quast et al. 2013) as the reference. Tables with OTUs and the corresponding taxonomy assignments were generated and used in subsequent analyses. The next step was to remove likely non-informative OTUs with an independent filtering process. Rare OTUs or taxa are strongly affected by MiSeq sequencing errors, and any statistical conclusions relying on them are typically unstable. Even in the univariate differential abundance analysis, the presence of such taxa increases the penalty from the multiple testing correction applied to the more abundant taxa. We used unbiased metadata-independent filtering at each level of the taxonomy by eliminating all features that did not pass these criteria. This included samples with less than 2000 reads and OTUs present in less than 10 samples.

Identification of phylogenetic groups in gut and oral microbiota

The phyloseq package version 1.16.2 in the R package version 3.2.3 was used for the microbiome census data analysis (McMurdie and Holmes 2013; Team 2015). The ordination analysis was performed using non-metric multidimensional scaling (NMDS) with the Bray–Curtis dissimilarity matrix (Bray and Curtis 1957). The data output was used for the generation of a heatmap using the plot_heatmap function in the phyloseq package (McMurdie and Holmes 2013). Differences in microbial richness (alpha diversity) were evaluated using different algorithms included in the phyloseq plot_richness function. For genus-level and OTU count matrices, we performed the following richness and diversity analyses using the R phyloseq

package. The plot_richness function was used to create a plot of alpha diversity index estimates for each sample. Heatmaps of taxonomic profiles clustered hierarchically using the plot_heatmap function in phyloseq were constructed (Rajaram and Oono 2010). A beta diversity Bray–Curtis dissimilarity matrix was used to compute the non-metric multidimensional scaling (NMDS). The ordination output was plotted in the form of heatmaps using the plot_heatmap function including a side bar where clinical variables associated with each sample were assigned to look for specific associations.

Statistical analyses of gut and oral microbiome data

To detect differential abundances in the gut microbiota at a genus or species level, the DESeq2 package version 1.12.3 in R was used. The phyloseq data are converted into a DESeq2 object using the function phyloseq_to_deseq2 function. DESeq2 (Love et al. 2014) is a method for the differential analysis of count data that uses shrinkage estimation for dispersions and fold changes to improve the stability and interpretability of estimates. The DESeq2 test uses a negative binomial model rather than simple proportion-based normalization or rarefaction to control for different sequencing depths, which may increase the power and also lower the false positive detection rate (McMurdie and Holmes 2014). Default options of DESeq2 were used for multiple testing adjustment applying the Benjamini and Hochberg method (Benjamini and Hochberg 1995).

Results

Cohort descriptions and study design

We recruited 65 human subjects in the age range of 70 to 82. The healthy aging (HA) and non-healthy aging (NHA) groups were discordant in the incidence of one or more major diseases based on self-reported data. Major disease categories defining NHA for this study's participants were at least one-time diagnosis for (1) cancer, (2) acute or chronic cardiovascular diseases, (3) acute or chronic pulmonary diseases, (4) diabetes, and (5) stroke or neurodegenerative disorders. Moreover, study participants were asked questions related to chronic pain, memory loss, recent hospitalizations (these factors were not among the criteria sufficient to place an individual in the NHA group) and nutrition. Data on the disease groups among the 65 participants is provided in Table 1. All demographic and medical data that were collected and the group assignments (HA vs. NHA) are provided in Suppl. File S1. The specimens to characterize microbial and other molecular signatures for the HA and NHA cohorts were blood plasma, urine, saliva, and stool. The specimens were collected for two timepoints, approximately 4 to 6 months apart, from all but 5 study participants. We analyzed 125 saliva and stool samples to characterize differences in the oral and gastrointestinal (GI) tract microbiomes, respectively. There were no instructions to fast or change diet prior to the specimen collection times or alter the regimen for therapeutic drug intake. Recent intake of antibiotic drugs was reported by one participant, which occurred 6 weeks prior to the 1st collection timepoint. These donor's samples were not excluded from the surveys. All medical conditions newly reported at the time of the 2nd clinical visit did not modify group assignments. No conclusive new diagnoses of a major disease pertaining to the subjects of the HA group occurred in the 4- to 6-month timeframe of specimen collection.

