Snider et al. *Clinical and Translational Gastroenterology* (2018)9:135
DOI 10.1038/s41424-018-0005-8

Clinical and Translational Gastroenterology

ORIGINAL CONTRIBUTIONS Open Access

Barrett's esophagus is associated with a distinct oral microbiome

Erik J. Snider[1], Griselda Compres[2], Daniel E. Freedberg[2], Marla J. Giddins[2,3], Hossein Khiabanian[4], Charles J. Lightdale[2], Yael R. Nobel[2], Nora C. Toussaint[5], Anne-Catrin Uhlemann[2,3] and Julian A. Abrams[2]

Abstract

Objectives: The esophageal microbiome is composed of predominantly oral flora and is altered in reflux-related conditions including Barrett's esophagus (BE). Changes to the esophageal microbiome may be reflected in the oral cavity. Assessing the oral microbiome thus represents a potential non-invasive method to identify patients with BE.

Methods: Patients with and without BE undergoing upper endoscopy were prospectively enrolled. Demographics, clinical data, medications, and dietary intake were assessed. 16S rRNA gene sequencing was performed on saliva samples collected prior to endoscopy. Taxonomic differences between groups were assessed via linear discriminant analysis effect size (LEfSe). Logit models were used to develop microbiome signatures to distinguish BE from non-BE, assessed by area under the receiver operating curve (AUROC).

Results: A total of 49 patients were enrolled (control = 17, BE = 32). There was no significant difference in alpha diversity comparing all BE patients vs. controls. At the phylum level, the oral microbiome in BE patients had significantly increased relative abundance of Firmicutes ($p = 0.005$) and decreased Proteobacteria ($p = 0.02$). There were numerous taxonomic differences in the oral microbiome between BE and controls. A model including relative abundance of *Lautropia*, *Streptococcus*, and a genus in the order Bacteroidales distinguished BE from controls with an AUROC 0.94 (95% CI: 0.85–1.00). The optimal cutoff identified BE patients with 96.9% sensitivity and 88.2% specificity.

Conclusions: The oral microbiome in BE patients was markedly altered and distinguished BE with relatively high accuracy. The oral microbiome represents a potential screening marker for BE, and validation studies in larger and distinct populations are warranted.

Introduction

The incidence of esophageal adenocarcinoma (EAC) continues to rise at an alarming rate in Western countries, and this malignancy is associated with a dismal prognosis[1,2]. Barrett's esophagus (BE) is the precursor lesion to the development of EAC, and patients diagnosed with BE can undergo endoscopic surveillance and endoscopic therapy for those who develop dysplasia or early cancer. This approach reduces the risk of EAC and potentially lowers EAC mortality[3–6], yet only 10% of patients with EAC receive a prior diagnosis of BE[6,7]. While widespread identification of patients with BE has the potential to reduce EAC mortality in the long run, broad screening with upper endoscopy is impractical and costly. Therefore, there is marked interest in the development of non-endoscopic, minimally invasive means to identify patients with BE that could be implemented in the primary care setting[8].

Coincident with the rise in EAC incidence, the gastrointestinal microbiome has undergone a significant shift

Correspondence: Julian A. Abrams (ja660@cumc.columbia.edu)
[1]Department of Medicine, Oregon Health Sciences University, Portland, OR, USA
[2]Department of Medicine, Columbia University Medical Center, New York, NY, USA
Full list of author information is available at the end of the article

© The Author(s) 2018

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, and provide a link to the Creative Commons license. You do not have permission under this license to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

with the advent of antibiotics and the progressive decline in *Helicobacter pylori* infection rates. This raises the possibility that the upper gastrointestinal microbiome may play a key role in the development of EAC. The esophageal microbiome is broadly similar to the oral microbiome; both contain an abundance of anaerobes as well as a high ratio of Firmicutes to Bacteroidetes[9,10]. This similarity may be due in part to distal migration via swallowed secretions and other mechanisms. The esophageal microbiome is altered in reflux-related conditions including Barrett's esophagus[11–13], and these changes may be reflected in the oral microbiome.

The assessment of the oral microbiome may allow for the identification of patients with BE. We therefore carried out a case–control study to test the hypothesis that patients with BE have a distinct oral microbiome compared to patients without BE.

Methods

Study population

We performed a case–control study of patients ≥18 years old, enrolling subjects without or with a diagnosis of Barrett's esophagus who were scheduled to undergo upper endoscopy for clinical indications. Subjects were prospectively enrolled over 18 months at a single academic medical center (Columbia University Medical Center, New York, NY). Barrett's esophagus subjects had a history of biopsy-proven BE (intestinal metaplasia from the tubular esophagus), BE length ≥2 cm, and taking at least once daily proton pump inhibitors (PPI) for the prior month. Controls were considered PPI-positive if taking at least once daily PPI for the prior month, or PPI-negative if not taking any acid suppression (PPIs or H2-receptor antagonists) for the prior month. Subjects were excluded for any of the following: use of antibiotics, steroids, or other immunosuppressants within the previous 3 months; use of H2-receptor antagonists as only acid suppression for the prior month (to better distinguish findings between acid suppression and no acid suppression); past history of gastric or esophageal cancer; history of gastric or esophageal surgery, including antireflux or bariatric surgery; uncontrolled HIV immunosuppressed states or conditions; and for BE patients only, prior endoscopic therapy for BE or esophageal cancer.

After obtaining informed consent and prior to the endoscopy, demographics and clinical data were collected. Height was recorded, and weight and waist and hip circumference were measured. A history of reflux symptoms was assessed using a modified version of the Mayo Gastro-Esophageal Reflux Questionnaire[14]. Subjects also completed a food frequency questionnaire derived from the National Health Interview Survey and validated for assessment of fat and fiber intake over the preceding 4 weeks[15,16]. All participants provided written informed consent. The Institutional Review Board of Columbia University approved the study on February 25, 2015.

