Tobacco Smoking and the Fecal Microbiome in a Large, Multi-ethnic Cohort

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ABSTRACT

Background: Increasing evidence suggests that tobacco smoking, a well-known driver of carcinogenesis, influences the gut microbiome; however, these relationships remain understudied in diverse populations. Thus, we performed an analysis of smoking and the gut microbiome in a subset of 803 adults from the multi-ethnic NYU FAMiLI study.

Methods: We assessed fecal microbiota using 16S rRNA gene sequencing, and clustered samples into Amplicon Sequence Variants using QIIME2. We evaluated inferred microbial pathway abundance using PICRUSt. We compared population β-diversity, and relative taxonomic and functional pathway abundance, between never smokers, former smokers, and current smokers.

Results: We found that the overall composition of the fecal microbiome in former and current smokers differs significantly from that of never smokers. The taxa *Prevotella* and *Veillonellaceae* were enriched in current and former smokers, whereas the taxa *Lachnospira* and Tenericutes were depleted, relative to never smokers. These shifts were consistent across racial and ethnic subgroups. Relative to never smokers, the abundance of taxa enriched in current smokers were positively correlated with the imputed abundance of pathways involving smoking-associated toxin breakdown and response to reactive oxygen species (ROS).

Conclusions: Our findings suggest common mechanisms of smoking associated microbial change across racial subgroups, regardless of initial microbiome composition. The correlation of these differentials with ROS exposure pathways may suggest a role for these taxa in the known association between smoking, ROS and carcinogenesis.

Impact: Smoking shifts in the microbiome may be independent of initial composition, stimulating further studies on the microbiome in carcinogenesis and cancer prevention.

Introduction

Recent work suggests that cigarette smoke may alter the microbiome (1–5). Cigarette smoke is a source of numerous toxicants (6), which can perturb the microbial ecology of the gut via antibiotic effects, oxygen deprivation, or other potential mechanisms (7–10). Loss of beneficial gut species due to smoking may then lead to pathogen colonization and ultimately to disease (11). Yet, these studies describe only phylum-level taxonomic shifts in the Firmicutes to Bacteroidetes ratio, and theoretical physiologic effects. Thus, the relationship between cigarette smoking and alterations in specific microbial taxa and functional characteristics of the gut microbiome remains under-investigated. Given the importance of both smoking and the gut microbiome in modifying cancer risk, understanding their inter-relationships may be critical to modification of those risks.

Thus, we hypothesized that smoking influences the fecal microbiome, resulting in shifts in specific taxonomic abundances and changes in imputed microbial metabolism. To study this hypothesis in a large multi-ethnic cohort of 803 adults from the NYU Food and Microbiome Longitudinal Investigation (FAMiLI), we examined the overall structure of the human gut microbiome and specific taxon abundances in current, former, and never smokers and identified related imputed microbial metabolic pathways.

Materials and Methods

Study population

Participants were recruited from the New York University Food and Microbiome Longitudinal Investigation study cohort (12). Briefly, participants over the age of 40 were recruited in the New York Metropolitan area, including nearby areas of New Jersey and Connecticut. Study participants submitted samples of stool, and completed a questionnaire describing demographic and lifestyle characteristics (13). From the recruited population, we selected an initial subset of 873 participants for microbiome sequencing who confirmed no use of antibiotics in the 2 weeks prior to provision of stool samples (12). Participants with missing or unknown demographic data (age, race, or gender; $n = 45$), BMI ($n = 16$), or smoking status ($n = 8$) were excluded from analysis. Some samples were excluded for missing data in multiple categories. One sample was excluded for insufficient richness, resulting in a final sample size of 803 subjects (**Table 1**), including 357 White, 98 Black, 269 Asian, and 79 other subjects.

Fecal microbiome assay

Stool samples were provided by all participants and stored in our biorepository in a −80°C freezer. As described previously, samples underwent 16S rRNA gene sequencing at the Environmental Sample Preparation and Sequencing Facility at Argonne National

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Table 1. Baseline subject characteristics.

	Never smokers $N = 543$	%	Former smokers $N = 181$	%	Current smokers $N = 79$	%	
Age							$P = 0.2398$[a]
Range	38–91		40–77		40–87		
Median	59		56		62		
Sex							$P = 1.67E-10$[b]
Female	386	71.09	84	46.41	37	46.84	
Male	157	28.91	97	53.59	42	53.16	
Race							$P = 2.90E-6$[b]
White	221	40.70	103	56.91	33	41.77	
Black	60	11.05	22	12.15	16	20.25	
Asian	214	39.41	39	21.55	16	20.25	
Other	48	8.84	17	9.39	14	17.72	
Ethnicity[c]							$P = 0.1940$[b]
Hispanic	78	14.42	26	14.36	19	24.05	
Non-Hispanic	463	85.58	155	85.64	60	75.95	
Weight category							$P = 0.0269$[b]
Underweight	13	2.39	3	1.66	6	7.59	
Normal Weight	175	32.23	47	25.97	25	31.65	
Overweight/obese	355	65.38	131	72.38	48	60.76	
Time since quitting							
<10 years ago	NA		37	20.44	NA		
10–29 years ago	NA		90	49.72	NA		
30+ years ago	NA		54	29.83	NA		
Median IQR: 21 years ago							
Cigarettes per day[d]							
1–10	NA		NA		41	52.56	
11+	NA		NA		37	47.44	
Median IQR: 10 cigarettes per day							

[a]*P* values based on Kruskal–Wallis test.
[b]*P* values based on Chi-square test.
[c]Ethnicity data unavailable for two subjects.
[d]Daily usage data unavailable for one subject.

