HHS Public Access

Author manuscript

Int J Cancer. Author manuscript; available in PMC 2023 March 15.

Published in final edited form as:

Int J Cancer. 2022 March 15; 150(6): 928–940. doi:10.1002/ijc.33848.

Oral and gastric microbiome in relation to gastric intestinal metaplasia

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Abstract

Evidence suggests that *Helicobacter pylori* plays a role in gastric cancer initiation. However, epidemiologic studies on the specific role of other bacteria in the development of gastric cancer are lacking. We conducted a case-control study of 89 cases with gastric intestinal metaplasia (IM) and 89 matched controls who underwent upper gastrointestinal endoscopy at three sites

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Data Availability Statement

Metagenomic sequencing data generated in this study is available in the database of Genotypes and Phenotypes (dbGaP) with accession code phs002566.v1.p1. Investigators who would like to access individual-level study data should submit an application to the NIH Data Access Committee (DAC) to request the datasets. Further information is available from the corresponding author upon request.

Ethics Statement

This study was approved by the NYU institutional review board and all participants provided written informed consent.

Conflict of interest: The authors disclose no conflicts.

affiliated with NYU Langone Health. We performed shotgun metagenomic sequencing using oral wash samples from 89 case-control pairs and antral mucosal brushing samples from 55 case-control pairs. We examined the associations of relative abundances of bacterial taxa and functional pathways with IM using conditional logistic regression with and without elastic-net penalty. Compared with controls, oral species *Peptostreptococcus stomatis*, *Johnsonella ignava*, *Neisseria elongata*, and *Neisseria flavescens* were enriched in cases (odds ratios [ORs] = 1.29–1.50, $P = 0.004–0.01$) while *Lactobacillus gasseri*, *Streptococcus mutans*, *S. parasanguinis*, and *S. sanguinis* were under-represented (ORs = 0.66–0.76, $P = 0.006–0.042$) in cases. Species *J. ignava* and *Filifactor alocis* in the gastric microbiota were enriched (ORs = 3.27 and 1.43, $P = 0.005$ and 0.035, respectively), while *S. mutans*, *S. parasanguinis*, and *S. sanguinis* were under-represented (ORs = 0.61–0.75, $P = 0.024–0.046$), in cases compared with controls. The lipopolysaccharide and ubiquinol biosynthesis pathways were more abundant in IM, while the sugar degradation pathways were under-represented in IM. The findings suggest potential roles of certain oral and gastric microbiota, which are correlated with regulation of pathways associated with inflammation, in the development of gastric precancerous lesions.

Keywords

oral microbiome; gastric microbiome; gastric intestinal metaplasia; case-control study; shotgun metagenomic sequencing

Introduction

Gastric cancer (GC) is the fifth most common cancer and the fourth leading cause of cancer deaths worldwide, with over 1 million new cases and 769,000 deaths in 2020[1]. Histologically the major type of GC is the intestinal type of non-cardia GC that occurs via a predictable progression from chronic gastritis to atrophic gastritis, intestinal metaplasia (IM), dysplasia, and gastric adenocarcinoma[2]. *Helicobacter pylori* (*H. pylori*), which causes mucosal inflammation and progressive destruction of the hydrochloric acid-secreting glands of the stomach, plays a crucial role in the initial steps of the carcinogenesis cascade[3]. However, only 3% of *H. pylori*+ individuals develop GC[4], implying that there are other risk factors. In addition, colonization of *H. pylori* decreases (and is eventually lost) under achlorhydric condition in the precancerous and cancerous lesions[5,6]. The loss of *H. pylori* and impairment of acid secretion in later steps of carcinogenesis may allow the stomach to be colonized by oral and intestinal microbes that are not ordinarily present under its normal acidic condition.

Oral health conditions and selected periodontal pathogens have been related to GC and precancerous lesions[7,8], suggesting that the oral microbiome may also contribute to the onset and progression of GC. In addition, oral bacteria can reach the stomach through swallowed saliva, nutrients, and drinks and change its microbiota and possibly immune defenses[9]. However, studies investigating the role of specific oral bacteria in GC development are limited.

Mechanistic studies have suggested that the presence of other bacteria following *H. pylori* infection promotes GC development[10,11]. Several studies also have provided evidence that

gastric microbiota other than *H. pylori* is altered during the progression from a healthy gastric mucosa to GC[12–14]. However, existing studies were mostly based on genetic analysis of 16S rRNA which is limited in characterizing the underlying microbial species and genes that might be involved in carcinogenesis[15]. Shotgun metagenomic sequencing provides higher-level taxonomic and functional resolution by targeting the entire genomic content of a sample[16].

In the present study, we performed shotgun metagenomics on the oral and gastric microbial communities to comprehensively evaluate the compositional and functional changes associated with gastric IM, an established precancerous lesion for GC with shared risk factors[17].

Materials and Methods

Study population and sample collection

We invited individuals who were scheduled for upper gastrointestinal endoscopy for clinically indicated reasons at three sites affiliated with NYU Langone Health, including Bellevue Hospital Center, a private group practice at New York City, and NYU Langone Gastroenterology Associates between 2009–2019, following protocols similar to those used in the Bellevue Hospital Center[7,8]. Exclusion criteria include: 1) prior gastric surgery, 2) use of antimicrobial agents within the prior 2 months, 3) current use of anticoagulants, 4) active gastrointestinal bleeding, and 5) having had or suspected to have esophageal varices. Information on demographic and lifestyle factors was collected with structured questionnaires administered by a trained interviewer. We collected biopsies from the antrum, cardia, corpus, and fundus of the stomach for standard pathology review, blinded to questionnaire data.

We collected stimulated saliva samples from participants recruited between 2009 and 2011, and oral wash samples from those recruited in later years. Briefly, participants were asked to chew a piece of paraffin wax to stimulate saliva production and to gently expectorate 2–5 mL of saliva directly into a sterile sample collection tube, on ice[8]. Participants recruited in later years were asked to swish with 10 ml saline and to expectorate into a sterile sample collection tube. Both saliva and oral wash samples were vortex-mixed thoroughly, immediately placed into a container with ice, transferred to the laboratory within 1 h, and stored at −80°C for further processing. In 2016, we started to collect a mucosal brushing sample from the gastric antrum during the endoscopy, using an endoscopic cytology brush.

A total of 1198 eligible individuals were approached, and 675 (56%) were recruited, including 348 from the Bellevue Hospital Center, 246 from the private group practice, and 81 from the NYU Langone Gastroenterology Associates. The most common reasons for declining participation and comparison on the distribution of sex, age, and race between participants and non-participants are described in detail in the Supplementary Material. We recruited a total of 125 cases with newly diagnosed IM in the gastric antrum or body/fundus and 550 non-cases with normal gastric histology or superficial gastritis without any atrophy, according to the pathologic review results. Saliva or oral wash samples were collected from

82%, serum samples from 91%, of the participants recruited. Antral brushing samples were collected from 91.5% of those recruited since 2016.

For the 106 IM cases with saliva/oral wash samples, we selected controls that were individually matched on sex, recruitment site, and sample types (stimulated saliva or oral wash), and frequency-matched on age categories (<35, 35–49, 50–64, 65+ years) and recruitment year (± 3 years). Based on the matching criteria, we finalized the selection of 89 IM cases (antrum: n = 84, body/fundus: n = 5) and 89 controls, and antral brushing samples were available for 55 case-control pairs.

