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ORIGINAL RESEARCH

The Role of Plasma Metabolites in Mediating the Effect of Gut Microbiota on Obstructive Sleep Apnea: A Two-Step, Two-Sample Mendelian Randomization Study

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Background: Recent research has increasingly underscored a significant correlation between gut microbiota and obstructive sleep apnea (OSA). Probiotics have emerged as promising adjunctive interventions for OSA. Metabolites and their related biochemical pathways have emerged as important contributors to the development of OSA. This study aimed to estimate the causal association between gut microbiota and OSA and to quantify the mediating effects of metabolites.

Methods: We employed two-step, two-sample Mendelian randomization techniques, utilizing single nucleotide polymorphisms as genetic instruments for exposures and mediators. Summary statistics were obtained from genome-wide association studies of gut microbiota (the Dutch Microbiome Project, n=7,738), plasma metabolites (the Canadian Longitudinal Study on Aging cohort, n=8,299), and OSA (FinnGen database, n=410,385). To ensure the robustness of our findings, sensitivity analyses and heterogeneity tests were systematically conducted.

Results: In the Dutch Microbiome Project, species Parabacteroides merdae, genus Faecalibacterium, species Faecalibacterium prausnitzii and species Bifidobacterium longum demonstrated a potential protective association with OSA. We included the top 10 metabolites with potential biological significance as candidate mediators. Among them, only 2-hydroxypalmitate was associated with a reduced risk of OSA. 2-hydroxypalmitate partially mediated the association between species Parabacteroides merdae and OSA, with a mediation proportion of 20.53%.

Conclusion: The study highlighted the protective effect of species Parabacteroides merdae against OSA. It also revealed the mediating role of 2-hydroxypalmitate in the relationship between species Parabacteroides merdae and OSA.

Keywords: gut microbiota, obstructive sleep apnea, plasma metabolites, Mendelian randomization

Background

Obstructive sleep apnea (OSA) is a common sleep disorder, with a prevalence of approximately 23% in women and nearly 50% in men.[1] The development of OSA is influenced by both anatomical and non-anatomical risk factors. Anatomical factors, such as obesity and craniofacial abnormalities, promote upper airway collapse and obstruction during sleep.[2] Non-anatomical factors include heightened airway collapsibility, a low respiratory arousal threshold, and unstable ventilatory control.[3] The hallmark feature of OSA is intermittent hypoxia during sleep, which significantly increases the risk of cardiovascular, neurological, and metabolic disorders.[4]

The potential role of the gut microbiota in human disease development has received increasing attention over the past decade. In particular, microbiota dysbiosis has been implicated in the pathogenesis of OSA.[5] Disruption of the gut microbiota has been associated with altered sleep-wake architecture[6] and reduced sleep efficiency.[7,8] It may also impair the ventilatory response to hypercapnia,[9] thereby contributing to unstable ventilatory control in patients with OSA. In addition, microbiota dysbiosis contributes to the development of obesity,[10] a key risk factor for OSA. Probiotics have emerged as promising adjunctive interventions for OSA, primarily through their ability to reduce systemic inflammation and enhance gut barrier integrity.[11] Faecal microbiota transplantation has also been shown to partially alleviate

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cardiovascular disturbances induced by chronic intermittent hypoxia.[12] Moreover, the probiotic strain Lactobacillus rhamnosus GG has demonstrated beneficial effects in experimental models of OSA, including improvements in metabolic disturbances,[13] mitigation of hypertension severity,[14] attenuation of cardiac remodeling and inflammation.[15] Accordingly, elucidating the role of gut microbiota in the pathogenesis of OSA holds substantial promise, and the identification of effective probiotic-based interventions may offer a novel therapeutic avenue.

