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Association of oral microbiome with Type 2 diabetes risk

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Abstract

Background and Objective—The oral microbiome may help to maintain systemic health, including how it affects blood glucose levels; however, direct evidence linking the oral microbiome with diabetes is lacking.

Material and Methods—We compared the oral microbiome profiles of 98 participants with incident diabetes, 99 obese non-diabetics, and 97 normal weight non-diabetics, via deep sequencing of the 16S rRNA gene.

Results—We found that the phylum *Actinobacteria* was present significantly less abundant among diabetes patients than among the controls ($P=3.9 \times 10^{-3}$); the odds ratio (OR) and 95% confidence interval (CI) was 0.27(0.11–0.66) for those individuals who had relative abundance higher than the median value. Within this phylum, five families and seven genera were observed, and most of them were less abundant among diabetes patients. Notably, genera *Actinomyces* and *Atopobium* were associated with 66% and 72% decreased risk of diabetes with P values of 8.9×10^{-3} and 7.4×10^{-3} , respectively. Stratified analyses by race showed that most taxa in this phylum were associated with diabetes in both black and white participants. This phylum was also less abundant among non-diabetic obese subjects compared to normal weight individuals, especially genera *Mobiluncus*, *Corynebacterium* and *Bifidobacterium*, which showed $P < 0.05$.

Conclusion—Our study revealed that multiple bacteria taxa in the phylum *Actinobacteria* are associated with risk of type 2 diabetes. Some are also associated with the prevalence of obesity, suggesting that the oral microbiome may play an important role in diabetes etiology.

Keywords

diabetes; oral microbiome; next-generation sequencing

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INTRODUCTION

Diabetes is a leading health problem in the US and in many other parts of the world. There is growing evidence suggesting a two-way relationship between diabetes and oral health. Multiple large population-based studies have reported that periodontal diseases were independently associated with an increased risk of diabetes (1,2). Treatment of oral diseases has been shown to be associated with improved glycemic control and a reduction in HbA1C (3,4). On the other hand, individuals with diabetes are more likely to have periodontitis with increased severity when their diabetes is uncontrolled or poorly controlled (5). It has been suggested that systemic inflammation may be the link between the pathogenesis of diabetes and oral diseases (6,7).

The human mouth is heavily colonized by microorganisms, with over 700 species detected (8). The oral microbiome plays a critical role in oral diseases such as dental caries and periodontal diseases. It has been suggested that the oral microbiome also plays a role in systemic health through pathogen inhibition, immune regulation, nutrition absorption and metabolism (9). Increasing evidence suggests that oral bacteria can migrate to extra-oral sites, causing infection and inflammation (8,10). Alteration in the oral microbial community structure has been suggested to be linked to systemic diseases, including cardiovascular disease, stroke, and Alzheimer's disease (11–15).

There is also growing evidence suggesting that the oral microbiome plays an important role in obesity and diabetes. Studies have suggested a direct link between periodontal pathogenic bacteria (such as *P. gingivalis* and *A. actinomycetemcomitans*) and glycemic control and diabetes risk (16–19). However, direct investigations of the relationship between the oral microbiome and diabetes have been very limited. In a study of 29 morbidly-obese subjects, including 13 diabetes patients, the genus *Bifidobacteria* in the phylum *Actinobacteria* was shown to have a lower abundance in the diabetes patients (20). In another study including 20 diabetes cases and 11 controls (21), two genera, *Streptococci* and *Lactobacilli*, in the phylum *Firmicutes* were found to be more abundant in the diabetes patients. However, the biological samples used in these two studies were collected after a diabetes diagnosis, which could have led to flawed conclusions because disease status and treatment are known to alter microbial profiles. In addition, these studies were limited by their small (20 cases) sample sizes and/or a limited number of bacteria species investigated. Studies are needed to prospectively investigate the associations of oral microbiota in diabetes etiology. In this report, we describe the results from a nested case-control study investigating the associations of the oral microbiome with incident diabetes using data and pre-diagnosis biological samples from a prospective cohort study, the Southern Community Cohort Study (SCCS).

