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ORIGINAL RESEARCH

Causal Associations Between Oral Microbiota and Gestational Diabetes Mellitus: A Two-Sample Mendelian Randomization Study

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Background: Gestational diabetes mellitus (GDM) is associated with adverse pregnancy outcomes. The oral microbiota, influenced by genetic factors, may play a role in GDM development, but the causal association remains unclear.

Methods: We employed a two-sample Mendelian randomization (MR) approach using Genome-Wide Association Study (GWAS) data on GDM from FINN cohort data (ID: finngen_R10_GEST_DIABETES) and GWAS data on the Oral microbiota from the Danish ADDITION-PRO cohort. We screened SNPs significantly associated with Oral microbiota abundance as instrumental variables (IVs) and assessed their association with GDM risk. The study primarily used an inverse variance weighting (IVW) approach and further applied MR-Egger regression, weighted median, and weighted mode methods for robustness testing. Sensitivity analyses were conducted to evaluate the impact of heterogeneity and pleiotropy, including MR-Egger, MR-PRESSO, Cochran's Q, and leave-one-out methods.

Results: We identified 267 IVs associated with Oral microbiota abundance. IVW analysis revealed a positive causal association between Genus *Schaalia* and GDM risk (OR = 1.03, 95% CI: 1.01–1.06, P = 0.02) and a negative association between Genus *Haemophilus* and GDM risk (OR = 0.96, 95% CI: 0.93–1.00, P = 0.034). Sensitivity analyses confirmed the robustness of these two results, showing no evidence of heterogeneity or pleiotropy.

Conclusion: Our study provides evidence for a causal association between Genus *Schaalia* and *Haemophilus* and GDM risk. This highlights the potential role of the Oral microbiota in GDM pathogenesis and suggests potential targets for GDM prevention and treatment.

Keywords: oral microbiota, gestational diabetes mellitus, Mendelian randomization, causal inference, European descent

Background

Gestational Diabetes Mellitus (GDM) is a disorder of glucose metabolism diagnosed for the first time during pregnancy and is among the most prevalent pregnancy complications, with a rising global prevalence.[1] Clinical characteristics of GDM include increased insulin resistance and impaired glucose control, which not only heighten the risk of complications such as preterm birth and preeclampsia in pregnant women but may also result in fetal macrosomia and neonatal hypoglycemia.[2] The development of GDM is associated with a multitude of factors, including genetics, obesity, age, ethnicity, and lifestyle.[3] However, given the complexity and multifactorial nature of gestational diabetes mellitus (GDM), the current understanding remains incomplete. It necessitates continued exploration to identify new and previously unrecognized risk factors.

Oral microbiota is the community of bacteria, fungi, viruses, and protozoa that reside within the oral cavity. Oral microbiota may contribute to the development of GDM through multiple pathways. It can disrupt blood glucose metabolism, for instance, by triggering inflammation through the Toll-like receptor 2 (TLR2) pathway with certain bacterial overgrowth, thus impairing insulin signaling and causing glucose level fluctuations.[4] Additionally, it can heighten systemic inflammation due to local oral inflammation, increasing GDM risk.[5] Observational studies have

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revealed significant differences in the composition of oral microbiota between GDM patients and healthy pregnant women.[6] Another study found that pregnant women with poor oral hygiene were at a higher risk of developing GDM.[7] These findings suggest that oral microbiota may play a significant role in the pathogenesis of GDM. Inflammatory cytokines are key mediators in the interplay between oral microbiota dysbiosis and the development of GDM. Alterations in the oral microbiota of pregnant women with GDM often create a pro-inflammatory environment, which is characterized by increased levels of specific cytokines. These cytokines can influence both local oral health and systemic metabolic processes, contributing to the pathogenesis of GDM.[8,9]

Mendelian Randomization (MR) is a method of causal inference based on genetic principles. The central assumption of MR is that genetic variants can be used as "instrumental variables (IVs)" which can randomly assign exposure factors, thereby avoiding the influence of confounding factors and reverse causality inherent in traditional observational studies, and providing a more reliable assessment of the causal association between exposure factors and disease.[10] Previous studies have used a two-sample MR to reveal causal associations between the gut microbiota and type 1 diabetes.[11] However, the associations by which the oral microbiota may influence the pathogenesis of GDM remain unclear, and further studies are needed to explore these associations.

This study employs a two-sample Mendelian Randomization (MR) approach, integrating Genome-Wide Association Study (GWAS) data, to investigate the causal relationship between the oral microbiome and gestational diabetes mellitus (GDM). By identifying specific oral microorganisms that influence GDM pathogenesis, the research aims to optimize prevention strategies and advance personalized medicine. It also enhances diagnostic tools and innovates treatment options, potentially introducing microbial interventions as novel therapeutic targets. Furthermore, this work provides a scientific basis for public health policies and fosters interdisciplinary collaboration, ultimately improving health outcomes for pregnant women and their offspring.

Methods

This study was reported following the STROBE-MR checklist.[12] The checklist is available as Supplementary Materials.

Study Design

In our study, we carefully selected single-nucleotide polymorphisms (SNPs) from Genome-Wide Association Studies (GWAS) to serve as IVs. As shown in Figure 1, our two-sample Mendelian Randomization (MR) investigation is specifically focused on exploring the causal associations between GDM and oral microbiota-related characteristics, based on three core assumptions:[13] The relevance assumption: There is a strong association between the IVs and the oral microbiota factor under investigation. The independence assumption: The IVs are not associated with any confounding variables that might affect the oral microbiota or the GDM. The exclusion restriction assumption: the IVs affect the GDM only through their influence on the oral microbiota factor, without any other causal pathways. The need for ethical approval was waived by the committee of the Second People's Hospital of Nantong because the study used publicly available aggregated data that did not allow the re-identification of the original participants, as supported by Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China, as well as various legislations around the globe.[14]

Data Sources

The outcome data for GDM were obtained from the FINN cohort data (ID: finngen_R10_GEST_DIABETES), which consisted of 14,718 cases and 215,592 controls. The exposure data were sourced from a published article[15] that detailed the association between 43 oral microbial communities and host genotypes. These data were based on saliva samples from 610 adults in the Danish ADDITION-PRO cohort, who had the V4 region of the 16S rRNA gene sequenced on the Illumina HiSeq 2500 platform. Forty-three GWAS statistical abstracts were validated after stringent quality control measures. All study data were from European populations, with more specific details provided in Table 1.

