Published in final edited form as:
Gut. 2012 April ; 61(4): 582–588. doi:10.1136/gutjnl-2011-300784.

Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer

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Abstract

Objective—The associations between oral diseases and increased risk of pancreatic cancer have been reported in several prospective cohort studies. In this study, we measured variations of salivary microbiota and evaluated their potential associations with pancreatic cancer and chronic pancreatitis.

Methods—This study was divided into three phases: (1) microbial profiling using the Human Oral Microbe Identification Microarray to investigate salivary microbiota variation between 10 resectable patients with pancreatic cancer and 10 matched healthy controls, (2) identification and verification of bacterial candidates by real-time quantitative PCR (qPCR) and (3) validation of bacterial candidates by qPCR on an independent cohort of 28 resectable pancreatic cancer, 28 matched healthy control and 27 chronic pancreatitis samples.

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Competing interests DTWW disclose ownership of intellectual property related to the saliva diagnostics field. The other authors disclosed no potential conflicts of interests.

Ethics approval UCLA IRB Committee. This study was approved by the UCLA Institutional Review Board.

Contributors LZ, JF and DW supervised all aspects of this study including study design, execution and data interpretation. LZ, JF and BP conducted the experiments and analysed experimental data. LZ, HZ, DE and BP contributed to data acquisition and data interpretation. JF provided human saliva samples. LZ, JF and KJ wrote the final manuscript. All authors reviewed the manuscript.

Provenance and peer review Not commissioned; externally peer reviewed.

Results—Comprehensive comparison of the salivary microbiota between patients with pancreatic cancer and healthy control subjects revealed a significant variation of salivary microflora. Thirty-one bacterial species/clusters were increased in the saliva of patients with pancreatic cancer (n=10) in comparison to those of the healthy controls (n=10), whereas 25 bacterial species/clusters were decreased. Two out of six bacterial candidates (*Neisseria elongata and Streptococcus mitis*) were validated using the independent samples, showing significant variation ($p<0.05$, qPCR) between patients with pancreatic cancer and controls (n=56). Additionally, two bacteria (*Granulicatella adiacens* and *S mitis*) showed significant variation ($p<0.05$, qPCR) between chronic pancreatitis samples and controls (n=55). The combination of two bacterial biomarkers (*N elongata* and *S mitis*) yielded a receiver operating characteristic plot area under the curve value of 0.90 (95% CI 0.78 to 0.96, $p<0.0001$) with a 96.4% sensitivity and 82.1% specificity in distinguishing patients with pancreatic cancer from healthy subjects.

Conclusions—The authors observed associations between variations of patients' salivary microbiota with pancreatic cancer and chronic pancreatitis. This report also provides proof of salivary microbiota as an informative source for discovering non-invasive biomarkers of systemic diseases.

INTRODUCTION

The poor outcome associated with pancreatic cancer stems from its propensity to rapidly disseminate to the lymphatic system and distant organs.[1–3] This aggressive biology, resistance to conventional and targeted therapeutic agents, and lack of biomarkers for early detection result in a 5-year survival rate of only 5% among patients diagnosed as having pancreatic cancer.[45] Around 15%–20% of patients have surgically resectable disease at the time of presentation, but only around 20% of these survive to 5 years.[3] Cigarette smoking is considered to be the only established modifiable risk factor for cancer of the pancreas, although some data also suggest an association of diabetes, obesity and insulin resistance with increased risk of developing pancreatic cancer. Additionally, the association of chronic pancreatitis with an extremely high risk of pancreatic cancer suggests that inflammation may be involved in the initiation and/or promotion of pancreatic cancer. Inflammation may enhance cellular proliferation and mutagenesis, reduce adaptation to oxidative stress, promote angiogenesis, inhibit apoptosis and increase secretion of inflammatory mediators.