Sample collection

All subjects were fasting at the time of sample collection. Saliva was collected using the drool technique and stored in Oragene DNA OG-500 collection kits (DNA Genotek). The adherent oral microbiome was sampled using oral swabs (Epicentre Catch-All Sample collection swabs) by broadly sampling five distinct sites (right and left buccal lining, tongue dorsum, hard palate, and superior labial frenulum). At the beginning of the upper endoscopy, the scope channel was flushed with 20 mL sterile water. The esophageal squamous microbiome was sampled with two separate brushes (Endoscopy Cytology Brush, model G22174; Cook Medical), by passing the brush back and forth 10 times in each of four quadrants. This was similarly performed of areas with BE tissue (in BE patients) or gastric cardia (within 1 cm of the squamocolumnar junction) in controls). Brush tips were cut using sterile wire cutters and placed in sterile Eppendorf tubes. All samples were then stored at −80 °C.

Microbiome characterization

DNA for all oral and esophageal samples was extracted using the MoBio PowerSoil kit (Qiagen, Carlsbad, CA) according to the manufacturer's instructions. The V4 hypervariable ribosomal RNA region was amplified using primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVHHHTWTCTAAT-3′)[17]. Sequencing of the 16S rRNA gene V4 region was performed using the Illumina HiSeq 2500 in Rapid Mode with 2×250 bp read length (Illumina, San Diego, CA). Read pairs were merged to yield 549,946,303 total sequence contigs or an average 1,851,700 contigs per sample using mothur. After downsampling the number of contigs per sample to 200,000, singleton contigs were discarded. The remaining contigs were trimmed and filtered for quality using mothur yielding an average of 101,359 contigs per sample (total: 30,103,626)[18]. Greengenes was used as reference database[19]. Clustering of taxonomic units was made at 97% sequence similarity using USEARCH and taxonomic assignments were made using mothur. FastTree version 2.1.7 was used to generate a phylogenetic tree of the contigs[20]. Using mothur and the phylogenetic tree, weighted and unweighted UniFrac distances as well as diversity indices were calculated[21]. Broad analyses were performed to identify differentially abundant taxa between groups. Differentially abundant taxa between groups were identified using linear discriminant analysis effect size (LEfSe)[22]. Differential abundance analysis was also performed using phyloseq with DESeq2.

Within-individual correlations were assessed between saliva and esophagus (both squamous and BE/cardia) and then repeated for oral swabs and esophagus, by

calculating Spearman rank correlation coefficients at the genus level for all genera with non-zero read counts in oral and esophageal samples. There was a non-significantly higher correlation between saliva and BE/cardia as compared to oral swab (mean rho 0.73 vs. 0.69, $p = 0.27$), with similar findings for comparisons with squamous esophagus (mean rho 0.71 vs. 0.69, $p = 0.56$). The decision was made to focus analyses on saliva; saliva may represent a better biomarker of BE status than do oral swabs, as bacteria in swallowed secretions (i.e., saliva) may be more likely than adherent oropharyngeal bacteria to influence the make-up of the esophageal microbiome.

Analyses

Continuous variables were analyzed using *t*-tests and rank-sum tests, and categorical variables were analyzed using Fisher's exact tests. Average linkage (UPGMA) method was used to cluster patients based on weighted UniFrac metric. Rank-sum test was used to compare pairwise distances within BE patients and controls, as well as across BE patients and controls. Differences between groups at the phylum level were assessed using ANOVA ($p = 0.03$ for comparison of BE vs. controls). Subsequently, *t*-tests were used to compare phylum relative abundances between groups. Each of the individual taxa at the family or genus level that were significantly differentially abundant between groups (identified by LEfSe or DESeq) were then analyzed. Logit models were generated for each taxon and its association with BE status. For logit models with multiple taxa (up to a maximum of three in taxa in a model), the beta coefficients were used to generate a score for each subject. This score was then used to generate areas under the receiver operating curve (AUROCs) to assess how well they discriminated between groups. Leave-one-out cross-validation was performed to assess model accuracy. Youden's index was used to identify optimal cut points. Multivariable logistic regression was performed to determine whether other factors (clinical characteristics, medications, smoking, dietary fat, and fiber intake) influenced the association between microbiome-based scores and BE status. Each variable was assessed, one at a time, to determine whether that particular variable was a potential confounder for the association between the microbiome signature and BE status. Covariates were defined as potential confounders if they altered the β coefficient for the microbiome score by >10%. Interaction was assessed by generating multiplicative terms in regression models. To assess the oral microbiome as a potential marker of advanced neoplastic progression, the above analyses were repeated comparing subjects with BE-associated high-grade dysplasia (HGD) or EAC to those with non-dysplastic BE. Subjects with low-grade dysplasia (LGD) were excluded from these comparisons; there are diverse estimates on the progression rate of LGD, and management guidelines do not recommend endoscopic eradication therapy for all patients with LGD[23–25]. Statistical significance was defined as $p < 0.05$. Analyses were performed using Stata 14.1 (StataCorp, College Station, TX).

PCR of candidate taxa

Semi-quantitative PCR (SsoAdvanced Universal SYBR Green Supermix, Bio-Rad, Hercules, CA) was performed for the taxa *Lautropia*, *Streptococcus*, and *Enterobacteriaceae* to validate key findings from 16S rRNA gene sequencing analyses. Previously published primer pairs were used for *Streptococcus*, *Enterobacteriaceae* und Eubacteria and newly designed for *Lautropia* (Laut_16s_F: 5′-GTCCTTTTCGTTCCCGCC-3′, Laut_16s_R: 5′-CAAGGCGACGATCTGTAGCTGG-3′)[26,27]. Samples were diluted to 5 ng/μL DNA and run on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). For each sample and taxon primer, ΔCt values were calculated using as a reference the Ct value for Eubacteria for the corresponding sample. qPCR for Eubacteria represents the entire bacterial DNA in the sample; thus, the ΔCt values were analogous to relative abundance data from 16S rRNA gene sequencing.