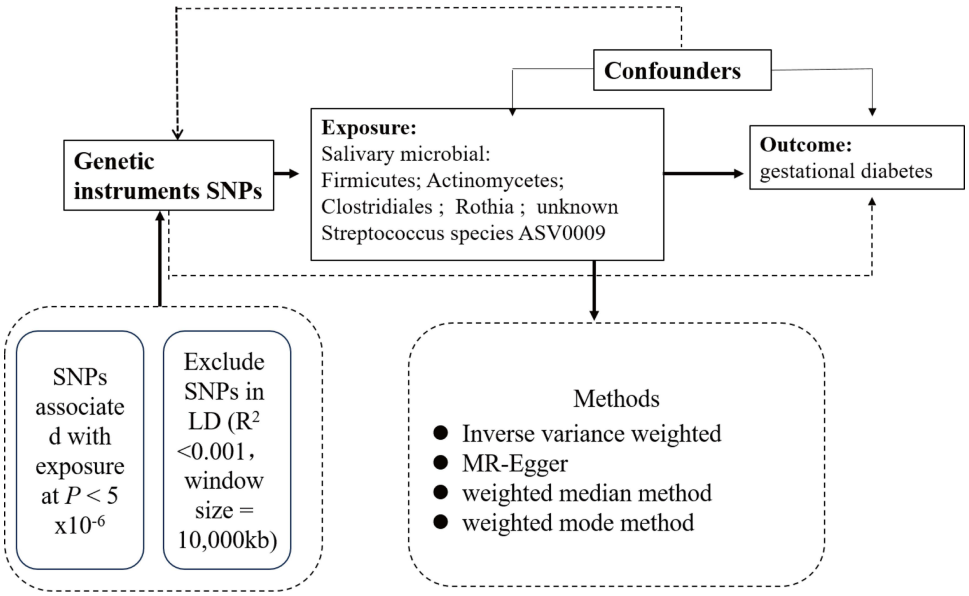

Figure 1 Flowchart of the two-sample Mendelian randomization analysis for the association between salivary microbial exposure and gestational diabetes. The flowchart illustrates the steps involved in conducting a two-sample Mendelian randomization (MR) analysis to investigate the causal relationship between specific salivary microbial taxa and the risk of gestational diabetes, while accounting for potential confounders. In this legend, **bold text** is used to emphasize and highlight the key components and crucial steps of the analytical process depicted in the flowchart, facilitating quick identification of the core elements of the MR analysis.

Instrumental Variable Selection

In this study, the selection of IVs was based on the following criteria: (1) SNPs significantly associated with the whole genome of the oral microbiota were first identified with a threshold of $P < 5 * 10^{-6}$.[16] (2) Only SNPs with a minor allele frequency (MAF) greater than 0.01 were retained. (3) SNPs showing linkage disequilibrium (LD) were excluded using a threshold of $R^2 < 0.001$ and a window size of 10,000kb. (4) If the selected IV was not present in the summary statistics of the results, a SNP with a strong LD ($R^2 > 0.8$) to the IV was identified as a substitute and replaced with it.[17] 5) An F value was calculated for each SNP in the IV to assess its strength and to mitigate possible bias caused by weak IVs associated with the exposure factor. The F value was calculated as $F = R^2 * (N-2) / (1-R^2)$, where R^2 is the exposure variance explained by the SNP in the IV. An F-value greater than 10 is required.[12] To ensure that the effective alleles are indeed from the same allele, we aligned the oral microbiota and GDM datasets, thereby removing any SNPs with discordant alleles and those with allele frequencies in the intermediate range.

MR Analyses

This study primarily employed the inverse variance weighted (IVW) method for analysis,[18] calculating the odds ratio (OR) and its 95% confidence interval (CI) to evaluate the causal association between exposure and outcome risk. The IVW method, as the preferred approach for interpreting MR results, calculates the weighted average of effect sizes using

Table 1 Detailed Information for the GWAS Data

Character	Trait	GWAS ID	Sample Size (Case/Control)	PMID
Outcome	Gestational diabetes (for exclusion)	Finngen_R10_GEST_DIABETES	14,718/215,592	NA
Expose	Salivary microbial abundance (Firmicutes)	GCST90429799	610	38926497
Expose	Salivary microbial abundance (Actinomycetes)	GCST90429804	610	38926497
Expose	Salivary microbial abundance (Clostridiales)	GCST90429805	610	38926497
Expose	Salivary microbial abundance (Rothia)	GCST90429818	610	38926497
Expose	Salivary microbial abundance (unknown Streptococcus species (ASV0009))	GCST90429831	610	38,926,497

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the inverse variance of each SNP as weights. To assess the robustness of the results, we also used MR-Egger regression,[19] weighted median,[20] and weighted mode methods.[21] MR-Egger regression considers the intercept term, providing accurate causal effect estimates even in the presence of pleiotropic bias. The weighted median method assumes that half of the IVs are valid, analyzing the causal association between exposure and outcome. All analyses were conducted using the "TwoSampleMR" in the R software package version 4.0.5, and the results were visualized with scatter plots and sensitivity analysis graphs.

Sensitivity and Pleiotropy Analysis

Sensitivity analysis was performed to detect potential pleiotropy in MR studies. We assessed the heterogeneity among IVs using Cochran's Q test,[22] with a P-value greater than 0.05 indicating low heterogeneity, suggesting that the estimates of IVs are randomly varied and have a minimal impact on the IVW results. Considering that the pleiotropy of genetic variants might affect the estimation of effects, we used MR-Egger regression to explore the presence of horizontal pleiotropy. An intercept close to zero or statistically insignificant in the MR-Egger regression suggests the absence of pleiotropy. Additionally, we employed the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method to detect possible outliers and re-estimated the causal associations after their removal to correct for horizontal pleiotropy.[23] A leave-one-out analysis was conducted to evaluate the robustness and consistency of the results.

Result

Selection of IVs

In this study, we used IV analysis to gain insight into the oral microbiota community. After screening, we identified a total of 267 IVs associated with the abundance of the oral microbiota community and computed the corresponding F-statistics for each taxonomic unit. The mean of these F-statistics ranged from 21.51 to 27.10, with the minimum values ranging from 21.15 to 22.63, and the maximum values ranging from 21.51 to 38.10. To ensure the robustness of our analytical results, we specifically replaced SNPs inconsistent with summary data with suitable proxy SNPs in the analysis of oral microbiota community abundance (Table 2). For more detailed information, please refer to Table S1.

Table 2 Information for Instrument Variable (IV)

Expose	Number of Total SNPs	Mean_F	Min_F	Max_F	Proxy.snp
Phylum Firmicutes	9	24.14	21.25	31.77	
Phylum Proteobacteria	8	23.57	21.59	26.24	
Class Bacilli	4	22.53	21.23	24.94	
Order Bacteroidales	13	23.49	21.18	27.48	
Order Fusobacteriales	7	23.67	21.18	27.38	
Order Actinomycetales	9	25.45	21.28	30.91	
Order Clostridiales	9	25.33	21.15	36.62	
Family Veillonellaceae	4	22.88	21.96	24.79	
Family Pasteurellaceae	9	23.37	21.38	28.02	
Family Prevotellaceae	8	24.66	21.91	28.21	
Family Actinomycetaceae	5	22.41	21.36	23.77	
Family Lachnospiraceae_[XIV]	9	22.82	21.66	24.99	
Genus Veillonella	6	22.31	21.26	23.75	
Genus Haemophilus	7	23.37	21.71	27.24	
Genus Streptococcus	3	21.64	21.18	22.26	
Genus Neisseria	5	23.03	21.25	26.23	
Genus Prevotella	11	23.32	21.2	28.62	

(*Continued*)

Table 2 (Continued).