Laboratory (12). DNA was extracted using the PowerSoil (MO BIO Laboratories) DNA Isolation Kit, following the manufacturer's protocol. The V4 region of the 16S rRNA gene was PCR amplified with the 515F/806R primer pair, which included Illumina flow cell adapter sequences with sample-specific barcodes (14). Sequence reads were processed using the QIIME2 pipeline (15). Briefly, sequence reads were demultiplexed and paired-end reads were joined, followed by quality filtering (16). Next the Deblur workflow was applied, which uses sequence error profiles to obtain putative error-free sequences, referred to as "sub" amplicon sequence variant (s-ASV; ref. 17). s-ASVs were assigned taxonomy using a naïve Bayes classifier pretrained on the Greengenes 13_8 99% ASVs (18), where the sequences have been trimmed to only include 250 bases from the 16S rRNA gene region V4, bound by the 515F/806R primer pair (14). A phylogenetic tree was constructed via sequence alignment with MAFFT (19), filtering the alignment, and applying FastTree (20) to generate the tree.

Statistical analysis

We identified current, former, and never smokers, and assessed number of cigarettes per day and duration of use in ever-smokers. The relationship between smoking status (never smokers, former smokers, or current smokers) and overall fecal microbiome community structure (β diversity) at the species level was assessed by analysis of Bray–Curtis dissimilarity (21), computed using the QIIME2 pipeline (15). Principal coordinate analysis plots were generated using the nonmetric multidimensional scaling rank method (22), and labeled according to smoking status. Within the vegan R package, permutational MANOVA ("adonis" function, refs. 23–25) of the Bray–Curtis distance was used to test differences in overall microbiome composition across the categories of smoking status, adjusting for age, sex, race, and BMI. Bray–Curtis distance from each smoking group centroid, both within and between each subgroup was also calculated ("meandist" function, vegan R package, refs. 23, 24) and analyzed by ANCOVA controlling for age, sex, race, and BMI. Race was categorized as White, Black, Asian, or Other. BMI was categorized as underweight (BMI < 18.5), normal weight (18.5–25), or overweight/obese (>25; ref. 26). Overweight/obese was defined as BMI > 23 for Asian subjects per global consensus guidelines based on cardiovascular risk (27).

For the primary analysis of bacterial relative abundance by smoking subgroups, the ASV tables of raw counts across all phylogenetic levels were normalized to relative abundances. We filtered taxa with fewer than two copies or present in fewer than 25% of subjects, similar to previous taxonomic analyses (5). Relative abundance of bacterial taxa were compared across the three categories of smoking status, current, former, or never, using the analysis of communities method (ANCOM-II package, R v1.1.463, refs. 28, 29), adjusting for age, sex, race, and BMI. ANCOM-II accounts for the compositionality and sparse nature of microbiome data matrices, in which nearly 80% of the matrix values may be zero (30), to analyze differential abundance between two or more groups with greater power and lower FDR than similar methodologies (29). Taxa were considered statistically

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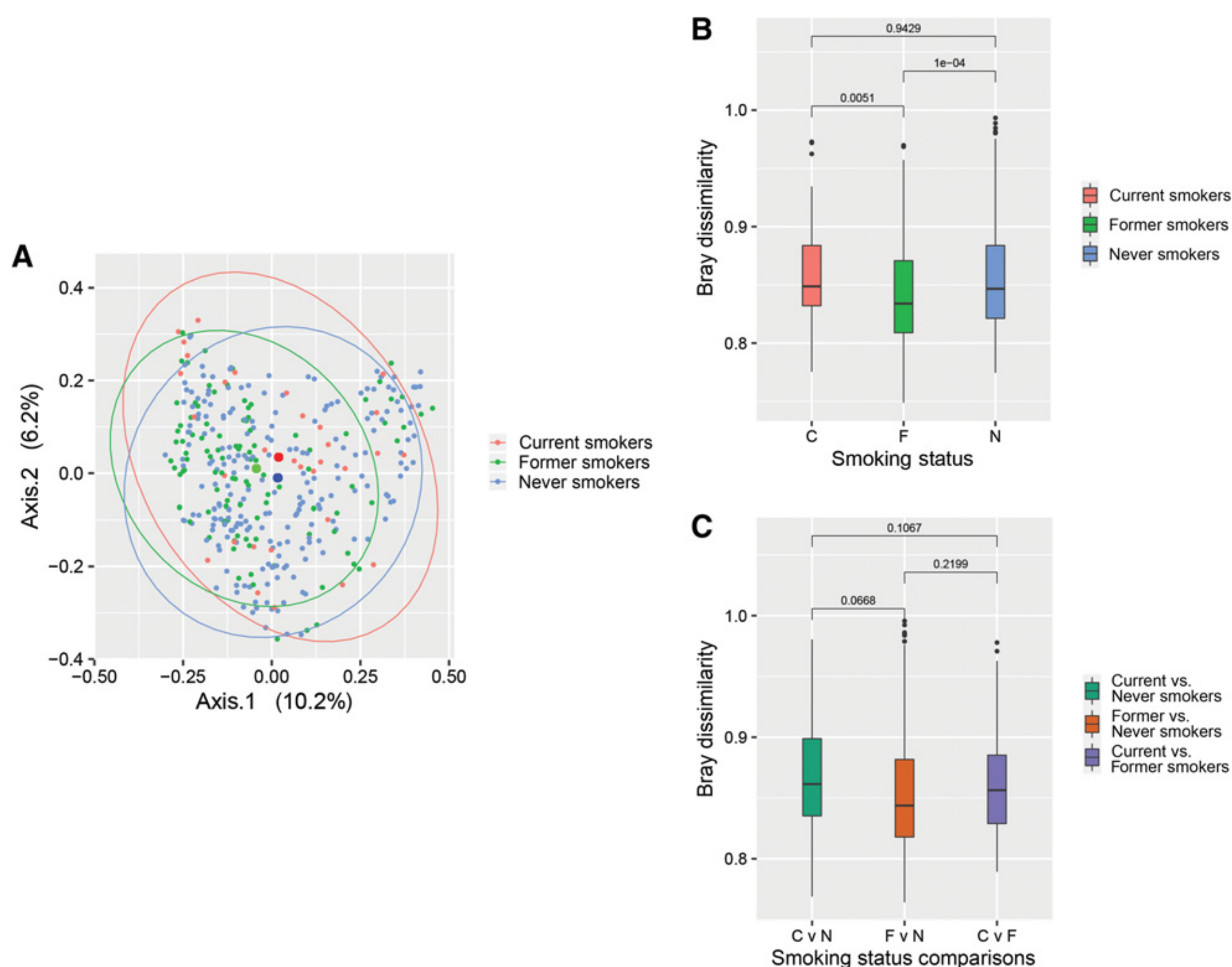