Abundance differences for oral microbial profiles comparing HA and NHA cohorts

The alpha-diversity parameters consisting of Shannon (species evenness) and Chao1 (species richness) diversity indices show that the oral microbiome alpha diversity of the HA group is significantly higher than that of the NHA group with p values of 0.003 (Shannon) and 0.004 (Chao1) (Fig. 1a). The most abundant phyla were Firmicutes followed by Actinobacteria (Fig. 1b). Most abundant among the top 10 genera in both HA and NHA groups were *Streptococcus* (> 40%), *Veillonella* (each belongs to phylum Firmicutes), *Rothia* (phylum Actinobacteria), *Prevotella* (phylum Bacteriodetes), and *Neisseria* (phylum Proteobacteria) (Fig. 1c). Next, we determined differentially abundant genera between the HA and NHA groups by using the negative binomial model-implemented DESeq2 package. In Fig. 2, we considered only those genera that make up at least 1% of the relative abundance of the entire community and have p values < 0.05 (HA vs. NHA). *Streptococcus*, *Veillonella*, and *Rothia* were increased in the NHA cohort, while *Neisseria* was increased in the HA cohort. Lower abundance genera with differential abundances increased in the HA cohort were *Haemophilus*, *Fusobacterium*, and *Capnocytophaga* (Suppl. File S2). We performed ordination with the Bray–Curtis

Table 1 Demographic and medical history data for HA and NHA cohorts

Gender	HA	NHA	Cancer	Diabetes	Cardio. diseases	Pulmo. diseases	Neuro. diseases	Memory loss	Frequent fever/ pain	Avg no. hosp/10 years[a]
Male	14	17	6	6	10	3	1	5	5	1.8
Female	19	15	5	2	5	4	0	3	4	1.4

Cardio. (cardiovascular) diseases include heart attack, chronic heart disease, and coronary bypass surgery; Pulmo. (pulmonary) diseases include COPD, emphysema, chronic bronchitis, and pneumoniae; Neuro. (neurodegenerative) diseases include stroke and Alzheimer's and Parkinson's diseases

[a] Average hospitalization events over last 10 years

dissimilarity matrix (PERMANOVA's $R^2 = 0.047$, $p = 0.001$) and the Bray–Curtis similarity matrix (presence and absence) (PERMANOVA's $R^2 = 0.048$, $p = 0.001$). Thus, we identified statistically significant differences between the HA and NHA groups. As shown in the ordination plots of Fig. 3, the separation of the two groups is weak. Given the small R^2 values, we infer that only a few species are differentially abundant as evident in the DESeq2 results.

Abundance differences for gut microbial profiles comparing HA and NHA cohorts

The gut microbiota is more diverse compared with other body sites including the oral cavity. This explains that, using statistical analyses for species evenness and richness, there were no significant differences in alpha-diversity comparing the HA and NHA groups (p values > 0.05) (Fig. 4a). The most abundant phylum was Firmicutes. Verrucomicrobia, Bacteriodetes, and Actinobacteria were less abundant (Fig. 4b). On the genus level, abundant in both HA and NHA groups were *Clostridium* sensu stricto followed by *Blautia*, *Eubacterium hallii* group, *Romboutsia*, *Peptoclostridium*, *Ruminococcus*, and *Anaerostipes* (all in the order Clostridiales) (Fig. 4c). We determined differentially abundant genera between the HA and NHA groups (via the DESeq2 tool). Interestingly, non-clostridial genera were most significantly changed in abundance, foremost *Akkermansia* (phylum Verrucomicrobia) with a more than 3-fold abundance increase in the HA group (Fig. 5). Erysipelotrichaceae (UCG-003), in the order Erysipelotrichiales, and Bacteroides were more abundant in the HA group, while *Streptococcus* and *Lactobacillus*, each in the order Lactobacillales, were less abundant in the HA group (Fig. 5). Lachnospiraceae (UCG-005) and the