Expose	Number of Total SNPs	Mean_F	Min_F	Max_F	Proxy.snp
Genus Porphyromonas	4	23.46	21.76	25.38	
Genus Fusobacterium	11	22.76	21.16	25.53	
Genus Rothia	6	26.69	22.39	38.1	
Genus Schaalia	12	22.6	21.15	24.41	
Genus Granulicatella	7	22.3	21.62	23	
Genus Leptotrichia	3	27.1	22.63	29.66	
Genus Alloprevotella	3	21.94	21.22	22.89	
Unknown Veillonella species (ASV0001)	5	24.14	21.97	28.45	
Species parainfluenzae	3	23	21.29	24.79	
Unknown Streptococcus species (ASV0003)	1	21.51	21.51	21.51	
Unknown Neisseria species (ASV0004)	4	22.29	21.26	24.72	
Species histicola	7	23.37	21.34	26.52	
Unknown Streptococcus species (ASV0006)	7	23.31	21.33	26.95	
Species parvula	2	25.76	21.67	29.85	rs113621445 replaced by rs4860383; rs9564412 replaced by rs9571821
Unknown Porphyromonas species (ASV0008)	4	23.96	21.54	25.85	
Unknown Streptococcus species (ASV0009)	5	24.06	21.52	31.36	
Species periodonticum	7	23.44	21.25	27.59	
Species dispar	4	23.57	21.91	27.52	
Unknown Rothia species (ASV0012)	2	21.89	21.59	22.19	
Species micronuciformis	6	22.47	21.2	24.52	
Species pallens	4	22.75	21.42	24.83	
Species mucilaginosa	11	22.51	21.49	24.86	rs117506041 replaced by rs186755051
Unknown Rothia species (ASV0016)	6	23.68	21.99	26.24	rs61026851 replaced by rs67487314; rs7247650 replaced by rs34837414
Unknown Schaalia species (ASV0017)	7	23.48	21.43	26.44	
Species rogosae	4	24.27	22.2	26.69	
Unknown Gemella	6	22.41	21.57	23.42	

Mendelian Randomization Analysis

This study employed an MR approach to investigate the causal association between oral microbiota and GDM. The IVW analysis revealed a positive causal association between Genus *Schaalia* and GDM risk (Table 3 and Figure 2, OR = 1.03, 95% CI: 1.01–1.06; P=0.02), and a negative association between Genus *Haemophilus* and GDM risk (Table 3 and Figure 3, OR = 0.96, 95% CI: 0.93–1.00, P=0.034). However, no significant causal associations were identified between other oral microbiota and the risk of GDM.

Table 3 Genetics Predicts Associations Between Oral Microbiota and the Risk of Gestational Diabetes

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P
Class Bacilli	Gestational diabetes (for exclusion)	4	IVW	1.04 (0.99–1.09)	0.112
Class Bacilli		4	MR Egger	0.89 (0.71–1.12)	0.429
Class Bacilli		4	Weighted median	1.03 (0.98–1.09)	0.276
Class Bacilli		4	Weighted mode	1.03 (0.96–1.1)	0.471
Family Actinomycetaceae		5	IVW	1.01 (0.96–1.07)	0.703
Family Actinomycetaceae		5	MR Egger	0.95 (0.61–1.5)	0.849
Family Actinomycetaceae		5	Weighted median	0.97 (0.92–1.02)	0.253

(*Continued*)

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Table 3 (Continued).

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P
Family Actinomycetaceae		5	Weighted mode	0.96 (0.88–1.05)	0.474
Family Lachnospiraceae_[XIV]		9	IVW	0.98 (0.95–1.01)	0.258
Family Lachnospiraceae_[XIV]		9	MR Egger	1.07 (0.95–1.19)	0.291
Family Lachnospiraceae_[XIV]		9	Weighted median	0.98 (0.94–1.02)	0.239
Family Lachnospiraceae_[XIV]		9	Weighted mode	0.97 (0.91–1.03)	0.375
Family Pasteurellaceae		9	IVW	0.97 (0.95–1)	0.087
Family Pasteurellaceae		9	MR Egger	0.9 (0.76–1.07)	0.264
Family Pasteurellaceae		9	Weighted median	0.97 (0.93–1.01)	0.104
Family Pasteurellaceae		9	Weighted mode	0.97 (0.91–1.03)	0.284
Family Prevotellaceae		8	IVW	1.02 (0.99–1.05)	0.227
Family Prevotellaceae		8	MR Egger	0.95 (0.84–1.08)	0.485
Family Prevotellaceae		8	Weighted median	1.02 (0.98–1.06)	0.37
Family Prevotellaceae		8	Weighted mode	1.01 (0.95–1.06)	0.858
Family Veillonellaceae		4	IVW	1.01 (0.97–1.05)	0.498
Family Veillonellaceae		4	MR Egger	1.01 (0.91–1.12)	0.84
Family Veillonellaceae		4	Weighted median	1.01 (0.97–1.06)	0.556
Family Veillonellaceae		4	Weighted mode	1.01 (0.96–1.07)	0.617
Genus Alloprevotella		2	IVW	1.02 (0.94–1.11)	0.609
Genus Fusobacterium		11	IVW	1.01 (0.98–1.03)	0.664
Genus Fusobacterium		11	MR Egger	0.92 (0.83–1.03)	0.172
Genus Fusobacterium		11	Weighted median	1 (0.97–1.04)	0.879
Genus Fusobacterium		11	Weighted mode	1 (0.95–1.05)	0.953
Genus Granulicatella		6	IVW	1.02 (0.98–1.07)	0.259
Genus Granulicatella		6	MR Egger	0.95 (0.8–1.11)	0.548
Genus Granulicatella		6	Weighted median	1 (0.95–1.05)	0.977
Genus Granulicatella		6	Weighted mode	0.99 (0.93–1.05)	0.786
Genus Haemophilus		7	IVW	0.96 (0.93–1)	0.034
Genus Haemophilus		7	MR Egger	0.9 (0.75–1.08)	0.303
Genus Haemophilus		7	Weighted median	0.96 (0.92–1)	0.078
Genus Haemophilus		7	Weighted mode	0.96 (0.91–1.01)	0.209
Genus Leptotrichia		3	IVW	1.01 (0.97–1.06)	0.621
Genus Leptotrichia		3	MR Egger	1.05 (0.9–1.23)	0.65
Genus Leptotrichia		3	Weighted median	1 (0.95–1.06)	0.917
Genus Leptotrichia		3	Weighted mode	0.99 (0.93–1.06)	0.816
Genus Neisseria		5	IVW	0.99 (0.92–1.06)	0.749
Genus Neisseria		5	MR Egger	0.77 (0.32–1.84)	0.593
Genus Neisseria		5	Weighted median	1.01 (0.95–1.07)	0.781
Genus Neisseria		5	Weighted mode	1 (0.9–1.11)	0.962
Genus Neisseria /After exclusion		3	IVW	0.99 (0.94–1.04)	0.689
Genus Neisseria /After exclusion		3	MR Egger	1.81 (0.79–4.17)	0.394
Genus Neisseria /After exclusion		3	Weighted median	1.01 (0.95–1.08)	0.764
Genus Neisseria /After exclusion		3	Weighted mode	1.02 (0.93–1.11)	0.715
Genus Porphyromonas		4	IVW	0.97 (0.89–1.05)	0.436
Genus Porphyromonas		4	MR Egger	1.22 (0.75–1.97)	0.504
Genus Porphyromonas		4	Weighted median	0.99 (0.92–1.05)	0.674
Genus Porphyromonas		4	Weighted mode	1 (0.91–1.1)	0.994
Genus Prevotella		11	IVW	1.02 (1–1.05)	0.097
Genus Prevotella		11	MR Egger	0.96 (0.87–1.05)	0.407
Genus Prevotella		11	Weighted median	1.02 (0.98–1.05)	0.348