H. pylori and CagA seropositivity measurements

Serum samples were available for 170 subjects in the 89 case-control pairs for determination of *H. pylori*/cytotoxin-associated gene A (CagA) seropositivity using enzyme-linked immunosorbent assay, as described[18,19] with slight modification. Briefly, IgG antibodies to *H. pylori* whole cell antigens or to a recombinant CagA fragment were tested in duplicate and in parallel with known positive controls. The cutoff for *H. pylori* and CagA positivity was an optical density ratio > 0.6 and > 0.3, respectively.

Shotgun metagenomic sequencing

Bacterial DNA from the 54 saliva samples was isolated using the MasterPure DNA purification kit (Epicentre, Madison, WI). DNA from the 124 oral wash and 110 antral mucosal brushing samples was extracted using the DNeasy PowerLyzer PowerSoil kit (Qiagen, Germantown, MD). Metagenomic DNA samples were quantified using the Qubit dsDNA HS Assay Kit with a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA) and normalized to a concentration of 5 ng/μl. Shotgun sequencing libraries were constructed using the automated KAPA HyperPrep kit (Roche, Wilmington, MA) and sequenced on an Illumina NovaSeq 6000 System (Illumina, San Diego, LA) at 2×100 bp paired-end with 96 samples pooled in each run by the NYU Genomics Core.

Sequencing data processing

Raw sequencing reads were demultiplexed, and Trimmomatic (v0.36) was used to trim low-quality sequences. Retained reads were first aligned to the human genome (GRCh38) by Bowtie2 (v_2.2.9) and the mapped reads were filtered out. Details on metagenomic sequencing data were summarized in Supplementary Methods and Supplementary Table S1, S2 and S3. The non-human reads were further used for taxonomic classification by Kraken2 (v_ 2.0.8-beta)[20] against the eHOMD reference database (http://www.homd.org/), which reduces a shotgun metagenome to a table of relative abundance for each taxon at the level from phylum to species. Gene family and pathway abundance of each sample was determined directly from the processed reads using HUMAnN2 (v_0.11.1)[21] with default parameters. HUMAnN2 maps reads to functionally annotated microbial species genomes and performs translated search to align non-human reads to UniRef90 protein clusters (gene families)[22]. Gene families are then grouped into MetaCyc pathways using MinPath. For a lower level of resolution, we also regrouped UniRef90 gene families into Gene Ontology (GO) categories using the “humann2_regroup_table” script. We removed

unintegrated/unmapped/unknown/ungrouped pathways, categories, and gene families prior to calculating relative abundance, using the "humann2_renorm_table" script.

Statistical analyses

α and β diversity—α-diversity (within-subject diversity) was assessed using richness (observed number of species and Chao1) and diversity (the Shannon and Simpson's diversity index) metrics. Species level read counts were rarefied to the 90% of the minimum sample depth in the dataset (611,771 and 33,287 reads per sample in the oral and gastric microbiota, respectively). We used conditional logistic regression models using matched sets as strata to determine whether α-diversity was associated with gastric IM, adjusting for age and race.

β-diversity (between-subject diversity) was assessed using the Jensen-Shannon Divergence (JSD) on the species level. Principal coordinate analysis (PCoA) was used for visualization. Non-parametric permutational multivariate analysis of variance (PERMANOVA; 'adonis' function, 'vegan' package, R) with 9999 permutations was used to test the association between community-level bacterial composition and gastric IM, using matched sets as strata and adjusting for age and race.

Identification of taxa associated with gastric IM

We applied the centered log-ratio (clr) transformation[23] to the relative abundance of taxa at each level (e.g. phylum, class, etc.) after adding a pseudo relative abundance (the minimal relative abundance in the whole dataset at each level), in order to remove compositional constraints of sequencing. Additionally, we excluded rare taxa with mean relative abundance ≤ 0.01%. These exclusions resulted in inclusion of 9 phyla, 18 classes, 29 orders, 40 families, 65 genera, and 265 species for the oral microbiota, and 9 phyla, 18 classes, 29 orders, 44 families, 78 genera, and 297 species for the gastric microbiota in the analyses. We fit standard conditional logistic regression models to assess the association between the relative abundance of each taxon and gastric IM, using matched pairs as strata and adjusting for age and race. Additional adjustment for ever smoking, ever drinking, and *H. pylori*/CagA status did not impact effect estimates (data not shown). *P* values from these models were adjusted for the false discovery rate (FDR)[24] at each taxonomic level (i.e., genus, species) separately. Taxa associated with gastric IM were also assessed using conditional logistic regression with elastic-net penalties, to allow selection of a set of representative taxa while considering their correlations[25]. We conducted leave-one-out cross-validation using the "cv.clogitL1" function in the clogitL1 R package[26] and covariates (age and race) were also penalized in each model. We also conducted stratified analyses to assess whether the association between bacterial taxa and gastric IM differed by seropositivity of *H. pylori*. We further conducted analyses separately for oral wash samples (62 pairs, n = 124) and saliva samples (27 pairs, n = 54), and combined the results using fixed-effect meta-analyses ('metagen' function, 'meta' package, R). Additional sensitivity analyses were conducted to exclude cases of IM in the gastric body and/or fundus (n = 5 and n = 3 for oral and gastric microbiome analyses, respectively) and their paired controls.

Identification of functional pathways and GO categories associated with gastric IM

We assessed associations of metagenomic functional pathways and GO categories' relative abundance with gastric IM using standard conditional logistic regression models as described above. Relative abundance of pathways and GO categories was clr transformed. We only considered pathways and GO categories with mean relative abundance > 0.03%[27] and largely explained by known species (< 25% unclassified in > 75% of individuals according to the species-specific pathway data)[28]. For GO categories analyses, in addition to the criteria above, we further focused on GO categories with variance > the 25th percentile of variances[27]. This resulted in inclusion of 111 and 69 pathways, as well as 266 and 143 GO categories in the oral and gastric microbiome, respectively. We presented pathways and GO categories related to gastric IM with a nominal $P < 0.05$. We also examined to what extent these pathways and GO categories were driven by specific species by calculating Spearman's correlation coefficients between pathway/GO relative abundance and species relative abundance and using heatmap for visualization.

Results

Participant characteristics

Compared with controls, cases were more likely to be older or Asians (Table 1, all $P < 0.05$), and more likely to carry antibody to *H. pylori*, particularly the CagA-positive strain ($P = 0.06$). There were no significant differences by case status in terms of educational attainment, smoking status and intensity, and alcohol consumption (all $P > 0.05$).

Overall oral and gastric microbiota diversity in relation to gastric IM

Cases did not differ significantly from matched controls in oral and gastric α-diversity (all $P > 0.05$; data not shown). We found significant differences in overall oral microbial composition between cases and controls ($P < 0.01$; Supplementary Figure S1A); overall gastric microbial composition was marginally related to gastric IM ($P = 0.067$; Supplementary Figure S1B).

Oral and gastric taxa associated with gastric IM

We identified 2 phyla, 6 classes, 8 orders, 9 families, 10 genera, and 50 species in the oral microbiota that were nominally associated with gastric IM (FDR-adjusted $P = 0.07$–0.26, Supplementary Table S4). In leave-one-out cross-validated elastic-net conditional logistic regression models for gastric IM, 2 phyla, 6 classes, 2 orders, 4 families, 5 genera, and 10 species were selected as the most important oral bacterial taxa associated with gastric IM (Table 2). *Oribacterium sinus*, *Peptostreptococcus stomatis*, *Neisseria elongata*, *N. flavescens*, and *SR1 bacterium oral taxon 874* were positively related to gastric IM (ORs = 1.24–1.43, $P = 0.004$–0.03). The higher-rank taxa of these species had consistent associations with gastric IM and were also retained in the model (Table 2). For instance, in the Clostridia-Clostridiales-*Peptostreptococcaceae*-*Peptostreptococcus*-*P. stomatis* lineage, Clostridia (class), *Peptostreptococcaceae (family), Peptostreptococcus (genus),* and *P. stomatis (species)* were all selected in the model. The procedure also selected several species with their relative abundance inversely associated with gastric IM. These

species included *Lactobacillus gasseri*, *Streptococcus sanguinis*, *Shuttleworthia satelles*, *Achromobacter xylosoxidans*, and *Kingella oralis* (odds ratios [ORs] = 0.66–0.80, P = 0.002–0.046). The higher-rank taxa to which *L. gasseri* belongs, such as Bacilli (class) and *Lactobacillus (genus)* were also selected by the model. The association patterns of the aforementioned species with gastric IM remained similar in meta-analyses of participants with oral wash and saliva samples separately (Supplementary Table S5).