Metabolites and their related biochemical pathways have emerged as important contributors to the development of OSA.[16] An increased prevalence of OSA has been reported in patients with neurodegenerative disorders, potentially related to impairments in central respiratory control and functional alterations of the upper airway.[17] The nervous system and gastrointestinal tract communicate through a bidirectional network of signaling pathways, collectively referred to as the microbiota–gut–brain axis, which involves the vagus nerve, immune mechanisms, and microbiota-associated metabolic and molecular products. Emerging evidence suggests that this axis plays a pivotal role in the pathogenesis of neurodegenerative diseases.[18] These findings highlight the potential importance of microbiota–gut–brain axis related pathways in the pathogenesis and progression of OSA, suggesting that further research in this area is warranted. Previous studies have reported the involvement of metabolites and microbiota-related metabolites in the development and progression of OSA. Xu et al reported correlations between alterations in the oral microbiome and disruptions in urinary metabolites in children with OSA.[19] Using Mendelian randomization (MR), Yan et al identified several gut microbiota and microbiota-related metabolites as potential independent risk factors for OSA.[20] Therefore, investigating the relationship between the gut microbiota and OSA, and further exploring the potential mediating role of metabolites, is of substantial significance.

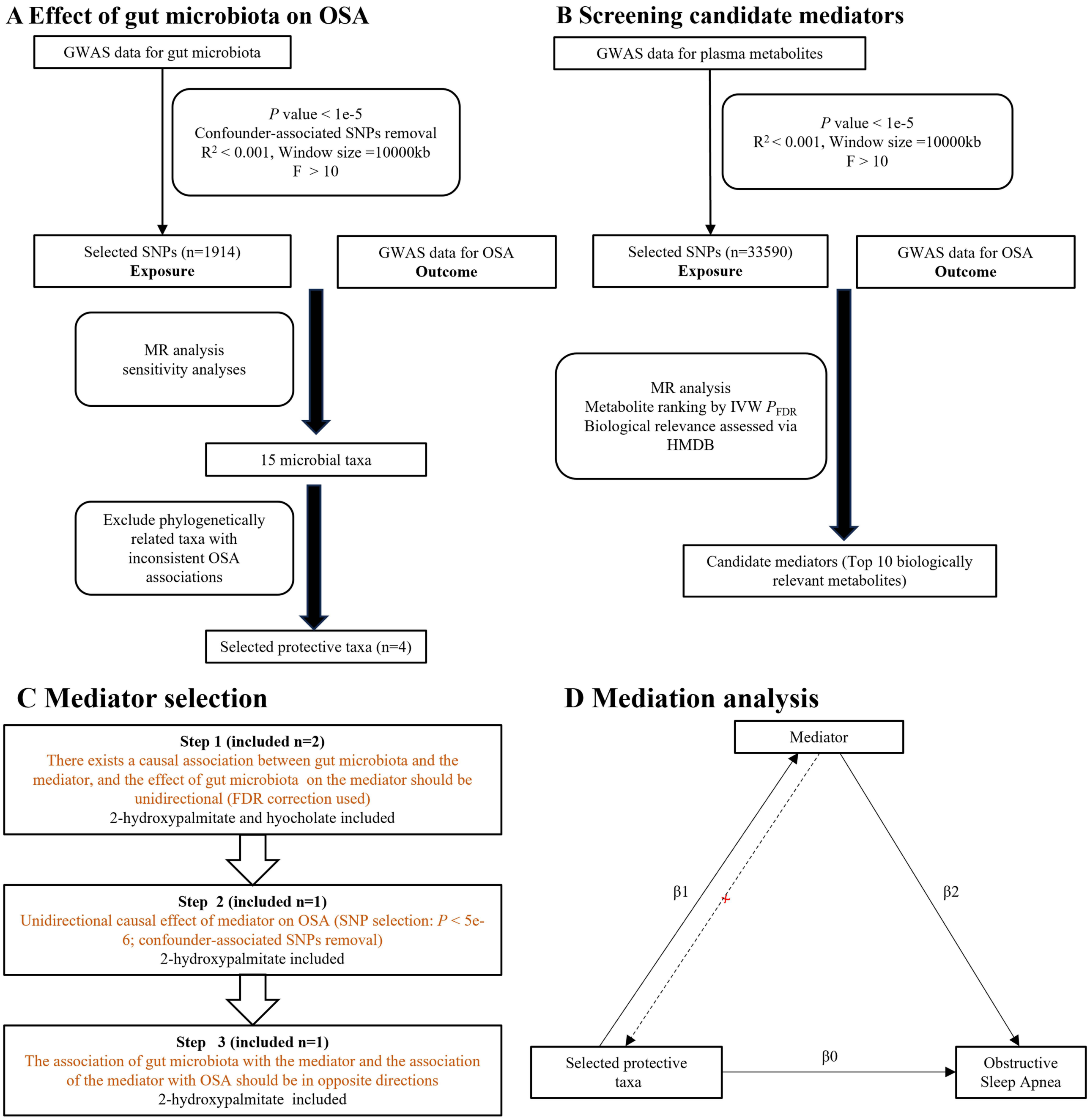
MR provides a powerful framework for investigating the causal relationship between gut microbiota and OSA.[21] In MR analysis, genetic variants are used as instrumental variables (IVs) for exposure traits, enabling the estimation of causal effects on clinical outcomes while minimizing confounding and reverse causation. In this study, we performed two-sample and two-step MR analyses using summary-level data from the genome-wide association studies (GWAS) on gut microbiota, plasma metabolites, and OSA. This study primarily focused on identifying gut microbiota and potential mediator metabolites that may confer protective effects against OSA, with the aim of uncovering novel therapeutic targets and providing preliminary insights into the underlying mechanisms.

