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ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, Dr. Tsolakis is an interventional endoscopist (Senior Consultant) and group leader for endoscopic ultrasound and duodenal polyps at Karolinska University Hospital, Stockholm, Sweden. Dr. Tsolakis became a Specialist in Internal Medicine (2010) and Gastroenterology (2011) at the Uppsala University Hospital, Sweden, where he was subsequently appointed as Consultant Gastroenterologist focusing on endoscopy and neuroendocrine tumors in the Departments of Gastroenterology and Endocrine Oncology. He completed training in advanced therapeutic endoscopy in Canada, initially at the University of Calgary (2014) and later at St. Michael's Hospital in Toronto (2015). His ongoing career research interests include advanced endoscopy and neuroendocrine tumors, yielding several peer-reviewed publications in the field of endoscopy and neuroendocrine tumor disease. (L-Editor: Filipodia)

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Observational Study

Oral microbiome and pancreatic cancer

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Abstract

BACKGROUND

Microbiota profiles differ between patients with pancreatic cancer and healthy people, and understanding these differences may help in early detection of pancreatic cancer. Saliva sampling is an easy and cost-effective way to determine microbiota profiles compared to fecal and tissue sample collection.

AIM

To investigate the saliva microbiome distribution in patients with pancreatic adenocarcinoma (PDAC) and the role of oral microbiota profiles in detection and risk prediction of pancreatic cancer.

METHODS

We conducted a prospective study of patients with pancreatic cancer ($n = 41$) and healthy individuals ($n = 69$). Bacterial taxa were identified by 16S ribosomal ribonucleic acid gene sequencing, and a linear discriminant analysis effect size algorithm was used to identify differences in taxa. Operational taxonomic unit values of all selected taxa were converted into a normalized Z-score, and logistic

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Institutional review board

statement: The Institutional Review Board of the West China Hospital, Sichuan University approved this prospective study.

Informed consent statement: All participants signed written informed consent.

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regressions were used to calculate risk prediction of pancreatic cancer.

RESULTS

Compared with the healthy control group, carriage of *Streptococcus* and *Leptotrichia* (z-score) was associated with a higher risk of PDAC [odds ratio (OR) = 5.344, 95% confidence interval (CI): 1.282-22.282, $P = 0.021$ and OR = 6.886, 95%CI: 1.423-33.337, $P = 0.016$, respectively]. *Veillonella* and *Neisseria* (z-score) were considered a protective microbe that decreased the risk of PDAC (OR = 0.187, 95%CI: 0.055-0.631, $P = 0.007$ and OR = 0.309, 95%CI: 0.100-0.952, $P = 0.041$, respectively). Among the patients with PDAC, patients reporting bloating have a higher abundance of *Porphyromonas* ($P = 0.039$), *Fusobacterium* ($P = 0.024$), and *Alloprevotella* ($P = 0.041$); while patients reporting jaundice had a higher amount of *Prevotella* ($P = 0.008$); patients reporting dark brown urine had a higher amount of *Veillonella* ($P = 0.035$). Patients reporting diarrhea had a lower amount of *Neisseria* and *Campylobacter* ($P = 0.024$ and $P = 0.034$), and patients reporting vomiting had decreased *Alloprevotella* ($P = 0.036$).

CONCLUSION

Saliva microbiome was able to distinguish patients with pancreatic cancer and healthy individuals. *Leptotrichia* may be specific for patients living in Sichuan Province, southwest China. Symptomatic patients had different bacteria profiles than asymptomatic patients. Combined symptom and microbiome evaluation may help in the early detection of pancreatic cancer.

Key Words: Oral microbiota; Dysbiosis; Pancreatic cancer; Cancer detection; 16s rRNA; High-throughput sequencing

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Core Tip: Pancreatic adenocarcinoma (PDAC) patients benefit from early detection. This study analyzed the composition and diversity of saliva microbiota in PDAC patients through 16S ribosomal ribonucleic acid sequencing. Normalized z-score of bacteria abundance associated clinical data were analyzed for PDAC risk prediction. Microbiome abundance differences were found between PDAC patients with symptoms and patients without symptoms. Combined symptom and microbiome evaluation may help in early detection and risk prediction of pancreatic cancer.

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INTRODUCTION

Pancreatic cancer or pancreatic adenocarcinoma (PDAC) is a lethal disease with a 5-year survival rate of about 6%^[1,2]. Early detection and diagnosis are essential for effective surgery treatment that improves cancer survival^[3,4], yet these remain a great challenge. A variety of diagnostic methods are available. For example, deoxyribonucleic acid (DNA) sequencing for detecting and diagnosing pancreatic cancer are limited in clinical use due to the need for fresh, high-quality specimens, tumor content, and tumor heterogeneity^[5,6]. Molecular markers, such as mutant DNA or DNA methylomes, are also limited in clinical use to enhance diagnostic sensitivity or early detection of pancreatic cancer recurrence^[7,8]. Biomarker Ca19-9 has been commonly used for diagnosis and prognosis of pancreatic cancer with diagnostic sensitivity of 0.78 and specificity of 0.77, but this biomarker test has limited sensitivity among patients with jaundice, pancreatitis, enteritis, and elevated blood glucose, since such patients usually have elevated Ca19-9 concentrations^[9-11]. In addition, 7%-10% Lewis (a-/b-) populations could not express Ca19-9^[12].

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The oral or fecal microbiota profile of gastrointestinal and colorectal cancer, oropharyngeal cancer, liver cancer, and lung cancer may be a novel and potential diagnostic biomarker^[13-19]. Accumulated studies have revealed that oral and gastrointestinal microbiomes differ in abundance in patients with pancreatic cancer compared with healthy individuals^[20-23]. Cancer risk increases with carriage of *Porphyromonas gingivalis*^[21], *Actinobacillus actinomycetemcomitans*^[21], and *Alloprevotella*^[21], while *Fusobacterium*^[21,24], *Leptotrichia*^[21,25,26], *Neisseria elongate*^[21,23], and *Streptococcus mitis*^[21,23] might be a protective factor for having pancreatic cancer. However, Olson *et al*^[22] did not find significant differences in the diversity of the oral microbiome among PDAC patients ($n = 40$), intraductal papillary mucinous neoplasms (IPMNs) ($n = 39$), and healthy participants ($n = 58$) in the United States^[22]. The conflicting findings in the prior studies may be due to the differences in methodological approach and sample collection. For example, some studies performed real-time quantitative polymerase chain reaction (PCR) for validation of bacterial candidates^[23], and some sequenced the microbiota profile in samples of tongue coating^[20] or oral wash samples^[21]. Tongue coating change is a major often-used approach of tongue diagnosis in traditional Chinese medicine, but tongue coating can only capture partial oral microbiota^[27,28]. The oral wash method is more complicated and relatively expensive.

Oral cavity contains nearly 619 taxa in 13 phyla (Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Spirochaetes, SR1, Synergistes, Tenericutes, and TM7), and 68% of these bacteria are uncultivated phylotypes^[29,30]. Advanced genomic sequencing for human oral microbiome distribution makes it possible to measure the proportions of bacterial species without relying on traditional culture methods^[31-33]. Saliva has been found to contain broad spectrum of bacteria with easy sampling method and is relatively cost-effective. Although there are some studies on oral flora and pancreatic cancer in non-Chinese population, the impact of geographical and medical factors, such as race and ethnicity, different dietary habits, antibiotic use, and cancer, may make the oral microbial profile differ among people from different geographic locations. In addition, there are few studies on oral saliva flora and pancreatic cancer in China. Thus, the purpose of our study was to: (1) Determine the saliva microbiome distribution of pancreatic cancer (including resectable PDAC and unresectable PDAC) among Chinese population using 16S rRNA sequencing; and (2) Select proper and specific microbiota for PDAC detecting.