Results

A total of 49 patients were enrolled, 32 with Barrett's esophagus and 17 controls. There were 16 BE patients without dysplasia, 6 with low-grade dysplasia, 5 with high-grade dysplasia, and 5 with EAC. Patient characteristics are shown in Table 1. A higher proportion of BE patients was male (87.5 vs. 52.9%; $p = 0.007$), had GERD (93.8 vs. 58.8%; $p = 0.005$), and used PPIs (100 vs. 35.3%; $p < 0.001$), and controls had higher mean BMI (32.9, SD 8.5 vs. 28.5, SD 5.2; $p = 0.03$).

Salivary microbiome

To test the potential utility of the salivary microbiome as a screening marker for Barrett's esophagus, all 32 BE patients were compared to the 17 controls. There was no difference in alpha diversity between the two groups (mean Shannon index: BE 2.73 vs. controls 2.89; $p = 0.10$). On beta diversity analyses, there was evidence of significant clustering. (Fig. 1) At the phylum level, BE patients had significantly greater relative abundance of Firmicutes (27.1 vs. 14.6%; $p = 0.005$) and decreased Proteobacteria (23.8 vs. 34.5%; $p = 0.02$) (Fig. 2).

Both broad and specific taxonomic differences were found comparing saliva from BE patients and controls (Fig. 3). Specific differentially abundant taxa are shown in Supplementary Table 1. Notable differences include increased relative abundance of *Streptococcus*, *Veillonella*,

Table 1 Characteristics of patients who underwent upper endoscopy, comparing those without to those with Barrett's esophagus (BE)

	Controls (n = 17)	BE (n = 32)	p
Age, mean (SD)	58.1 (16.5)	63.1 (11.4)	0.22
Sex, male	9 (53%)	28 (88%)	0.01
BMI[a], mean (SD)	32.9 (8.2)	28.5 (5.2)	0.03
WHR, mean (SD)	0.95 (0.08)	0.97 (0.05)	0.17
GERD	10 (59%)	30 (94%)	0.005
Ever smoker	7 (42%)	21 (66%)	0.13
PPI use	6 (35%)	32 (100%)	<0.001
Aspirin use	3 (18%)	11 (34%)	0.32
Dietary fiber[b] (grams per day), mean	15.2 (5.2)	17.6 (5.6)	0.16
Dietary fat[b] (% daily calories), mean	33.8 (2.4)	34.2 (3.1)	0.68

[a]BMI data missing in three subjects
[b]Dietary data missing in one subject

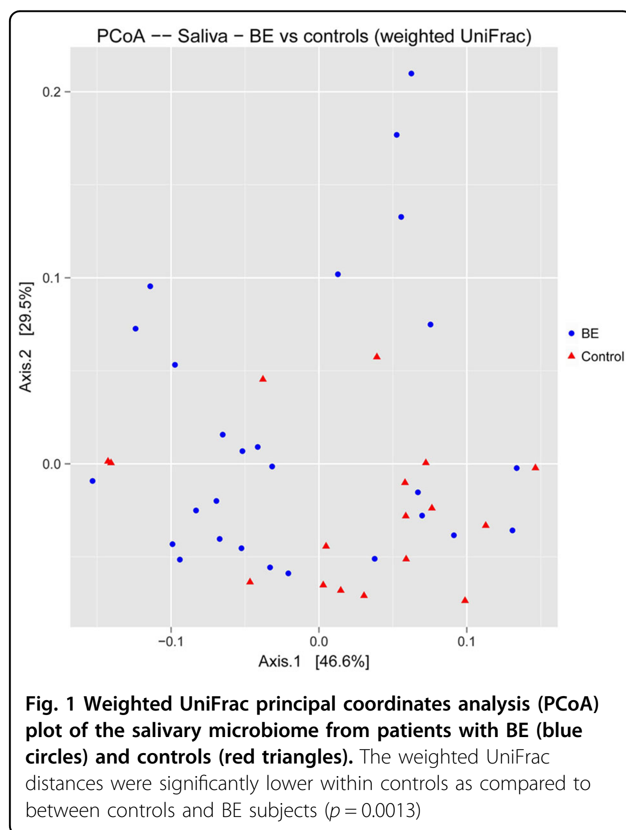

Fig. 1 Weighted UniFrac principal coordinates analysis (PCoA) plot of the salivary microbiome from patients with BE (blue circles) and controls (red triangles). The weighted UniFrac distances were significantly lower within controls as compared to between controls and BE subjects ($p = 0.0013$)

and *Enterobacteriaceae* in patients with BE. Controls had increased relative abundance of numerous taxa, including *Neisseria*, *Lautropia*, and *Corynebacterium*.

AUROCs were then calculated for the differentially abundant genera and families. Relative abundance of *Lautropia* produced the highest AUROC of any individual taxon (0.86, 95% CI: 0.73–0.98). A three-taxon model with *Lautropia*, *Streptococcus*, and *Enterobacteriaceae* discriminated between BE and controls with an AUROC of 0.90 (95% CI: 0.81–0.99; $p = 0.30$ vs. *Lautropia* alone). The model with the greatest discrimination between BE and controls included *Lautropia*, *Streptococcus*, and an unspecified genus of the order Bacteroidales, with an AUROC of 0.94 (95% CI: 0.85–1.00; $p = 0.04$ vs. *Lautropia* alone) (Fig. 4; relative abundance of these taxa shown in Supplementary Figure 1). Using the optimal cutoff, a score produced by this model discriminated BE patients from controls with 96.9% sensitivity and 88.2% specificity, and produced positive and negative likelihood ratios of 8.23 and 0.035, respectively. By altering the cutoff, 100% sensitivity could be achieved with an associated 70.6% specificity. In leave-one-out cross-validation analysis, the AUROC was 0.92 for the three-taxon model of *Lautropia*, *Streptococcus*, and an unspecified genus of the order Bacteroidales. Qualitatively similar results were found in secondary analyses restricted to BE subjects without dysplasia compared to controls (Supplementary Table 2).