We identified 1 class, 1 order, 2 families, 2 genera, and 17 species in the gastric microbiota that were nominally associated with gastric IM, including a highly significant species *Johnsonella ignava* (P = 0.005; Supplementary Table S6). In leave-one-out cross-validated elastic-net conditional logistic regression models, 9 taxa were selected as the most important taxa related to gastric IM (Table 3). Specifically, higher relative abundance of species *Actinomyces sp. oral taxon 448*, *Prevotella baroniae*, *Filifactor alocis*, *Veillonella sp. oral taxon 780*, and *Leptotrichia goodfellowii* was associated with higher odds of gastric IM (ORs = 1.42–1.67, P = 0.015–0.04) while higher relative abundance of *L. gasseri* and *S. mutans* was related to lower odds of gastric IM (ORs = 0.75 and 0.61, P = 0.046 and 0.024, respectively). Genus *Filifactor* to which *F. alocis* belongs was also selected with consistent association with gastric IM.

Stratified analyses of the associations between the aforementioned species and gastric IM by serum *H. pylori* status (Supplementary Table S7) indicated that many of the associations were stronger among those tested positive for *H. pylori* or CagA antibodies. The associations between the aforementioned species and gastric IM did not materially change with exclusion of cases of IM in the gastric body and/or fundus (Supplementary Table S8).

Several taxa in both the oral and gastric microbiota showed consistent associations with gastric IM (Figure 1). These included class Bacilli and species *L. gasseri*, *S. mutans*, *S. parasanguinis*, and *S. sanguinis*, that were associated with lower odds of gastric IM, as well as species *J. ignava*, that was associated with higher odds of gastric IM. In addition, *P. stomatis*, which was positively related to gastric IM in the oral data, was marginally associated with gastric IM in the gastric data (P = 0.058).

Oral and gastric functional pathways and GO categories associated with gastric IM

We identified 16 pathways in the oral microbiome that were associated with gastric IM at the nominal level (P = 0.002–0.05) (Figure 2A). Some of the IM-enriched pathways were involved in lipopolysaccharide (LPS) biosynthesis (PWY0–1241: ADP-L-glycero-β-D-manno-heptose biosynthesis, NAGLIPASYN-PWY: lipid IVA biosynthesis) and ubiquinol biosynthesis (PWY-5855/5856/5857/6708: ubiquinol-7/9/10/8 biosynthesis (prokaryotic)). Those under-represented pathways in IM were mainly involved in sugar degradation (PWY-5384: sucrose degradation IV (sucrose phosphorylase), LACTOSECAT-PWY: lactose and galactose degradation I).

We estimated pair-wise correlations of the relative abundance between the selected species (Table 2) and the IM-associated pathways that they contributed to (Figure 2B). IM-enriched pathways tended to be positively correlated with IM-enriched species (*N. elongata* and *N. flavescens*). Pathways under-represented in IM were positively correlated

with protective species (*L. gasseri*, *S. mutans*, *S. sanguinis*, and *S. parasanguinis*). Average contributions by pathway-correlated oral species to overall pathway abundances were shown in Supplementary Figure S2.

We identified 15 pathways in the gastric microbiome that were associated with gastric IM at the nominal level ($P = 0.003–0.05$) (Figure 3A). Several of these pathways were related to sugar degradation (LACTOSECAT-PWY: lactose and galactose degradation I, PWY66–422: D-galactose degradation V (Leloir pathway), PWY-6317: galactose degradation I (Leloir pathway)). Most of the under-represented pathways in IM were positively associated with under-represented species (*S. parasanguinis* and *S. sanguinis*) (Figure 3B). Average contributions by pathway-correlated gastric species to overall pathway abundances were shown in Supplementary Figure S3.

Associations between oral and gastric GO categories and gastric IM (Supplementary Tables S9 and S10) largely corresponded to the associations between functional pathways and IM (details in the Supplementary Materials). Correlations between IM-associated oral and gastric species and GO categories are shown in Supplementary Figure S4 and S5.

Discussion

In this study of oral and gastric microbiome and gastric premalignant lesions (IM), we identified species related to periodontal disease (*P. stomatis*, *J. ignava*, *F. alocis*)[29,30] and opportunistic pathogens (*N. elongata*, *N. flavescens*) that were enriched in gastric IM, as well as probiotic species (*L. gasseri)* and commensals (*S. mutans*, *S. parasanguinis*, *S. sanguinis*) that were under-represented in gastric IM. Several species (*J. ignava*, *L. gasseri*, *S. mutans*, *S. parasanguinis*, *S. sanguinis*) in both the oral and gastric microbiota were consistently associated with gastric IM. Further, we identified metagenomic functions as potential mechanism by which these bacteria influence disease risk, including via LPS and ubiquinol biosynthesis and sugar degradation.

Previous prospective studies have reported positive associations of tooth loss and periodontal disease with gastric cancer risk[31]. However, studies investigating specific periodontal pathogens are limited[8,32]. In our previous study with 37 cases of gastric precancerous lesions, we observed that DNA levels of periodontal pathogen *Aggregatibacter actinomycetemcomitans* was related to a non-significant elevated OR of gastric precancerous lesions (OR: 1.36, $P = 0.17$)[8]. Using metagenomic sequencing in the present study, we also observed a positive albeit non-significant association between *A. actinomycetemcomitans* and gastric IM (OR: 1.22; $P = 0.088$) which was stronger in those carrying *H. pylori* antibodies (OR: 2.69; $P = 0.002$). In addition, we observed positive associations of several newly appreciated species related to periodontal disease (*P. stomatis*, *J. ignava*, and *F. alocis*)[29,30] with gastric IM. A previous case-control study with 16S rRNA gene analysis identified increased abundance of *P. stomatis* in gastric biopsies of gastric cancer patients compared with individuals with superficial gastritis[12], and another study found the enrichment of *Peptostreptococcus* in biopsies of gastric atrophy and IM[33]. *P. stomatis* may contribute to the acidic and hypoxic tumor microenvironment, which promotes bacterial colonization[34]. *F. alocis* can induce the secretion of proinflammatory cytokines from

gingival epithelial cells[35]. Taken together, the data suggest a role of these highly host-interactive organisms in gastric cancer and warrant further investigations.