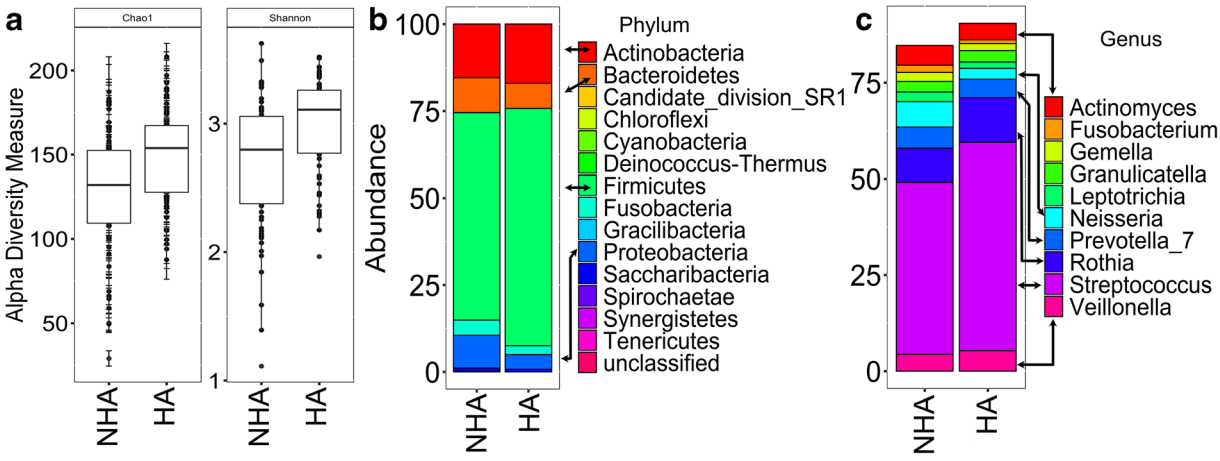

Fig. 1 **a** Alpha-diversity comparing the oral HA and NHA groups. **b** Phyla and **c** top 10 genera in the saliva microbiota averaged from the data of HA and NHA groups. Bacterial alpha-diversity using calculations are based on the Chao1 (emphasis on OTU richness) and Shannon (emphasis on OTU abundance/evenness) indices. The cohorts consisted of 33 HA and 32 NHA subjects. The data for the two timepoints were merged to perform the statistical analyses which were based on Wilcoxon rank sum tests (p value < 0.05). Arrows connect specific bar segments in **b** and **c** with the names of the phyla and genera, respectively

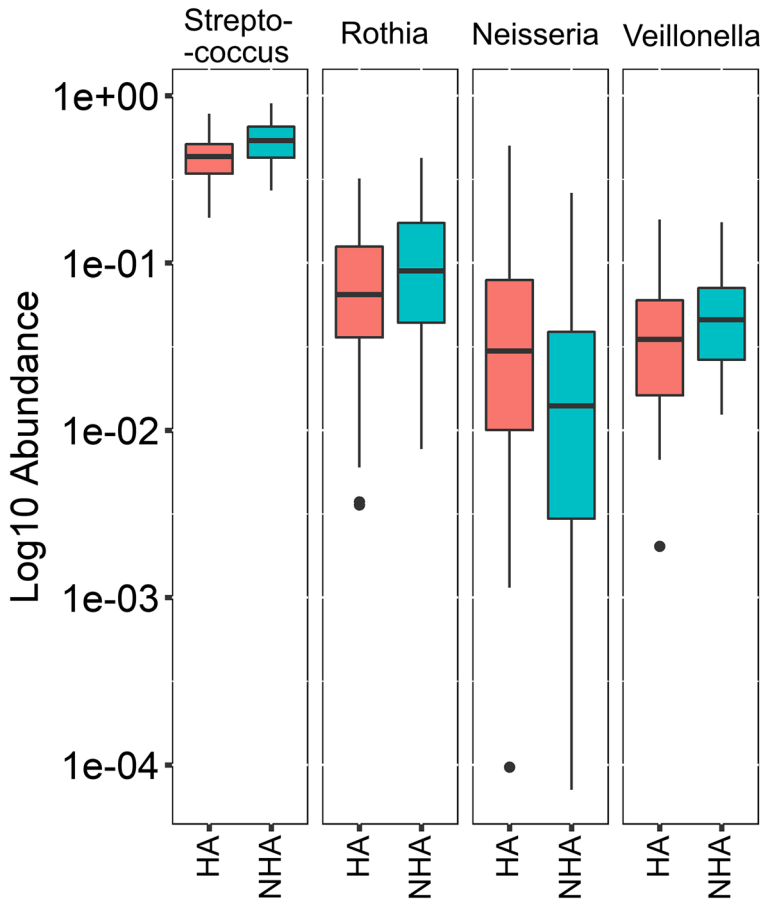

Fig. 2 Differentially abundant genera in the oral microbiome with average abundances greater than 1%. We converted the relative abundance value of each genus into a log10 values for a better comparative display in box plots. The data for the two timepoints were merged to perform the statistical analyses which were based on Wilcoxon rank sum tests (p value < 0.05)