Figure 1.

β-Diversity and ordination of microbiome by smoking status. Bray–Curtis dissimilarity by smoking status demonstrated by PCoA ordination controlling for age, sex, race, and BMI (**A**). Significant dissimilarity from never smokers noted for both Current ($P = 0.0006$) and former ($P = 0.0186$) smokers. Bray dissimilarity from centroid within (**B**) and between (**C**) smoking subgroups annotated with ANCOVA analysis controlling for age, sex, race, and BMI.

significant if they were differentially abundant, as measured by rejection of the pairwise null hypothesis, from more than 90% of taxa at the same phylogenetic level (FDR < 0.001, ref. 28). Differences in the relative abundance of taxa between subgroups was termed "differentials."

Analysis of differential taxonomic abundance by time since smoking cessation and cigarettes smoked daily was performed with the subset of taxa which were found to be significant by ANCOM analysis. Taxonomic abundances were normalized by centered log-ratio (CLR) transformation (31, 32). This accounts for the compositional nature of microbiome data by taking the ratio of each observation with the geometric mean of the whole composition, thus producing more precise relative abundances than other methodologies (31). CLR normalized abundance of taxa in former and current smokers was compared with never smokers. For analysis of smoking cessation, subjects were binned into current smokers, and former smokers who quit smoking less than 10 years ago, 10 to 29 years ago, or greater than 29 years ago (30+). For analysis of cigarette dose, subjects were binned into smokers of 1 to 10 cigarettes daily or greater than 10 cigarettes daily (11+).

Bacterial metabolic genes and pathways were imputed from 16S rRNA gene-based microbial compositions using the PICRUSt2 algorithm (33), with reference to the MetaCyc pathway catalog (https://metacyc.org/; ref. 34). A total of 391 MetaCyc pathways were imputed across all of our samples. Relative imputed abundance of MetaCyc pathways across the three categories of smoking were analyzed by ANCOM-II, controlling for age, sex, race, and BMI (28). We noted that these pathways were largely clustered within the Biosynthesis and Degradation/Utilization/Assimilation ("Oxidation/Degradation") MetaCyc superpathways, with the few pathways which fell outside these classes termed "Other." The correlation between imputed pathways and taxonomic abundance was evaluated by Spearman correlation analysis and visualized using the pheatmap R package (https://CRAN.R-project.org/package=pheatmap).

Results

Study population

Our population includes 803 subjects, of which 79 (9.9%) were current smokers, 181 (22.5%) were former smokers, and 543 (67.6%)

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were never smokers (**Table 1**). Within these groups, we found that former and current smokers were more likely to be men than women, compared with never smokers. We also found that current smokers were less likely to be overweight or obese, and more likely to identify as Black.

Diversity of the fecal microbiome by current, former, and never smoking status

There were no significant differences in subject α-diversity between smoking subgroups (Supplementary Table S1). However, we found significant differences in β-diversity by smoking status (**Fig. 1A** and **B**). This differential is suggested by PCoA visualization (**Fig. 1A**) of Bray–Curtis dissimilarity, and confirmed by PERMANOVA analysis by smoking status (**Fig. 1B**). We found that β-diversity, as measured by Bray–Curtis dissimilarity, differed significantly between never smokers and both current ($P = 0.0006$) and former smokers ($P = 0.0186$). No significant changes were noted when additionally controlling for ethnicity or fiber in our model (Supplementary Table S1). We then evaluated microbiome dissimilarity from centroid, which acts as a measure of the actual microbial variation demonstrated by β-diversity, and found that it varied both within and between smoking subgroups. Specifically, current and never smokers showed significantly greater Bray–Curtis dissimilarity than former smokers (**Fig. 1B**). We observed that the dissimilarity between current and never smokers was greater than that between former and current smokers or never and former smokers (**Fig. 1C**).