Several *Neisseria* species were enriched in gastric IM, such as *N. elongata* and *N. flavescens*. *Neisseria* species are oral cavity commensals and have been recognized as opportunistic pathogens. A recent small metagenomics study revealed enrichment of *Neisseriaceae-Neisseria-N. sicca* in gastric wash samples of gastric cancer patients compared with individuals with superficial gastritis[36]. In our collaborative prospective study of oral microbiome and gastric cancer that was also based on metagenomics data, the order Neisseriales, family *Neisseriaceae*, and genus *Neisseria* were enriched in oral wash samples collected before cancer occurrence, compared with controls[37] (Supplementary Table S11). The relative abundance of *N. elongata* was positively related to an increased risk of gastric cancer in one of the cohorts. However, a 16S rRNA-based study observed significant depletion of *Neisseria* in gastric cancer[13,38,39]. This discrepancy could reflect that 16S rRNA gene sequencing typically provides only family- or genus-level taxonomy. *Neisseria* species were correlated with pathways and GO categories for LPS and ubiquinol biosynthesis (Figure 2B and Supplementary Figure S4). LPS is a gram-negative bacterial antigen that increases inflammation in the tumor microenvironment and drives tumorigenesis[40]. LPS-related pathways were enriched in gastric cancer[36]. Most Gram-negative bacteria produce ubiquinone, which can form a microbial environment characteristic of inflammation[41]. Additional research is warranted to investigate these potential mechanisms by which *Neisseria* species may influence gastric cancer risk.

In our study, several commensals in the oral cavity and digestive tract, including *L. gasseri*, *S. mutans*, *S. parasanguinis*, and *S. sanguinis*, were associated with lower odds of gastric IM, with consistent associations across the oral and gastric microbiota. *S. mutans* is a major pathogen causing human dental caries[42]. *S. parasanguinis* is an early colonizer of dental surfaces and is related to a healthy microbiota[43]. *S. sanguinis*, a member of the oral biofilm community, is considered benign, or even beneficial, with regard to dental caries[44]. Dental caries-associated bacteria such as *Streptococcus* species elicit potent Th1 immune responses and promote $CD8^+$ T-cell responses[45] that may decrease cancer development[46]. The abundances of *Lactobacillus* and *Streptococcus* species were correlated with pathways for sugar degradation that were under-represented in gastric IM, suggesting a role of fermentation of sugars and production of lactic acid in gastric cancer. Lactic acid produced by *Lactobacillus* can lower the gastrointestinal tract pH, thus creating a hostile environment for resident pathogenic bacteria and eliciting antibacterial effects[47].Probiotics can induce the coccoid conversion of *H. pylori* and suppress *H. pylori* colonization and multiplication[48,49]. Supporting our results, significant reduction in the abundance of *Streptococcus* was observed in gastric microbiota of gastric cancer compared with chronic gastritis[13]. However, several studies using 16S rRNA gene sequencing reported significantly higher abundance of *Lactobacillus* and *Streptococcus* in gastric carcinoma relative to chronic gastritis[13,14]. Again, differences in sequencing methods and study design may explain the discrepancy.

Although seropositivity of *H. pylori* or CagA was positively associated with gastric IM (Table 1), the relative abundance of *H. pylori* in gastric microbiome was not (data not

shown). This observation is consistent with the observation that *H. pylori* is absent in gastric tissues in the large majority of patients with advanced atrophy, IM or gastric cancer[5] even when serology is positive, suggesting the disappearance of active *H. pylori* infection during the later stages of gastric cancer development[6]. The loss of *H. pylori* and impairment of acid secretion in these lesions may facilitate the colonization of other bacteria in the stomach that may play a role in gastric cancer development. Many of the associations between the non-*H. pylori* bacteria and gastric IM we found were stronger among individuals carrying *H. pylori* antibodies, suggesting their additive effects on the *H. pylori*-initiated gastric cancer development. Some experimental studies suggested that *H. pylori* can act synergistically with a community of bacteria to promote gastric neoplasia, and the gastric cancer risk may depend on the microbiota following *H. pylori* infection[10,11]. Future larger studies should be conducted to investigate interaction between *H. pylori* and specific taxa in gastric cancer risk.

Strengths and limitations

Strengths of our study included the matched design, comprehensive shotgun metagenomic sequencing, inclusion of both oral and gastric microbiome profiling, and adjustment for gastric cancer risk factors throughout analysis. Several case-control studies of gastric premalignant lesions, predominantly gastric IM, have identified shared risk factors and molecular alterations for gastric cancer[17] under the premise that a risk factor's association with the precancerous lesion parallels its association with cancer. Thus, IM can be used to identify risk factors for gastric cancer and elucidate the underlying carcinogenesis. However, although our study is the largest of its kind, case sample sizes (n=89 oral and n=55 gastric) remained small, limiting statistical power and our ability to investigate race-specific associations. Although we only study the compositions at a single time, the abundance of core members of the oral and gut microbiota are stable over time at the genus level[50].

Conclusion

We found evidence that individuals with gastric IM exhibited different microbial composition and functions compared with healthy individuals. Future studies are needed to confirm our findings and investigate the underlying mechanisms. Given that bacterial profiles may be modified, identification of bacterial risk factors of malignancy might enable interventions and more cost-effective cancer screening by risk stratification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by the National Institutes of Health grants [R01 CA204113, R01 DK110014, P42 ES010349, P30 ES000260, and P30 ES009089], and by the C & D and Sergei Zlinkoff funds.

Abbreviations:

CagA	cytotoxin-associated gene A
CI	confidence interval
clr	centered log-ratio
FDR	false discovery rate
GC	gastric cancer
GO	Gene Ontology
H. pylori	*Helicobacter pylori*
IM	intestinal metaplasia
JSD	Jensen-Shannon Divergence
LPS	lipopolysaccharide
OR	odds ratio
PCoA	principal coordinate analysis
PERMANOVA	permutational multivariate analysis of variance
SCCS	Southern Community Cohort Study
SMHS	Shanghai Men's Health Study
SWHS	Shanghai Women's Health Study

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Novelty and impact:

The colonization of bacteria other than *H. pylori* in precancerous and cancerous lesions of the stomach may play a role in the development of gastric cancer, but the evidence is not well established. This study identified species related to periodontal disease (*P. stomatis*, *J. ignava*, *F. alocis*) and opportunistic pathogens (*N. elongata*, *N. flavescens*) that were enriched in gastric IM, as well as probiotic species (*L. gasseri)* and commensals (*S. mutans*, *S. parasanguinis*, *S. sanguinis*) that were under-represented in gastric IM. Several species (*J. ignava*, *L. gasseri*, *S. mutans*, *S. parasanguinis*, *S. sanguinis*) in both the oral and gastric microbiota were consistently associated with gastric IM. Further, we identified metagenomic functions as potential mechanism by which these bacteria influence disease risk, including via LPS and ubiquinol biosynthesis and sugar degradation. The findings on potential roles of certain oral and gastric microbiota in the development of gastric precancerous lesions, if confirmed by future studies, may be considered in interventions and more cost-effective cancer screening.