MATERIALS AND METHODS

Ethical consideration

The Institutional Review Board of the West China Hospital, Sichuan University approved this prospective study. All participants signed written informed consent.

Research design and participants

This was a prospective study. We consecutively recruited 80 patients who were over age 18 years and suspected to have pancreatic tumor prior to biopsy or surgery. Histopathological results confirmed 45 patients with primary PDAC and 35 patients with non-cancer pancreatic tumors, including 9 IPMN, 11 pancreatic serous cystadenoma, 5 solid pseudopapillary neoplasm, and 10 neuroendocrine tumors. We also recruited 69 healthy participants from the community as a comparison group. Healthy adults had normal liver and renal function, normal cardio-pulmonary function, no history of cancer, and no viral infection. Participants were excluded if they had: (1) A history of prior malignancy and chemotherapy or radiotherapy; (2) Metastatic PDAC or PDAC with other cancer; (3) A history of viral infection (*i.e.* hepatitis B virus, hepatitis C virus, human immunodeficiency virus); (4) Use of antibiotics (including oral, intravenous, or intramuscular) and probiotics within 4 wk prior to enrollment; and (5) Use of corticosteroids (nasal or inhaled) or other immunosuppressants. In addition, we excluded participants with insufficient saliva sample ($n = 12$) for sequencing analysis and patients with non-cancer pancreatic tumors ($n = 35$).

Demographic and clinical phenotype

The demographic information collected included age, gender, body mass index (BMI), smoking history, alcohol consumption, dietary habit, and chronic diseases (hypertension and type II diabetes). Clinical information was also collected to include cancer site, surgery type, and cancer stages using the American Joint Commission on

Cancer, seventh edition staging manual^[34].

Symptom phenotype

Since there is no measure or checklist for symptoms specific to pancreatic cancer, we developed a checklist based on literature review to assess symptoms specific to pancreatic cancer, such as bloating, jaundice, nausea, vomiting, dark brown urine, diarrhea, constipation, pale stools, pruritus, lack of appetite, pain, fatigue, and disturbed sleeping. Patients reported the presence and absence of symptoms by checking “Yes” or “No.”

Saliva sample collection

Before the patients had surgery to confirm pancreatic cancer diagnosis, saliva samples were collected by trained professionals (Wang X and Li GQ). All the participants were instructed to not eat and drink for 0.5 h prior to saliva sample collection. Participants were also instructed not to brush their teeth at least 8 h prior to saliva sample collection, since brushing teeth may remove part of the oral flora. Participants were asked to rinse their mouths to remove debris from the oral cavity before saliva collection. To ensure all sample collection was at a similar time period in a day, we collected patient samples around 4:00 pm on the day of admission prior to biopsy or surgery for cancer diagnosis. Healthy subjects’ saliva samples were also collected around 4:00 pm in the afternoon. About 3 mL saliva was collected in a sterile tube after it accumulated on the mouth floor. The fresh samples were placed on ice and transported to the laboratory. Samples were divided into 1.5 mL aliquots and stored immediately at -80 °C.

Genome DNA extraction

We used the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, United States) to extract bacterial genomic DNA from saliva samples. DNA concentration and purity was quantified by Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Wilmington, DE, United States) and agarose gel electrophoresis. Genome DNA with strong smear or with concentration lower than 5 µg/mL (by Qubit) was excluded for library construction.

16S rRNA gene sequencing

The third and fourth hypervariable regions (V3-V4) of the 16S rRNA gene of bacteria were amplified by PCR with a domain-specific primer: 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTA ATCC-3'). PCR reactions were performed with a 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, United States), 0.2 µmol/L of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s. Finally, samples were incubated at 72 °C for 5 min. The library quality was assessed by Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, United States). Sequencing was performed on an Illumina Novaseq6000 sequencing platform (Illumina, San Diego, CA, United States), and 250 bp paired-end reads were generated.

Statistical analysis

Phenotype data analysis: Statistical analyses were performed using SPSS (v23.0, SAGE IBM, Armonk, NY, United States). Continuous variables (age and BMI) were estimated as average ± standard error, and categorical variables were analyzed in terms of frequencies and percentages. Chi square analysis and Fisher’s exact tests were used for categorical variables; *t*-test and Mann-Whitney *U* test were used for continuous variables. All tests were two-sided, and *P* values < 0.05 were considered statistically significant with 95% confidence interval (CI).

Microbiome data analysis

Profile and quality assurance: Raw sequences were denoised *via* FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>)^[35]. Quality filtering was performed on raw sequences using QIIME quality control process (v1.9.1_ <http://qiime.org/index.html>) and then high quality clean tags were obtained^[36]. Tags were compared with gold database (http://drive5.com/uchime/uchime_download.html), and chimeras were removed with the UCHIME algorithm (v11.0, http://www.drive5.com/usearch/manual/uchime_algo.html)^[37]. Effective Tags were finally obtained. All effective sequence analysis was performed by Uparse software (v7.0.1001,

<http://drive5.com/uparse/>)^[38]. The optimized, high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity.

Microbiome diversity: According to the results of OTUs clustering analysis and the research requirements, the Venn diagram was constructed to illustrate the number of unique and shared species in saliva samples between PDAC and healthy groups. The Venn diagram was made using R program (Package_VennDiagram). We applied alpha diversity to analyze complexity of species diversity for a sample. Four indices were used: “Chao1” and “Abundance-based coverage estimator (ACE)” estimate the species abundance; “Shannon index” and “Simpson” account for the richness and evenness. The value of Simpson index was calculated as Simpson’s index of diversity 1-D. Thus, higher Shannon and Simpson indices mean higher species diversity. All indices were calculated with QIIME (v1.9.1) and R software (V2.15.3, Auckland, New Zealand). We compared four indices between PDAC and healthy control group using Mann-Whitney U test. Mann-Whitney U test was used to compare the alpha diversity indices between groups of resectable PDAC (rPDAC) and unresectable PDAC (unrPDAC). The bacterial taxonomic compositions were evaluated with a linear discriminant analysis effect size algorithm (<https://huttenhower.sph.harvard.edu/>). $P < 0.05$ and an LDA score ≥ 2.0 were recognized as significant in Kruskal-Wallis and pairwise Wilcoxon evaluation, respectively.

Abundance of bacteria and symptom: We used Wilcoxon rank-sum test to compare the abundance of bacteria (top 10 positively expressed flora) in PDAC patients with and without typical symptoms of PDAC, including bloating, jaundice, nausea, vomiting, dark brown urine, diarrhea, constipation, pale stools, pruritus, lack of appetite, pain, fatigue, and disturbed sleeping.

Risk prediction for PDAC

Logistic regressions were used to explore the association of significant taxa with clinical covariates (age, BMI, smoking status, alcohol consumption status, history of blood hypertension, and eating habits). To avoid the occurrence of false negative diagnosis, we focused on the top 20 species (OTUs abundance) and the flora associated with PDAC that has been reported^[20-23]. Finally, *Streptococcus*, *Prevotella*, *Porphyromonas*, *Neisseria*, *Veillonella*, *Leptotrichia*, *Lactobacillus*, *Actinomyces*, *Haemophilus*, *Rothia*, and *Fusobacterium* were selected for analysis. To make the values comparable, we converted the OTU values of all selected taxa into a normalized z-score. The tetranucleotide-derived z-score, superior to (G + C) content differences, was calculated according to the previous methods^[39,40]. Odds ratio with 95% CIs were calculated.