Escherichia/Shigella group were increased and decreased, respectively, with statistically significant differences in the HA cohort (Suppl. File S2). The ordination plots do not show a separation of the two groups, using the Bray–Curtis dissimilarity matrix (PERMANOVA's $R^2 = 0.009$, $p = 0.2$) and Bray–Curtis similarity matrix (presence and absence) (PERMANOVA's $R^2 = 0.167$, $p = 0.013$) (Suppl. File S3). The overall microbial diversity of the two groups was similar. To determine if the gut microbial profiles clustered for any individual disease group, a principal component analysis was performed to separately visualize the main five NHA groups. As shown in Fig. 6, there was no separation among the disease groups.

Eating habits in the context of HA vs. NHA group associations

Using the Rapid Eating Assessment for Participants (REAP) survey with 16 questions of diets, differences between the HA and NHA cohorts were assessed. Moderate differences were observed for five questions. REAP 3 pertained to the intake of high-fiber/whole-grain foods, REAP 4 the intake of fruit/fruit juices, REAP 6 to dairy products, REAP 10 to processed foods high in fat contents, and REAP 12 to dessert foods high in sugar and fat contents. The strongest difference pertained to REAP 4. Seventy-five percent of the HA subjects reported rarely missing the consumption of two servings of fruit per day, while only 43% of the NHA subjects did (Suppl. File S4).

Discussion

Research during the last two decades has demonstrated evidence that a large subset of the microbes colonizing the human GI tract, oral cavity, and skin has a mutualistic relationship with the host. Especially in the GI tract, these microbiota influence and are influenced by the immune system via interactions with mucosal tissues

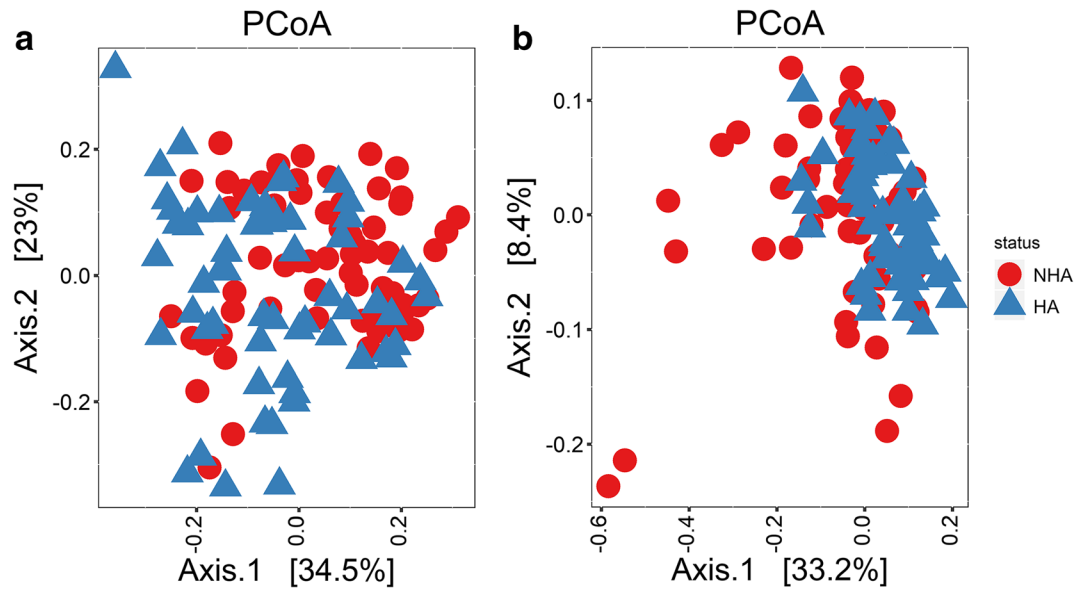

Fig. 3 Principal component analysis based on ordination with **a** Bray–Curtis dissimilarity and **b** Bray–Curtis similarity matrices using saliva microbial profiles. There is a weak separation of the two cohorts