MATERIAL AND METHODS

Study participants and data collection

The SCCS is an ongoing prospective cohort study investigating risk factors for cancer and other chronic diseases including diabetes. Details on the methodology of the study have been described elsewhere (22). In brief, approximately 86,000 adults, two-thirds of whom are African Americans (Blacks), were recruited between 2002 and 2009 from 12 southeastern

states. Approximately 86% of them were recruited from community health centers (CHCs), institutions providing basic health care and preventative services in underserved areas, so that the cohort includes large numbers of individuals of low income and educational status. The remaining 14% of the cohort members were recruited through mail-based general population sampling. At the time of enrollment, a mouth rinse sample was collected from ~34,100 participants. The SCCS was reviewed and approved by the institutional review boards at Vanderbilt University Medical Center and Meharry Medical College. Written informed consent was obtained from all study participants.

For the baseline survey, participants completed a comprehensive questionnaire, which collected information on anthropometric characteristics, lifestyle factors, disease history, medication use, and other characteristics. Passive cohort follow-up by record linkage to state tumor registries and the National Death Index registry started immediately after the completion of the baseline survey. Active follow-up surveys started in 2008. In the active follow-up surveys, participants were asked about their personal medical history and medication use. Information on diabetes was obtained from the baseline and follow-up surveys with the question: "Has a doctor ever told you that you have had diabetes or high blood sugar?" For those who responded "yes," follow-up questions were asked regarding age at diagnoses and use of diabetes medications. In the SCCS, 70% of the surviving cohort participants successfully completed the first round follow-up survey.

In the research presented here, we conducted a nested case-control study that included 300 participants grouped into three sets of 100 individuals, with each set comprising an incident diabetes case, a non-diabetic obese participant, and a normal weight non-diabetic control. All individuals in the present study were selected only from those who completed active follow up. Diabetes cases were defined as incident cases (for those with no prior history of diabetes and had never used diabetic medication at the time of the baseline survey) and a diagnosis of diabetes by a physician, or the reported use of diabetes medication on the follow-up survey. For each incident diabetes case, we selected one control (normal weight group) and one non-diabetic obese group by doing individual matching. The non-diabetic obesity group was selected to investigate whether the associations between bacteria taxa with diabetes were through association with obesity. Normal weight controls were selected from participants who had a normal weight of $25 < \text{BMI} < 18.5$ at cohort entry and follow-up, did not have a history of diabetes, and did not use diabetic medication. The 100 non-diabetic obese participants were selected from those participants who had a $\text{BMI} \geq 30$, did not have a prior history of diabetes, and did not use diabetic medication at the time of the baseline, during follow-up, or up to the time when the index diabetes case was diagnosed. We individually matched controls and obesity cases to index diabetes cases by age (± 2 years), race, sex, smoking status (current, former, or never), date of mouth rinse sample collection (± 90 days), recruitment source, and the CHC recruitment site. Participants who used antibiotics during the year prior to sample collection were excluded. Participants with a history of HIV infection, cancer (except skin cancer), stroke, or myocardial infarction were excluded as well. To minimize batch effects during the experiment, we included three samples from each set in the same 96-well plate during DNA preparation and sequencing.

DNA extraction and 16S rRNA gene sequencing

DNA was isolated from mouth rinse samples using Qiagen's QIAmp DNA kit. Sequencing libraries were prepared using the NEXTflex 16S V4 Amplicon-Seq Kit (Bioo Scientific 4201-05), following the manufacturer's protocol. This kit was designed to sequence approximately 253bp of the fourth hypervariable (V4) domain of the bacterial 16S rRNA gene (23,24). Sequencing was performed at pair-end 150bp using Illumina MiSeq 300 at the VANDerbilt Technologies for Advanced Genomics (VANTAGe) Core. Each 96-well plate was sequenced on one MiSeq run with two duplicated quality controls (QCs) and one negative control sample included.