The oral cavity is a large reservoir of bacteria composed of more than 700 species or phylotypes, of which approximately 35% have not been cultured.[6] Periodontitis is an inflammatory disease of the oral cavity due to bacteria. Several prospective studies have shown positive associations between oral inflammation (periodontitis) and an increased risk of pancreatic cancer.[7–9] Additional studies have also illustrated the potential role of periodontal disease as a risk factor for cardiovascular and cerebrovascular diseases,[10–12] preterm birth[13] and certain cancers.[14] In addition, bacteria have been implicated in the pathogenesis of pancreatic diseases including autoimmune pancreatitis and pancreatic ductal adenocarcinoma.[15–28]

Assessing bacterial flora composition appears to be of increasing importance in order to unravel bacterial role or to better understand flora changes upon disease onset or between different disease stages. The role of oral microbiota composition on chronic disease development and progression is important to evaluate, especially in the context of developing non-invasive diagnostic tests. A recently developed 16S rRNA-based oligonucleotide microarray, the Human Oral Microbe Identification Microarray (HOMIM) (http://mim.forsyth.org/index.html), made it possible to profile and monitor the oral microbial changes. HOMIM allows the simultaneous detection of about 300 of the most prevalent oral bacterial species, including those that cannot yet be grown in vitro.[29]

In this study, we performed a comprehensive comparison of the oral microbiota in human saliva from healthy control subjects and patients with either pancreatic cancer or chronic pancreatitis using HOMIM array and quantitative real-time PCR (qPCR). Furthermore, we evaluated the performance and potential translational utilities of salivary microbial signatures as an additional biomarker source for non-invasive detection of pancreatic cancer.

PATIENTS AND METHODS

Study design, populations and samples

This study was approved by the UCLA Institutional Review Board. The study design followed the principle of PRoBE design (prospective specimen collection before outcome ascertainment and retrospective blinded evaluation).[30] All subjects were recruited from the UCLA Medical Center prospectively. The saliva bank of pancreatic diseases at the UCLA Dental Research Institute had collected 283 saliva samples. Of these, 103 saliva pellet samples, including 38 pancreatic cancer, 38 matched healthy control and 27 chronic pancreatitis samples, were selected for the discovery and validation phase of this study. Inclusion criteria of disease patients consisted of confirmed diagnosis of pancreatic cancer confined to the pancreas, either resectable or borderline resectable (due to superior mesenteric vein or portal vein involvement), and chronic pancreatitis. Exclusion criteria included evidence of locally advanced pancreatic cancer due to arterial involvement or direct extension into adjacent organs, metastatic pancreatic cancer, chemotherapy or radiation therapy prior to saliva collection and a diagnosis of other malignancies within 5 years from the time of saliva collection. Written informed consents and questionnaire data sheets were obtained from all patients who agreed to serve as saliva donors. The information on individual characteristics, such as age, gender, ethnicity, smoking and drinking history (current or past), is presented in table 1. Healthy control individuals were matched for age, gender and ethnicity to the cancer group. Unstimulated saliva samples were consistently collected, stabilised and preserved as previously described.[31] The sample pellets were preserved at −80°C prior to assay.

This study consisted of a discovery phase and a verification phase, followed by an independent validation phase. The salivary microflora in the pellet samples from 10 patients with pancreatic cancer and 10 healthy control subjects were profiled using the HOMIM array.[32] Biomarkers identified from the microarray study were first verified using qPCR on the discovery sample set (10 cancers and 10 healthy controls). An independent sample set, including 28 patients with pancreatic cancer, 28 matched healthy controls and 27 patients with chronic pancreatitis, was used for the biomarker validation phase (figure 1). The validated biomarkers were evaluated within three levels of clinical discrimination categories: pancreatic cancer versus healthy control, pancreatic cancer versus chronic pancreatitis and pancreatic cancer versus combined non-cancer (healthy control + chronic pancreatitis). The purpose of including the patients with chronic pancreatitis in the validation is to evaluate whether the discovered biomarkers can also differentiate patients with cancer from patients with chronic pancreatitis, which has phenotypic overlap with early pancreatic cancer.

Salivary microflora profiling and microbial biomarker validation

Bacterial DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories Inc, Carlsbad, California, USA). PCR amplification was performed using 16S universal primers (forward primer, 5′-GAG AGT TTG ATY MTG GCT CAG-3′; reverse primer, 5′-GAA GGA GGT GWT CCA RCC GCA-3′),[33] followed by hybridisation to HOMIM array.[32] Selection of bacterial candidates was based on Present detection call and p value by Mann–Whitney U test (P call ≥20%, $p<0.05$). Quantities of bacterial species in the

original DNA samples were determined by qPCR. Specific primers were designed for the 16S rRNA genes of the bacterial biomarker candidates (table 2). qPCR was carried out in duplicate in reaction volumes of 10 μl using power SYBR-Green Master Mix (Applied Biosystems, Foster City, California, USA) for 15 min at 95°C for initial denaturing, followed by 40 cycles of 95°C for 30 s and 60°C for 30 s in the ABI 7900HT Fast Real Time PCR system (Applied Biosystems). Verified microbial biomarkers were then subjected to independent validation by qPCR using the validation samples.