(*Continued*)

Table 3 (Continued).

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P
Genus Prevotella		11	Weighted mode	1 (0.95–1.06)	0.904
Genus Rothia		6	IVW	0.99 (0.96–1.02)	0.45
Genus Rothia		6	MR Egger	1.02 (0.92–1.13)	0.736
Genus Rothia		6	Weighted median	0.99 (0.95–1.03)	0.573
Genus Rothia		6	Weighted mode	0.99 (0.94–1.04)	0.712
Genus Schaalia		12	IVW	1.03 (1–1.06)	0.02
Genus Schaalia		12	MR Egger	1 (0.92–1.09)	0.963
Genus Schaalia		12	Weighted median	1.03 (1–1.07)	0.061
Genus Schaalia		12	Weighted mode	1.04 (0.99–1.1)	0.148
Genus Streptococcus		2	IVW	1.01 (0.95–1.08)	0.676
Genus Veillonella		6	IVW	1.02 (0.98–1.05)	0.299
Genus Veillonella		6	MR Egger	1.02 (0.92–1.13)	0.754
Genus Veillonella		6	Weighted median	1.02 (0.97–1.06)	0.503
Genus Veillonella		6	Weighted mode	1.01 (0.96–1.07)	0.668
Order Actinomycetales		9	IVW	0.97 (0.95–1)	0.054
Order Actinomycetales		9	MR Egger	1.04 (0.92–1.18)	0.517
Order Actinomycetales		9	Weighted median	0.98 (0.95–1.02)	0.32
Order Actinomycetales		9	Weighted mode	0.98 (0.93–1.03)	0.41
Order Bacteroidales		13	IVW	1 (0.98–1.03)	0.794
Order Bacteroidales		13	MR Egger	1.03 (0.95–1.12)	0.478
Order Bacteroidales		13	Weighted median	1 (0.97–1.03)	0.898
Order Bacteroidales		13	Weighted mode	1 (0.96–1.04)	0.905
Order Clostridiales		9	IVW	0.98 (0.95–1.01)	0.106
Order Clostridiales		9	MR Egger	1.02 (0.91–1.14)	0.714
Order Clostridiales		9	Weighted median	0.97 (0.94–1.01)	0.157
Order Clostridiales		9	Weighted mode	0.98 (0.93–1.02)	0.344
Order Fusobacteriales		7	IVW	1 (0.97–1.04)	0.857
Order Fusobacteriales		7	MR Egger	1 (0.89–1.13)	0.956
Order Fusobacteriales		7	Weighted median	1.01 (0.97–1.05)	0.636
Order Fusobacteriales		7	Weighted mode	1.01 (0.96–1.07)	0.698
Phylum Firmicutes		8	IVW	0.98 (0.95–1.01)	0.151
Phylum Firmicutes		8	MR Egger	1.04 (0.9–1.2)	0.647
Phylum Firmicutes		8	Weighted median	0.98 (0.94–1.02)	0.354
Phylum Firmicutes		8	Weighted mode	0.96 (0.9–1.02)	0.224
Phylum Proteobacteria		7	IVW	0.98 (0.95–1.01)	0.275
Phylum Proteobacteria		7	MR Egger	0.9 (0.77–1.06)	0.255
Phylum Proteobacteria		7	Weighted median	0.98 (0.94–1.02)	0.355
Phylum Proteobacteria		7	Weighted mode	0.98 (0.91–1.04)	0.507
Species dispar		4	IVW	0.99 (0.95–1.03)	0.653
Species dispar		4	MR Egger	0.94 (0.82–1.07)	0.432
Species dispar		4	Weighted median	1 (0.95–1.05)	0.871
Species dispar		4	Weighted mode	1 (0.94–1.06)	0.983
Species histicola		7	IVW	1.01 (0.97–1.04)	0.604
Species histicola		7	MR Egger	1.12 (0.85–1.47)	0.446
Species histicola		7	Weighted median	1.02 (0.97–1.07)	0.477
Species histicola		7	Weighted mode	1.03 (0.95–1.11)	0.519
Species micronuciformis		6	IVW	0.97 (0.92–1.02)	0.25
Species micronuciformis		6	MR Egger	0.84 (0.61–1.17)	0.365
Species micronuciformis		6	Weighted median	0.97 (0.92–1.02)	0.216

(*Continued*)

Table 3 (Continued).