(Round and Mazmanian 2009) and contribute to the metabolism of foods, thus enhancing the utilization of nutrients not digestible by the host enzymatic repertoire (Krajmalnik-Brown et al. 2012). In that process, the microbiome generates unique molecules, for example SCFAs, that are absorbed by mucosal enterocytes and modify signaling processes and ultimately organ function in the human host (Rios-Covian et al. 2016; Tan et al. 2014). Under conditions of dysbiosis, opportunistic pathogens often expand, and commensal microbes are diminished in abundance. The imbalance can trigger various human pathologies (Round and Mazmanian 2009; Sun and Kato 2016; Yang et al. 2009). Given that human aging is defined by immunosenescence and inflamm-aging, mechanisms that are considered to have adverse health effects (Franceschi and Campisi 2014; Fulop et al. 2017; Gruver et al. 2007), it is of interest to assess whether

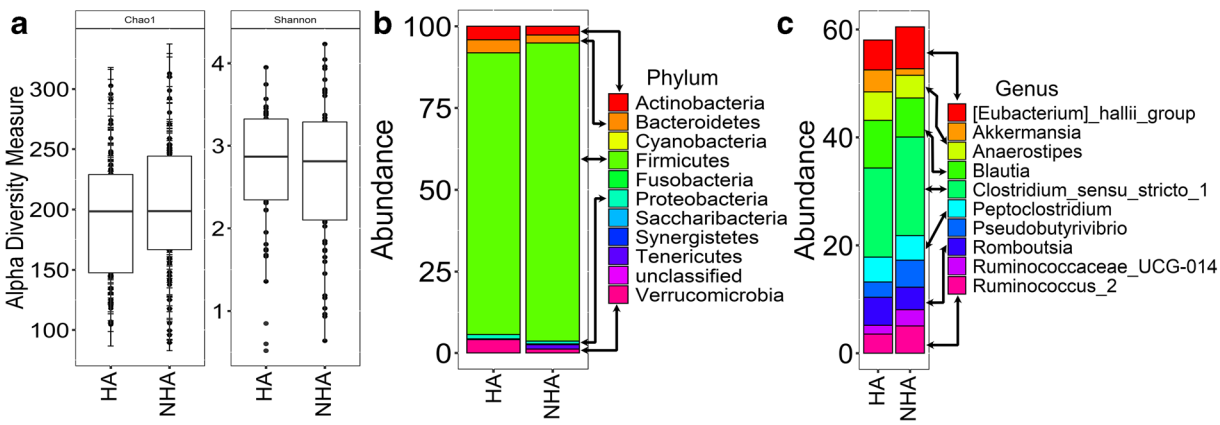

Fig. 4 **a** Alpha-diversity comparing the GI tract HA and NHA groups. **b** Phyla and **c** top 10 genera in the stool microbiota averaged from the data of HA and NHA groups. Bacterial alpha-diversity using calculations are based on the Chao1 (emphasis on OTU richness) and Shannon (emphasis on OTU abundance/evenness) indices. The cohorts consisted of 33 HA and 32 NHA subjects. The data for the two timepoints were merged to perform the statistical analyses which were based on Wilcoxon rank sum tests (p value < 0.05). Arrows connect specific bar segments in **b** and **c** with the names of the phyla and genera, respectively