RESULTS

Phenotypic characteristics

Between November 2017 and December 2018, a total of 157 participants were enrolled in this study; four PDAC patients and eight healthy participants were eventually excluded due to the insufficient saliva sample for sequencing analysis. A final sample of 110 included patients in PDAC ($n = 41$) and healthy individuals ($n = 69$). **Table 1** shows the demographic characteristics of PDAC patients and healthy participants. Compared with the healthy group, the PDAC had lower BMIs (22.76 *vs* 24.44, $P < 0.0001$). As for eating habits, more PDAC patients (61%) preferred oily and fatty foods compared to the healthy control group ($P = 0.002$). More healthy control participants had hypertension ($P = 0.006$). Among the 41 patients with PDAC, 31 (76%) had head pancreatic cancer, and 20 (49%) patients had resectable pancreatic cancer.

Bacteria profile

Alpha-diversity analysis of the study participant groups: From 110 samples, we filtered 6356399 qualified reads. We randomly chose 2235200 reads (110 samples multiplied by 20320 reads/sample, the minimum number of reads/sample). Finally, we obtained 1975 OTUs for further analysis. A Venn diagram (**Figure 1**) shows the details of the OTUs at 97% identity for PDAC patients and healthy participants. The two groups had 690 shared species, 231 unique species for PDAC patient, and 389 for healthy control group. As **Table 2** shows, compared with the healthy group, the PDAC group had significantly increased microbial abundance estimated by the Chao1 index and ACE index while decreased microbial diversity estimated by Shannon and Simpson indices ($P < 0.0001$). Patients with rPDAC had lower bacteria abundance and

Table 1 Demographic characteristics of participants

Variables	PDAC group, <i>n</i> = 41	Healthy control group, <i>n</i> = 69	<i>P</i> value
Age, average \pm standard error	61.17 \pm 1.79	64.64 \pm 1.04	0.098
Gender, <i>n</i> (%)			
Male	24 (59)	50 (72)	0.132
Female	17 (41)	19 (28)	
BMI, average \pm standard error	22.76 \pm 0.94	24.44 \pm 0.39	< 0.0001
Smoking history, <i>n</i> (%)	17 (41)	37 (54)	0.217
Alcohol consumption, <i>n</i> (%)	16 (39)	30 (43)	0.647
Dietary habit, <i>n</i> (%)			
Oily and fatty food	25 (61)	21 (31)	0.002
Salty food	6 (15)	8 (11)	0.664
Light diet	10 (24)	40 (58)	0.001
Chronic disease, <i>n</i> (%)			
Hypertension	1 (2)	15 (22)	0.006
Type II diabetes	2 (5)	6 (9)	0.714
Both	3 (7)	5 (7)	1.000
Loss of weight, <i>n</i> (%)	23 (56)	3 (4)	0.0001
Primary cancer site, <i>n</i> (%)			
Head	31 (76)	NA	NA
Body and tail	10		
Surgery, <i>n</i> (%)			
Pancreaticoduodenectomy	14 (34)	NA	NA
Distal pancreatectomy	6 (15)		
Palliative intervention techniques	21 (51)		
AJCC staging		NA	NA
I-IIIB	20 (49)		
III-IV	21		

AJCC: American Joint Commission on Cancer; BMI: Body mass index; PDAC: Pancreatic adenocarcinoma.

Table 2 α -diversity indices of two groups

	PDAC group, <i>n</i> = 41	Healthy control group, <i>n</i> = 69	<i>P</i> value
Shannon	5.14 \pm 0.67	5.67 \pm 0.51	0.0001
Simpson	0.90 \pm 0.08	0.95 \pm 0.02	0.0001
Chao1	423.48 \pm 55.69	295.00 \pm 54.05	0.0001
ACE	424.00 \pm 55.72	293.97 \pm 50.09	0.0001

PDAC: Pancreatic adenocarcinoma.

diversity than patients with unrPDAC estimated by Chao1, ACE, Shannon indices, and Simpson indices. However, Shannon ($P = 0.273$), Simpson ($P = 0.715$), Chao1 ($P = 0.159$), and ACE ($P = 0.137$) were not able to distinguish rPDAC and unrPDAC.

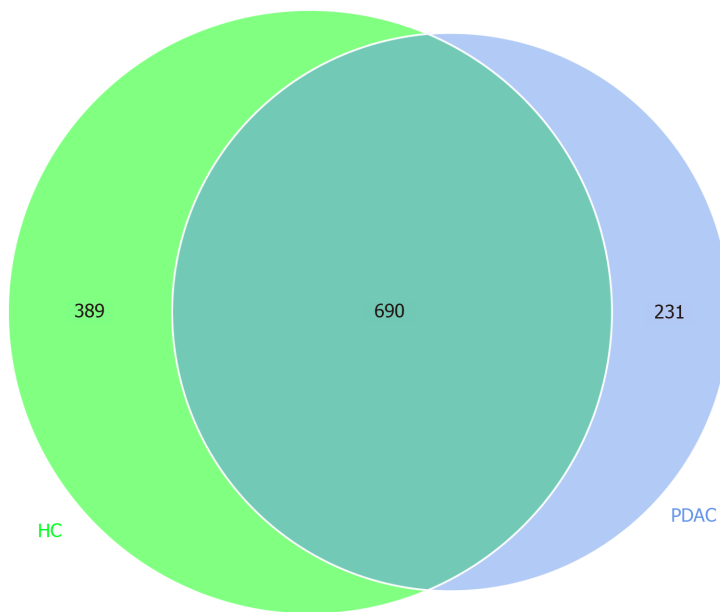


Figure 1 Microbial profiles of two groups. Venn diagram showing shared and unique operational taxonomic units (OTUs) at 97% identity among pancreatic adenocarcinoma (PDAC) group ($n = 41$) and healthy controls (HC) group ($n = 69$). PDAC group is blue; HC group is green. OTUs (690) are shared by two groups. Unique OTUs of 231 and 389 were found in PDAC and HC, respectively.

Bacterial taxonomic alterations in PDAC

We used a linear discriminant analysis effect size algorithm to assess the bacterial taxonomic compositions and differences between PDAC group and healthy control subjects. Compared with the healthy group, PDAC patients were significantly enriched in order_Lactobacillales, class_Bacilli, genus_*Streptococcus*, phylum_Firmicutes, genus_*Actinomyces*, genus_*Rothia*, genus_*Leptotrichia*, genus_*Lactobacillus*, species_*Escherichia coli*, and order_Enterobacteriales (Figure 2A). Conversely, PDAC patients had significantly reduced abundances of *Selenomonas*, *Porphyromonas*, *Prevotella*, *Capnocytophaga*, *Alloprevotella*, *Tannerella*, and *Neisseria* at genus level. We also compared the bacterial distributions between rPDAC and unrPDAC patients. Figure 2B shows that species_*Escherichia coli*, genus_*Peptostreptococcus*, genus_*Asteroleplasma*, and species_*Tannerella forstythia* were more prevalent in the unrPDAC group, whereas we found reduced occurrence of species_*Bacteroides stercoris*, genus_*Megasphaera*, and genus_*Veillonella* (Figure 2).