Sequence data processing and QC

Sequencing data were processed using QIIME (Quantitative Insights Into Microbial Ecology) package v1.8. The pair-end reads were stitched together using `join_paired_ends.py` with parameters of `-j 45 -p 5` (45bp overlapped and 5% unmatched bp between the paired reads). Sequencing reads were then de-multiplexed, with barcode and primers removed using `split_libraries_fastq.py`. Low-quality reads with phred score <30 were filtered out. Chimeric sequences were removed using ChimeraSlayer (25) and implemented in `identify_chimeric_seqs.py`. Sequence reads were then clustered into Operational Taxonomic Units (OTUs) at 97% sequence identity by using `uclust` (26) against the GreenGenes database release `gg_13_5` (27). OTUs with very low abundance (fraction <0.0005%) were discarded using `filter_otus_from_otu_table.py`. OTU tables were rarefied to 5,000 sequences per sample to account for variations in sequencing depth. The most abundant sequence in each OTU was selected as a representative. Taxonomy was then assigned for each OTU using the GreenGenes taxonomy map (28). Samples with sequencing reads <5,000 were removed. A total of 98 incident diabetes cases, 99 obesity cases and 97 normal weight controls were included in the final analyses.

Statistical analysis

Associations between bacteria taxa and diabetes risk or obesity prevalence were identified through conditional logistic regression analyses in each matched set. Subjects were categorized into two groups according to the median value of relative abundance for each taxon among control subjects. Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for measuring the association of taxon abundance with diabetes risk or obesity prevalence. The group with relative abundance lower than the median values was the reference. Potential confounder factors that were not matched between cases and controls, such as physical exercise, total energy intake, oral health condition (such as tooth loss) and BMI (when investigating associations between microbiome and diabetes risk) were adjusted.

RESULTS

Characteristics of the study subjects

Table 1 presents the distribution of selected baseline demographic characteristics for participants. Diabetes cases, non-diabetic obese participants, and normal weight controls

were matched with respect to race, sex, age, and smoking status. Control participants had higher education levels (with an especially high percentage of participants with education college), higher annual household income, better oral health (with a higher proportion of people having a full set of teeth), and were more likely to be drinkers than in diabetes cases. The above characteristics for the non-diabetic obese cases resembled the diabetes group more closely than the controls except that non-diabetic obese cases were more likely to be drinkers than diabetes cases.

Sequencing summary

Among the 294 samples, we obtained 22.8 million sequence reads, with an average of 77,473 reads per sample. A total of 682 OTUs were observed, with a mean of 140 OTUs and a range of 60–269 OTUs per sample. Most of these OTUs were rare, with only 10% having a relative abundance >0.1%. These OTUs were classified into 11 phyla and 95 genera. The phylum *Firmicutes* predominated, representing approximately 73% of the bacteria. The other three common phyla were *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, with average relative abundances of 11.8%, 7.2%, and 6.5%, respectively (Figure 1).