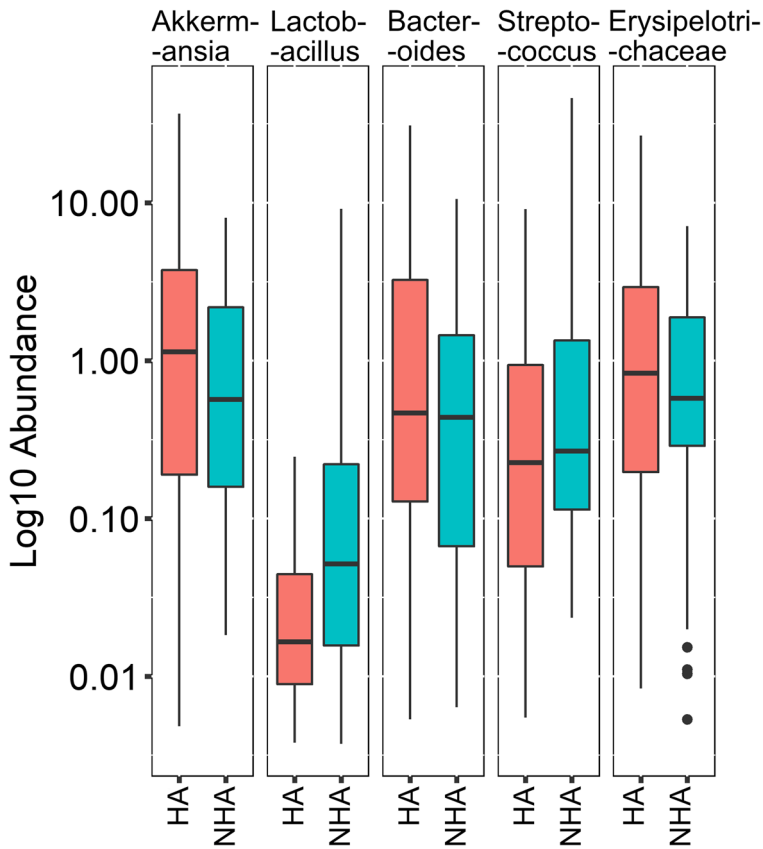

Fig. 5 Differentially abundant genera in the GI tract microbiome with average abundance greater than 1%. We convert the relative abundance value of each genera into log10 values for a better comparative display as box plots. The data for the two timepoints were merged to perform the statistical analyses which were based on Wilcoxon rank sum tests (p value < 0.05)

the altered composition of microbiota in elderly populations can provide insights into pathologies affecting older people and into healthy vs. non-healthy aging.

By surveying 65 people ranging in age from 70 to 82 years, approximately half of whom had not suffered from a major chronic disease (HA group) and half of whom had (NHA group), we quantitatively profiled their salivary and stool microbiomes via 16S rDNA metagenomic sequencing and identified genera that were differentially abundant in HA vs. NHA cohorts. In contrast to the salivary microbiome, the gut microbiome did not reveal statistically significant differences in alpha-diversity. The HA cohort revealed higher taxonomic diversity in the oral microbiome than the NHA cohort. In related studies, Claesson et al. (2011) found core gut microbiota of elderly subjects to be distinct from those of younger adults, with a greater proportion of *Bacteroides* spp., and gut microbiota of elderly in long-term care facilities to be less diverse than that of community dwellers (Claesson et al. 2012). Jiang et al. (2018) reported that the oral microbiota of elderly patients with dental caries were not significantly different in bacterial richness and diversity compared with age-matched healthy controls.

Our metagenomic data on gut microbiomes suggest that *Clostridium* sensu stricto (Clostridiaceae), *Blautia* (Lachnospiraceae), and the *Eubacterium hallii* group (Eubacteriaceae) were abundant for both the HA and NHA groups. The data aligned with one of the previously reported three gut microbial enterotypes, the enterotype dominated by *Ruminococcus* and other Clostridiales (for example, *Blautia*) that also harbors *Akkermansia* as one of the more abundant members (Arumugam et al. 2011). In our comparative analysis, *Akkermansia* was 3-fold more abundant in the HA vs. NHA cohort. It was the eighth most abundant genus in the HA group overall. *Akkermansia*, named after Antoon Akkermans, has been discovered as an intestinal mucin-degrading bacterial genus and is a member of the phylum Verrucomicrobia (Belzer and de Vos 2012). One species, *A. muciniphila*, is by far the best characterized one, although metagenomic data suggest that eight additional species of this genus that can colonize humans (Belzer and de Vos 2012). *A. muciniphila* accounts for up to 1–4% of the bacteria in the human colon (Derrien et al. 2008), strengthens the intestinal epithelial cell integrity (Reunanen et al. 2015; Collado et al. 2007),