Microbiome profile and symptoms

Table 3 presented flora abundance differences between the PDAC patients with symptoms and without symptoms. Patient reporting bloating had greater abundance of *Porphyromonas* (660.4 ± 461.0 , $P = 0.039$), *Fusobacteria* (490.0 ± 186.6 , $P = 0.024$), and *Alloprevotella* (155.4 ± 124.1 , $P = 0.041$) compared to those without bloating (412.0 ± 394.3 , 361.8 ± 184.4 and 99.3 ± 81.9 , respectively). *Prevotella* presented greater abundance in patients without jaundice (669.4 ± 384.3 , $P = 0.008$) compared to those with jaundice (403.2 ± 310.8). *Veillonella* presented greater abundance in patients without dark brown urine (1863.8 ± 1449.2 , $P = 0.035$) compared to those with dark brown urine (1018.6 ± 766.7). *Alloprevotella* presented greater abundance in patients without vomiting (130.3 ± 100.9 , $P = 0.036$) compared to those with vomiting (91.8 ± 134.4), while *Neisseria* presented greater abundance in patients with vomiting (3343.3 ± 1829.9 , $P = 0.024$) compared to those without vomiting (1360.3 ± 1256.6). *Campylobacter* presented greater abundance in patients with diarrhea (130.5 ± 59.7 , $P = 0.034$) compared to those without diarrhea (74.9 ± 87.2).

Logistic regression for microbiota profile

We explored the PDAC risk in relation to selected bacteria abundances (normalized z-score). As shown in Table 4, compared with healthy control group, carriage of *Streptococcus* (OR = 5.344, 95%CI: 1.282-22.282, $P = 0.021$) and *Leptotrichina* (OR = 6.886, 95%CI: 1.423-33.337, $P = 0.016$) were associated with a higher risk of PDAC. With each increase of z-score of *Streptococcus* and *Leptotrichina* in PDAC patients, the risk of pancreatic cancer increased by 5.344 odds and 6.886 odds, respectively. Carriage of

Table 3 Flora abundance differences in pancreatic adenocarcinoma patients with symptomatic phenotype

Symptoms	Microbiome	Without symptoms	With symptoms	P value
Bloating	<i>Porphyromonas</i>	412.0 ± 394.3	660.4 ± 461.0	0.039
	<i>Fusobacteria</i>	361.8 ± 184.4	490.0 ± 186.6	0.024
	<i>Alloprevotella</i>	99.3 ± 81.9	155.4 ± 124.1	0.041
Jaundice	<i>Prevotella</i>	669.4 ± 384.3	403.2 ± 310.8	0.008
Dark brown urine	<i>Veillonella</i>	1863.8 ± 1449.2	1018.6 ± 766.7	0.035
Vomiting	<i>Alloprevotella</i>	130.3 ± 100.9	91.8 ± 134.4	0.036
Diarrhea	<i>Neisseria</i>	1360.3 ± 1256.6	3343.3 ± 1829.9	0.024
	<i>Campylobacter</i>	74.9 ± 87.2	130.5 ± 59.7	0.034

Table 4 Oral bacteria distribution and risk of pancreatic adenocarcinoma

	Odds ratio	95%CI		P value
Healthy control group	Base outcome			
PDAC group				
Age	0.956	0.875	1.046	0.327
BMI	0.973	0.708	1.338	0.866
Oily and fatty food	0.759	0.122	4.730	0.768
<i>Streptococcus</i>	5.344	1.282	22.282	0.021
<i>Veillonella</i>	0.187	0.055	0.631	0.007
<i>Neisseria</i>	0.309	0.100	0.952	0.041
<i>Lactobacillus</i>	0.713	0.357	1.425	0.338
<i>Leptotrichia</i>	6.886	1.423	33.337	0.016
<i>Actinomyces</i>	4.515	0.444	45.887	0.203
<i>Haemophilus</i>	1.185	0.513	2.738	0.691
<i>Prevotella</i>	0.673	0.298	1.519	0.341
<i>Porphyromonas</i>	0.294	0.084	1.033	0.056
<i>Rothia</i>	1.257	0.467	3.384	0.650
<i>Fusobacterium</i>	1.006	0.335	3.017	0.576

BMI: Body mass index; CI: Confidence interval; PDAC: Pancreatic adenocarcinoma.

Veillonella and *Neisseria* were protective factors of having PDAC (OR = 0.187, 95%CI: 0.055-0.631, $P = 0.007$ and OR = 0.309, 95%CI: 0.100-0.952, $P = 0.041$, respectively). With each decrease of z-score of *Veillonella* and *Neisseria* in PDAC patients, the risk of pancreatic cancer decreased by 0.187 odds and 0.309 odds, respectively.

DISCUSSION

This prospective study found dysbacteriosis of the oral microbiota existed in patients with PDAC. Fecal bacteria flora has been the main sample method for research on pancreatic cancer^[41,42]. Our study used saliva sample method, which is convenient and the quality of sample is easy to control during sample collection. When comparing bacteria profiles from our saliva samples and fecal samples from other research on Chinese PDAC patients^[20,42], salivary and intestinal bacteria flora consistently had low Shannon index and high Chao1 index, and *Lactobacillus*, *Enterobacter*, and *Leptotrichia* at the genus level was significantly increased. This provides supporting evidence that

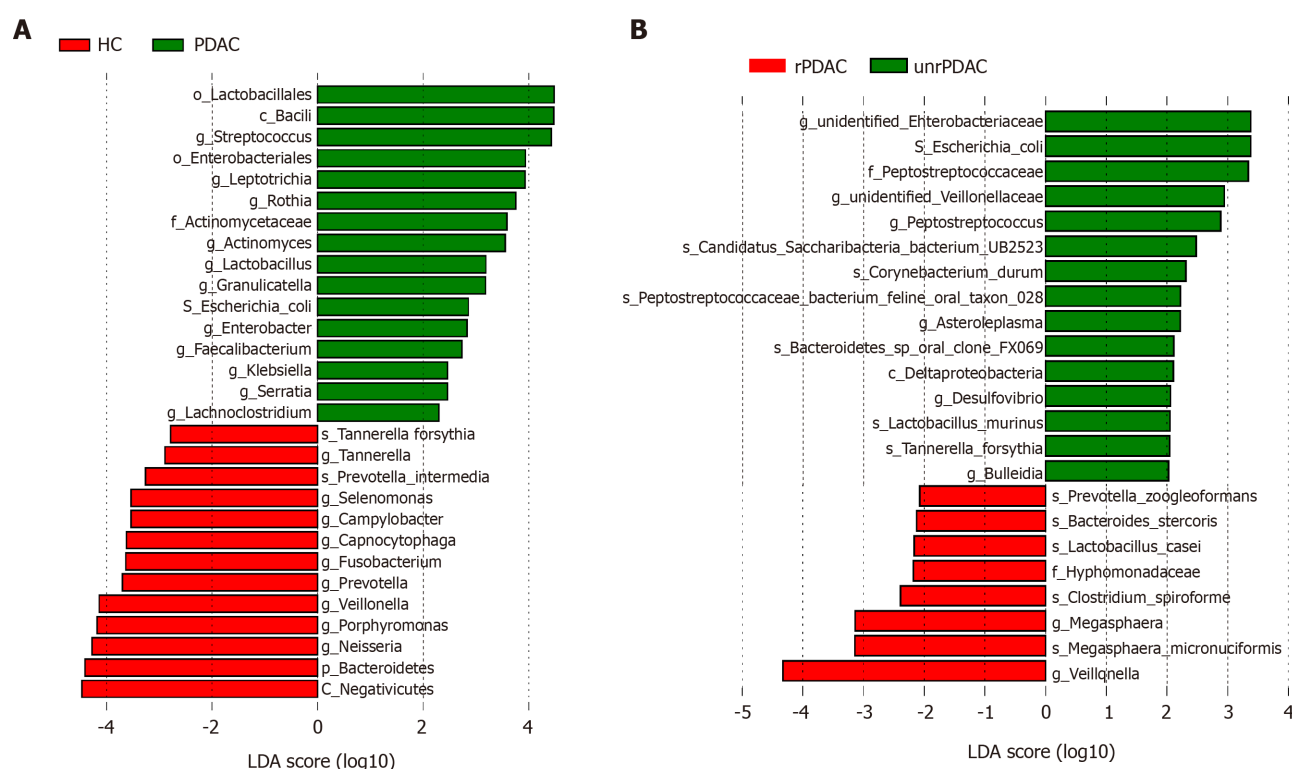


Figure 2 Linear discriminant analysis effect size and latent dirichlet allocation analysis based on operational taxonomic units. A: Shows a list of specific oral bacteria that enable discrimination between pancreatic adenocarcinoma (PDAC) patients and healthy controls (HC). Differences in oral microbial communities between PDAC patients and HC. The horizontal line with red and green denotes the means of the HC and PDAC groups, respectively; B: Resectable PDAC (rPDAC) and unresectable PDAC (unrPDAC). Differences in oral microbial communities between rPDAC group and unrPDAC. The horizontal line with red and green denotes the means of the rPDAC and unrPDAC groups, respectively.