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P
Species micronuciformis		6	Weighted mode	0.96 (0.89–1.05)	0.443
Species micronuciformis /After exclusion		5	IVW	0.99 (0.95–1.04)	0.77
Species micronuciformis /After exclusion		5	MR Egger	0.98 (0.7–1.36)	0.907
Species micronuciformis /After exclusion		5	Weighted median	0.98 (0.93–1.03)	0.405
Species micronuciformis /After exclusion		5	Weighted mode	0.97 (0.89–1.05)	0.463
Species mucilaginosa		11	IVW	1 (0.98–1.02)	0.845
Species mucilaginosa		11	MR Egger	1 (0.94–1.08)	0.901
Species mucilaginosa		11	Weighted median	1 (0.98–1.03)	0.792
Species mucilaginosa		11	Weighted mode	1.02 (0.98–1.06)	0.409
Species pallens		3	IVW	0.97 (0.92–1.02)	0.257
Species pallens		3	MR Egger	0.92 (0.71–1.19)	0.628
Species pallens		3	Weighted median	0.96 (0.9–1.02)	0.185
Species pallens		3	Weighted mode	0.95 (0.88–1.03)	0.323
Species parainfluenzae		3	IVW	0.99 (0.94–1.05)	0.826
Species parainfluenzae		3	MR Egger	1.17 (0.79–1.74)	0.576
Species parainfluenzae		3	Weighted median	0.99 (0.92–1.05)	0.65
Species parainfluenzae		3	Weighted mode	0.98 (0.91–1.05)	0.612
Species parvula		2	IVW	1.02 (0.96–1.08)	0.513
Species periodonticum		7	IVW	1 (0.97–1.03)	0.948
Species periodonticum		7	MR Egger	0.93 (0.79–1.1)	0.455
Species periodonticum		7	Weighted median	0.99 (0.95–1.03)	0.562
Species periodonticum		7	Weighted mode	0.98 (0.92–1.04)	0.557
Species rogosae		4	IVW	1.01 (0.97–1.05)	0.628
Species rogosae		4	MR Egger	1.03 (0.85–1.24)	0.8
Species rogosae		4	Weighted median	1.01 (0.96–1.06)	0.677
Species rogosae		4	Weighted mode	0.99 (0.91–1.07)	0.788
Unknown Gemella		6	IVW	0.99 (0.94–1.04)	0.616
Unknown Gemella		6	MR Egger	1.09 (0.9–1.31)	0.435
Unknown Gemella		6	Weighted median	0.99 (0.94–1.05)	0.817
Unknown Gemella		6	Weighted mode	0.99 (0.92–1.07)	0.897
Unknown Neisseria species (ASV0004)		4	IVW	1.02 (0.98–1.06)	0.322
Unknown Neisseria species (ASV0004)		4	MR Egger	1.16 (0.86–1.56)	0.438
Unknown Neisseria species (ASV0004)		4	Weighted median	1.03 (0.98–1.08)	0.242
Unknown Neisseria species (ASV0004)		4	Weighted mode	1.03 (0.97–1.1)	0.397
Unknown Porphyromonas species (ASV0008)		4	IVW	0.98 (0.93–1.02)	0.324
Unknown Porphyromonas species (ASV0008)		4	MR Egger	1.15 (0.89–1.47)	0.398
Unknown Porphyromonas species (ASV0008)		4	Weighted median	0.98 (0.93–1.03)	0.389
Unknown Porphyromonas species (ASV0008)		4	Weighted mode	0.97 (0.9–1.04)	0.445
Unknown Rothia species (ASV0012)		2	IVW	1.01 (0.94–1.09)	0.819
Unknown Rothia species (ASV0016)		6	IVW	0.99 (0.96–1.02)	0.452
Unknown Rothia species (ASV0016)		6	MR Egger	1.04 (0.93–1.15)	0.544
Unknown Rothia species (ASV0016)		6	Weighted median	1 (0.97–1.04)	0.856
Unknown Rothia species (ASV0016)		6	Weighted mode	1.01 (0.96–1.06)	0.73
Unknown Schaalia species (ASV0017)		7	IVW	1.02 (0.99–1.06)	0.155
Unknown Schaalia species (ASV0017)		7	MR Egger	1.09 (0.91–1.31)	0.379
Unknown Schaalia species (ASV0017)		7	Weighted median	1.02 (0.98–1.06)	0.407
Unknown Schaalia species (ASV0017)		7	Weighted mode	1.01 (0.95–1.09)	0.69
Unknown Streptococcus species (ASV0003)		1	Wald ratio	0.98 (0.9–1.07)	0.617
Unknown Streptococcus species (ASV0006)		7	IVW	0.98 (0.95–1.02)	0.34
Unknown Streptococcus species (ASV0006)		7	MR Egger	0.95 (0.79–1.14)	0.606

(*Continued*)

Table 3 (Continued).

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P
Unknown Streptococcus species (ASV0006)		7	Weighted median	0.98 (0.94–1.03)	0.435
Unknown Streptococcus species (ASV0006)		7	Weighted mode	0.96 (0.89–1.03)	0.337
Unknown Streptococcus species (ASV0009)		5	IVW	0.98 (0.92–1.04)	0.438
Unknown Streptococcus species (ASV0009)		5	MR Egger	1.18 (0.91–1.55)	0.306
Unknown Streptococcus species (ASV0009)		5	Weighted median	0.96 (0.91–1.01)	0.121
Unknown Streptococcus species (ASV0009)		5	Weighted mode	0.96 (0.9–1.01)	0.205
Unknown Veillonella species (ASV0001)		5	IVW	0.99 (0.93–1.04)	0.619
Unknown Veillonella species (ASV0001)		5	MR Egger	1.16 (1.02–1.31)	0.103
Unknown Veillonella species (ASV0001)		5	Weighted median	0.98 (0.93–1.04)	0.532
Unknown Veillonella species (ASV0001)		5	Weighted mode	0.96 (0.87–1.06)	0.512

Sensitivity Analysis

A sensitivity analysis was performed to determine the reliability of the IVW results. The MR-Egger indicated no pleiotropic effects in the analysis (Table 4, $P > 0.05$). The Cochran Q test (Table 4) for heterogeneity showed no heterogeneity in the MR analysis between Genus *Schaalia* and Genus *Haemophilus* and GDM ($P > 0.05$) (Table 2). However, the MR analysis between Genus *Neisseria*, Genus *Porphyromonas*, and Species *micronuciformis* and GDM exhibited heterogeneity ($P<0.05$) (Table 4). Pleiotropy and heterogeneity disappeared after the exclusion of outlier SNPs for Species *micronuciformis* and Genus *Neisseria* identified by MR-PRESSO (Tables 4 and 5). Notably, after the removal of abnormal SNPs, the results did not change, remaining consistent with those obtained before the sensitivity analysis (Table 3).

Discussion

This study employed a two-sample MR approach to reveal the potential causal association between the oral microbiota and gestational GDM. The findings revealed a positive association between the genus *Schaalia* and the risk of GDM, while the genus *Haemophilus* was negatively associated with GDM risk. This finding suggests potential targets for future preventive and therapeutic strategies.