Taxonomic composition of the fecal microbiome by current, former, and never smoking status

Fecal taxonomic composition analysis identified 48 taxa differentially prevalent in current smokers versus never smokers, 33 differentially prevalent taxa in former smokers versus never smokers, and 19 taxa in current versus former smokers (Supplementary Table S2). We noted that the Tenericutes, *Lachnospira, Veillonellaceae*, and *Prevotella* taxa were significantly altered at the highest ANCOM significance threshold (0.9) between all smoking subgroups. Analysis of mean differences in abundances following centered log-ratio normalization confirmed differences in taxonomic abundance between these taxa, which is visualized using cladogram across three smoking groups and ethnic subgroups (**Fig. 2**). We noted that the relative abundance of the Firmicutes genus *Lachnospira* was lower in current relative to never smokers (**Fig. 2A** and **D**), whereas the relative abundance of members of the Bacteroidetes genera *Prevotella*, and the Clostridia family *Veillonellaceae* were higher in current smokers. These relative differentials in abundance were largely preserved when comparing current smokers to former smokers (**Fig. 2C**), with depletion of *Lachnospira* and enrichment of *Veillonellaceae* and *Prevotella* members. However, smaller relative differentials were noted between former and never smokers (**Fig. 2B**) in most significantly differential taxa. Of note, unlike current smokers, the microbiome of former smokers demonstrated even lower relative abundance of *Prevotella* and *Veillonellaceae Phascolarcobacterium* than never smokers.

Shifts in relative abundance across significant taxa were largely consistent across all ethnic and racial subgroups, defined as non-Hispanic White or Black, Asian, and Hispanic (**Fig. 2D**). Notably, although we observed similar directional shifts in taxa between current smokers and never smokers in Asian subjects (Supplementary Table S3), these differences were more muted than in other ethnic subgroups. *Post hoc* subgroup ANCOM analysis showed only *Phascolarcobacterium* genus and *Eubacterium biforme* species to be significantly differentially abundant between current and never smokers

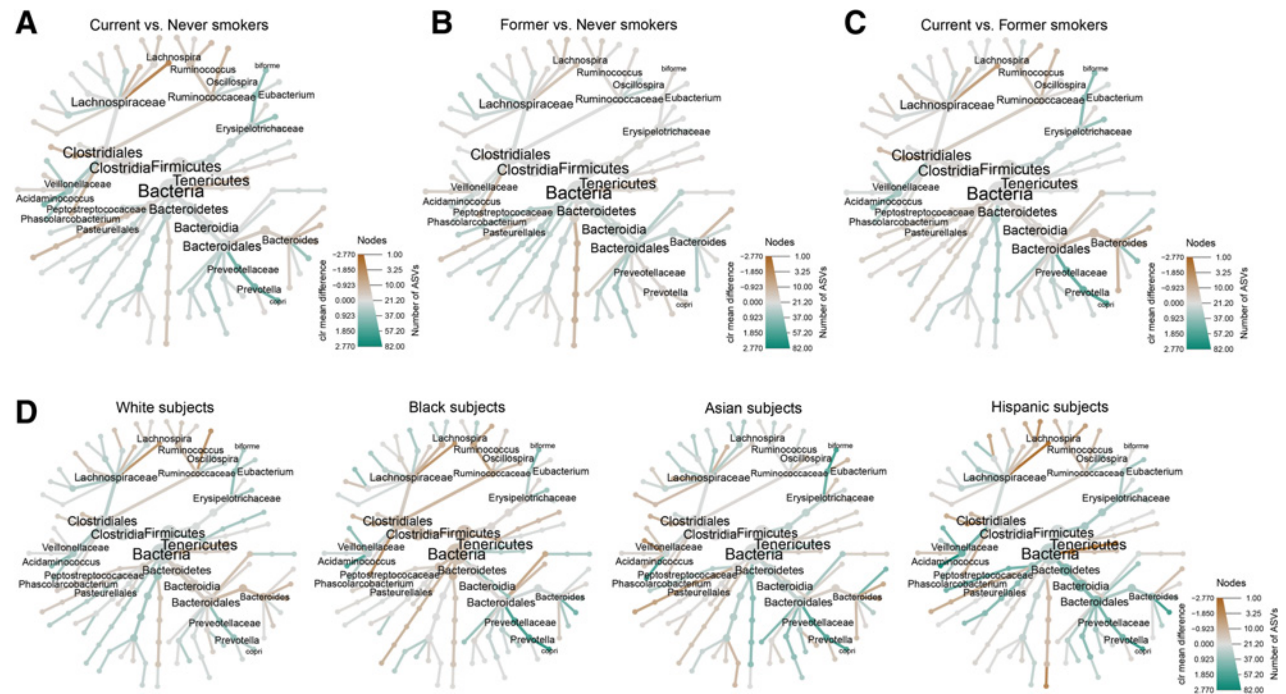

Figure 2.

Normalized relative taxonomic abundance by smoking status. Cladograms visualizing the CLR-normalized relative abundance of all 48 taxa found to be significantly differentially abundant in current vs. normal smokers. Relative taxonomic abundance in current versus never smokers (**A**), former versus never smokers (**B**), and current versus former smokers (**C**). Node size indicates number of ASV's associated with the node, while node color indicates relative abundance from blue-green (enriched) to brown (depleted. **D,** CLR normalized relative abundance of the same taxa subset in current versus never smokers across racial and ethnic subgroups.

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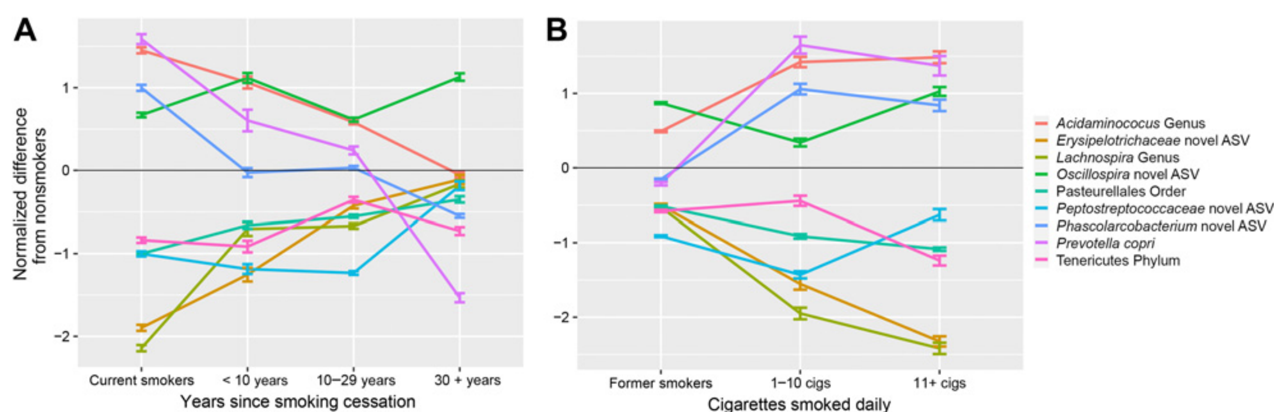

Figure 3.