Methods

Study Design

This study was conducted using a two-sample MR framework, with single nucleotide polymorphisms (SNPs) selected as IVs. MR relies on three key assumptions: i the IVs are strongly associated with the exposure; ii the IVs are not associated with any confounders; and iii the IVs affect the outcome solely through the exposure, without exerting a direct effect on the outcome.

An overview of the study design is provided in Figure 1. First, a two-sample MR analysis was performed to explore the potential causal relationship between gut microbiota and OSA. To reduce potential functional heterogeneity, microbial taxa within the same genus or family that showed divergent associations with the OSA were excluded. Only microbial taxa associated with a reduced risk of OSA were retained for further analysis. Second, potential mediators were identified according to the following steps. Step 1: An exploratory two-sample MR analysis was conducted to identify metabolites potentially involved in the development and progression of OSA. To account for multiple testing, the false discovery rate (FDR) was controlled using the Benjamini–Hochberg procedure. Metabolites were prioritized based on inverse variance weighted (IVW) P values adjusted for FDR (P_{FDR}). The biological relevance of these metabolites was further assessed using the Human Metabolome Database (HMDB) (Version 5.0). Ultimately, the top 10 metabolites with potential biological significance were selected as candidate mediators for subsequent analyses (Supplementary Table 1). Step 2: Potential mediators of the association between gut microbiota and OSA were identified based on the following criteria: i a two-sample MR analysis was conducted to assess the causal relationship between each selected protective taxon and candidate mediator. P values were adjusted for multiple comparisons using the FDR correction. The directionality of the associations was confirmed to be unidirectional. ii a two-sample MR analysis was performed to evaluate the causal effect of each



Step 1 (included n=2)
There exists a causal association between gut microbiota and the mediator, and the effect of gut microbiota on the mediator should be unidirectional (FDR correction used)
2-hydroxypalmitate and hyocholate included

Step 2 (included n=1)
Unidirectional causal effect of mediator on OSA (SNP selection: P < 5e-6; confounder-associated SNPs removal)
2-hydroxypalmitate included

Step 3 (included n=1)
The association of gut microbiota with the mediator and the association of the mediator with OSA should be in opposite directions
2-hydroxypalmitate included

Figure 1 Overview of the study design. (**A**) Protective gut microbiota associated with reduced OSA risk were identified using two-sample MR analysis. (**B**) The top 10 candidate metabolites were selected based on FDR-adjusted IVW results and biological relevance derived from the HMDB. (**C**) Potential mediators were identified through a three-step process involving causal links between gut microbiota and metabolites, metabolites-OSA associations, and consistent directional effects. (**D**) A two-step MR analysis was conducted to quantify the mediation effect linking gut microbiota to OSA through the selected metabolites. MR analyses were primarily conducted using the IVW method, with complementary approaches including MR-Egger, weighted median, simple mode, and weighted mode. Sensitivity analyses included Cochran's Q statistic, MR-Egger intercept test, MR-Pleiotropy Residual Sum and Outlier method, MR Steiger directionality test, and leave-one-out approach.