Schaalia bacteria, Gram-positive and facultative anaerobic microorganisms, predominantly colonize the oral ecosystems of humans and other animals. They play a pivotal role in biofilm formation, modulating host immune responses, and interacting with other microbes, which closely ties them to the pathogenesis of various infectious diseases.[24] *Schaalia* bacteria are commonly found as commensals in the oral cavity and other mucosal sites. While typically harmless, *Schaalia* species can become pathogenic when mucosal barriers are breached, leading to inflammatory responses, inducing the production of inflammatory cytokines.[25,26] Recent research indicates that in women with impaired glucose tolerance (IGT), a precursor to GDM, there is a significant reduction in gut microbiota diversity, along with an increase in the abundance of specific microbial species such as *Schaalia turicensis*. These alterations in microbial composition are closely associated with the development of IGT.[27] This finding is consistent with our findings suggesting that the dysbiosis of the oral *Schaalia* genus may be associated with an increased risk of GDM. Therefore, future research should delve into the specific role of *Schaalia* bacteria in the development of GDM and reveal more about the biological mechanisms of the disease, while also assessing the potential of the *Schaalia* genus as a target for the prevention and treatment of GDM.

Haemophilus, a group of gram-negative, facultative anaerobic bacteria, resides on the mucosal surfaces of humans.[28] They are well-known for their role in both localized and systemic infections, and their involvement in inflammation is central to their disease-causing potential and to trigger inflammation.[29,30] The study of the association between GDM and the oral microbiota has revealed a connection, with significant differences in β-diversity observed in the gut microbiota of pregnant women with GDM compared to controls, characterized by an increase in *Haemophilus* species and a decrease in the α-diversity of the oral microbiota.[31] Other research has shown a notable rise in the relative abundance of *Haemophilus* among women with GDM.[32] This increase may be related to the metabolic status of patients with GDM,

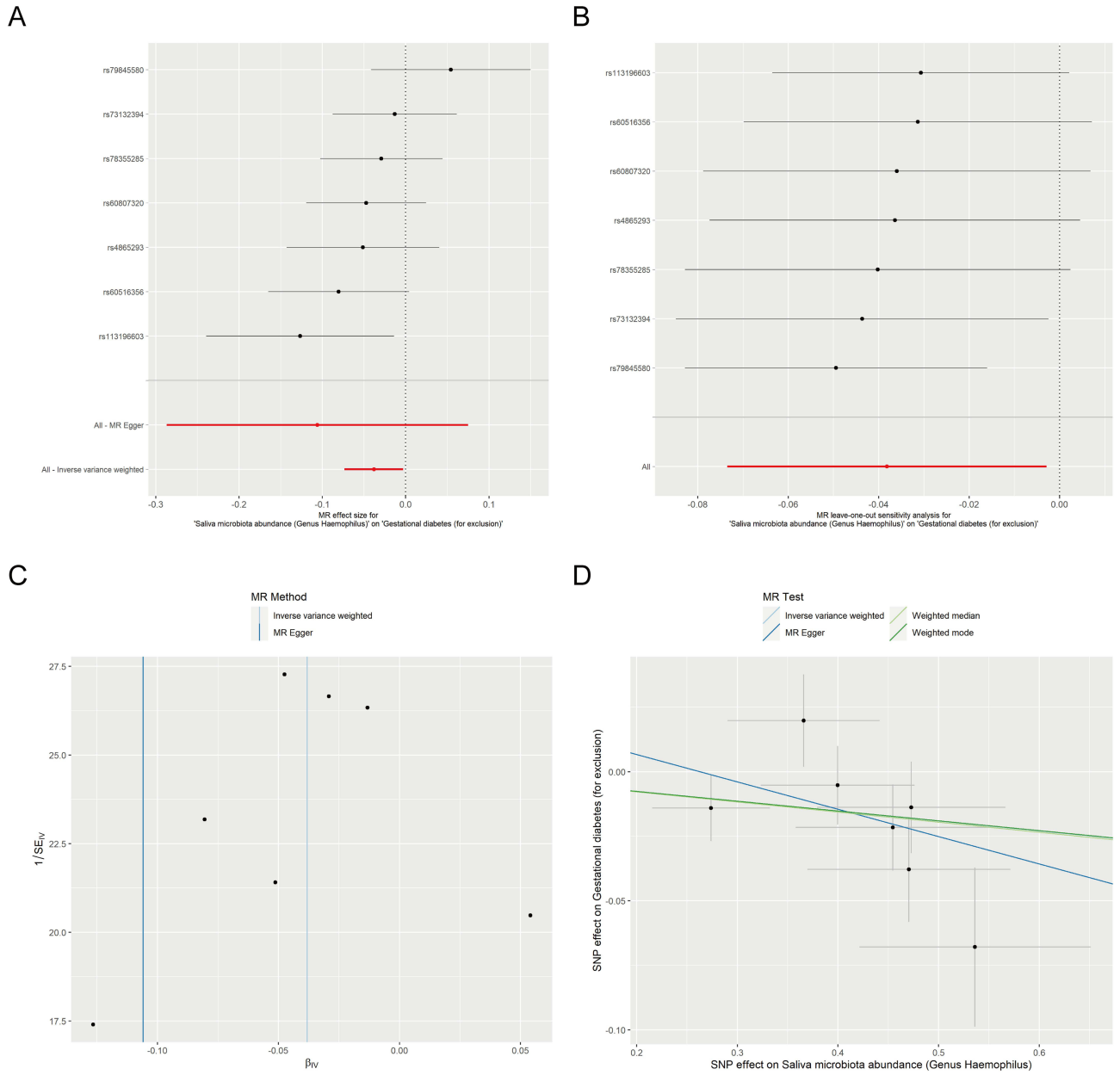

Figure 2 The association between Genus *Schaalia* and the risk of gestational diabetes is presented in (**A**) a forest plot, (**B**) a leave-one-out sensitivity analysis, (**C**) a scatter plot, and (**D**) a funnel plot.

as *Haemophilus* was associated with fasting blood glucose and lipid levels in participants.[33] Specifically, the rise in *Haemophilus* may be linked to the production of short-chain fatty acids, changes in incretin hormones, bile acid homeostasis, and the deficiency of peroxisome proliferator-activated receptor γ, which may affect insulin sensitivity and glucose metabolism.[34] These findings suggest that changes in the oral microbial *Haemophilus* profile could serve as a non-invasive biomarker for monitoring GDM during pregnancy. Future research needs to further explore the mechanism of *Haemophilus* in GDM and how oral microbiota regulation can prevent and treat GDM.