Patient characteristics and test performance

All three models (*Lautropia* alone; *Lautropia*, *Streptococcus*, and *Enterobacteriaceae*; *Lautropia*, *Streptococcus*, and an unspecified genus in the order Bacteroidales) remained significantly associated with BE status after individual adjustment for typical BE risk factors including age, sex, BMI, and GERD status. The association between the microbiome models and BE status was also not influenced by dietary fat or fiber intake. There was evidence of significant interaction by ever smoking status and the microbiome models. In stratified analyses, the AUROCs were higher in never smokers and also with BE compared to controls who used PPIs, although there was broad overlap in the confidence intervals (Supplementary Table 3). Comparing BE patients to GERD controls, the AUROC for the microbiome signature (*Lautropia*, *Streptococcus*, and an unspecified genus in the order Bacteroidales) was 0.95 (95% CI: 0.88–1.00).

Advanced neoplasia

Exploratory analyses were performed comparing patients with non-dysplastic BE to BE with HGD or EAC to gain insight into the potential utility of the salivary microbiome as a marker of progression. There was no difference in diversity between no dysplasia and HGD or EAC (mean Shannon index 2.71 vs. 2.82; $p = 0.38$). Patients with HGD/EAC had increased relative abundance of *Enterobacteriaceae* and decreased *Veillonella*

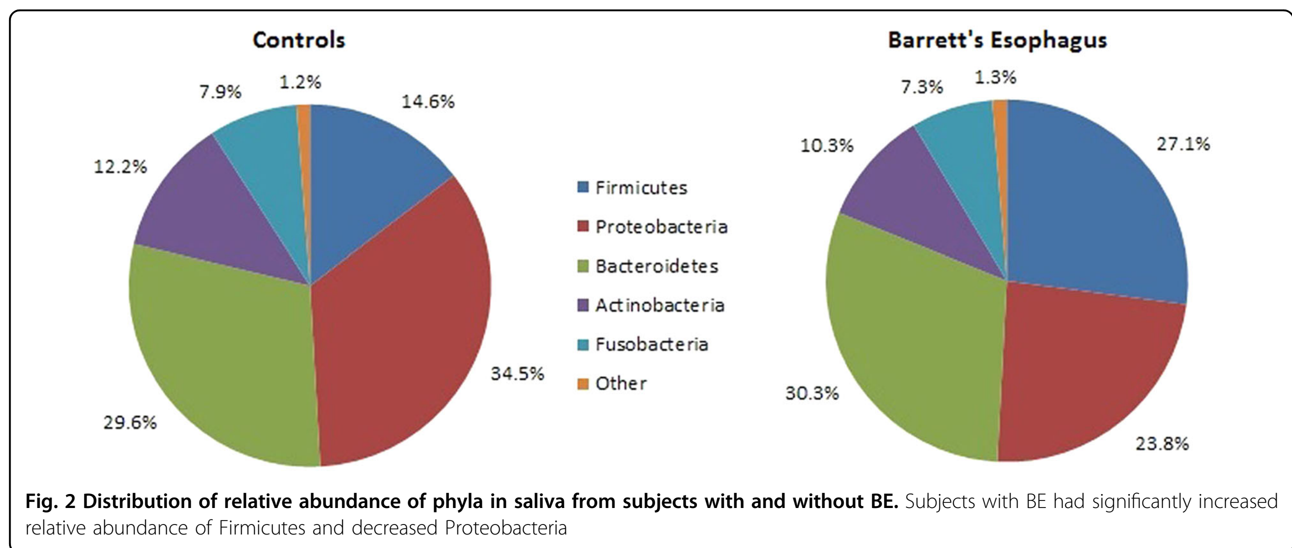

Fig. 2 Distribution of relative abundance of phyla in saliva from subjects with and without BE. Subjects with BE had significantly increased relative abundance of Firmicutes and decreased Proteobacteria