Abbreviations: OSA, obstructive sleep apnea; GWAS, genome-wide association study; SNPs, single nucleotide polymorphisms; MR, mendelian randomization; IVW, inverse variance weighted; FDR, false discovery rate; P_{FDR}, P values adjusted for FDR; HMDB, Human Metabolome Database.

candidate mediator on the OSA, using a more stringent SNP selection threshold (P < 5e-6) and excluding SNPs associated with known confounders. iii the direction of the association between the mediator and OSA had to be opposite to that between the selected protective taxa and the mediator. Third, the mediating effects of the identified metabolites on the causal pathway linking gut microbiota to OSA were quantified using a two-step MR approach. In the initial step, we employed a two-sample MR analysis to assess the total direct effect of gut microbiota on OSA risk (β0). In the subsequent step, we estimated the effect of gut microbiota on plasma metabolites (β1) and the effect of plasma metabolites on OSA (β2), allowing us to calculate the indirect effect (β1 × β2). The proportion of the mediated effect was determined by dividing β1 × β2 by the total effect (β0).

Data Sources

We garnered the GWAS data for the gut microbiota from the Dutch Microbiome Project.[22] The Dutch Microbiome Project analyzed feces from 7,738 individuals of European descent, involving 207 taxa.[22] The GWAS data for plasma metabolites were obtained from the Canadian Longitudinal Study on Aging cohort, comprising 8,299 participants.[23] This analysis encompassed 1,091 metabolic features and 309 metabolite ratios.

The GWAS data for OSA were obtained from the FinnGen database (R10) with 410385 individuals of European ancestry. The data contained 43901 OSA cases and 366484 controls. OSA diagnostic criteria was depended on ICD codes (ICD-10: G47.3; ICD-9: 3472), which were obtained from the Finnish National Hospital Discharge Registry and the Causes of Death Registry. The ICD-10 code G47.3 encompasses both OSA and central sleep apnea (CSA). Nevertheless, Strausz et al validated the registry-based OSA diagnosis, reporting a positive predictive value of >98%, indicating a high level of diagnostic accuracy.[24] The FinnGen study is a large-scale genomics project that has analyzed more than 500,000 Finnish biobank samples, linking genetic variations with health data to uncover disease mechanisms and predispositions.[25]

Instrumental Variables Selection

In this study, SNPs were selected as IVs, and their selection and validation followed the criteria outlined below. Firstly, due to the limited number of genome-wide significant SNPs available for gut microbiota, we applied a significance threshold of $P < 1e\text{-}5$ to identify potentially relevant SNPs. For the selected mediator metabolites, MR analyses were performed using OSA as the outcome, with SNPs selected based on a stricter threshold of $P < 5e\text{-}6$. Secondly, to minimize bias from linkage disequilibrium (LD), we performed SNP clumping using an LD threshold of $R^2 < 0.001$ within a 10,000 kb window. Thirdly, to reduce the risk of weak instrument bias, we calculated the F-statistic for each SNP and retained only those with $F > 10$ as valid IVs. Functional annotation of SNPs was conducted using functional mapping and gene annotation (FUMA) (Version 1.8.0), based on the most recent release of the GWAS Catalog.[26] To reduce the risk of potential pleiotropy, SNPs previously associated with OSA-related traits, such as body mass index, waist circumference, hip circumference, or waist-to-hip ratio, were excluded (Supplementary Table 2).

Statistical Analysis

We conducted a two-sample MR analysis to evaluate the causal relationships between gut microbiota and OSA, as well as between plasma metabolites and OSA independently. We evaluated the causal association between gut microbiota and the plasma metabolites using bidirectional MR. We used the IVW method as the primary MR approach. To enhance the robustness and reliability of our findings, we also applied complementary MR methods, including MR-Egger regression, weighted median, simple mode, and weighted mode. Apart from the MR methods outlined above, we also carried out various supplementary sensitivity analyses. Firstly, we evaluated heterogeneity in causal inference through the calculation of Cochran's Q statistic. Secondly, we applied the MR-Egger intercept test to detect horizontal pleiotropy. Moreover, the MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) method was employed to detect horizontal pleiotropy and identify potential outlier SNPs. A global test *P*-value < 0.05 was considered indicative of significant distortion in the causal estimates due to pleiotropy. Additionally, we employed the MR Steiger directionality test to ascertain the causal direction between the exposure and the outcome. Furthermore, the reliability of the findings was assessed through a leave-one-out approach for validation.