Oral dysbiosis promotes local inflammation in periodontal tissues, leading to the production and release of pro-inflammatory cytokines such as TNF-α, IL-6, and CRP. They play central roles in mediating the association between oral microbiota dysbiosis and GDM. These cytokines are produced in response to pathogenic oral bacteria, enter systemic circulation, and contribute to insulin resistance and glucose intolerance. A feedback loop exists where hyperglycemia and inflammation perpetuate each other, worsening both oral and metabolic health in pregnancy. TNF-α and IL-6 are known

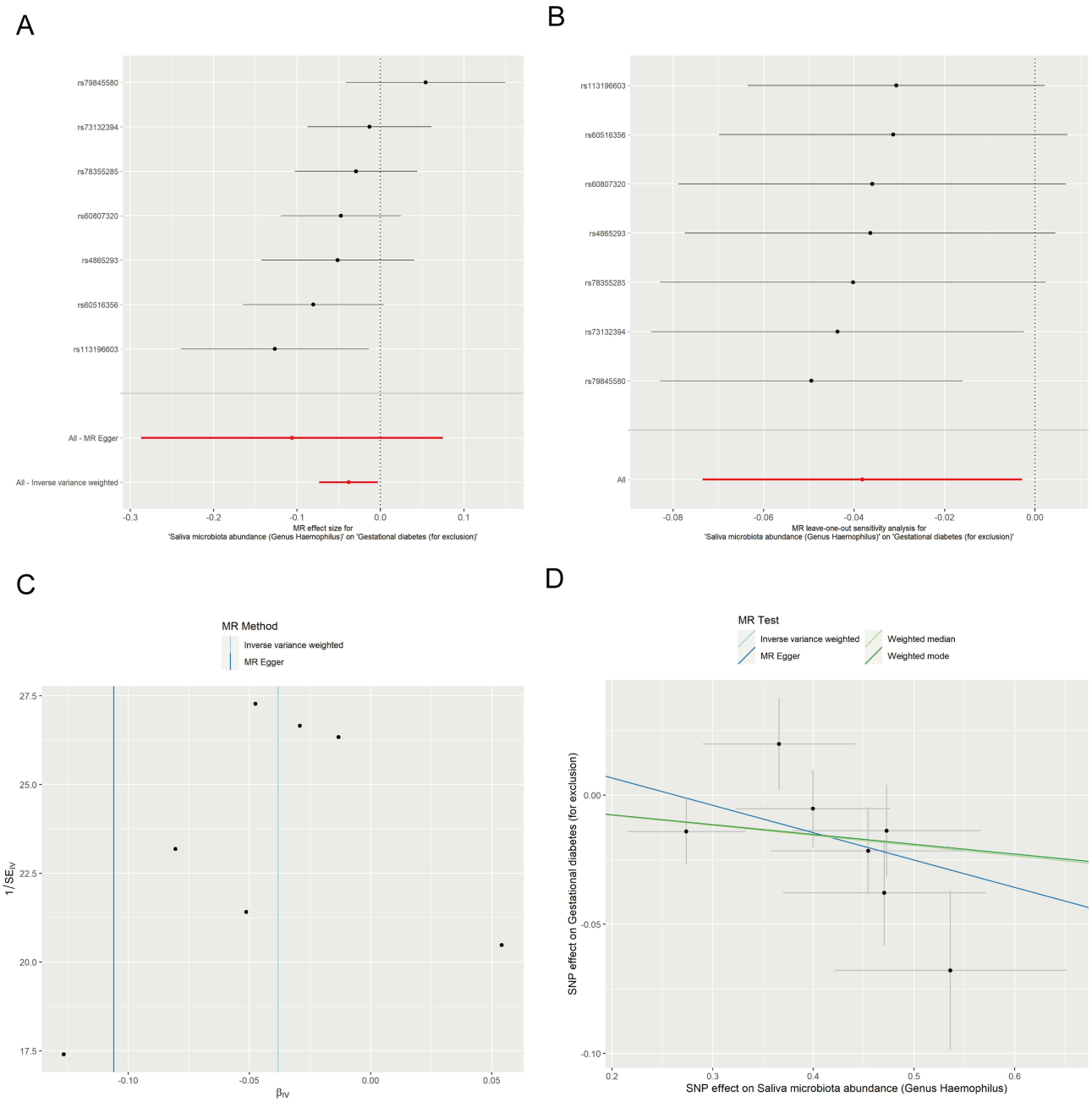

Figure 3 The association between Genus *Haemophilus* and the risk of gestational diabetes is presented in (**A**) a forest plot, (**B**) a leave-one-out sensitivity analysis, (**C**) a scatter plot, and (**D**) a funnel plot.

to antagonize insulin signaling, impairing glucose uptake and promoting insulin resistance (a central feature of GDM). Chronic elevation of these cytokines disrupts carbohydrate metabolism, increasing the risk of glucose intolerance and the development of GDM.[8] This mechanistic understanding highlights the importance of oral health and inflammation control in the prevention and management of GDM.[8,9]

This study used SNPs significantly associated with oral microbiota abundance as instrumental variables, which are controlled by genetic factors and unaffected by lifestyle and environmental influences.[15] Thus, the MR method can effectively circumvent confounding biases inherent in traditional observational studies, providing a more accurate assessment of the causal association between the oral microbiota and GDM. However, MR also has its limitations, as its validity depends on the choice of instrumental variables. Weak associations or pleiotropy between the selected instruments and the exposure can lead to biased results.[35] In

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Table 4 Heterogeneity Tests and Pleiotropy Tests for Instrumental Variables

Exposure	Outcome	Heterogeneity		Pleiotropy	
		Q Statistic (IVW)	*P* Value	MR-Egger Intercept	*P* Value
Class Bacilli	Gestational diabetes (for exclusion)	1.959	0.581	0.051	0.314
Family Actinomycetaceae		7.106	0.13	0.029	0.816
Family Lachnospiraceae_[XIV]		7.689	0.464	−0.033	0.178
Family Pasteurellaceae		8.99	0.343	0.03	0.388
Family Prevotellaceae		2.203	0.948	0.032	0.332
Family Veillonellaceae		0.242	0.97	0	0.984
Genus Alloprevotella		2.052	0.152		
Genus Fusobacterium		6.128	0.804	0.034	0.138
Genus Granulicatella		7.095	0.214	0.032	0.384
Genus Haemophilus		7.531	0.274	0.028	0.487
Genus Leptotrichia		1.556	0.459	−0.016	0.708
Genus Neisseria		11.814	0.019	0.078	0.606
Genus Neisseria /After exclusion		2.315	0.314	−0.192	0.388
Genus Porphyromonas		9.025	0.029	−0.085	0.441
Genus Prevotella		3.69	0.96	0.026	0.195
Genus Rothia		1.898	0.863	−0.014	0.566
Genus Schaalia		6.751	0.819	0.014	0.458
Genus Streptococcus		0.325	0.569		
Genus Veillonella		1.031	0.96	0	0.997
Order Actinomycetales		5.19	0.737	−0.025	0.288
Order Bacteroidales		6.766	0.873	−0.013	0.504
Order Clostridiales		6.201	0.625	−0.017	0.431
Order Fusobacteriales		1.806	0.937	0	0.994
Phylum Firmicutes		2.173	0.95	−0.02	0.444
Phylum Proteobacteria		4.811	0.568	0.032	0.328
Species dispar		2.998	0.392	0.024	0.469
Species histicola		5.581	0.472	−0.033	0.478
Species micronuciformis		13.1	0.022	0.053	0.449
Species micronuciformis /After exclusion		5.946	0.203	0.005	0.937
Species mucilaginosa		8.699	0.561	−0.003	0.848
Species pallens		0.542	0.763	0.023	0.741
Species parainfluenzae		2.493	0.288	−0.055	0.559
Species parvula		0.059	0.808		
Species periodonticum		6.909	0.329	0.026	0.452
Species rogosae		2.055	0.561	−0.007	0.867
Unknown Gemella		10.285	0.068	−0.034	0.353
Unknown Neisseria species (ASV0004)		1.071	0.784	−0.043	0.491
Unknown Porphyromonas species (ASV0008)		1.774	0.621	−0.057	0.333
Unknown Rothia species (ASV0012)		1.877	0.171		
Unknown Rothia species (ASV0016)		4.901	0.428	−0.024	0.413
Unknown Schaalia species (ASV0017)		4.474	0.613	−0.027	0.501
Unknown Streptococcus species (ASV0006)		5.031	0.54	0.013	0.721
Unknown Streptococcus species (ASV0009)		7.773	0.1	−0.061	0.25
Unknown Veillonella species (ASV0001)		8.368	0.079	−0.056	0.075