Statistical analysis

Fisher's exact test and the Wilcoxon rank sum test were used to compare the distributions of the clinical characteristics across groups. The Wilcoxon test was also used to compare the biomarkers between groups. For each biomarker, we constructed the receiver operating characteristic (ROC) curve and computed the area under the curve (AUC) value by numerical integration. Next, the validated salivary biomarkers were fit into logistic regression models (separately for each group comparisons). The sensitivity and specificity for the biomarker combinations were estimated by identifying the cut-off point of the predicted probability that yielded the highest sum of sensitivity and specificity.[3435]

RESULTS

Significant variation of microflora profiles in the saliva of patients with pancreatic cancer versus matched healthy controls

Out of 410 oligonucleotide probes on HOMIM, 149 probes targeting different species or higher taxa showed detectable signals after hybridisation. In all, 56 predominant species or clusters were defined as showing a mean signal intensity >10% of the positive control signal (16S rRNA universal probe on the HOMIM array), of which 31 species/clusters were increased in the saliva pellets of patients with pancreatic cancer (n=10) in comparison to those of the healthy controls (n=10), whereas 25 species/clusters were decreased. Predominant species/clusters detected in the saliva pellets belonged to five different bacterial phyla, namely, the Firmicutes (eg, *Streptococcus* and *Granulicatella*), Proteobacteria (eg, *Campylobacter* and *Neisseria*), CFB group bacteria (eg, *Prevotella* and *Porphyromonas*) and Actinobacteria (eg, *Atopobium* and *Rothia*). Firmicutes was the most diverse phylum, comprising 34 different genus/clusters, and *Streptococcus* was the most diverse genus, comprising 13 different species/groups (figure 2).

Identification and independent validation of bacterial biomarkers

Based on the HOMIM data, 16 species/clusters showing significant difference between pancreatic cancer and matched healthy controls ($p<0.05$, n=20; mean signal intensity >20% of the positive control signal) were selected as biomarker candidates. These 16 species/clusters represented six different genera, including *Streptococcus* (3 species/groups), *Prevotella* (4 species/groups), *Campylobacter* (4 species/groups), *Granulicatella* (2 species), *Atopobium* (1 species) and *Neisseria* (2 species). qPCR was performed to verify the HOMIM array results. Using the original sample set of 10 pancreatic cancer samples and 10 matched healthy controls, 6 out of 16 species were confirmed by qPCR. All six microbial biomarker candidates showed significant differences between patients with pancreatic cancer and healthy controls ($p<0.05$, n=20). These candidates were then subjected to independent validation by qPCR (28 pancreatic cancer, 28 matched healthy controls and 27 chronic pancreatitis). Two microbial biomarkers (*N elongata* and *S mitis*) showed significant difference between patients with pancreatic cancer and healthy controls ($p<0.05$, n=56), yielding ROC-plot AUC values of 0.657 and 0.680, respectively (table 3). The levels of both bacterial markers were decreased in pancreatic cancer as shown by the results of qPCR, which were consistent with the results obtained by HOMIM array. Interestingly, the levels

of one increased species (*G adiacens*) and one decreased species (*S mitis*) were significantly different between pancreatic cancer and chronic pancreatitis ($p<0.05$, n=55). The levels of *G adiacens* and *S mitis* were also significantly different between pancreatic cancer (n=28) and non-cancer subjects (chronic pancreatitis and healthy controls, n=55) ($p<0.05$) (table 3).