Effect sizes were expressed as odds ratios (OR), β-coefficients, and corresponding 95% confidence intervals (CI). All statistical analyses were conducted using the "TwoSampleMR" package (version 0.6.6), "MRPRESSO" package, and "ggplot2" packages within R software (version 4.4.1).

Results

Instrument Variables Included in Analysis

Based on the predefined selection criteria, we identified valid IVs from GWAS summary statistics of gut microbiota and plasma metabolites. Supplementary Tables 3 and 4 provide detailed characteristics of these IVs, including their corresponding F statistics. All included SNPs had F statistics greater than 10, supporting adequate instrument strength.

Effects of Gut Microbiota on Obstructive Sleep Apnea

Using the IVW method, this study identified 4 microbial taxa that were associated with a reduced risk of the OSA, including species Parabacteroides merdae, genus Faecalibacterium, species Faecalibacterium prausnitzii and species Bifidobacterium longum (Table 1). Species Parabacteroides merdae exhibited the most pronounced protective effect (OR = 0.909, 95% CI = 0.828~0.999, *P* value = 0.047). Species Faecalibacterium prausnitzii and genus Faecalibacterium showed the second strongest protective effects (species Faecalibacterium prausnitzii, OR = 0.921, 95% CI = 0.860~0.986, *P* value = 0.019; genus Faecalibacterium, OR = 0.923, 95% CI = 0.857~0.994, *P* value = 0.034). The protective effect of species Bifidobacterium longum was comparatively weaker (OR = 0.930, 95% CI = 0.869~0.997, *P* value = 0.040).

Effects of Gut Microbiota on Mediators

Following the established criteria and selection workflow, we identified two candidate mediators, 2-hydroxypalmitate and hyocholate, from the top 10 biologically relevant metabolites. As shown in Table 2 and Supplementary Table 5, species Parabacteroides merdae was positively associated with the levels of 2-hydroxypalmitate (β = 0.254, 95% CI = 0.117~0.391, IVW P value < 0.001, IVW P_{FDR} value = 0.003). Since MR analyses were performed between the selected microbial taxa and the top 10 metabolites with potential biological significance, FDR correction was applied across these 10 tests using the Benjamini–Hochberg method. After FDR correction, no statistically significant associations were observed between genus Faecalibacterium and 2-hydroxypalmitate, species Faecalibacterium prausnitzii and 2-hydroxypalmitate, or species Bifidobacterium longum and hyocholate.

Table 1 Effects of Gut Microbiota on the Risk of Obstructive Sleep Apnea

Exposure	Method	nSNPs	OR	95% CI	*P* value
Species Parabacteroides merdae	MR Egger	6	1.005	0.695~1.453	0.982
	Weighted median	6	0.957	0.867~1.055	0.377
	Inverse variance weighted	6	0.909	0.828~0.999	0.047
	Simple mode	6	1.003	0.825~1.219	0.981
	Weighted mode	6	1.003	0.863~1.164	0.975
Genus Faecalibacterium	MR Egger	7	0.804	0.557~1.160	0.296
	Weighted median	7	0.925	0.842~1.017	0.106
	Inverse variance weighted	7	0.923	0.857~0.994	0.034
	Simple mode	7	0.936	0.816~1.075	0.385
	Weighted mode	7	0.953	0.836~1.087	0.500
Species Faecalibacterium prausnitzii	MR Egger	8	0.832	0.593~1.167	0.327
	Weighted median	8	0.910	0.833~0.993	0.035
	Inverse variance weighted	8	0.921	0.860~0.986	0.019
	Simple mode	8	0.915	0.804~1.041	0.219
	Weighted mode	8	0.924	0.818~1.044	0.245
Species Bifidobacterium longum	MR Egger	7	0.706	0.514~0.970	0.085
	Weighted median	7	0.935	0.852~1.027	0.163
	Inverse variance weighted	7	0.930	0.869~0.997	0.040
	Simple mode	7	0.943	0.819~1.086	0.448
	Weighted mode	7	0.943	0.824~1.080	0.429

Abbreviations: nSNPs, number of Single Nucleotide Polymorphisms; OR, odds ratio; CI, confidence interval.