Normalized relative abundance by quit duration and smoking dose. CLR-normalized taxonomic abundance relative to never smokers of taxa noted to be significantly altered by ANCOM analysis. **A,** Relative abundance of current smokers and former smokers based on years since quitting smoking (<10, 10–29, and 30+). **B,** Relative abundance of former smokers, and current smokers by cigarettes-per-day (1–10 and 11+). Error bars denote standard error of the mean normalized abundance relative to never smokers.

in Asian subjects, with increased abundance in current smokers (**Fig. 2D**). Shifts in taxonomic abundance with smoking were directionally similar across sexes, BMI subgroups, and fiber intake subgroups (Supplementary Table S4).

Difference in taxonomic abundance by smoking dose and time of cessation

We evaluated whether differences in taxonomic abundance varied by smoking dose or time since smoking cessation, selecting taxa noted to be significantly differentially abundant by our ANCOM analysis (**Fig. 3**; Supplementary Table S2). When we examined the normalized differential taxonomic abundance between either current or former smokers and never smokers based on years since quitting (**Fig. 3A**; **Table 2**), we found that the taxa which were relatively enriched in the microbiota of current smokers, depleted to nearly the level of nonsmokers in those who reported smoking cessation of over 30 years. This included depletion in *Veillonellaceae* taxa *Acidaminococcus* and *Phascolarcobacterium*, and *Prevotella copri*. Only an ASV identified within *Oscillospira* remained persistently enriched in all former smokers. Similarly, we found that taxa which were depleted in current smokers relative to never smokers, like *Lachnospira* and *Erysipelotrichaceae*, enriched to near nonsmoker abundances in formers smokers who reported cessation of over 30 years. The Tenericutes phylum remained persistently depleted in all former smokers.

Similar differentials in relative taxonomic abundance were noted when comparing current smokers by cigarettes smoked per day with nonsmokers (**Fig. 3B**; **Table 2**). We again noted depletion of *Veillonellaceae* and *Prevotellaceae*, and enrichment of *Lachnospiraceae* and *Erysipelotrichaceae* with decreasing cigarettes-per-day dosing. *Oscillospira* and *Peptostreptococcaceae* abundance did not differ significantly with cigarettes smoked daily (**Table 2**).

Inferred metagenomics pathways and smoking

We performed an ANCOM analysis of imputed metabolic pathways relative to smoking status to further understand how alterations in gut microbiome affected gut function. In current smokers versus former, or never smokers, we found relative enrichment of oxidative and degradative metabolism superpathway members, such as lactose and galactose degradation or formaldehyde oxidation, and depletion of biosynthesis superpathway members, such as peptidoglycan biosynthesis (**Fig. 4A**). Pathways outside of these superpathway classes, like glycolysis and reductive acetyl coenzyme A pathway, were also increased in current smokers relative to former/never smokers.

We then correlated normalized imputed pathways with normalized taxonomic abundance of taxa previously identified as significantly differentially abundant by ANCOM (**Fig. 4B**), as in our smoking cessation and dose analysis. We found that taxa depleted in current

Table 2. Taxa differential *Q* values relative to nonsmokers by smoking status.

	Smoking status						
		Time since smoking cessation				Cigs/day smoked	
Taxa and phylogenetic level	**Current smokers**	**<10 years**	**10–29 years**	**30+ years**	**Former Smokers**	**1–10 cigarettes**	**11+ cigarettes**
Acidaminococcus Genus	8.75E−09	0.0094	0.0687	1	0.0392	5.27E−05	4.70E−05
Erysipelotrichaceae novel ASV	1.70E−06	0.1250	0.9353	1	0.2148	0.0117	5.31E−05
Lachnospira Genus	1.75E−08	0.5552	0.1066	1	0.0687	0.0007	1.15E−05
Oscillospira novel ASV	0.0062	0.0022	0.0279	0.0006	1.52E−06	0.6919	0.0053
Pasteurellales Order	0.0004	0.3109	0.0687	1	0.0110	0.0329	0.0145
Peptostreptococcaceae novel ASV	0.0008	0.0251	7.01E-05	1	4.27E−05	0.0013	0.1080
Phascolarcobacterium novel ASV	0.0011	1	0.9720	0.2553	0.6176	0.0329	0.0427
Prevotella copri	0.0031	1	0.9720	0.0517	0.6176	0.0329	0.1080
Tenericutes Phylum	0.0171	0.3109	0.9353	1	0.1095	0.6919	0.0355