Biomarker combination analysis

Logistic regression was used to evaluate different combinations of two biomarkers for three levels of clinical discrimination: pancreatic cancer versus healthy control, pancreatic cancer versus chronic pancreatitis and pancreatic cancer versus non-cancer (healthy control + chronic pancreatitis). For pancreatic cancer versus healthy control, the combination of two microbial biomarkers (*N elongata* and *S mitis*) yielded an ROC-plot AUC value of 0.90 (95% CI 0.78 to 0.96, $p<0.0001$) with 96.4% sensitivity and 82.1% specificity in distinguishing patients with pancreatic cancer from healthy subjects (figure 3A). For pancreatic cancer versus chronic pancreatitis, the combination of two microbial biomarkers (*G adiacens* and *S mitis*) yielded an ROC-plot AUC value of 0.70 (95% CI 0.56 to 0.81, $p=0.0047$) with 85.7% sensitivity and 55.6% specificity in distinguishing patients with pancreatic cancer from healthy subjects (figure 3B). For the discrimination of pancreatic cancer versus non-cancer, the combination of the same two microbial biomarkers as pancreatic cancer versus chronic pancreatitis (*G adiacens* and *S mitis*) yielded an ROC-plot AUC value of 0.68 (95% CI 0.57 to 0.78, $p=0.0063$) with 85.7% sensitivity and 52.7% specificity (figure 3C).

DISCUSSION

Our study is among the first systematic surveys profiling the microbiome in saliva samples of patients with pancreatic cancer or chronic pancreatitis. We applied the HOMIM array profiling technology to assess salivary microflora alterations in pancreatic cancer and chronic pancreatitis, and possible discriminatory salivary microbial biomarkers that can be validated for these systemic diseases. By addressing both questions, our profiling results and further prevalidation of detection biomarkers open new research directions supporting the idea of systemic inflammation contributing to pancreatic diseases and that saliva is a scientifically feasible and credible biomarker source for non-oral diseases. The early detection of cancer can significantly improve survival rates, especially for pancreatic cancer which, unlike some cancers such as colon cancer, has no clear symptoms or screening methods. Cancer detection tools need to be sufficiently non-invasive and inexpensive to allow widespread applicability. The harnessing of valuable disease-specific biomarkers using less invasive methods such as salivary microflora alterations supports this concept.

The HOMIM profiling of microflora in saliva revealed that microbial composition shifts significantly between patients with pancreatic cancer and healthy controls. The validated bacterial signatures discovered in our study can be linked to pancreatic cancer in multiple aspects. Recent prospective studies showed associations between periodontal disease/tooth loss and an increased risk of pancreatic cancer.[7–9] The oral cavity is a large reservoir of bacteria composed of more than 700 species or phylotypes, of which approximately 35% have not been cultured.[6] The study of oral bacteria extends beyond the focus of oral disease to systemic diseases. Several studies have illustrated the potential role of periodontal disease as a risk factor for cardiovascular and cerebrovascular diseases,[10–12] preterm birth[13] and certain cancers.[14] Additionally, researchers have found that certain bacteria or variation of the microbiota diversity is associated with atheromas,[36] preterm birth, low birth weight[37] and human cancers.[38–44] *P gingivalis* is associated with periodontal disease and has been shown to accelerate atheroma deposition in animal models[45] by activating host innate immune responses associated with atherosclerosis. *P gingivalis*, *Actinobacillus actinomycetemcomitans* and *Treponema denticola* were detected in atheromatous plaques of

humans with atherosclerosis.[46–48] Serum antibodies to *P gingivalis* have also been associated with elevated risk of coronary heart disease.[4950]

In our study, the levels of *N elongata* and *S mitis* were significantly decreased in patients with pancreatic cancer relative to healthy controls. The level of *G adiacens* was significantly elevated in patients with pancreatic cancer relative to all non-cancer subjects. These results validate an association between *N elongata* and *G adiacens* with periodontal disease.[51–53] In addition, *G adiacens* isolates have been detected in bacteraemia/septicaemia in patients with infective endocarditis/atheroma and in primary bacteraemia.[5455] Together, these observations indicate that *G adiacens*, often considered opportunistic pathogens, may be associated with systemic inflammations. An elevation of *G adiacens* may be related to a decrease in *S mitis* levels. It has been indicated that *S mitis* plays a protective role against the adhesion of cariogenic bacteria[56] and the loss of colonisation by *Streptococcus* spp. may contribute to aggressive periodontitis.[57]