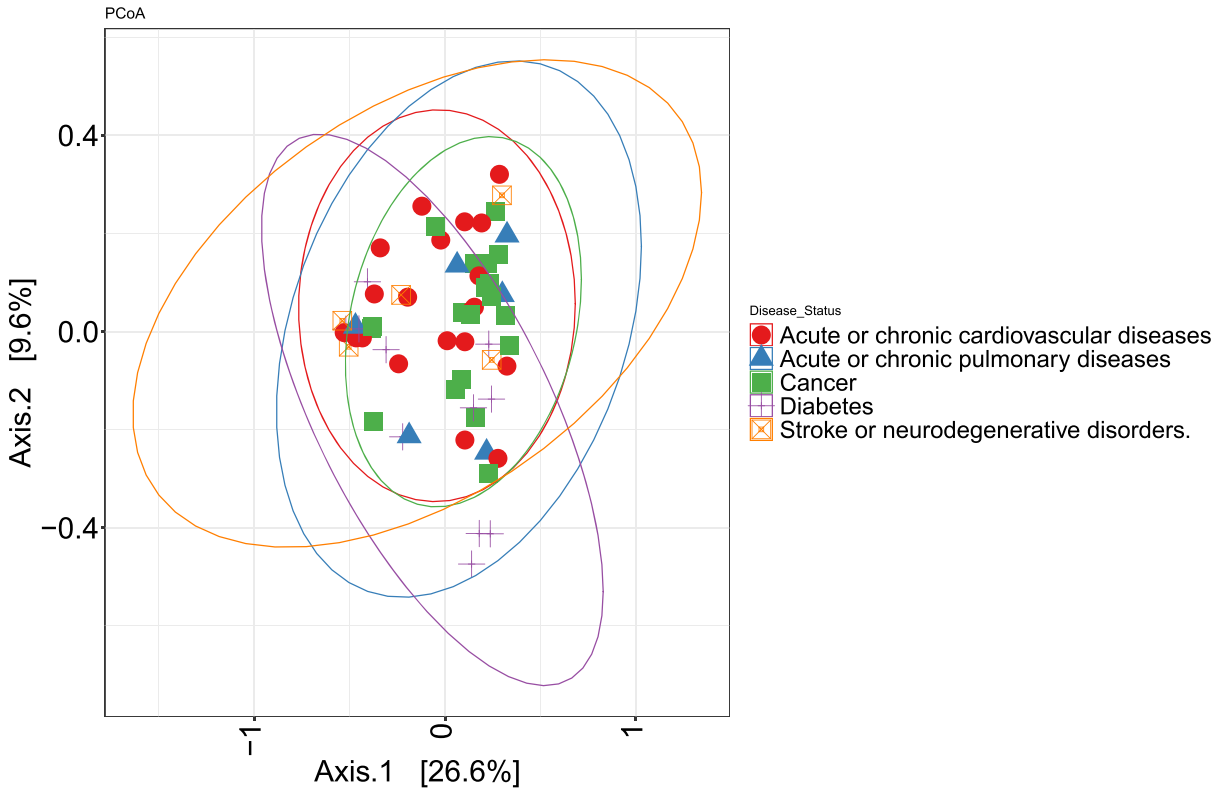

Fig. 6 Principal component analysis based on ordination separating gut microbiota into 5 distinct disease groups

and has a competitive advantage during nutrient deprivation in the intestine due to its unique mucin-catabolism function (Belzer and de Vos 2012). Consistent with our data revealing a 1–4% abundance in the HA cohort, *A. muciniphila* may promote health and act in an anti-inflammatory role in the GI tract of the elderly and may have "anti-inflamm-aging" properties. Its abundance in the gut has been reported to inversely correlate with the onset of inflammation during obesity (Schneeberger et al. 2015), and high abundance of *Akkermansia* has been associated with improved metabolic health during dietary intervention in obesity (Dao et al. 2016). In a murine model, the presence of *A. muciniphila* in the gut microbiome enhanced glucose tolerance in an IFN-γ-dependent pathway (Greer et al. 2016). In a NOD mouse model, *A. muciniphila* promoted regulatory immunity and delayed diabetes development, supporting the notion of positive effects of robust colonization with *A. muciniphila* on metabolic diseases such as diabetes and obesity.

Erysipelotrichaceae UCG-003 were similarly increased in abundance in the HA vs. NHA cohort. Erysipelotrichi are Firmicutes (Kaakoush 2015). Their increased abundance in the GI tract has been associated with effects detrimental to human health, e.g., colorectal cancer (Chen et al. 2012) and TNF-α-induced gastrointestinal inflammation, which is a pathway implicated in irritable bowel disease (IBD) and Crohn's disease (Schaubeck et al. 2016). Erysipelotrichi also appear to affect cholesterol and lipid metabolism in the GI tract (Parmentier-Decrucq et al. 2009). Distinct functional roles for the UCG-003 subtype have not been reported. Among the taxa that have metabolic activities to digest complex carbohydrates and metabolize them to SCFAs, Lachnospiraceae UCG-005 and *Bacteroides* were moderately increased in the HA cohort. *Bacteroides* spp. are well-known fermenters of complex carbohydrates and produce hundreds of glycosyl hydrolases (Wexler 2007). Most *Bacteroides* are commensals that produce volatile SCFAs and attach to mucus glycans via their surface structures, processes that likely benefit mucosal integrity in the colon, and result in outcompetition of harmful microbes in the gut. For example, *B. thetaiotaomicron* stimulates the production of