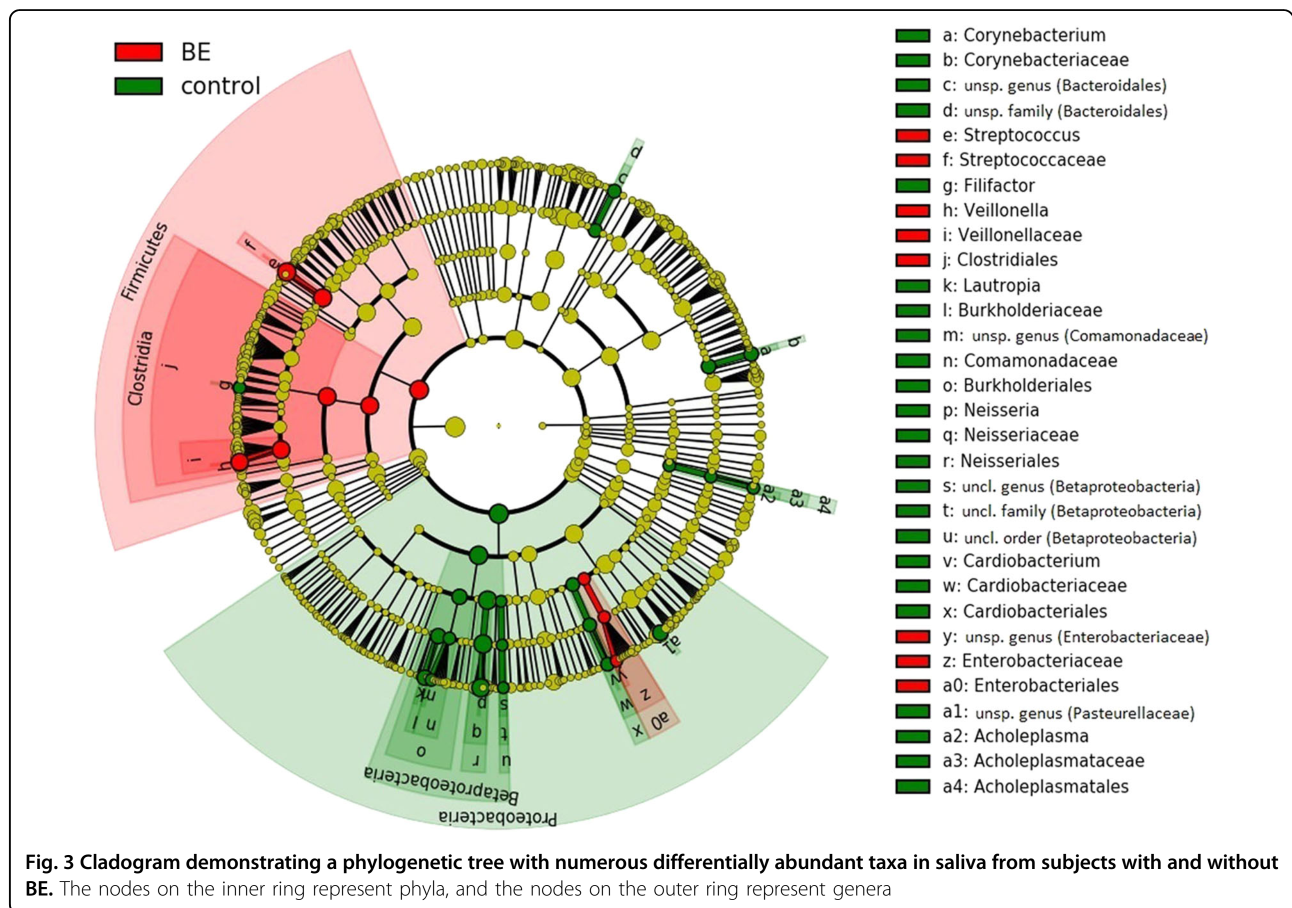

Fig. 3 Cladogram demonstrating a phylogenetic tree with numerous differentially abundant taxa in saliva from subjects with and without BE. The nodes on the inner ring represent phyla, and the nodes on the outer ring represent genera

(full table of differentially abundant taxa is shown in Supplementary Table 4). A model with a combination of the two taxa produced an AUROC of 0.93 (95% CI: 0.81–1.00) (Supplementary Figure 2). The optimal cutoff for this model was associated with 90.0% sensitivity and 87.5% specificity.

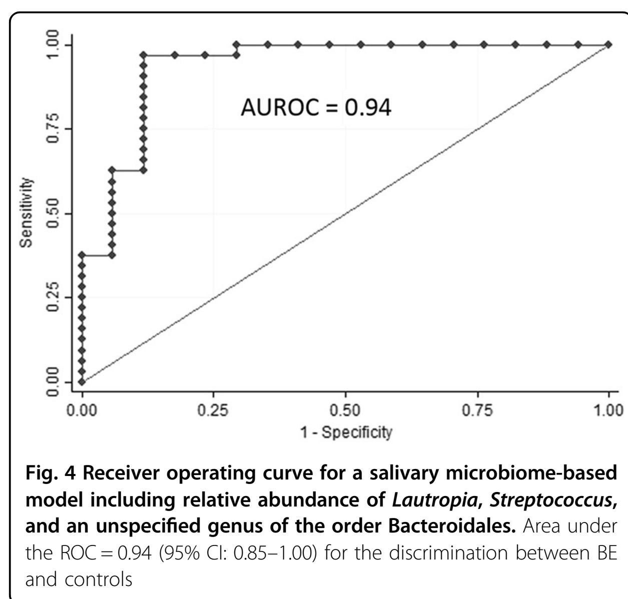

Fig. 4 Receiver operating curve for a salivary microbiome-based model including relative abundance of *Lautropia*, *Streptococcus*, and an unspecified genus of the order Bacteroidales. Area under the ROC = 0.94 (95% CI: 0.85–1.00) for the discrimination between BE and controls

qPCR analyses of saliva

To validate the findings from 16S rRNA gene sequencing, qPCR was performed for *Lautropia*, *Streptococcus*, and *Enterobacteriaceae* and assessed relative to Eubacteria for each sample. Similar to results from 16S analyses, saliva from Barrett's esophagus subjects had significantly decreased *Lautropia* ($p = 0.002$) and increased *Streptococcus* ($p = 0.009$) compared to controls. Saliva from subjects with advanced neoplasia had significantly increased *Enterobacteriaceae* compared to non-dysplastic BE ($p = 0.04$) (Supplementary Figure 3).

Discussion

In this case–control study of patients with and without Barrett's esophagus, there were marked differences in the oral microbiome of patients with BE, with significantly increased Firmicutes and decreased Proteobacteria. An oral microbiome signature model including relative abundance of *Lautropia*, *Streptococcus*, and an unspecified genus of order Bacteroidales distinguished patients with BE from controls with relatively high accuracy, and this signature remained significantly associated with BE status after adjustment for potential confounders. Additional changes in the oral microbiome were also noted in patients with HGD or EAC, with decreased *Veillonella* and increased *Enterobacteriaceae* compared to non-dysplastic BE patients.