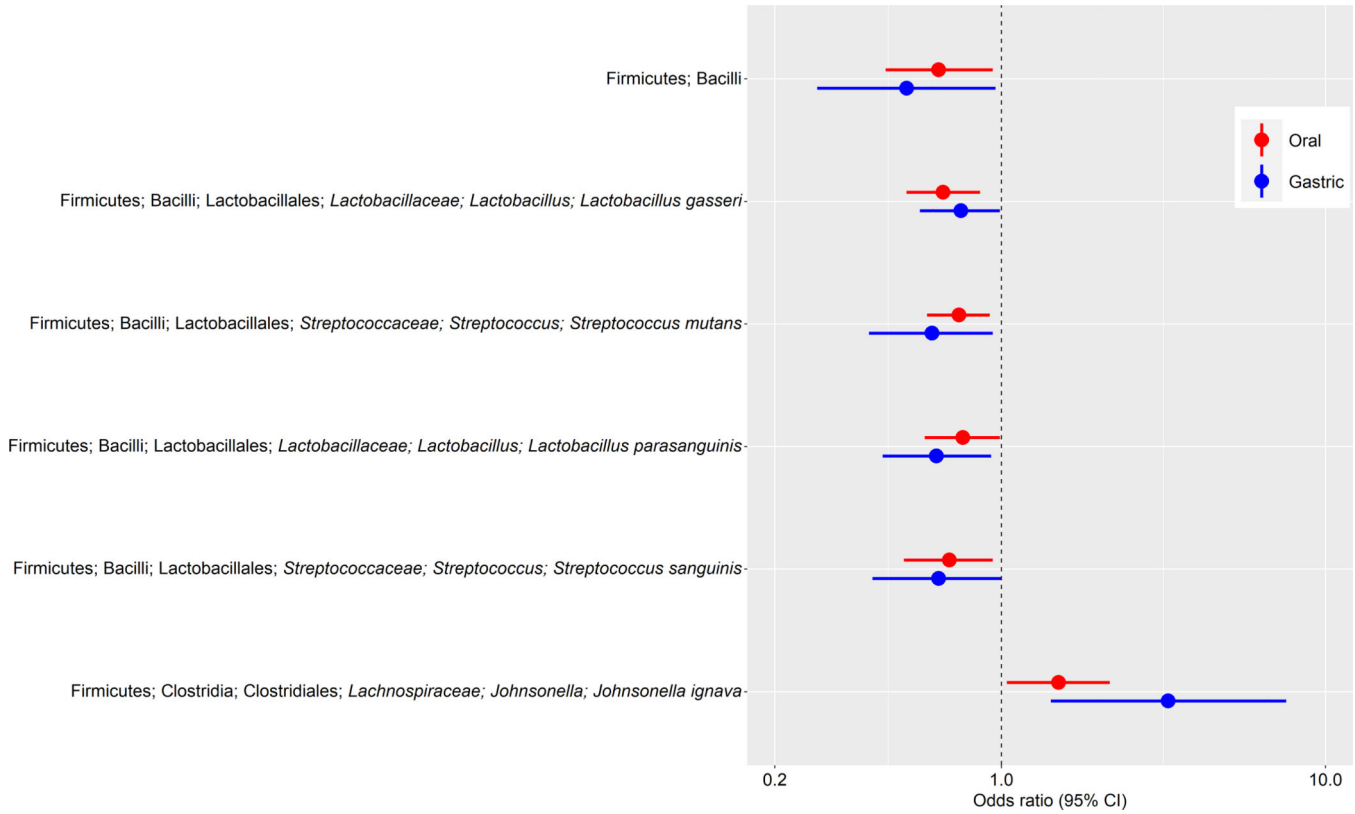
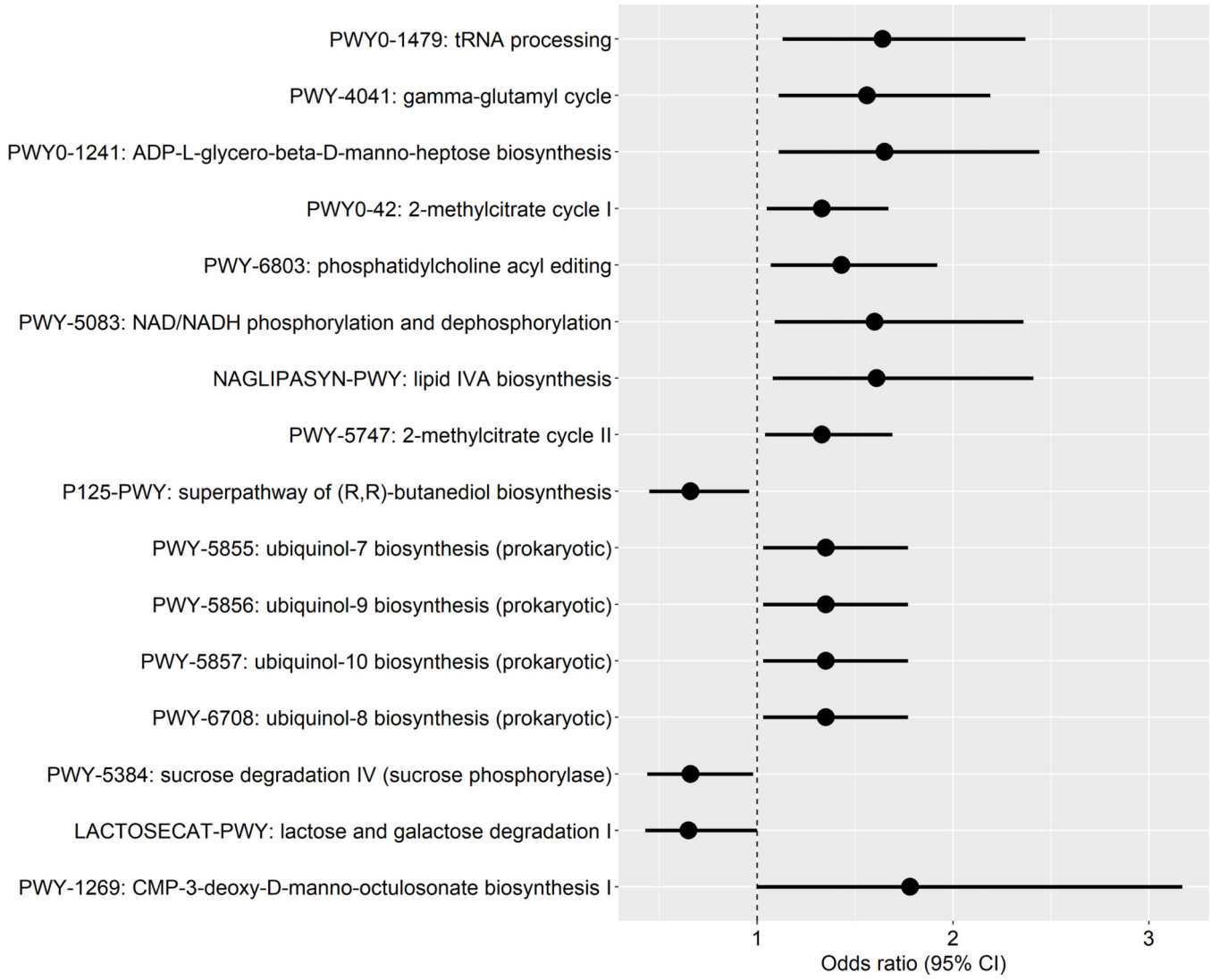


Figure 1.

Oral and gastric taxa associated with gastric intestinal metaplasia. Forest plot of odds ratios (ORs) and 95% confidence intervals (95% CI) for associations between clr-transformed taxa relative abundance and gastric IM in standard conditional logistic regression models. These taxa in both the oral and gastric microbiota were consistently associated with gastric IM.