saliva sample method yields similar bacteria flora profiles compared to the fecal sample method, which is very often difficult to collect the samples. Findings of our study also provided additional evidence to confirm that *Neisseria* and *Streptococcaceae* are risk factors for pancreatic cancer^[21,23]. Currently, no studies have focused on comparing the advantages and disadvantages of using different sample collection techniques, and studies are necessary to compare the effectiveness of using different sample collection techniques, such as saliva, tongue coating, and oral wash, on sample quality for microbiota profiles and preference of patients.

In terms of microbiota abundance and species diversity, our study found that the PDAC group had significantly increased microbial abundance as estimated by the Chao1 and ACE indices and decreased microbial diversity as estimated by Shannon and Simpson indices. Lu *et al*^[20] also had similar findings from a study on Chinese pancreatic cancer patients using tongue coating samples^[20]. However, studies of non-Chinese population did not find any differences of alpha diversity indices of oral microbiota composition between pancreatic cancer patients and healthy individuals^[22,23]. Findings of our study and Lu *et al*^[20] demonstrated that seven of fourteen bacterial families (*Leptotrichiaceae*, *Actinomycetaceae*, *Lachnospiraceae*, *Micrococcaceae*, *Erysipelotrichaceae*, *Coriobacteriaceae*, *Moraxellaceae*) were consistently significantly increased, and *Porphyromonadaceae* was significantly decreased in Chinese PDAC patients. However, our study found that the abundance of three of fourteen bacterial families (*Fusobacteriaceae*, *Campylobacteraceae*, *Spirochaetaceae*) were significantly decreased in PDAC patients, while Lu *et al*^[20] found significantly more abundance^[20]. Both our study and the study by Lu *et al*^[20] found significant increase in the genus of *Leptotrichia*, *Actinomyces*, *Rothia*, *Rothia*, *Solobacterium*, *Peptostreptococcus*, and *Oribacterium*. Yet, decreased abundance in *Selenomonas*, *Tannerella*, and *Campylobacter* was found in our study using saliva sample method but was increased in the study by Lu *et al*^[20] using tongue coating sample method^[20]. There are four known main periodontal disease contributors: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Prevotella intermedia* were more prevalent in PDAC patients in Fan *et al*^[21]. However, except for *Actinomyces*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Prevotella intermedia* were significantly reduced in our study. *Leptotrichia* also showed different distribution in

our study comparing to Fan *et al*^[21]. Torres *et al*^[43] found higher *Leptotrichia* and lower *Porphyromonas* in the saliva of patients with pancreatic cancer, but no significant differences were found in the expression of *Streptococcus mitis* and *Granulicatella adiacens*. The conflicting findings between our study and other studies may be due to different sample collection methods, *e.g.*, saliva *vs* tongue coating method. Future research should compare different sample collection methods for microbiome research, *e.g.*, saliva *vs* tongue coating method. The other factor for the conflicting findings may geographic food preferences of Chinese population. For example, subjects in Lu *et al*^[20]'s study were enrolled from Zhejiang University, which is located in Hangzhou (southeast of China). Generally, people in Hangzhou have different diet preferences, such as preferences for milder taste and more sugar. Subjects in our study from Sichuan Province preferred adding a large amount of different herbs and spices and more fat and salt in food, which may lead to a high incidence of digestive system tumors^[44,45]. Future research should focus on the effects of geographical location, race, diet, antibiotic usage (including consuming meat products containing antibiotics), injury, illness, and hormonal change on flora analysis^[46].

One important finding of our study was that bacteria flora was able to differentiate patients with rPDAC and unrPDAC. This is important because patients with rPDAC usually have better prognosis with timely surgical treatment. We found that species_ *Escherichia coli* and species_ *Tannerella forstythia* were increased significantly in unrPDAC, and these bacteria may be able to predict a tumor that is already advanced. In contrast, the expression of *Veillonella* demonstrated a gradual decline in saliva samples from healthy people, rPDAC, and advanced PDAC (Figure 3), which indicates that *Veillonella* may be protective bacteria for PDAC development.

Our study is the first to investigate the associations between bacteria profiles and symptoms related to pancreatic cancer. Symptomatic patients had different bacteria profiles than asymptomatic patients in our study. For examples, PDAC with bloating have a higher abundance of *Porphyromonas*, *Fusobacterium*, and *Alloprevotella*, and *Alloprevotella* is decreased in patients with vomiting. In addition, PDAC with jaundice had a higher amount of *Prevotella* compared with the PDAC without jaundice. There was a higher amount of *Veillonella* in patients with dark brown urine. PDAC with diarrhea had a lower amount of *Neisseria* and *Campylobacter* compared with PDAC without diarrhea. One benefit of having symptoms is that patients will seek medical help earlier, leading to the diagnosis of early PDAC and improved survival. The exact microbiome mechanism for symptoms is unknown, and more studies are needed to investigate the associations between microbiota and symptoms. Perhaps, combined symptom and microbiome evaluation may help in early detection of pancreatic cancer.

Our study had limitations. We did not include the data of other pancreatic diseases because the sample size was very small. Second, we used only 16S rRNA sequencing to analyze bacterial distributions; future research should include metagenomic sequencing to enhance accuracy of bacterial distributions. It should be noted that the rapid, inexpensive tests of 16S rRNA sequencing can have advantages for clinical implementation by using bacterial distribution test for early detection or prevention of PDAC. Some studies found the association between microbiome profile and dental disease^[47]. Another limitation of our study was that we were not able to exclude participants with dental disease since our participants were not able to provide accurate history of dental disease, and there were no medical record regarding dental disease for us to verify participant dental disease status. In the future, it may be beneficial to have a professional dentist examine participant's oral health status so as to ascertain the potential impact of oral health status on microbiome flora profile among patients with pancreatic cancer. One strength of the study is that we compared the bacteria abundances in patients with positive symptoms to find the relative association between the occurrence of symptoms and potential functions of flora.