PPI use and a history of GERD symptoms were more common in BE patients. Thus, the question is raised as to whether the observed associations between specific oral microbiome alterations and BE status were merely a reflection of PPI use and/or GERD. In secondary analyses comparing BE patients to controls who were taking PPIs, the association between the microbiome signature and BE status was strengthened. If the observed alterations in the oral microbiome were in fact secondary to PPI use, then the association comparing BE patients to controls who were taking PPIs should have been weaker, not stronger. Similarly, in the analyses of the three-taxon microbiome signature comparing BE patients to GERD controls, the AUROC remained high.

The current findings raise the possibility that oral microbiome analyses could be used to screen for Barrett's esophagus. The test characteristics of the three-taxon model from the current study compare favorably with other non-endoscopic screening tests for BE. The Cytosponge, a tethered cell sampling device coupled with immunohistochemical staining for trefoil factor 3, is the best studied non-endoscopic test for BE. In a case–control study of 1110 patients, the Cytosponge had 80% sensitivity and 92% specificity for the diagnosis of BE[28]. In a recent study, an electronic nose device had 82% sensitivity and 80% specificity for the diagnosis of BE, with an associated AUROC of 0.79[29]. Interestingly, this device measures volatile organic compounds, which may be produced and influenced by oropharyngeal bacteria. In light of the high sensitivity, a major strength of an oral microbiome-based test may lie in its ability to rule out BE due to a very low negative likelihood ratio. Even in a population with a high BE prevalence (e.g., 15%), a negative test would lower the probability of BE to <1%.

Saliva collection is non-invasive and can be performed in any setting. However, prior to adoption into clinical practice, the current findings need to be validated in larger and distinct populations. There was evidence of possible effect modification by ever smoking status, although there were not enough current smokers enrolled to fully assess the impact of smoking. The effects of medications, diet, smoking, and oral health all need to be assessed further, as these factors may impact the salivary microbiome test characteristics[30,31]. Also, presently 16S rRNA gene sequencing and analyses are relatively expensive and labor intensive. Ultimately, a multiplex PCR test of key taxa may be more robust, faster, and cheaper.

Some of the current findings are consistent with prior oral microbiome studies and associations with inflammatory or neoplastic conditions. In a study of head and neck squamous cell cancer, the saliva from non-cancer controls had significantly increased relative abundance of *Lautropia*, whereas cancer patients had increased *Streptococcus*[32]. Relative abundance of *Lautropia* has also been found to be decreased in the saliva of esophageal squamous cell cancer patients and in subgingival samples of patients with periodontitis[33–36]. Abundance of *Lautropia* subsequently increases after successful treatment of periodontitis[37]. Relatively little is known with regard to the function of this genus. *Lautropia* may represent a keystone bacteria in the mouth, and its loss may lead to

proliferation of other proinflammatory bacteria, analogous to the role of Clostridia in the colon[38,39].

Interestingly, there were also differences in the oral microbiome between patients with high-grade dysplasia or EAC and non-dysplastic BE. Notably, there was significantly increased relative abundance of *Enterobacteriaceae* in HGD and EAC patients. Relative abundance of *Enterobacteriaceae* in the saliva may serve as a surveillance marker for patients with BE, although these findings should be interpreted with caution given the relatively small number of subjects. This family contains numerous genera and species of Gram-negative bacteria, including *Escherichia coli*, *Salmonella*, *Shigella*, and *Citrobacter*, which have been associated with gastrointestinal infection, inflammation, and cancer[40–45]. There was moderate within-individual correlation of *Enterobacteriaceae* between saliva and BE tissue (data not shown), which raises the possibility that some of these taxa may play a pathogenic role in progression from BE to EAC.

The current study has several strengths. There was excellent sequencing coverage, which allowed for the identification and analysis of potentially important but low abundance taxa such as *Lautropia* and *Enterobacteriaceae*. Data were collected with regard to medication use, smoking history, reflux symptoms, and dietary intake, which allowed for the assessment how these factors could impact the association between oral microbiome taxa and BE status. The findings from 16S rRNA gene sequencing were further validated by qPCR of three key taxa from the microbiome signatures.

There were certain limitations. This was a relatively small study, which limits the generalizability of the findings. Only three subjects were current smokers, and recent antibiotic use was an exclusion criterion. Thus, additional studies are needed to determine whether active smoking or antibiotic use impacts the relationship between the oral microbiome and BE status. Pre- and probiotics could alter the microbiome, and data on their use were not captured. Thus, we are unable to determine whether or how pre- or probiotic use may have impacted the association between the microbiome signature and BE status. Variability in the oral microbiome has been reported across geographic regions as well as ethnically distinct populations[46,47]. It is unknown whether geographic variation exists within populations at highest risk for EAC (e.g., older white males). While the current study showed associations between various taxa and BE status, no clear insight can be gleaned with regard to a potential biological role for these microbiome alterations.

In conclusion, patients with Barrett's esophagus have a highly distinct oral microbiome. Barrett's esophagus patients had increased relative abundance of Firmicutes and decreased Proteobacteria. Numerous differences in lower-level taxa were also found. A model including relative abundance of *Lautropia*, *Streptococcus*, and an unspecified genus of order Bacteroidales distinguished patients with and without BE with high accuracy. These findings warrant further validation studies in distinct populations in order to assess whether an oral microbiome-based test can be used to screen for Barrett's esophagus.

Study Highlights

What is current knowledge?

- Roughly, 90% of patients with esophageal adenocarcinoma are not previously diagnosed with Barrett's esophagus, and thus the opportunity to intervene and potentially prevent cancer is missed.
- Widespread endoscopic screening for Barrett's esophagus is not practical, and there is a need to develop non-endoscopic techniques to diagnose Barrett's esophagus.
- The esophageal microbiome is altered in Barrett's esophagus, and these changes may be due in part to changes to the oral microbiome with subsequent distal migration.