RegIII-γ, an antimicrobial protein that kills gram-positive bacteria (Wexler 2007). Far less well characterized are Lachnospiraceae, a bacterial family with select members able to degrade dietary fibers with deoxy sugars in the gut and metabolize those to SCFAs. An example is *Coprococcus catus* (Reichardt et al. 2014). Volatile SCFAs produced by these fermenting bacteria result in toxicity for other, potentially pathogenic bacteria. Diminished competitiveness of the latter contain their abundance in the colon and the production of toxic bacteriocins, e.g., those produced by *Escherichia/Shigella* and *Streptococci* (Schippa and Conte 2014). Both *Streptococcus* and *Escherichia/Shigella* were moderately decreased in abundance in stool of the HA vs. NHA cohort, indeed contrasting with the parallel increase of anaerobic commensals such as *Bacteroides* and subgroups of Lachnospiraceae and Erysipelotrichaceae. To assess this in the context of the intake of complex carbohydrates, the percentage of study participants in the HA group, compared with the NHA group, who reported rarely missing days with high consumption of high-fiber foods (whole grain products, fruit) was higher. Using a mouse model, dietary fiber-deprived gut microbial communities were reported to degrade the mucosal barrier integrity (Desai et al. 2016).

Streptococcal species are diverse in the intestinal microbial communities (van den Bogert et al. 2013) and diverse and dominant bacteria in the oral cavity (Aas et al. 2005). Interestingly, *Streptococci* were the only at least moderately abundant bacterial taxon in saliva and stool samples and featured statistically significant abundance increases in the NHA vs. HA microbiomes. Some *Streptococci* such as *S. mitis* and *S. pneumoniae* are pathogens and can cause tooth decay and pneumonia. Both species evolved to have mutualistic and pathogenic lifestyles (Kilian et al. 2014). We did not have the data resolution to speciate the *Streptococci*. Higher taxonomic resolution for *Streptococci* is of interest to investigate the role of this bacterial genus in the context of healthy aging. This requires the analysis of the gut and saliva microbiota by in depth shotgun sequencing.

The abundances of *Veillonella* and *Rothia*, known to be dominant genera in the oral cavity (Aas et al. 2005; Tsuzukibashi et al. 2017), were increased in abundance in the NHA vs. HA cohort and, together with *Streptococci*, responsible for decreased alpha-diversity in salivary samples of the NHA cohort. *Neisseria* and *Fusobacterium* were among the two genera higher in abundance for the HA cohort. Similar to *Streptococci*, many *Neisseria* species are identified as oral commensal organisms, but a few species are pathogens (Liu et al. 2015). *Fusobacteria* are more moderately abundant among the oral cavity microbiota. One species, *Fusobacterium nucleatum*, has been associated with inflammation in the oral cavity (Shang and Liu 2018), colorectal cancer (Shang and Liu 2018), and esophageal cancer (Yamamura et al. 2016). Although the increased diversity of oral microbiota in the HA cohort is noteworthy, it is difficult to draw clear conclusions from the genus-level abundance differences because most genera in oral microbiomes profiled here are represented by multiple species, some of which are opportunistic pathogens that can also live a mutualistic lifestyle in the human host and others that are commensal organisms. Shotgun metagenomic analyses need to be performed to identify distinct species, or even strains, that allow deeper insights into their prevalence, including pathogens, within microbial profiles of the HA vs. NHA groups. Due to the absence of data on diseases affecting the oral cavity, we were not able to correlate microbial profiles with oral mucosal and dental morbidities.

Concluding remarks

In a comparison of oral and gut microbiomes from a cohort with evidence of healthy aging (HA) and a cohort with a medical history of serious chronic diseases (NHA), we identified higher alpha diversity in the HA oral microbiome and increased abundances of *Akkermansia* and Erysipelotrichaceae (UCG-003) in the HA gut microbiome. We hypothesize that these fermentative, complex carbohydrate-digesting bacteria promote a healthy intestinal barrier function and thus contribute to the healthy aging process.

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Author contributions Harinder Singh: microbiome data analysis and interpretation, manuscript writing; Manolito G Torralba, led DNA library preparation work; K. Moncera, generated DNA libraries; Joann Petrini, directed human subject enrolment, questionnaires on medical history, sample collections, and human subject protocols; Lauren DiLello, coordinated human subject enrolment; Karen E. Nelson, project conception and design and

manuscript review; Rembert Pieper, project conception, directed the study over all, wrote manuscript, and interpreted data.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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