CONCLUSION

Saliva microbiome are able to distinguish PDAC and healthy individuals. Higher *Streptococcus* and *Leptotrichia* abundances were associated with increased risk of PDAC. *Veillonella* and *Neisseria* were protective factors for detecting PDAC. *Neisseria* was recognized by all studies to reduce the risk of pancreatic cancer while *Leptotrichia* was identified in our study as a potential specific detector of PDAC in patients living in Sichuan Province, southwest China. Symptomatic patients had different bacteria profiles than asymptomatic patients. As symptoms of PDAC are usually nonspecific, combined symptom and microbiome evaluation may help in early detection of

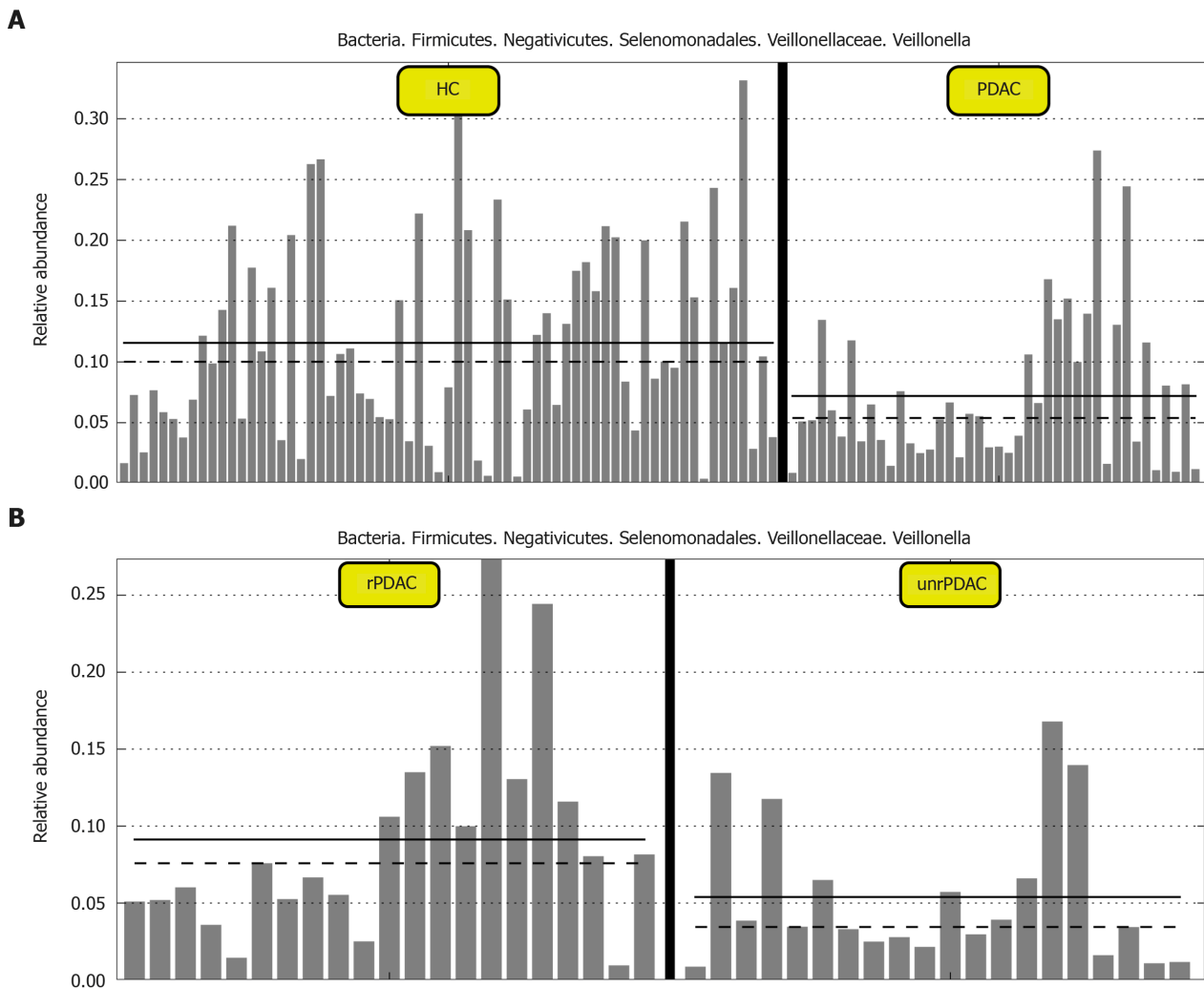


Figure 3 The abundance of *Veillonella* in different groups. The relative abundance of *Veillonella* in pancreatic adenocarcinoma (PDAC) patients is shown by the straight, and dotted lines plot the means and medians of the relative abundance. The abundance of *Veillonella* showed a gradual decline in saliva samples from healthy people, resectable PDAC (rPDAC), and unresectable PDAC (unrPDAC). HC: Healthy controls.

pancreatic cancer. Understanding the distribution of bacteria flora is essential step for developing probiotic treatment plans for reducing the risk of pancreatic cancer.

ARTICLE HIGHLIGHTS

Research background

Understanding the distribution of bacteria flora is essential step for developing probiotic treatment plans for reducing the risk of pancreatic cancer.

Research motivation

The impact of geographical and medical factors, such as race and ethnicity, different dietary habits, antibiotic use, and cancer, may make the oral microbial profile differ among people from different geographic locations.

Research objectives

To investigate the saliva microbiome distribution in patients with pancreatic adenocarcinoma and the role of oral microbiota profiles in detection and risk prediction of pancreatic cancer.

Research methods

A prospective design was utilized with 16S ribosomal ribonucleic acid gene sequencing to identify differences in bacterial taxa using a linear discriminant analysis

effect size algorithm. Operational taxonomic unit values of all selected taxa were converted into a normalized Z-score, and logistic regressions were used to calculate risk prediction of pancreatic cancer.

Research results

Saliva microbiome was able to distinguish patients with pancreatic cancer and healthy individuals. Symptomatic patients had different bacteria profiles than asymptomatic patients.

Research conclusions

Combined symptom and microbiome evaluation may help in early detection of pancreatic cancer.

Research perspectives

Further work may focus on specific microbiota verification and diagnostic ability *via* large sample studies.