What is new here?

- The oral microbiome in patients with Barrett's esophagus is markedly different from patients without Barrett's esophagus.
- A panel of taxa, including *Lautropia* and *Streptococcus*, can discriminate patients with Barrett's esophagus with relatively high accuracy.
- *Enterobacteriaceae*, a family associated with infection and inflammation, are increased in the saliva of patients with high-grade dysplasia and cancer.

Author details
[1]Department of Medicine, Oregon Health Sciences University, Portland, OR, USA. [2]Department of Medicine, Columbia University Medical Center, New York, NY, USA. [3]Microbiome & Pathogen Genomics Core, Department of Medicine, Columbia University Medical Center, New York, NY, USA. [4]Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, USA. [5]New York Genome Center, New York, NY, USA

Competing interests
Guarantors of the article: Julian Abrams.
Specific authors' contributions: E.J.S.—study concept and design, study conduct, interpretation of data, manuscript preparation, critical revision of manuscript, approval of final draft submitted. G.C.—study conduct, critical revision of manuscript, approval of final draft submitted. D.E.F.—analysis and interpretation of data, critical revision of manuscript, approval of final draft submitted. M.J.G.—analysis of data, critical revision of manuscript, approval of final draft submitted. H.K.—analysis and interpretation of data, critical revision of manuscript, approval of final draft submitted. C.J.L.—study conduct, interpretation of data, critical revision of manuscript, approval of final draft submitted. Y.R.N.—interpretation of data, critical revision of manuscript,

approval of final draft submitted. N.C.T.—analysis and interpretation of data, critical revision of manuscript, approval of final draft submitted. A.C.U.—analysis and interpretation of data, manuscript preparation, critical revision of manuscript, approval of final draft submitted. J.A.A.—study concept and design, study conduct, analysis and interpretation of data, manuscript preparation, critical revision of manuscript, approval of final draft submitted.

Financial support: The authors were supported in part by a Columbia Physician's and Surgeon's Dean's Research Fellowship (E.J.S.), a Career Development Award from NIDDK (K23 DK111847; D.E.F.), a U54 award from NCI (U54 CA163004; J.A.A.), a R01 from NIAID (AI116939; A.C.U.), and the Price Family Foundation.

Potential competing interests: None.

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41424-018-0005-8.

Received: 8 December 2017 Accepted: 15 January 2018
Published online: 20 February 2018