Associations of microbiome composition with diabetes

At the phylum level, *Actinobacteria* was significantly associated with a decreased risk of diabetes ($P=3.9 \times 10^{-3}$). This phylum was common, observed among all participants, with a median abundance of 5.1% in diabetes cases and 6.8% in controls. Compared with the reference group (participants with relative abundance lower than the median value), the OR and 95% CI was 0.5127(0.11–0.66) for participants with relative abundance higher than median value (Table 2). Within this phylum, five families were observed, *Actinomycetaceae*, *Bifidobacteriaceae*, *Coriobacteriaceae*, *Corynebacteriaceae*, and *Micrococcaceae*, and all of them were less abundant among diabetes cases compared to normal weight controls, with ORs (95% CIs) being 0.29(0.10 – 0.83), 0.87(0.35 – 2.13), 0.17(0.02 – 1.34), 0.73(0.38 – 1.40) and 0.56(0.29 – 1.07), respectively (Table 2). At the genus level, a total of seven taxa were clearly defined. With the exception of *Scardovia*, all the other 6 genera were less abundant among diabetes cases than among controls. Notably, genera *Actinomyces* and *Atopobium* were significantly associated with a decreased risk of diabetes with ORs (95% CIs) being 0.34(0.15 – 0.76) and 0.28(0.11 – 0.71), and P values of 8.9×10^{-3} and 7.4×10^{-3} , respectively (Table 2). In addition to the relative abundance, genera *Mobiluncus*, *Atopobium* and *Corynebacterium* were less prevalent among diabetes cases than among controls, with 7.1% vs. 11.3%, 65.3% vs. 74.2% and 79.6% vs. 83.5%, respectively. Stratified analyses by race showed that most taxa in the phylum *Actinobacteria* were associated with diabetes in both black and white participants (Table 3); however, the associations did not reach statistical significance with the exception of the phylum *Actinobacteria* among Blacks with OR (95% CI) being 0.39(0.16 – 1.00) and a P value of 0.05.

We found that the family *Gemellaceae* in the phylum *Firmicutes* was associated with an increased risk of diabetes. This family was observed to be present in all of the diabetes cases and 99% of the controls. Participants having abundance higher than the median value has a 77% increased risk of developing diabetes. In this family, only one genus, *Gemella*, was

clearly defined. It was associated with an increased risk of diabetes, with OR (95% CI) being 1.77(0.92 – 3.43). In this family, there was one unclearly-defined genus also associated with an increased risk of diabetes, with OR (95% CI) being 2.70(1.26 – 5.79) and having a P value of 0.01.

We further investigated whether those bacterial taxa that were associated with diabetes risk were correlated to obesity, a well-established risk factor for diabetes, by comparing non-diabetic obese and normal weight participants for these bacterial taxa. We found that the phylum *Actinobacteria* was presented with lower abundance among obese subjects compared to normal weight individuals. Especially, genera *Mobiluncus*, *Corynebacterium* and *Bifidobacterium* showed significant associations with ORs (95% CIs) being 0.15(0.03–0.77), 0.38(0.16–0.93), 0.25(0.07–0.94), and having P values of 0.02, 0.03 and 0.04, respectively. Similarly, the family *Gemellaceae* and the genus *Granulicatella* in the phylum *Firmicutes* were more abundant among non-diabetic obese participants than among normal weight controls; however the difference did not reach significance.

DISCUSSION

Increasing evidence suggests that oral bacteria may play an important role in the pathogenesis of chronic diseases, including diabetes, via its role in regulating systemic inflammation (11–15). In this nested case-control study, we found that higher abundance of the phylum *Actinobacteria* and almost all taxa in this phylum were associated with a decreased risk of diabetes. At the genus level, *Actinomyces* was the most abundant with a median abundance >1%, and showed a strong association with diabetes risk. On the other hand, the family *Gemellaceae* in the phylum *Firmicutes* showed higher abundance among diabetes patients than among normal weight controls.

The phylum *Actinobacteria* (a synonym for *Actinomycetes*) is a group of gram-positive bacteria. These bacteria are the producers of many bioactive metabolites, and are present in two-thirds of the antibiotics used in the world (29). They also produce inhibitors for enzymes, such as Amylase and N-Acetyl-b-D-glucosaminidase. Amylase, present in the saliva, is the major enzyme in catalyzing hydrolysis of dietary starch into disaccharides and trisaccharides. Amylase inhibitors produced by *Actinobacteria* may be useful in controlling carbohydrate-dependent diseases such as diabetes (30). N-Acetyl-glucosaminidase is higher in diabetes patients and is correlated with glycemic control (31). Inhibitors of this enzyme, produced by *Actinobacteria*, could potentially be developed as a therapeutic agent for diabetes (32).