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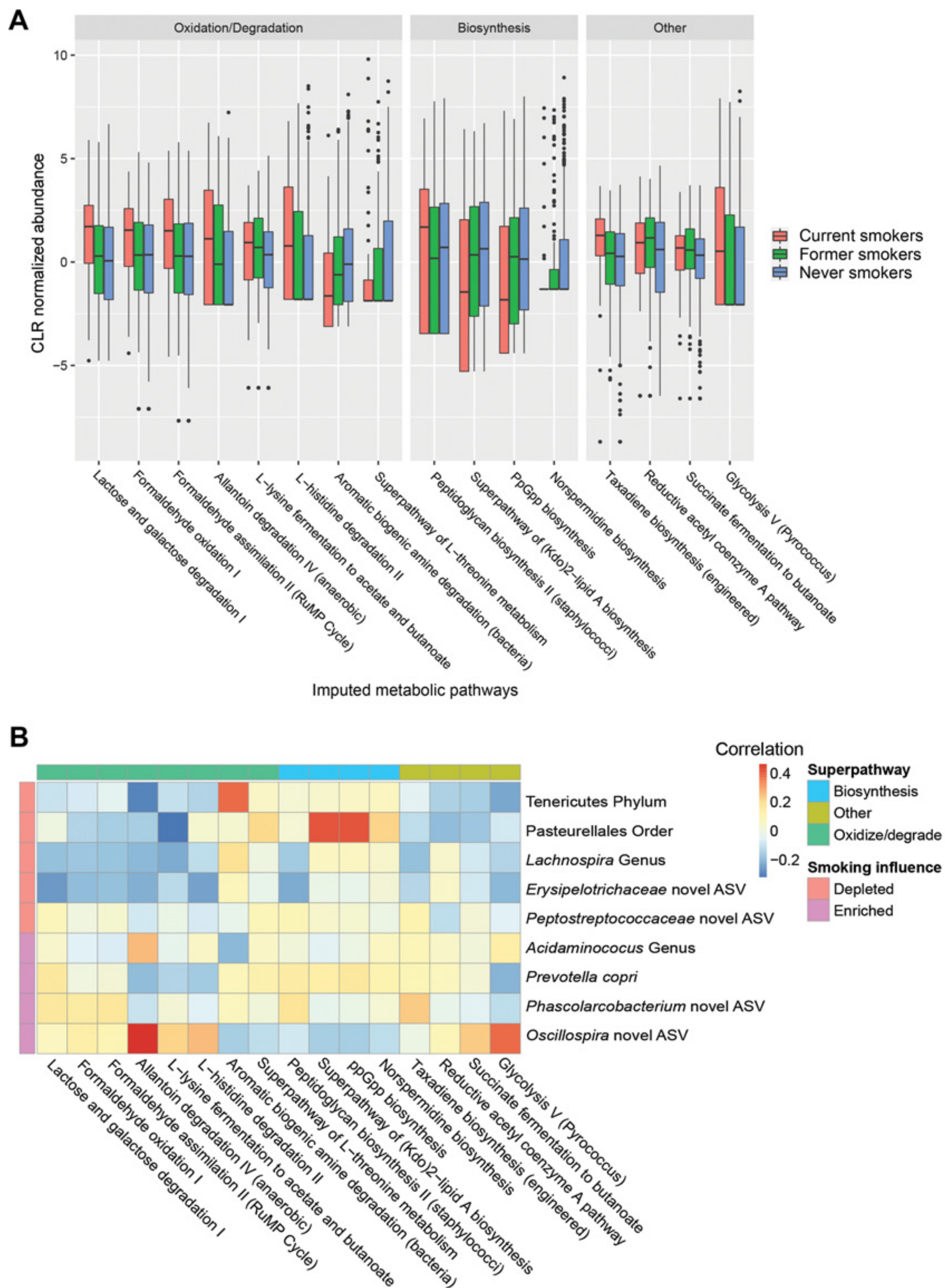

Figure 4.

Inferred metabolic pathway abundance by smoking status. CLR-normalized inferred abundance of MetaCyc pathways as calculated by PICRUSt2. **A,** Normalized pathway abundance in current (red), former (green), and never smokers (blue). Pathways grouped by MetaCyc SuperPathway definition as oxidative/degradative or biosynthetic. Unclassified pathways noted as "Other." **B,** Correlation between imputed pathways and ANCOM-identified significant taxa, annotated with superpathway function and smoking influence on taxonomic abundance. Positive correlation noted in red, whereas negative correlation noted in blue.

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smokers relative to never or former smokers negatively correlated with imputed oxidative/degradative superpathway members and unclassified pathways. An ASV from *Peptostreptococcaceae* did not conform to this trend in pathway correlation, despite showing significant depletion in current smokers. Of smoking depleted taxa, only the order Pasteurellales demonstrated significant positive correlation with biosynthetic pathways. Taxa enriched in current smokers, specifically an *Oscillospira* ASV and the genus *Acidaminococcus*, demonstrated positive correlation with oxidative/degradative superpathway members and unclassified pathways, including allantoin degradation and glycolysis (**Fig. 4B**).

Discussion

This work demonstrates that the β-diversity of the fecal microbiome differs in current and former smokers from never smokers. We found that previously observed phylum-wide shifts in Bacteroidetes and Firmicutes were broadly similar across racial subgroups. These data suggest that smoking associated shifts persist much longer than previously hypothesized, with most taxa normalizing only after approximately 30 years cessation of tobacco use. These suggest underlying long-term changes in the microbial niche, which have not previously been described. Further, these data demonstrated novel smoking differentials in fecal taxonomic abundance, especially in the *Veillonellaceae* family and Tenericutes phylum.