Bacteria have been implicated in the pathogenesis of pancreatic diseases including autoimmune pancreatitis and pancreatic ductal adenocarcinoma. A role of *Helicobacter pylori* infection in the pathogenesis of autoimmune pancreatitis has been suggested.[15–19] In a recent study of patients with autoimmune pancreatitis, the peptide $AIP_{1–7}$, which is homologous to amino acid sequence of PBP of *H pylori*, was identified from the majority of patients with autoimmune pancreatitis.[22] However, this peptide was also identified in a small number of patients with pancreatic adenocarcinoma. *H pylori* was recently isolated from a human cirrhotic liver,[58] suggesting that microorganisms may infect the pancreas and associated tissues by ascending gastric infections or retrograde transfer from the small bowel.[2021] Other data support an association between *H pylori* colonisation and pancreatic cancer.[23–28] Whether a variation in bacterial abundance is a causative factor for cancer carcinogenesis or a derivational reflection of cancer onset due to the change of oral niches needs to be further explored in longitudinal studies. Meanwhile, the link between chronic inflammation and the development of pancreatic ductal adenocarcinoma is becoming clearer. Chronic pancreatitis is now considered a risk factor for the development of pancreatic cancer.[59]

Taken together, these data suggest that the association between variations in oral microbiota and pancreatic disease may likely be causative rather than reactive. However, this study does not explore changes in oral flora after the surgical resection of pancreatic cancer to address this question. Whether and how local oral infection without bacteria entering the blood stream could potentially result in systemic diseases such as chronic inflammation or neoplasia are currently under active investigation. For example, the immune system recognises the presence of bacterial pathogens through the expression of a family of membrane receptors known as Toll-like receptors (TLRs). Lipopolysaccharide (LPS) on bacteria is specifically recognised by TLR4. Recognition of microbial components by TLRs initiates signal transduction pathways, which upregulate genes involved in innate immune responses and further instruct development of antigen-specific acquired immunity. These pathways are further regulated by TLR domain-containing adaptors such as TIRAP/Mal, TRIF, TRAM and MyD88.

In addition to its effects on immune cells, LPS can also act on certain epithelial cells including cancer cells and promote their phenotypic transformation. For example, nuclear factor-κB is a transcriptional factor that controls the expression of numerous genes involved in inflammation and genes encoding growth factors and cellular invasion-related molecules.[6061] It is constitutively activated in several types of cancers, including pancreatic cancer, and can be induced by several types of inflammatory cytokines including interleukin-1b in pancreatic cancer.[62–65] In addition, it has also been shown that LPS,

released from the surface of the cell membrane of gram-negative bacteria, promotes nuclear factor-κB activation in pancreatic cancer, providing a possible link between inflammation and cancer development and progression.[66] Given the limited understanding of pancreatic cancer aetiology, further investigation into the role of bacterial associated systemic inflammation in pancreatic carcinogenesis is warranted. Finally, additional risk factors for pancreatic cancer should be further researched, including obesity and type 2 diabetes that are associated with inflammation, gastric acidity and high nitrosamines which are caused by nitrate-reducing bacteria.[67]

Screening for pancreatic cancer carries two major challenges. First is the need to detect early small pancreatic cancers confined to the pancreas or even precancerous stages, also known as PanIN stages. The second is in the ability to differentiate pancreatic cancer from the phenotypically similar chronic pancreatitis, a benign pancreatic disease. The determination of specific profiles of microflora changes in specific cancer types is important because it is possible that the different cancers may have overlapping signatures. We have evaluated the specificity of the validated microbial biomarkers against another HOMIM profiling study that had been performed in our laboratory using lung cancer. None of the bacterial biomarkers validated in this study was significantly altered in the microflora profile of lung cancer. This cross-disease comparison indicated that the validated microbial biomarkers in saliva are likely to be specific for pancreatic cancer detection. This is a discovery study with an initial validation of the statistically significant markers. Hence, in the absence of developing and testing of a prediction panel, this is a prevalidation study, and the biomarker model will need to be tested in an independent clinically relevant cohort in order to be ‘validated’.