Table 5 MR-PRESSO Results

Exposure	Outcome	Raw		Outlier Corrected		Global P	Number of Outliers	Distortion P
		OR (CI%)	P	OR (CI%)	P			
Class Bacilli	Gestational diabetes (for exclusion)	1.04 (1–1.08)	0.144	NA	NA	0.665	0	NA
Family Actinomycetaceae		1.01 (0.96–1.07)	0.723	NA	NA	0.145	0	NA
Family Lachnospiraceae_[XIV]		0.98 (0.95–1.01)	0.281	NA	NA			NA
Family Pasteurellaceae		0.97 (0.95–1)	0.125	NA	NA	0.369	0	NA
Family Prevotellaceae		1.02 (1–1.04)	0.068	NA	NA	0.957	0	NA
Family Veillonellaceae		1.01 (1–1.02)	0.097	NA	NA	0.972	0	NA
Genus Fusobacterium		1.01 (0.99–1.02)	0.592	NA	NA	0.811	0	NA
Genus Granulicatella		1.02 (0.98–1.07)	0.31	NA	NA	0.22	0	NA
Genus Haemophilus		0.96 (0.93–1)	0.078	NA	NA	0.375	0	NA
Genus Neisseria		0.99 (0.92–1.06)	0.765	0.99 (0.94–1.04)	0.728	0.044	2	1
Genus Porphyromonas		0.97 (0.89–1.05)	0.492	NA	NA	0.092	0	NA
Genus Prevotella		1.02 (1.01–1.04)	0.021	NA	NA	0.954	0	NA
Genus Rothia		0.99 (0.97–1.01)	0.275	NA	NA	0.893	0	NA
Genus Schaalia		1.03 (1.01–1.05)	0.013	NA	NA	0.831	0	NA
Genus Veillonella		1.02 (1–1.03)	0.071	NA	NA	0.966	0	NA
Order Actinomycetales		0.97 (0.95–1)	0.044	NA	NA	0.757	0	NA
Order Bacteroidales		1 (0.99–1.02)	0.734	NA	NA	0.877	0	NA
Order Clostridiales		0.98 (0.95–1)	0.103	NA	NA	0.689	0	NA
Order Fusobacteriales		1 (0.98–1.02)	0.754	NA	NA	0.948	0	NA
Phylum Firmicutes		0.98 (0.96–0.99)	0.036	NA	NA	0.933	0	NA
Phylum Proteobacteria		0.98 (0.95–1.01)	0.269	NA	NA	0.577	0	NA
Species dispar		0.99 (0.95–1.03)	0.683	NA	NA	0.473	0	NA
Species histicola		1.01 (0.98–1.04)	0.61	NA	NA	0.499	0	NA
Species micronuciformis		0.97 (0.92–1.02)	0.302	0.99 (0.95–1.04)	0.784	0.048	1	0.032
Species mucilaginosa		1 (0.98–1.02)	0.839	NA	NA	0.572	0	NA
Species periodonticum		1 (0.97–1.03)	0.95	NA	NA	0.342	0	NA
Species rogosae		1.01 (0.98–1.04)	0.6	NA	NA	0.586	0	NA
Unknown Gemella		0.99 (0.94–1.04)	0.637	NA	NA	0.088	0	NA
Unknown Neisseria species (ASV0004)		1.02 (1–1.04)	0.196	NA	NA	0.791	0	NA
Unknown Porphyromonas species (ASV0008)		0.98 (0.94–1.01)	0.29	NA	NA	0.665	0	NA
Unknown Rothia species (ASV0016)		0.99 (0.96–1.02)	0.482	NA	NA	0.435	0	NA
Unknown Schaalia species (ASV0017)		1.02 (1–1.05)	0.151	NA	NA	0.639	0	NA
Unknown Streptococcus species (ASV0006)		0.98 (0.95–1.02)	0.338	NA	NA	0.551	0	NA
Unknown Streptococcus species (ASV0009)		0.98 (0.92–1.04)	0.481	NA	NA	0.188	0	NA
Unknown Veillonella species (ASV0001)		0.99 (0.93–1.04)	0.645	NA	NA	0.109	0	NA

addition, this study, which is based primarily on data from European populations, may have limitations when applied to other populations. Therefore, subsequent studies should aim for validation and in-depth analysis in larger sample sizes.

Future research could further investigate the causal association between the oral microbiota and GDM and delve into its underlying mechanisms. For example, mechanistic studies through cellular and animal experiments could explore the specific pathways by which *Schaalia* and *Haemophilus* influence GDM. Moreover, the development of drugs targeting specific bacterial species for the prevention and treatment of GDM could be pursued. Clinical trials could assess the impact of probiotic or antibiotic interventions on the prevention and treatment of GDM and explore the optimal intervention strategies.

Conclusions

The results of this study indicate a causal association between the oral microbiota and GDM risk, particularly involving the genera *Haemophilus* and *Schaalia*. This provides new insights for the prevention and treatment of GDM and suggests that oral health may play a significant role in the occurrence and progression of GDM.

Abbreviations

GDM, Gestational diabetes mellitus; MR, Mendelian randomization; GWAS, Genome-Wide Association Study; IVs, Instrumental variables; IVW, Inverse variance weighting; TLR2, Toll-like receptor 2; SNPs, Single-nucleotide polymorphisms; MR, Mendelian Randomization; MAF, Minor allele frequency; LD, Linkage disequilibrium.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethics Approval and Consent to Participate

The study was submitted to the ethics committee of the Second People's Hospital of Nantong, China. The need for ethical approval was waived by the committee because the study used publicly available aggregated data that did not allow the re-identification of the original participants, as supported by Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China, as well as various legislations around the globe.[14]

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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