Enrichment in oral *Veillonellaceae* in current smokers has been reported in multiple studies (2, 35, 36), yet no prior work had demonstrated changes in this taxa in the fecal microbiome. Our results are consistent with oral-fecal bacterial transfer, especially in the presence of environmental stressors such as tobacco use (37). Prior studies have also demonstrated an enrichment in *Veillonellaceae* family members in colon and lung cancer tissue (38, 39), suggesting an important role as a marker of dysbiosis and a possible role in carcinogenesis. The relationship between tobacco smoking and Tenericutes abundance is less clear. Two prior studies found enrichment of this taxa with smoking (3, 40), whereas we observed depletion. Although this may be due to differences in populations, further studies are necessary to clarify the effect of smoking on Tenericutes phylum abundance, and to determine whether these shifts play a role in smoking-associated gut pathogenesis.

Although several studies have demonstrated the importance of race and immigrant acculturation to microbiome composition (12, 41, 42), few have evaluated effect of smoking on the microbiome in a multiethnic population. Our findings, which show that smoking leads to similar shifts across these populations, suggest a commonality of mechanisms by which smoking impacts the gut microbiome. That these shifts are present in all racial and ethnic subgroups, with documented variations in diet and culture (43, 44), reinforces the magnitude of smoking-associated microbial differentials. Thus, common mechanisms of tobacco smoking related shifts may include the influence of smoking-associated nitrous oxide compounds (4), smoking-associated immune changes (45), and possible pathogenic content of cigarette tobacco (8).

Evaluation of the imputed metabolic pathways in current and former smokers suggests that relative taxonomic differentials may arise due to exposure to smoking-associated toxins and free-radical species. Taxa enriched in current smokers, specifically the genus *Acidaminococcus* and an *Oscillospira* ASV, were positively correlated with formaldehyde and allantoin degradation, and glycolysis V. Given the known formaldehyde content of cigarette smoke (46), documented elevations in plasma allantoin in smokers (47), and upregulation of glycolysis in response to reactive oxygen species (48, 49), our data suggest a potential selection mechanism for these taxa based on available metabolic inputs. These suggest that smoking-related toxins ROS may be present in the colon in sufficient quantities to affect microbial ecology, and that these taxa may play a role in the known association between smoking, ROS, and carcinogenesis (50).

A limitation of our investigation, as a cross-sectional study, is that the temporal relationship between smoking-related exposures and gut microbial outcomes cannot be directly tested. However, given that smoking is a behavior and the gut microbiome is an observed state, it is unlikely that alteration of the gut microbiome precedes or causes smoking. The study may also have a limitation in that smoking behavior is ascertained through questionnaire, which may be subject to recall and reporting bias, and that racial/ethnic differentials were evaluated with relatively small numbers in certain groups.

In summary, our research demonstrates that smoking is associated with profound and long-lasting differences in the gut microbiome, across a diverse population, which likely persist following smoking cessation. The observed differentials may also be associated with microbial upregulation of pro-inflammatory and carcinogenic pathways, and downregulation of biosynthetic pathways and homeostatic taxa. Longitudinal and direct-transcriptional follow-up studies are warranted to evaluate the mechanistic implications of our findings.

Authors' Disclosures

R.B. Hayes reports grants from NCI during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

A. Prakash: Conceptualization, data curation, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **B.A. Peters:** Conceptualization, supervision, methodology, writing–review and editing. **E. Cobbs:** Resources, data curation, writing–review and editing. **D. Beggs:** Resources, data curation, writing–review and editing. **H. Choi:** Resources, data curation, writing–review and editing. **H. Li:** Methodology, writing–review and editing. **R.B. Hayes:** Conceptualization, resources, supervision, funding acquisition, project administration, writing–review and editing. **J. Ahn:** Conceptualization, resources, supervision, funding acquisition, methodology, project administration, writing–review and editing.

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References

1. Biedermann L, Zeitz J, Mwinyi J, Sutter-Minder E, Rehman A, Ott SJ, et al. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. PLoS One 2013;8:e59260.
2. Kato I, Vasquez AA, Moyerbrailean G, Land S, Sun J, Lin HS, et al. Oral microbiome and history of smoking and colorectal cancer. J Epidemiol Res 2016; 2:92–101.