This study has some limitations. Primarily, the cross-sectional nature of the study does not enable us to understand the mechanisms and time sequence of the associations. Additional large cohort studies are needed to establish the time sequence and evaluate changes in the oral microbiome from early to later stages of pancreatic cancer. Furthermore, the small sample size does not allow for subgroup analysis to assess whether the associations are consistent across different populations defined by factors such as race, ethnicity and smoking status. For example, none of the patients in our discovery group and very few of the patients in our validation group had a history of smoking. However, smoking is clearly a risk factor for pancreatic cancer, and cigarettes themselves may represent a source for exposure to a wide range of potentially pathogenic microbes.[68] However, this does not detract from the potential value of these markers for diagnostic testing, which is currently being evaluated in a nested, case-controlled study using a population-based cohort.

Acknowledgments

We thank Ali Ammar for collecting and processing saliva samples. We also thank Susan Boches for technical assistance.

Funding Funding support was provided by the National Institute of Health (RO1DE017170 and R21CA126733).

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Significance of this study What is already known about this subject?

What is already known about this subject?

▶ Previous studies suggest a link between oral disease, especially periodontitis, and systemic disease, including pancreatic cancer.

▶ Chronic inflammation of the pancreas is associated with an increased risk of developing pancreatic cancer.

▶ Bacteria have been implicated in the pathogenesis of autoimmune pancreatitis and pancreatic ductal adenocarcinoma.

What are the new findings?

▶ First study showing how variation of oral microbiota diversity is associated with pancreatic cancer.

▶ Oral microbiota may function as non-invasive diagnostic biomarkers of pancreatic disease.

How it might impact on clinical practice in the foreseeable future?

▶ Although unclear if the association is causative or reactive, this research may allow for intervention in altering the natural history of pancreatic cancer pathogenesis, especially in high-risk populations, through manipulation of the oral flora.

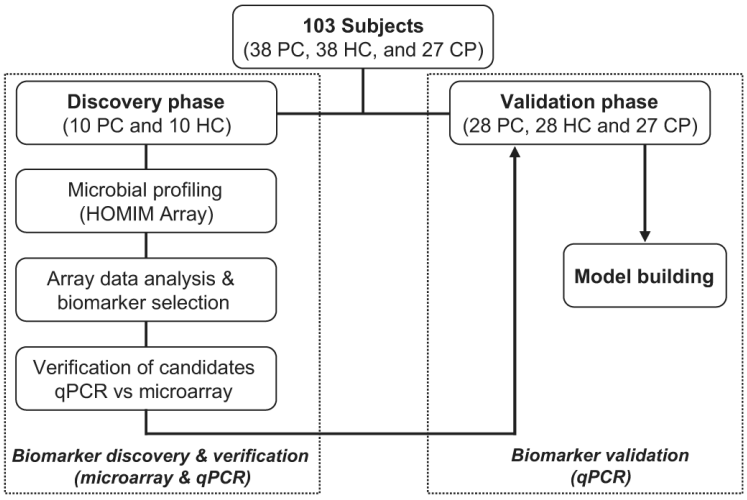

Figure 1.

chematic of the strategy used for the discovery (including verification) and validation of salivary bacterial biomarkers. PC, pancreatic cancer; HC, healthy control; CP, chronic pancreatitis.

Figure 2.

16S rRNA gene-based phylogenetic tree of 56 varied clusters/genera between patients with pancreatic cancer and healthy controls. Thirty-one clusters/species increased in the saliva of pancreatic cancer patients were marked with triangles. The phylogenetic tree was inferred by a minimum evolution analysis of 16S rRNA sequences.

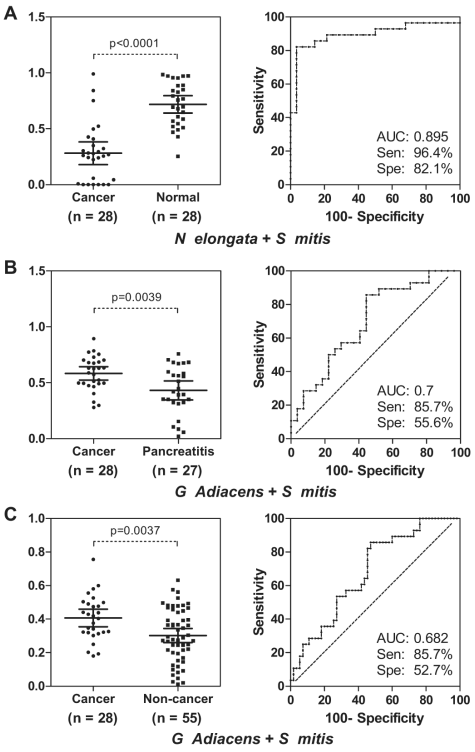

Figure 3.