The genus *Actinomyces*, one of the two common genera in the phylum *Actinobacteria*, showed a strong inverse association with diabetes risk in the present study. It was observed among 100% of participants, with median abundances of 1.9% among diabetes cases, 2.1% among non-diabetic obese and 2.7% among normal weight controls. Similar prevalence and abundance were observed in the oral samples from the human microbiome project (HMP), present among 99.2% subjects with a median abundance of 2.9%. Interestingly, gut microbiome studies have shown that the genus *Bifidobacterium* in the phylum *Actinobacteria* was associated with a decreased risk of diabetes and obesity (33,34). Similar

but non-significant associations were observed between the oral *Bifidobacterium* and diabetes in the present study and in another oral microbiome study (20). This genus was only observed among ~33% of subjects in the present study, with an abundance of only ~0.1%. Therefore, the statistical power for assessing this genus in association with diabetes risk in the present study is low. Human intervention studies of probiotic yogurt containing *Bifidobacterium lactis* resulted in an improvement in glucose-tolerance and glucose-induced insulin secretion, HbA1C, and antioxidant activities, as well as a decrease in inflammation (35–39). These findings highlight the importance of investigating bacteria taxa in the phylum *Actinobacteria* on the development of diabetes and serving as a potential therapeutic target in diabetes. If the results in the present study can be validated in independent studies, the findings are easy to translate because oral microbiota are easy to assess and modify.

The phylum *Firmicutes* was the most common phylum in oral samples. In the present study, associations with diabetes were observed for multiple taxa within this phylum. The genus *Granulicatella*, in the family *Gemellaceae*, showed higher abundance among diabetic and non-diabetic obese participants than among normal weight controls. The family *Gemellaceae* has been reported to be more abundant in saliva samples from obese participants than in normal weight individuals (40). This family was also reported to be more prevalent in Crohn's disease mucosa (41). The genus *Gemella* has recently been reported to be among the genera most predictive of dysbiosis (42). This genus has been shown to be positively associated with dental cavities (43), but two species within this genus were inversely associated with periodontitis in another study (44).

Limited research has been conducted to investigate the possible etiologic connections between the oral microbiome composition and diabetes (20,21). Although multiple bacteria taxa were suggested to be associated with diabetes, only a few of these were replicated in subsequent studies. As mentioned above, the genus *Bifidobacteria* in the phylum *Actinobacteria* showed lower levels in diabetes patients (20), an association that we replicated in the present study. A previous study including 20 prevalent diabetes patients reported that two genera in the phylum *Firmicutes*, including *Streptococcus* and *Lactobacillus*, were more abundant in diabetes patients (21). We did not find these taxa were associated with diabetes.

To our best knowledge, the present study is the largest study, and the only prospective study, to investigate the role of the oral microbiome in the development of diabetes. Inclusion of both White and Black participants, the ability to match multiple socio-demographic factors and smoking behavior, and the inclusion of both normal weight and non-diabetic obese control subjects are the noticeable strengths of this study. One of the limitations of the present study is that diabetes cases were self-reported. We have previously conducted a study to validate self-reported diabetes in a subset of 800 study participants and found that 94% of self-reported cases were validated (45). This is consistent with prior studies and support that self-reported incident diabetes is largely accurate and can be used in epidemiology studies (46–48).

Another limitation is the lack of a systematic assessment of oral health at the baseline exam when the samples were collected. Data on tooth decay and tooth loss were collected during

the follow-up, and were adjusted in the analyses of the association of the microbiome with diabetes and obesity. Previous studies showed that periodontal pathogenic bacteria, such as *P. gingivalis*, *T. denticola* and *T. forsythia* were associated with diabetes risk and glycemic control (16–19,49). Our study was limited because we can only classify the bacteria at the genus level and cannot investigate these specific periodontal pathogenic bacteria species in relation to diabetes risk. Whole metagenome shotgun sequencing is needed for a more comprehensive investigation of the microbiome at the species/strain level. In the present study, most of the associations with diabetes were observed in both Blacks and Whites. However, our study did not have adequate statistical power to detect potential modification effects by race.