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3. Lee SH, Yun Y, Kim SJ, Lee EJ, Chang Y, Ryu S, et al. Association between cigarette smoking status and composition of gut microbiota: population-based cross-sectional study. J Clin Med 2018;7:282.
4. Savin Z, Kivity S, Yonath H, Yehuda S. Smoking and the intestinal microbiome. Arch Microbiol 2018;200:677–84.
5. Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, et al. Cigarette smoking and the oral microbiome in a large study of American adults. ISME J 2016;10: 2435–46.
6. Stabbert R, Dempsey R, Diekmann J, Euchenhofer C, Hagemeister T, Haussmann HJ, et al. Studies on the contributions of smoke constituents, individually and in mixtures, in a range of in vitro bioactivity assays. Toxicol In Vitro 2017;42: 222–46.
7. Stone WL, Krishnan K, Campbell SE, Palau VE. The role of antioxidants and prooxidants in colon cancer. World J Gastrointest Oncol 2014;6:55–66.
8. Sapkota AR, Berger S, Vogel TM. Human pathogens abundant in the bacterial metagenome of cigarettes. Environ Health Perspect 2010;118:351–6.
9. Verschuere S, Bracke KR, Demoor T, Plantinga M, Verbrugghe P, Ferdinande L, et al. Cigarette smoking alters epithelial apoptosis and immune composition in murine GALT. Lab Invest 2011;91:1056–67.
10. Claus SP, Guillou H, Ellero-Simatos S. The gut microbiota: a major player in the toxicity of environmental pollutants? NPJ Biofilms Microbiomes 2016;2:16003.
11. Jung YS, Kim NH, Yang HJ, Park SK, Park JH, Park DI, et al. The impact of passive smoking on the risk of colorectal neoplasia in never, former, and current smokers. J Gastroenterol Hepatol 2018;33:1023–30.
12. Peters BA, Yi SS, Beasley JM, Cobbs EN, Choi HS, Beggs DB, et al. US nativity and dietary acculturation impact the gut microbiome in a diverse US population. ISME J 2020;14:1639–50.
13. 07/13/19. FAMiLI Study: Food and Microbiome Longitudinal Investigation. NYU Langone Medical Center <nyufamilu.org>. Accessed July 13, 2019.
14. Nossa CW, Oberdorf WE, Yang L, Aas JA, Paster BJ, Desantis TZ, et al. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J Gastroenterol 2010;16:4135–44.
15. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852–7.
16. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, et al. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 2013;10:57–9.
17. Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, et al. Deblur rapidly resolves single-nucleotide community sequence patterns. mSystems 2017;2:e00191–16.
18. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 2006;72:5069–72.
19. Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 2002;30:3059–66.
20. Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 2010;5:e9490.
21. Bray JR, Curtis JT. An ordination of the upland forest communities of southern wisconsin. Ecol Monogr 1957;27:326–49.
22. Legendre P, Borcard D, Peres-Neto PR. Analyzing beta diversity: partitioning the spatial variation of community composition data. Ecol Monogr 2005;75: 435–50.
23. McArdle BH, Anderson MJ. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 2001;82:290–7.
24. Warton DI, Wright ST, Wang Y. Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol Evol 2012;3:89–101.
25. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol 2001;26:32–46.
26. Flegal KM, Graubard BI. Estimates of excess deaths associated with body mass index and other anthropometric variables. Am J Clin Nutr 2009;89:1213–9.
27. Consultation WHOE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157–63.
28. Mandal S, Van Treuren W, White RA, Eggesbo M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microb Ecol Health Dis 2015;26:27663.
29. Kaul A, Mandal S, Davidov O, Peddada SD. Analysis of microbiome data in the presence of excess zeros. Front Microbiol 2017;8:2114.
30. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature 2012; 486:222–7.
31. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome datasets are compositional: and this is not optional. Front Microbiol 2017;8:2224.
32. Aitchison J. The statistical analysis of compositional data. Journal of the Royal Statistical Society 1982;44:139–77.
33. Douglas GM, Maffei VJ, Zaneveld J, Yurgel SN, Brown JR, Taylor CM, et al. PICRUST2: an improved and extensible approach for metagenome inference. BioRxiv 672295 [Preprint]. March 20, 2020.
34. Caspi R, Altman T, Dreher K, Fulcher CA, Subhraveti P, Keseler IM, et al. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res 2012;40:D742–53.
35. Lim MY, Yoon HS, Rho M, Sung J, Song YM, Lee K, et al. Analysis of the association between host genetics, smoking, and sputum microbiota in healthy humans. Sci Rep 2016;6:23745.
36. Shanahan ER, Shah A, Koloski N, Walker MM, Talley NJ, Morrison M, et al. Influence of cigarette smoking on the human duodenal mucosa-associated microbiota. Microbiome 2018;6:150.
37. Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, et al. Ectopic colonization of oral bacteria in the intestine drives TH1 cell induction and inflammation. Science 2017;358:359–65.
38. Yan X, Yang M, Liu J, Gao R, Hu J, Li J, et al. Discovery and validation of potential bacterial biomarkers for lung cancer. Am J Cancer Res 2015;5:3111–22.
39. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, et al. Comparison of human gut microbiota in control subjects and patients with colorectal carcinoma in adenoma: Terminal restriction fragment length polymorphism and next-generation sequencing analyses. Oncol Rep 2016;35:325–33.
40. Valles Y, Inman CK, Peters BA, Ali R, Wareth LA, Abdulle A, et al. Types of tobacco consumption and the oral microbiome in the United Arab Emirates Healthy Future (UAEHFS) Pilot Study. Sci Rep 2018;8:11327.
41. Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, et al. US immigration westernizes the human gut microbiome. Cell 2018; 175:962–72.
42. Hoffman KL, Hutchinson DS, Fowler J, Smith DP, Ajami NJ, Zhao H, et al. Oral microbiota reveals signs of acculturation in Mexican American women. PLoS One 2018;13:e0194100.
43. Brooks AW, Priya S, Blekhman R, Bordenstein SR. Gut microbiota diversity across ethnicities in the United States. PLoS Biol 2018;16:e2006842.
44. Gupta VK, Paul S, Dutta C. Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. Front Microbiol 2017;8: 1162.
45. Schwartz J, Weiss ST. Cigarette smoking and peripheral blood leukocyte differentials. Ann Epidemiol 1994;4:236–42.
46. Godish T. Formaldehyde exposures from tobacco smoke: a review. Am J Public Health 1989;79:1044–5.
47. Seet RC, Lee CY, Loke WM, Huang SH, Huang H, Looi WF, et al. Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress? Free Radic Biol Med 2011;50:1787–93.
48. Zhang Y, Kouril T, Snoep JL, Siebers B, Barberis M, Westerhoff HV. The peculiar glycolytic pathway in hyperthermophylic archaea: understanding its whims by experimentation in silico. Int J Mol Sci 2017;18:876.
49. Ghanbari Movahed Z, Rastegari-Pouyani M, Mohammadi MH, Mansouri K. Cancer cells change their glucose metabolism to overcome increased ROS: one step from cancer cell to cancer stem cell? Biomed Pharmacother 2019;112: 108690.
50. Carini F, Mazzola M, Rappa F, Jurjus A, Geagea AG, Al Kattar S, et al. Colorectal carcinogenesis: role of oxidative stress and antioxidants. Anticancer Res 2017;37: 4759–66.

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