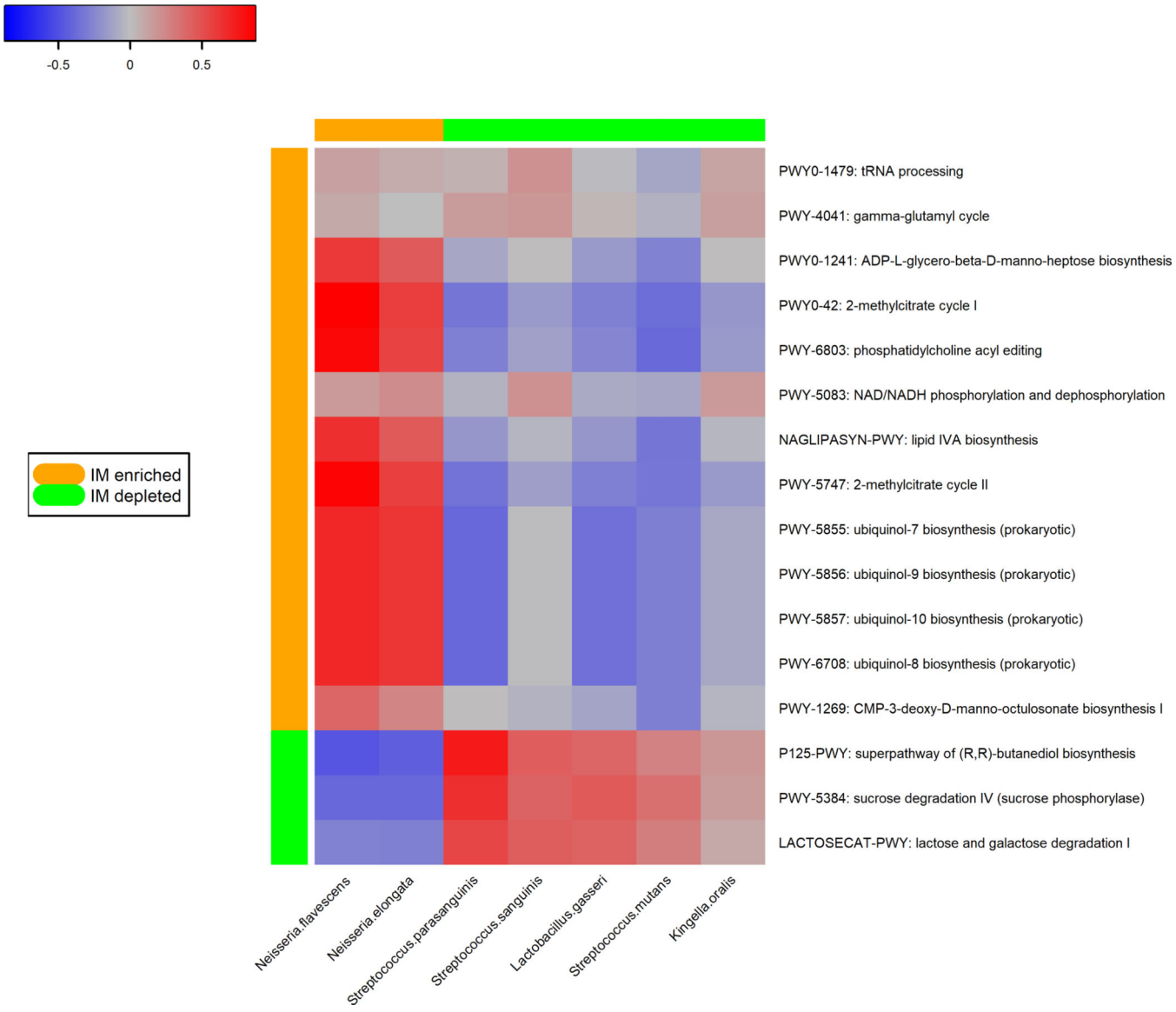
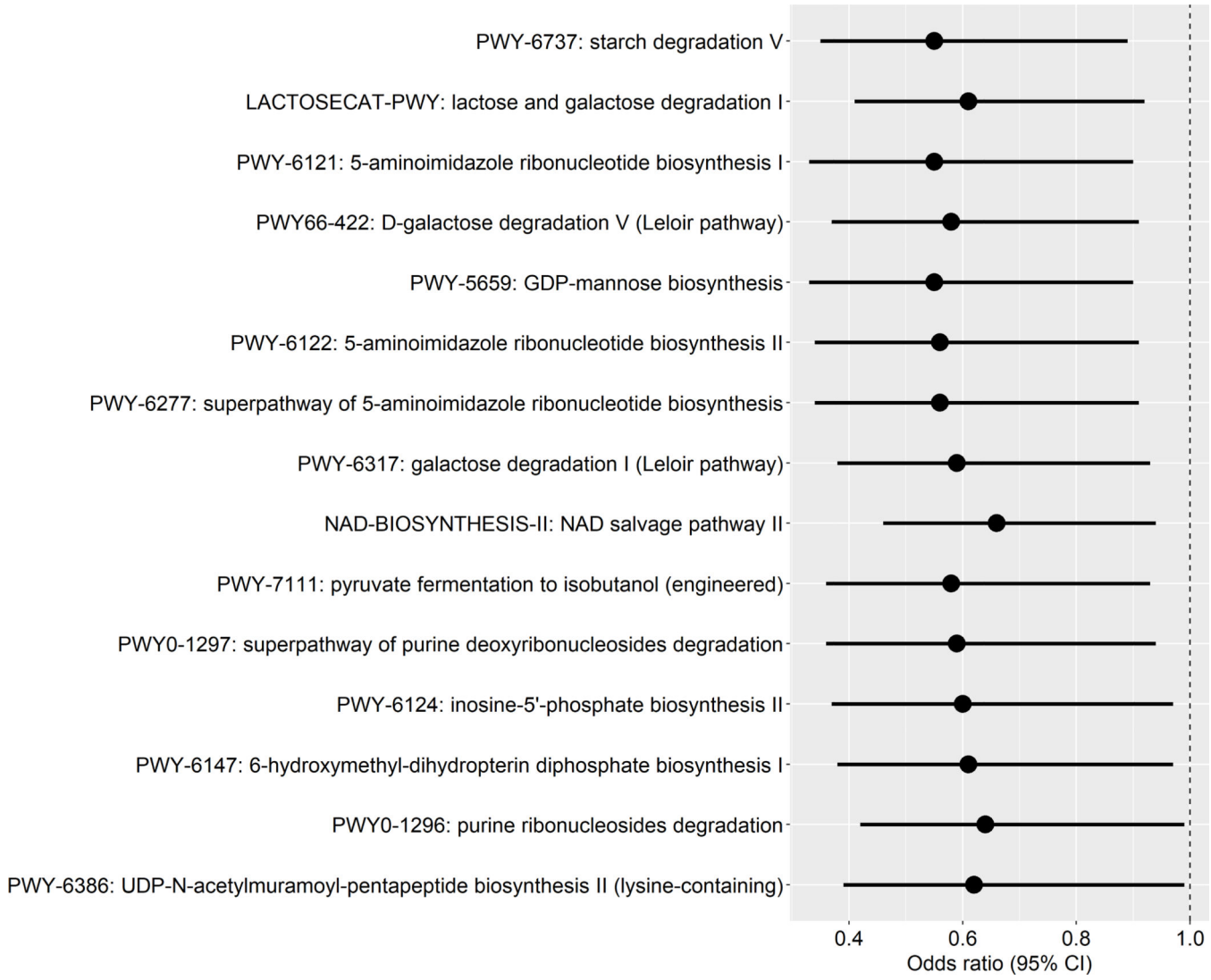


Figure 2.

Pathways in the oral microbiome associated with gastric intestinal metaplasia. (A) Forest plot of odds ratios (ORs) and 95% confidence intervals (95% CI) for associations between clr-transformed pathway relative abundance and gastric IM in standard conditional logistic regression models. (B) Correlations between IM-associated oral species and functional pathways. Spearman correlation coefficient values were estimated for each pairwise comparison of clr-transformed species and pathway relative abundance. Here we show only species in Table 2 and Figure 1 with known contribution to each pathway according to the species-specific pathway data.