In the present study, 11 phyla, 57 families and 95 genera were observed. The significant associations reported above would lose significance if the Bonferroni correction for multiple comparisons were applied. However, these taxa were not independent. The Bonferroni correction is likely to be too conservative and lead to a false negative conclusion. In addition, most of the bacterial taxa had very low prevalence and abundance, and our study does not have sufficient power to carry out a systematic evaluation of this. Further investigation with a large sample size is warranted.

In summary, we found that multiple bacterial taxa were associated with diabetes in this prospective study. Higher abundances of most taxa in the phylum *Actinobacteria* were associated with a decreased risk of diabetes. These results suggest that the oral microbiome may play an important role in diabetes etiology. A more comprehensive investigation with a larger sample size and with metagenome sequencing is warranted.

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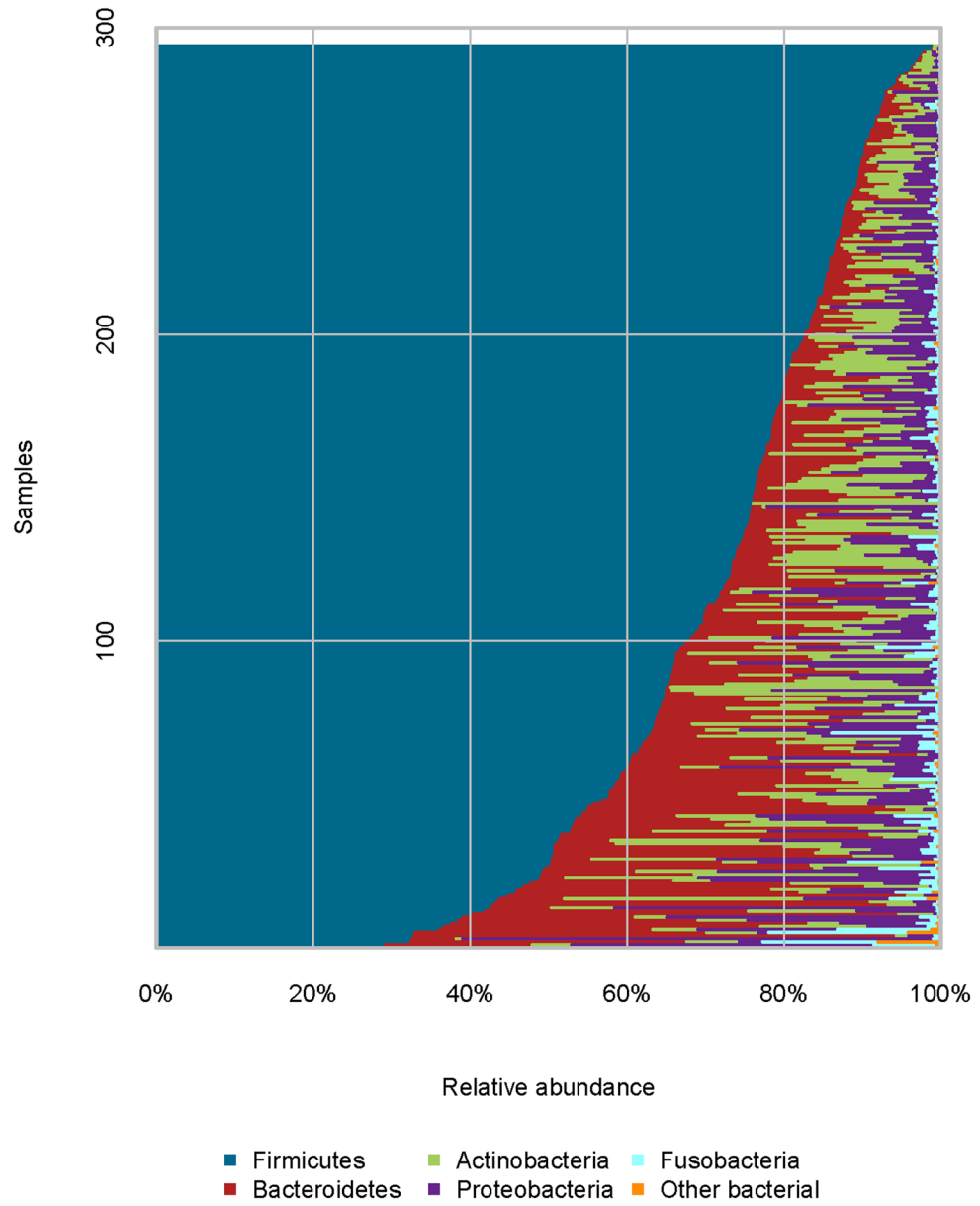