nteractive dot diagram analysis and receiver operating characteristic (ROC) curve analysis for the predictive power of combined salivary bacterial biomarkers. The validated biomarkers were evaluated by logistic regression within three levels of clinical discrimination categories: pancreatic cancer versus healthy control (A), pancreatic cancer versus chronic pancreatitis (B) and pancreatic cancer versus non-cancer (healthy control + chronic pancreatitis) (C). The sensitivity and specificity for each model were obtained by identifying the cut-off point in the predicted probabilities from the logistic regression that maximised the sum of the sensitivity plus specificity. In general, these cut-off points correspond well with the proportion of patients with cancer evaluated in each model.

Table 1

Demographic information of subjects in the discovery and validation phases

		Discovery phase			Validation phase			
Demographic variable	**Characteristics**	**Pancreatic cancer (n=10)**	**Healthy control (n=10)**	**p Value**	**Pancreatic cancer (n=28)**	**Healthy control (n=28)**	**Chronic pancreatitis (n=27)**	**p Value***
Age (years)	Mean±SD	66.5±8.9	66.4±10.5	0.98	69.9±11.6	65.1±10.1	57.8±11.0	0.10
Sex	Male	8	8	1	17	18	15	1
	Female	2	2		11	10	12	
Ethnicity	Caucasian	10	10	1	19	19	18	1
	African American	0	0		2	2	2	
	Asian	0	0		4	4	3	
	Hispanic	0	0		3	3	4	
Smoking		0	0	1	5	2	11	0.23
Drinking		0	0	1	2	3	2	0.65

* For the validation samples, p value was calculated between pancreatic cancer and healthy control

Table 2

16S rRNA primers for the six verified bacterial biomarkers

Strains	16S rRNA primer sequences (59′–39′)
Atopobium parvulum	F: CGAATACTTCGAGACTTCCGCA
	R: CAATCTGGCTGGTCGGTCTC
Granulicatella adiacens	F: CAAGCTTCTGCTGATGGATGGA
	R: CTCAGGTCGGCTATGCATCAC
Neisseria elongata	F: CATGCCGCGTGTCTGAAGAA
	R: CCGTCAGCAGAAACGGGTATT
Prevotella nigrescens	F: GACGGCATCCGATATGAAACA
	R: TGCACGCTACTTGGCTGGT
Streptococcus australis	F: AGAACGCTGAAGGAAGGAGCTT
	R: CAATAGTTATCCCCCGCTACCA
Streptococcus mitis	F: CCGCATAATAGCAGTTRTTGCA
	R: ACAACGCAGGTCCATCTGGTA

Table 3

Quantitative PCR results of six bacterial biomarkers using the validation samples (n=83)

	Pancreatic cancer versus healthy control			Pancreatic cancer versus chronic pancreatitis			Pancreatic cancer versus non-cancer		
Strain	**p Value**	**AUC**	**Fold change**	**p Value**	**AUC**	**Fold change**	**p Value**	**AUC**	**Fold change**
Atopobium parvulum	0.84	0.55		0.11	0.59		0.31	0.59	
Granulicatella adiacens	0.17	0.58		0.04	0.61	3.50 (+)	0.02	0.64	2.30 (+)
Neisseria elongata	0.02	0.66		2.84 (−)	0.77	0.52	0.10	0.59	
Prevotella nigrescens	0.09	0.60		0.15	0.63		0.82	0.52	
Streptococcus australis	0.29	0.55		0.12	0.61		0.65	0.53	
Streptococcus mitis	0.02	0.68	2.45 (−)	0.01	0.69	2.06 (−)	0.002	0.68	2.25 (−)

qPCR was performed to validate the HOMIM microarray findings of an independent clinical cohort, including saliva from 28 patients with pancreatic cancer, 28 healthy control subjects and 27 patients with chronic pancreatitis.

Wilcoxon test: validated if $p<0.05$. (+): increased risk in pancreatic cancer; (−): decreased risk in pancreatic cancer.

Fold change is only shown for the validated biomarkers.

AUC, area under the curve.