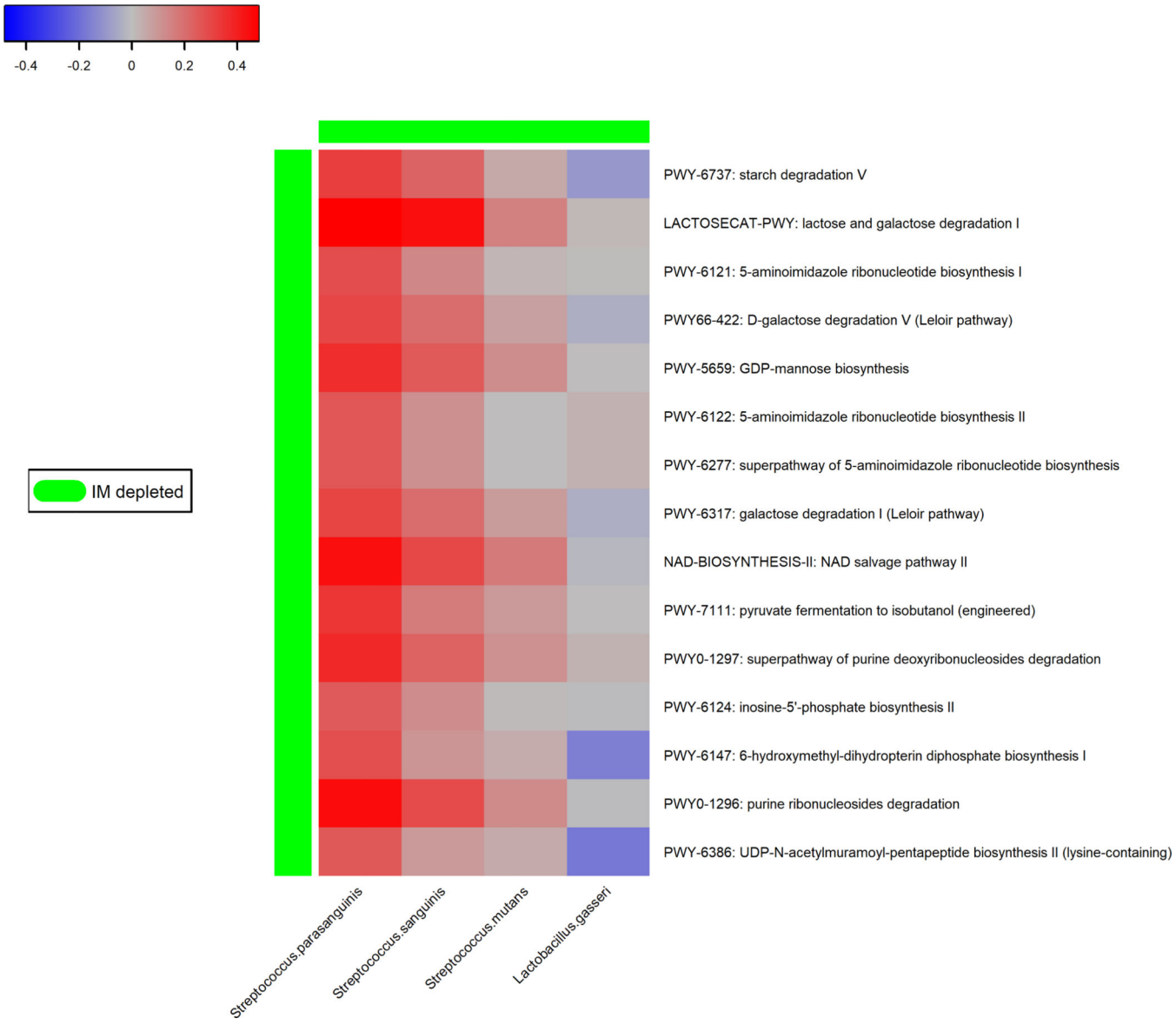


Figure 3.

Pathways in the gastric microbiome associated with gastric intestinal metaplasia. (A) Forest plot of odds ratios (ORs) and 95% confidence intervals (95% CI) for associations between clr-transformed pathway relative abundance and gastric IM in standard conditional logistic regression models. (B) Correlations between IM-associated gastric species and functional pathways. Spearman correlation coefficient values were estimated for each pairwise comparison of clr-transformed species and pathway relative abundance. Here we show only species in Table 3 and Figure 1 with known contribution to each pathway according to the species-specific pathway data.

Table 1.

Characteristics of study participants by case status of intestinal metaplasia

Characteristics	Intestinal metaplasia			
	Cases (n=89)	Controls (n=89)	OR[a] (95% CI)	*P*
Women, n (%)	52 (58.4)	52 (58.4)	–	–
Age (y), mean (SD)	58.3 (10.9)	57.3 (10.5)	1.13 (1.01–1.27)	0.04
BMI (kg/m^2), mean (SD)	25.0 (4.5)	26.5 (5.6)	0.93 (0.86–1.02)	0.12
Ever smoking, n (%)	30 (33.7)	26 (29.2)	1.64 (0.77–3.47)	0.20
Pack-years, mean (SD)	83.1 (183.9)	77.2 (159.5)	1.01 (0.99–1.03)[b]	0.54
Ever drinking, n (%)	48 (53.9)	50 (56.2)	1.45 (0.68–3.08)	0.33
Daily alcohol intake[c], mean (SD)	0.2 (0.6)	0.5 (1.3)	0.75 (0.51–1.10)	0.14
Education, n (%)				
<College	48 (53.9)	50 (56.2)	1.55 (0.46–5.18)	0.48
College	28 (31.5)	19 (21.4)	2.50 (0.71–8.75)	0.15
Graduate	13 (14.6)	20 (22.5)	Ref	
Race, n (%)				
White	8 (9.0)	20 (22.5)	Ref	
Black	5 (5.6)	16 (18.0)	0.89 (0.23–3.44)	0.86
Hispanic	23 (25.8)	21 (23.6)	2.45 (0.63–9.58)	0.20
Asian	53 (59.6)	32 (36.0)	4.22 (1.48–12.1)	0.007
H. pylori status (in serum), n (%)				
Negative	50 (61.7)	64 (71.9)	Ref	
Positive, CagA negative	9 (11.1)	5 (5.6)	3.51 (0.80–15.4)	0.10
Positive, CagA positive	22 (27.2)	20 (22.5)	2.80 (0.98–8.04)	0.06
Recruitment year			–	–
2009–2011	27 (30.3)	27 (30.3)		
2013–2015	8 (9.0)	0		
2016–2018	54 (60.7)	62 (69.7)		
Recruitment location			–	–
Bellevue Hospital	40 (44.9)	40 (44.9)		
Private clinic	46 (51.7)	46 (51.7)		
NYU Ambulatory Center	3 (3.4)	3 (3.4)		

[a]ORs were calculated using logistic regression conditional on matching factors including sex, recruitment site (Bellevue Hospital, Private clinic, Ambulatory Care Center), age categories (<35, 35–49, 50–64, 65+ years), and recruitment year (± 3 years), adjusting for age (continuous) and race (White, African American, Hispanic, Asian).

[b]Per 10 pack-years.

[c]Daily consumption of total standard drinks of alcoholic beverages (a 12-oz can of beer, 4-oz glass of wine, and 1.5-oz shot of hard liquor).

Table 2.