Figure 1. Relative abundance of bacterial phyla in individual samples.

Table 1
 Characteristics of the study participants in the Southern Community Cohort Study^a

Characteristic	Group	Diabetes (N=98)	Normal weight non-diabetics (N=97)	Obese non-diabetics (N=99)
Race	Whites	51.0	50.5	50.5
	Blacks	49.0	49.5	49.5
Sex	Male	51.0	50.5	50.5
	Female	49.0	49.5	49.5
Age	40–49	40.8	37.1	39.4
	50–59	44.9	48.5	45.5
	60–69	14.3	14.4	15.2
BMI	Underweight, <18.5	1.0		
	Normal, 18.5–24.9	14.4	100.0	
	Overweight, 25–29.9	28.9		
	Obese, ≥30	55.7		100
Education	<high school	11.2	7.2	11.1
	High/vocational school	30.6	27.8	24.2
	Some college	25.5	25.8	26.3
	≥college	32.7	39.2	38.4
Annual household income (\$)	<15,000	24.5	26.3	21.4
	15,000–24,999	18.1	12.6	13.3
	25,000–49,999	22.3	21.1	28.6
	≥50,000	35.1	40.0	36.7
Tobacco smoking	Current	27.6	24.7	26.3
	Former	24.5	25.8	25.3

Characteristic	Group	Diabetes (N=98)	Normal weight non-diabetics (N=97)	Obese non-diabetics (N=99)
Alcohol drinking	Never	48.0	49.5	48.5
	None	44.2	28.7	31.3
	Light, <1 drink per day	33.7	41.5	40.6
	Moderate, 1–2 drink/day	13.7	16.0	13.5
Tooth loss	Heavy, >2 drinks per day	8.4	13.8	14.6
	None	24.0	38.9	30.5
	1 to 10	53.1	47.4	53.7
	>10, not all	13.5	9.5	8.4
	All	9.4	4.2	7.4

^aPercentage was presented in the table

Table 2

Associations between Actinobacteria and diabetes risk

Taxa	% Positive (Carriage) ^a			Abundance ^b		
	Case	Control	OR(95% CI)	P value	OR(95% CI)	P value
Phylum Actinobacteria	100.0	100.0			0.27(0.11 – 0.66)	3.9E-03
Family Actinomycetaceae	100.0	100.0			0.29(0.10 – 0.83)	0.02
Genus Actinomyces	100.0	100.0			0.34(0.15 – 0.76)	8.9E-03
Genus Mobiluncus	7.1	11.3	0.48(0.16 – 1.48)	0.20	0.48(0.16 – 1.48)	0.20
Family Bifidobacteriaceae	52.0	48.5	1.05(0.53 – 2.10)	0.88	0.87(0.35 – 2.13)	0.76
Genus Bifidobacterium	33.7	34.0	0.91(0.46 – 1.80)	0.79		
Genus Scardovia	25.5	21.6	1.16(0.53 – 2.54)	0.70	1.25(0.48 – 3.22)	0.65
Family Coriobacteriaceae	68.4	75.3	0.42(0.17 – 1.02)	0.05	0.17(0.02 – 1.34)	0.09
Genus Atopobium	65.3	74.2	0.43(0.19 – 0.96)	0.04	0.28(0.11 – 0.71)	7.4E-03
Family Corynebacteriaceae	79.6	83.5	0.60(0.15 – 2.35)	0.46	0.73(0.38 – 1.40)	0.34
Genus Corynebacterium	79.6	83.5	0.60(0.15 – 2.35)	0.46	0.73(0.38 – 1.40)	0.34
Family Micrococcaceae	100.0	100.0			0.56(0.29 – 1.07)	0.08
Genus Rothia	100.0	100.0			0.56(0.29 – 1.07)	0.08