References

1. Abrams, J. A., Sharaiha, R. Z. & Gonsalves, L. et al. Dating the rise of esophageal adenocarcinoma: analysis of Connecticut Tumor Registry data, 1940-2007. *Cancer Epidemiol. Biomark. Prev.* **20**, 183–186 (2011).
2. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2017. *CA Cancer J. Clin.* **67**, 7–30 (2017).
3. Shaheen, N. J., Sharma, P. & Overholt, B. F. et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N. Engl. J. Med* **360**, 2277–2288 (2009).
4. Phoa, K. N., van Vilsteren, F. G. & Weusten, B. L. et al. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. *JAMA* **311**, 1209–1217 (2014).
5. El-Serag, H. B., Naik, A. D. & Duan, Z. et al. Surveillance endoscopy is associated with improved outcomes of oesophageal adenocarcinoma detected in patients with Barrett's oesophagus. *Gut* **65**, 1252–1260 (2016).
6. Verbeek, R. E., Leenders, M. & Ten Kate, F. J. et al. Surveillance of Barrett's esophagus and mortality from esophageal adenocarcinoma: a population-based cohort study. *Am. J. Gastroenterol.* **109**, 1215–1222 (2014).
7. Tramontano, A. C., Sheehan, D. F., Yeh, J. M., et al. The impact of a prior diagnosis of Barrett's esophagus on esophageal adenocarcinoma survival. *Am. J. Gastroenterol.* **112**, 1256–1264 (2017).
8. di Pietro, M., Chan, D. & Fitzgerald, R. C. et al. Screening for Barrett's esophagus. *Gastroenterology* **148**, 912–923 (2015).
9. Segata, N., Haake, S. K. & Mannon, P. et al. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biol.* **13**, R42 (2012).
10. Yang, L., Francois, F. & Pei, Z. Molecular pathways: pathogenesis and clinical implications of microbiome alteration in esophagitis and Barrett esophagus. *Clin. Cancer Res.* **18**, 2138–2144 (2012).
11. Yang, L., Lu, X. & Nossa, C. W. et al. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology* **137**, 588–597 (2009).
12. Amir, I., Konikoff, F. M. & Oppenheim, M. et al. Gastric microbiota is altered in oesophagitis and Barrett's oesophagus and further modified by proton pump inhibitors. *Environ. Microbiol.* **16**, 2905–2914 (2014).
13. Gall, A., Fero, J. & McCoy, C. et al. Bacterial composition of the human upper gastrointestinal tract microbiome is dynamic and associated with genomic instability in a Barrett's esophagus cohort. *PLoS ONE* **10**, e0129055 (2015).
14. Locke, G. R., Talley, N. J. & Weaver, A. L. et al. A new questionnaire for gastroesophageal reflux disease. *Mayo Clin. Proc.* **69**, 539–547 (1994).
15. Thompson, F. E., Midthune, D. & Subar, A. F. et al. Performance of a short tool to assess dietary intakes of fruits and vegetables, percentage energy from fat and fibre. *Public Health Nutr.* **7**, 1097–1105 (2004).
16. Thompson, F. E., Midthune, D. & Subar, A. F. et al. Dietary intake estimates in the National Health Interview Survey, 2000: methodology, results, and interpretation. *J. Am. Diet. Assoc.* **105**, 352–363 (2005). quiz 487.
17. Nossa, C. W., Oberdorf, W. E. & Yang, L. et al. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. *World J. Gastroenterol.* **16**, 4135–4144 (2010).
18. Schloss, P. D., Westcott, S. L. & Ryabin, T. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol* **75**, 7537–7541 (2009).
19. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010).
20. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**, e9490 (2010).
21. Lozupone, C. & Knight, R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228–8235 (2005).
22. Galaxy/Hutlab. [cited 2017 April 27]. Available from: https://huttenhower.sph.harvard.edu/galaxy/
23. Sharma, P., Falk, G. W. & Weston, A. P. et al. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. *Clin. Gastroenterol. Hepatol.* **4**, 566–572 (2006).
24. Montgomery, E., Goldblum, J. R. & Greenson, J. K. et al. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus: a follow-up study based on 138 cases from a diagnostic variability study. *Hum. Pathol.* **32**, 379–388 (2001).
25. Spechler, S. J., Sharma, P. & Souza, R. F. et al. American Gastroenterological Association technical review on the management of Barrett's esophagus. *Gastroenterology* **140**, e18–e52 (2011). quize13.
26. Fan, D., Coughlin, L. A. & Neubauer, M. M. et al. Activation of HIF-1alpha and LL-37 by commensal bacteria inhibits Candida albicans colonization. *Nat. Med.* **21**, 808–814 (2015).
27. Picard, F. J., Ke, D. & Boudreau, D. K. et al. Use of tuf sequences for genus-specific PCR detection and phylogenetic analysis of 28 streptococcal species. *J. Clin. Microbiol.* **42**, 3686–3695 (2004).
28. Ross-Innes, C. S., Debiram-Beecham, I. & O'Donovan, M. et al. Evaluation of a minimally invasive cell sampling device coupled with assessment of trefoil factor 3 expression for diagnosing Barrett's esophagus: a multi-center case-control study. *PLoS Med.* **12**, e1001780 (2015).
29. Chan, D. K., Zakko, L. & Visrodia, K. H. et al. Breath testing for Barrett's esophagus using exhaled volatile organic compound profiling with an electronic nose device. *Gastroenterology* **152**, 24–26 (2017).
30. Wu, J., Peters, B. A. & Dominianni, C. et al. Cigarette smoking and the oral microbiome in a large study of American adults. *ISME J.* **10**, 2435–2446 (2016).
31. Yu, G., Phillips, S. & Gail, M. H. et al. The effect of cigarette smoking on the oral and nasal microbiota. *Microbiome* **5**, 3 (2017).
32. Guerrero-Preston, R., Godoy-Vitorino, F. & Jedlicka, A. et al. 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment. *Oncotarget* **7**, 51320–51334 (2016).
33. Chen, X., Winckler, B. & Lu, M. et al. Oral microbiota and risk for esophageal squamous cell carcinoma in a high-risk area of China. *PLoS ONE* **10**, e0143603 (2015).
34. Tsai, C. Y., Tang, C. Y., Tan, T. S., et al. Subgingival microbiota in individuals with severe chronic periodontitis. *J. Microbiol. Immunol. Infect.* (2016). https://doi.org/10.1016/j.jmii.2016.04.007
35. Colombo, A. P., Boches, S. K. & Cotton, S. L. et al. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. *J. Periodontol.* **80**, 1421–1432 (2009).
36. Boutin, S., Hagenfeld, D. & Zimmermann, H. et al. Clustering of subgingival microbiota reveals microbial disease ecotypes associated with clinical stages of periodontitis in a cross-sectional study. *Front. Microbiol.* **8**, 340 (2017).
37. Colombo, A. P., Bennet, S. & Cotton, S. L. et al. Impact of periodontal therapy on the subgingival microbiota of severe periodontitis: comparison between good responders and individuals with refractory periodontitis using the human oral microbe identification microarray. *J. Periodontol.* **83**, 1279–1287 (2012).
38. Desai, M. S., Seekatz, A. M. & Koropatkin, N. M. et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* **167**, 1339–1353 (2016). e21.
39. Vincent, C., Stephens, D. A. & Loo, V. G. et al. Reductions in intestinal clostridiales precede the development of nosocomial Clostridium difficile infection. *Microbiome* **1**, 18 (2013).
40. Deng, Z., Mu, J. & Tseng, M. et al. Enterobacteria-secreted particles induce production of exosome-like S1P-containing particles by intestinal epithelium to drive Th17-mediated tumorigenesis. *Nat. Commun.* **6**, 6956 (2015).

41. Dalmasso, G., Cougnoux, A. & Delmas, J. et al. The bacterial genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment. *Gut Microbes* **5**, 675–680 (2014).
42. Nougayrede, J. P., Homburg, S. & Taieb, F. et al. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. *Science* **313**, 848–851 (2006).
43. Raisch, J., Rolhion, N. & Dubois, A. et al. Intracellular colon cancer-associated Escherichia coli promote protumoral activities of human macrophages by inducing sustained COX-2 expression. *Lab Invest.* **95**, 296–307 (2015).
44. Cougnoux, A., Dalmasso, G. & Martinez, R. et al. Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype. *Gut* **63**, 1932–1942 (2014).
45. Roy, B. C., Subramaniam, D. & Ahmed, I. et al. Role of bacterial infection in the epigenetic regulation of Wnt antagonist WIF1 by PRC2 protein EZH2. *Oncogene* **34**, 4519–4530 (2015).
46. Li, J., Quinque, D. & Horz, H. P. et al. Comparative analysis of the human saliva microbiome from different climate zones: Alaska, Germany, and Africa. *BMC Microbiol.* **14**, 316 (2014).
47. Mason, M. R., Nagaraja, H. N. & Camerlengo, T. et al. Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome. *PLoS ONE* **8**, e77287 (2013).