Taxa in the oral microbiome[a] associated with gastric intestinal metaplasia

Taxon (class; order; family; genus; species)	Mean relative abundance, %		OR[b] (95% CI)	P[c]
	Cases	Controls		
Bacteroidetes	26.9	23.3	1.59 (1.03–2.47)	0.037
Bacteroidia; Bacteroidales; *Porphyromonadaceae (family)*	5.34	3.60	1.56 (1.12–2.18)	0.009
Firmicutes				
Bacilli (class)	17.2	21.0	0.64 (0.44–0.94)	0.021
Bacilli; Lactobacillales; *Lactobacillaceae*; *Lactobacillus (genus)*	0.07	0.19	0.56 (0.40–0.80)	0.001
Bacilli; Lactobacillales; *Lactobacillaceae*; *Lactobacillus*; *Lactobacillus gasseri (species)*	0.003	0.04	0.66 (0.51–0.86)	0.002
Bacilli; Lactobacillales; *Streptococcaceae*; *Streptococcus*; *Streptococcus sanguinis (species)*	0.62	0.78	0.69 (0.50–0.94)	0.018
Clostridia (class)	2.02	1.67	1.71 (1.00–2.93)	0.048
Clostridia; Clostridiales; *Lachnospiraceae*; *Oribacterium*; *Oribacterium sinus (species)*	0.32	0.24	1.33 (1.03–1.72)	0.030
Clostridia; Clostridiales; *Lachnospiraceae*; *Shuttleworthia*; *Shuttleworthia satelles (species)*	0.01	0.02	0.76 (0.59–0.96)	0.023
Clostridia; Clostridiales; *Peptostreptococcaceae (family)*	0.24	0.15	1.59 (1.13–2.24)	0.008
Clostridia; Clostridiales; *Peptostreptococcaceae*; *Peptostreptococcus (genus)*	0.13	0.08	1.56 (1.16–2.10)	0.003
Clostridia; Clostridiales; *Peptostreptococcaceae*; *Peptostreptococcus*; *Peptostreptococcus stomatis (species)*	0.11	0.07	1.35 (1.08–1.70)	0.008
Proteobacteria				
Alphaproteobacteria (class)	0.03	0.05	0.81 (0.67–0.99)	0.043
Betaproteobacteria; Burkholderiales; *Alcaligenaceae (family)*	0.27	0.29	0.74 (0.59–0.92)	0.008
Betaproteobacteria; Burkholderiales; *Alcaligenaceae*; *Achromobacter (genus)*	0.27	0.29	0.75 (0.60–0.93)	0.008
Betaproteobacteria; Burkholderiales; *Alcaligenaceae*; *Achromobacter*; *Achromobacter xylosoxidans (species)*	0.27	0.29	0.76 (0.62–0.93)	0.008
Betaproteobacteria; Neisseriales (order)	12.3	9.66	1.27 (1.02–1.57)	0.033
Betaproteobacteria; Neisseriales; *Neisseriaceae (family)*	12.3	9.66	1.28 (1.03–1.59)	0.026
Betaproteobacteria; Neisseriales; *Neisseriaceae*; *Kingella*; *Kingella oralis (species)*	0.10	0.23	0.80 (0.65–1.00)	0.046
Betaproteobacteria; Neisseriales; *Neisseriaceae*; *Neisseria (genus)*	12.0	9.23	1.27 (1.04–1.56)	0.020
Betaproteobacteria; Neisseriales; *Neisseriaceae*; *Neisseria*; *Neisseria elongata (species)*	0.49	0.27	1.43 (1.12–1.83)	0.004
Betaproteobacteria; Neisseriales; *Neisseriaceae*; *Neisseria*; *Neisseria flavescens (species)*	3.56	2.04	1.29 (1.06–1.56)	0.010
Epsilonproteobacteria (class)	0.87	0.72	1.57 (1.01–2.46)	0.046
Gammaproteobacteria (class)	7.38	7.07	1.38 (1.05–1.82)	0.023

Taxon (class; order; family; genus; species)	Mean relative abundance, %		OR[b] (95% CI)	P[c]
	Cases	Controls		
Gammaproteobacteria; Enterobacterales (order)	0.04	0.05	0.70 (0.52–0.96)	0.026
Gammaproteobacteria; Pasteurellales; *Pasteurellaceae*; *Aggregatibacter (genus)*	0.75	0.56	1.41 (1.09–1.82)	0.009
Phylum no rank				
SR1 bacterium oral taxon 874 (species)	0.05	0.03	1.24 (1.05–1.47)	0.010
Synergistetes	0.07	0.07	0.81 (0.66–0.99)	0.038
Synergistia (class)	0.08	0.08	0.83 (0.69–1.00)	0.048

[a]Taxa relative abundances were normalized with the clr transformation. All taxa (phyla, classes, orders, families, genera, species) and covariates (age and race) were selected using conditional logistic regression with elastic-net penalties; leave-one-out cross-validation was conducted using the "cv.clogitL1" function in the clogitL1 R package. Taxa selected by the penalized model and with a nominal $P < 0.05$ based on standard conditional logistic regression models were shown in the table.

[b]ORs were calculated using standard logistic regression models conditional on matching factors including sex, recruitment site (Bellevue Hospital, Private clinic, Ambulatory Care Center), age categories (<35, 35–49, 50–64, 65+ years), and recruitment year (± 3 years), adjusting for age (continuous) and race (White, African American, Hispanic, Asian).

[c]*P* values from conditional logistic regression models.

Table 3.

Taxa in the gastric microbiome[a] associated with gastric intestinal metaplasia

Taxon (class; order; family; genus; species)	Mean relative abundance, %		OR[b] (95% CI)	*P*[c]
	Cases	Controls		
Actinobacteria				
Actinobacteria; Actinomycetales; *Actinomycetaceae*; *Actinomyces*; *Actinomyces sp. oral taxon 448 (species)*	0.11	0.10	1.42 (1.02–1.99)	0.040
Bacteroidetes				
Bacteroidia; Bacteroidales; *Prevotellaceae*; *Prevotella*; *Prevotella baroniae (species)*	0.20	0.09	1.61 (1.03–2.51)	0.037
Firmicutes				
Bacilli; Lactobacillales; *Lactobacillaceae*; *Lactobacillus*; *Lactobacillus gasseri (species)*	0.02	0.04	0.75 (0.56–0.99)	0.046
Bacilli; Lactobacillales; *Streptococcaceae*; *Streptococcus*; *Streptococcus mutans (species)*	0.03	0.03	0.61 (0.39–0.94)	0.024
Clostridia; Clostridiales; *Peptostreptococcaceae*; *Filifactor (genus)*	0.12	0.05	1.47 (1.03–2.11)	0.036
Clostridia; Clostridiales; *Peptostreptococcaceae*; *Filifactor*; *Filifactor alocis (species)*	0.11	0.04	1.43 (1.03–2.01)	0.035
Negativicutes; Veillonellales; *Veillonellaceae*; *Veillonella*; *Veillonella sp. oral taxon 780 (species)*	0.05	0.02	1.53 (1.09–2.16)	0.015
Fusobacteria				
Fusobacteriia; Fusobacteriales; *Leptotrichiaceae*; *Pseudoleptotrichia*; *Leptotrichia goodfellowii (species)*	0.01	0.01	1.67 (1.09–2.57)	0.019
Proteobacteria				
Deltaproteobacteria; Desulfovibrionales; *Desulfomicrobiaceae (family)*	0.02	0.01	1.50 (1.04–2.17)	0.031

[a] Taxa relative abundances were normalized with the clr transformation. All taxa (phyla, classes, orders, families, genera, species) and covariates (age and race) were selected using conditional logistic regression with elastic-net penalties; leave-one-out cross-validation was conducted using the "cv.clogitL1" function in the clogitL1 R package. Taxa selected by the penalized model and with a nominal $P < 0.05$ based on standard conditional logistic regression models were shown in the table.

[b] ORs were calculated using standard logistic regression models conditional on matching factors including sex, recruitment site (Bellevue Hospital, Private clinic, Ambulatory Care Center), age categories (<35, 35–49, 50–64, 65+ years), and recruitment year (± 3 years), adjusting for age (continuous) and race (White, African American, Hispanic, Asian).

[c] *P* values from conditional logistic regression models.