Note

^aFor those taxa present in all participants, no statistical analyses regarding carriage were conducted.

^bParticipants with relative abundance lower than the median values among controls were the reference group.

Table 3

Associations between Actinobacteria and diabetes risk in Blacks and Whites

Taxa	Race	% Positive (Carriage) ^d				Abundance ^b	
		Case	Control	OR(95% CI)	P value	OR(95% CI)	P value
Phylum Actinobacteria	Black	100.0	100.0			0.39(0.16 – 1.00)	0.05
	White	100.0	100.0			0.34(0.01 – 34.75)	0.65
Family Actinomycetaceae	Black	100.0	100.0				
	White	100.0	100.0			0.33(0.04 – 2.50)	0.28
Genus Actinomyces	Black	100.0	100.0				
	White	100.0	100.0			0.32(0.08 – 1.28)	0.11
Genus Mobiluncus	Black	8.3	14.6	0.38(0.06 – 2.65)	0.33	0.38(0.06 – 2.65)	0.33
	White	6.0	8.2	0.38(0.01 – 27.95)	0.66	0.38(0.01 – 27.95)	0.66
Family Bifidobacteriaceae	Black	43.8	50.0	0.76(0.30 – 1.90)	0.56	1.02(0.40 – 2.60)	0.97
	White	60.0	46.9	1.60(0.56 – 4.59)	0.38		
Genus Bifidobacterium	Black	29.2	37.5	0.71(0.28 – 1.85)	0.49	0.90(0.27 – 2.98)	0.87
	White	38.0	30.6	1.32(0.46 – 3.78)	0.61		
Genus Scardovia	Black	29.2	22.9	1.42(0.50 – 4.00)	0.51	0.71(0.18 – 2.83)	0.63
	White	22.0	20.4				
Family Coriobacteriaceae	Black	68.8	70.8	0.17(0.01 – 2.45)	0.19		
	White	68.0	79.6	0.17(0.03 – 1.14)	0.07	0.27(0.05 – 1.46)	0.13
Genus Atopobium	Black	62.5	70.8				
	White	68.0	77.6	0.31(0.06 – 1.51)	0.15		
Family Corynebacteriaceae	Black	66.7	77.1			0.54(0.15 – 1.87)	0.33
	White	92.0	89.8	0.49(0.06 – 4.21)	0.51	0.63(0.14 – 2.79)	0.54
Genus Corynebacterium	Black	66.7	77.1			0.54(0.15 – 1.87)	0.33
	White	92.0	89.8	0.49(0.06 – 4.21)	0.51	0.63(0.14 – 2.79)	0.54
Family Micrococcaceae	Black	100.0	100.0			0.58(0.21 – 1.62)	0.30
	White	100.0	100.0			0.65(0.09 – 4.80)	0.67
Genus Rothia	Black	100.0	100.0			0.58(0.21 – 1.62)	0.30
	White	100.0	100.0			0.65(0.09 – 4.80)	0.67

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Note

^aFor those taxa present in all participants, no statistical analyses regarding carriage were conducted.

^bParticipants with relative abundance lower than the median values among controls were the reference group.