REFERENCES

- 1 **McGuigan A**, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol* 2018; **24**: 4846-4861 [PMID: [30487695](#) DOI: [10.3748/wjg.v24.i43.4846](#)]
- 2 **Rawla P**, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. *World J Oncol* 2019; **10**: 10-27 [PMID: [30834048](#) DOI: [10.14740/wjon1166](#)]
- 3 **Neal RD**, Tharmanathan P, France B, Din NU, Cotton S, Fallon-Ferguson J, Hamilton W, Hendry A, Hendry M, Lewis R, Macleod U, Mitchell ED, Pickett M, Rai T, Shaw K, Stuart N, Tørring ML, Wilkinson C, Williams B, Williams N, Emery J. Is increased time to diagnosis and treatment in symptomatic cancer associated with poorer outcomes? *Br J Cancer* 2015; **112** Suppl 1: S92-107 [PMID: [25734382](#) DOI: [10.1038/bjc.2015.48](#)]
- 4 **Matsubayashi H**, Ishiwatari H, Sasaki K, Uesaka K, Ono H. Detecting Early Pancreatic Cancer: Current Problems and Future Prospects. *Gut Liver* 2020; **14**: 30-36 [PMID: [31009958](#) DOI: [10.5009/gnl18491](#)]
- 5 **Rothwell DG**, Ayub M, Cook N, Thistlethwaite F, Carter L, Dean E, Smith N, Villa S, Dransfield J, Clipson A, White D, Nessa K, Ferdous S, Howell M, Gupta A, Kilerci B, Mohan S, Frese K, Gulati S, Miller C, Jordan A, Eaton H, Hickson N, O'Brien C, Graham D, Kelly C, Aruketty S, Metcalf R, Chiramel J, Tinsley N, Vickers AJ, Kurup R, Frost H, Stevenson J, Southam S, Landers D, Wallace A, Marais R, Hughes AM, Brady G, Dive C, Krebs MG. Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study. *Nat Med* 2019; **25**: 738-743 [PMID: [31011204](#) DOI: [10.1038/s41591-019-0380-z](#)]
- 6 **Shen SY**, Singhanian R, Fehringer G, Chakravarthy A, Roehrl MHA, Chadwick D, Zuzarte PC, Borgida A, Wang TT, Li T, Kis O, Zhao Z, Spreafico A, Medina TDS, Wang Y, Roulois D, Ettayebi I, Chen Z, Chow S, Murphy T, Arruda A, O'Kane GM, Liu J, Mansour M, McPherson JD, O'Brien C, Leighl N, Bedard PL, Fleshner N, Liu G, Minden MD, Gallinger S, Goldenberg A, Pugh TJ, Hoffman MM, Bratman SV, Hung RJ, De Carvalho DD. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature* 2018; **563**: 579-583 [PMID: [30429608](#) DOI: [10.1038/s41586-018-0703-0](#)]
- 7 **Cohen JD**, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, Douville C, Javed AA, Wong F, Mattox A, Hruban RH, Wolfgang CL, Goggins MG, Dal Molin M, Wang TL, Roden R, Klein AP, Ptak J, Dobbys L, Schaefer J, Silliman N, Popoli M, Vogelstein JT, Browne JD, Schoen RE, Brand RE, Tie J, Gibbs P, Wong HL, Mansfield AS, Jen J, Hanash SM, Falconi M, Allen PJ, Zhou S, Bettegowda C, Diaz LA Jr, Tomasetti C, Kinzler KW, Vogelstein B, Lennon AM, Papadopoulos N. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 2018; **359**: 926-930 [PMID: [29348365](#) DOI: [10.1126/science.aar3247](#)]
- 8 **Sausen M**, Phallen J, Adleff V, Jones S, Leary RJ, Barrett MT, Anagnostou V, Parpart-Li S, Murphy D, Kay Li Q, Hruban CA, Scharpf R, White JR, O'Dwyer PJ, Allen PJ, Eshleman JR, Thompson CB, Klimstra DS, Linehan DC, Maitra A, Hruban RH, Diaz LA Jr, Von Hoff DD, Johansen JS, Drebin JA, Velculescu VE. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. *Nat Commun* 2015; **6**: 7686 [PMID: [26154128](#) DOI: [10.1038/ncomms8686](#)]
- 9 **Gu J**, Wang D, Huang Y, Lu Y, Peng C. Diagnostic value of combining CA 19-9 and K-ras gene mutation in pancreatic carcinoma: a meta-analysis. *Int J Clin Exp Med* 2014; **7**: 3225-3234 [PMID: [25419353](#)]
- 10 **Ballehaninna UK**, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. *J Gastrointest Oncol* 2012; **3**: 105-119 [PMID: [22811878](#) DOI: [10.3978/j.issn.2078-6891.2011.021](#)]
- 11 **Zhang D**, Hou W, Liu F, Yin J, Lu W, Li M, Zheng T, Lu F, Bao Y, Jia W. Metformin reduces serum

- CA199 levels in type 2 diabetes Chinese patients with time-effect and gender difference. *Diabetes Technol Ther* 2015; **17**: 72-79 [PMID: 25548963 DOI: 10.1089/dia.2014.0176]
- 12 **Lamerz R.** Role of tumour markers, cytogenetics. *Ann Oncol* 1999; **10** Suppl 4: 145-149 [PMID: 10436809]
 - 13 **Yachida S,** Mizutani S, Shiroma H, Shiba S, Nakajima T, Sakamoto T, Watanabe H, Masuda K, Nishimoto Y, Kubo M, Hosoda F, Rokutan H, Matsumoto M, Takamaru H, Yamada M, Matsuda T, Iwasaki M, Yamaji T, Yachida T, Soga T, Kurokawa K, Toyoda A, Ogura Y, Hayashi T, Hatakeyama M, Nakagama H, Saito Y, Fukuda S, Shibata T, Yamada T. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* 2019; **25**: 968-976 [PMID: 31171880 DOI: 10.1038/s41591-019-0458-7]
 - 14 **Lu H,** Ren Z, Li A, Zhang H, Jiang J, Xu S, Luo Q, Zhou K, Sun X, Zheng S, Li L. Deep sequencing reveals microbiota dysbiosis of tongue coat in patients with liver carcinoma. *Sci Rep* 2016; **6**: 33142 [PMID: 27605161 DOI: 10.1038/srep33142]
 - 15 **Zeller G,** Tap J, Voigt AY, Sunagawa S, Kultima JR, Costea PI, Amiot A, Böhm J, Brunetti F, Habermann N, Herczeg R, Koch M, Luciani A, Mende DR, Schneider MA, Schrott-King P, Tournigand C, Tran Van Nhieu J, Yamada T, Zimmermann J, Benes V, Kloor M, Ulrich CM, von Knebel Doeberitz M, Sobhani I, Bork P. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol Syst Biol* 2014; **10**: 766 [PMID: 25432777 DOI: 10.15252/msb.20145645]
 - 16 **Börnigen D,** Ren B, Pickard R, Li J, Ozer E, Hartmann EM, Xiao W, Tickle T, Rider J, Gevers D, Franzosa EA, Davey ME, Gillison ML, Huttenhower C. Alterations in oral bacterial communities are associated with risk factors for oral and oropharyngeal cancer. *Sci Rep* 2017; **7**: 17686 [PMID: 29247187 DOI: 10.1038/s41598-017-17795-z]
 - 17 **Gao S,** Li S, Ma Z, Liang S, Shan T, Zhang M, Zhu X, Zhang P, Liu G, Zhou F, Yuan X, Jia R, Potempa J, Scott DA, Lamont RJ, Wang H, Feng X. Presence of Porphyromonas gingivalis in esophagus and its association with the clinicopathological characteristics and survival in patients with esophageal cancer. *Infect Agent Cancer* 2016; **11**: 3 [PMID: 26788120 DOI: 10.1186/s13027-016-0049-x]
 - 18 **Zhang W,** Luo J, Dong X, Zhao S, Hao Y, Peng C, Shi H, Zhou Y, Shan L, Sun Q, Li Y, Zhao X. Salivary Microbial Dysbiosis is Associated with Systemic Inflammatory Markers and Predicted Oral Metabolites in Non-Small Cell Lung Cancer Patients. *J Cancer* 2019; **10**: 1651-1662 [PMID: 31205521 DOI: 10.7150/jca.28077]
 - 19 **Yan X,** Yang M, Liu J, Gao R, Hu J, Li J, Zhang L, Shi Y, Guo H, Cheng J, Razi M, Pang S, Yu X, Hu S. Discovery and validation of potential bacterial biomarkers for lung cancer. *Am J Cancer Res* 2015; **5**: 3111-3122 [PMID: 26693063]
 - 20 **Lu H,** Ren Z, Li A, Li J, Xu S, Zhang H, Jiang J, Yang J, Luo Q, Zhou K, Zheng S, Li L. Tongue coating microbiome data distinguish patients with pancreatic head cancer from healthy controls. *J Oral Microbiol* 2019; **11**: 1563409 [PMID: 30728915 DOI: 10.1080/20002297.2018.1563409]
 - 21 **Fan X,** Alekseyenko AV, Wu J, Peters BA, Jacobs EJ, Gapstur SM, Purdue MP, Abnet CC, Stolzenberg-Solomon R, Miller G, Ravel J, Hayes RB, Ahn J. Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut* 2018; **67**: 120-127 [PMID: 27742762 DOI: 10.1136/gutjnl-2016-312580]
 - 22 **Olson SH,** Satagopan J, Xu Y, Ling L, Leong S, Orlow I, Saldia A, Li P, Nunes P, Madonia V, Allen PJ, O'Reilly E, Pamer E, Kurtz RC. The oral microbiota in patients with pancreatic cancer, patients with IPMNs, and controls: a pilot study. *Cancer Causes Control* 2017; **28**: 959-969 [PMID: 28762074 DOI: 10.1007/s10552-017-0933-8]
 - 23 **Farrell JJ,** Zhang L, Zhou H, Chia D, Elashoff D, Akin D, Paster BJ, Joshipura K, Wong DT. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. *Gut* 2012; **61**: 582-588 [PMID: 21994333 DOI: 10.1136/gutjnl-2011-300784]
 - 24 **Michaud DS,** Izard J, Wilhelm-Benartzi CS, You DH, Grote VA, Tjønneland A, Dahm CC, Overvad K, Jenab M, Fedirko V, Boutron-Ruault MC, Clavel-Chapelon F, Racine A, Kaaks R, Boeing H, Foerster J, Trichopoulou A, Laggiou P, Trichopoulos D, Sacerdote C, Sieri S, Palli D, Tumino R, Panico S, Siersema PD, Peeters PH, Lund E, Barricarte A, Huerta JM, Molina-Montes E, Dorronsoro M, Quirós JR, Duell EJ, Ye W, Sund M, Lindkvist B, Johansen D, Khaw KT, Wareham N, Travis RC, Vineis P, Bueno-de-Mesquita HB, Riboli E. Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. *Gut* 2013; **62**: 1764-1770 [PMID: 22990306 DOI: 10.1136/gutjnl-2012-303006]
 - 25 **Maisonneuve P,** Lowenfels AB. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. *Int J Epidemiol* 2015; **44**: 186-198 [PMID: 25502106 DOI: 10.1093/ije/dyu240]
 - 26 **Wei MY,** Shi S, Liang C, Meng QC, Hua J, Zhang YY, Liu J, Zhang B, Xu J, Yu XJ. The microbiota and microbiome in pancreatic cancer: more influential than expected. *Mol Cancer* 2019; **18**: 97 [PMID: 31109338 DOI: 10.1186/s12943-019-1008-0]
 - 27 **Zhao Y,** Mao YF, Tang YS, Ni MZ, Liu QH, Wang Y, Feng Q, Peng JH, Hu YY. Altered oral microbiota in chronic hepatitis B patients with different tongue coatings. *World J Gastroenterol* 2018; **24**: 3448-3461 [PMID: 30122883 DOI: 10.3748/wjg.v24.i30.3448]
 - 28 **Hu J,** Han S, Chen Y, Ji Z. Variations of Tongue Coating Microbiota in Patients with Gastric Cancer. *Biomed Res Int* 2015; **2015**: 173729 [PMID: 26457297 DOI: 10.1155/2015/173729]
 - 29 **Kilian M,** Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, Tonetti MS, Wade WG, Zaura E. The oral microbiome - an update for oral healthcare professionals. *Br Dent J* 2016; **221**: 657-666 [PMID: 27857087 DOI: 10.1038/sj.bdj.2016.865]

- 30 **Dawes C**, Wong DTW. Role of Saliva and Salivary Diagnostics in the Advancement of Oral Health. *J Dent Res* 2019; **98**: 133-141 [PMID: [30782091](#) DOI: [10.1177/0022034518816961](#)]
- 31 **Johnson JS**, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, Leopold SR, Hanson BM, Agresta HO, Gerstein M, Sodergren E, Weinstock GM. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun* 2019; **10**: 5029 [PMID: [31695033](#) DOI: [10.1038/s41467-019-13036-1](#)]
- 32 **Caporaso JG**, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 2012; **6**: 1621-1624 [PMID: [22402401](#) DOI: [10.1038/ismej.2012.8](#)]
- 33 **Kozich JJ**, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* 2013; **79**: 5112-5120 [PMID: [23793624](#) DOI: [10.1128/AEM.01043-13](#)]
- 34 **Washington K**. 7th edition of the AJCC cancer staging manual: stomach. *Ann Surg Oncol* 2010; **17**: 3077-3079 [PMID: [20882416](#) DOI: [10.1245/s10434-010-1362-z](#)]
- 35 **Zhang J**, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 2014; **30**: 614-620 [PMID: [24142950](#) DOI: [10.1093/bioinformatics/btt593](#)]
- 36 **Caporaso JG**, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; **7**: 335-336 [PMID: [20383131](#) DOI: [10.1038/nmeth.f.303](#)]
- 37 **Edgar RC**, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011; **27**: 2194-2200 [PMID: [21700674](#) DOI: [10.1093/bioinformatics/btr381](#)]
- 38 **Edgar RC**. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013; **10**: 996-998 [PMID: [23955772](#) DOI: [10.1038/nmeth.2604](#)]
- 39 **Schbath S**, Prum B, de Turckheim E. Exceptional motifs in different Markov chain models for a statistical analysis of DNA sequences. *J Comput Biol* 1995; **2**: 417-437 [PMID: [8521272](#) DOI: [10.1089/cmb.1995.2.417](#)]
- 40 **Teeling H**, Meyerdierks A, Bauer M, Amann R, Glöckner FO. Application of tetranucleotide frequencies for the assignment of genomic fragments. *Environ Microbiol* 2004; **6**: 938-947 [PMID: [15305919](#) DOI: [10.1111/j.1462-2920.2004.00624.x](#)]
- 41 **Staley C**, Weingarden AR, Khoruts A, Sadowsky MJ. Interaction of gut microbiota with bile acid metabolism and its influence on disease states. *Appl Microbiol Biotechnol* 2017; **101**: 47-64 [PMID: [27888332](#) DOI: [10.1007/s00253-016-8006-6](#)]
- 42 **Ren Z**, Jiang J, Xie H, Li A, Lu H, Xu S, Zhou L, Zhang H, Cui G, Chen X, Liu Y, Wu L, Qin N, Sun R, Wang W, Li L, Wang W, Zheng S. Gut microbial profile analysis by MiSeq sequencing of pancreatic carcinoma patients in China. *Oncotarget* 2017; **8**: 95176-95191 [PMID: [29221120](#) DOI: [10.18632/oncotarget.18820](#)]
- 43 **Torres PJ**, Fletcher EM, Gibbons SM, Bouvet M, Doran KS, Kelley ST. Characterization of the salivary microbiome in patients with pancreatic cancer. *PeerJ* 2015; **3**: e1373 [PMID: [26587342](#) DOI: [10.7717/peerj.1373](#)]
- 44 **Tian D**, Mo SJ, Han LK, Cheng L, Huang H, Hao S, Guan YL, Jiang KY, Deng JY, Feng HH, Wen HY, Fu MY. Investigation of Dietary Factors and Esophageal Cancer Knowledge: Comparison of Rural Residents in High- and Low-incidence Areas. *Sci Rep* 2018; **8**: 4914 [PMID: [29559669](#) DOI: [10.1038/s41598-018-23251-3](#)]
- 45 **Chen YH**, Zou XN, Zheng TZ, Zhou Q, Qiu H, Chen YL, He M, Du J, Lei HK, Zhao P. High Spicy Food Intake and Risk of Cancer: A Meta-analysis of Case-control Studies. *Chin Med J (Engl)* 2017; **130**: 2241-2250 [PMID: [28875961](#) DOI: [10.4103/0366-6999.213968](#)]
- 46 **Mills S**, Stanton C, Lane JA, Smith GJ, Ross RP. Precision Nutrition and the Microbiome, Part I: Current State of the Science. *Nutrients* 2019; **11**: 923 [PMID: [31022973](#) DOI: [10.3390/nu11040923](#)]
- 47 **Bracci PM**. Oral Health and the Oral Microbiome in Pancreatic Cancer: An Overview of Epidemiological Studies. *Cancer J* 2017; **23**: 310-314 [PMID: [29189325](#) DOI: [10.1097/PPO.0000000000000287](#)]



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