Table 2 Effects of Gut Microbiota on Mediators

Exposure	Outcome	Method	nSNPs	β	95% CI	*P* value	P_{FDR} value	N Tests
2-hydroxypalmitate	Species Parabacteroides merdae	MR Egger	6	0.235	−0.236~0.707	0.383	0.767	10
		Weighted median	6	0.256	0.079~0.433	0.005	0.046	10
		Inverse variance weighted	6	0.254	0.117~0.391	<0.001	0.003	10
		Simple mode	6	0.254	0.024~0.483	0.082	0.588	10
		Weighted mode	6	0.251	0.022~0.480	0.085	0.595	10
	Genus Faecalibacterium	MR Egger	7	0.759	0.143~1.375	0.060	0.605	10
		Weighted median	7	0.162	−0.036~0.360	0.109	0.901	10
		Inverse variance weighted	7	0.200	0.056~0.345	0.007	0.065	10
		Simple mode	7	0.099	−0.191~0.389	0.528	0.950	10
		Weighted mode	7	0.097	−0.187~0.381	0.529	0.907	10
	Species Faecalibacterium prausnitzii	MR Egger	8	0.814	0.236~1.392	0.033	0.328	10
		Weighted median	8	0.106	−0.068~0.279	0.232	0.966	10
		Inverse variance weighted	8	0.166	0.026~0.305	0.020	0.198	10
		Simple mode	8	0.054	−0.221~0.328	0.714	0.789	10
		Weighted mode	8	0.051	−0.247~0.349	0.746	0.787	10
Hyocholate	Species Bifidobacterium longum	MR Egger	10	0.268	−0.307~0.843	0.388	0.970	10
		Weighted median	10	0.197	0.019~0.375	0.030	0.297	10
		Inverse variance weighted	10	0.151	0.012~0.290	0.033	0.331	10
		Simple mode	10	0.206	−0.070~0.482	0.177	0.829	10
		Weighted mode	10	0.211	−0.047~0.470	0.144	0.867	10

Abbreviations: nSNPs, number of Single Nucleotide Polymorphisms; CI, confidence interval; P_{FDR}, *P* values adjusted for false discovery rate.

Effects of Selected Mediators on the OSA and Mediation Analysis

Table 3 and Supplementary Table 5, demonstrates that elevated levels of 2-hydroxypalmitate were associated with a reduced risk of OSA (OR = 0.926, 95% CI = 0.865~0.991, *P* value = 0.027). As presented in Figure 2, through two-step MR analysis, 2-hydroxypalmitate was found to mediate 20.53% of the association between the species Parabacteroides merdae and OSA.

MR Sensitivity Analyses

According to the results presented in Supplementary Table 6, no evidence of heterogeneity was observed, as indicated by Cochran's Q statistic (all *P* values > 0.05). Both the MR-Egger intercept test and the MR-PRESSO global test indicated no signs of horizontal pleiotropy, with *P* values greater than 0.05. In addition, the MR-PRESSO method did not identify any outlier variants. The MR-Steiger directionality test provided no evidence supporting a reverse causal relationship from the OSA to the four protective taxa or to 2-hydroxypalmitate. As shown in Supplementary Figures 1–3, the leave-

Table 3 Effects of Mediators on Obstructive Sleep Apnea

Exposure	Method	nSNPs	OR	95% CI	*P* value
2-hydroxypalmitate	MR Egger	8	0.899	0.766~1.055	0.239
	Weighted median	8	0.923	0.841~1.012	0.086
	Inverse variance weighted	8	0.926	0.865~0.991	0.027
	Simple mode	8	0.889	0.786~1.005	0.102
	Weighted mode	8	0.906	0.809~1.015	0.132

Abbreviations: nSNPs, number of Single Nucleotide Polymorphisms; OR, odds ratio; CI, confidence interval.

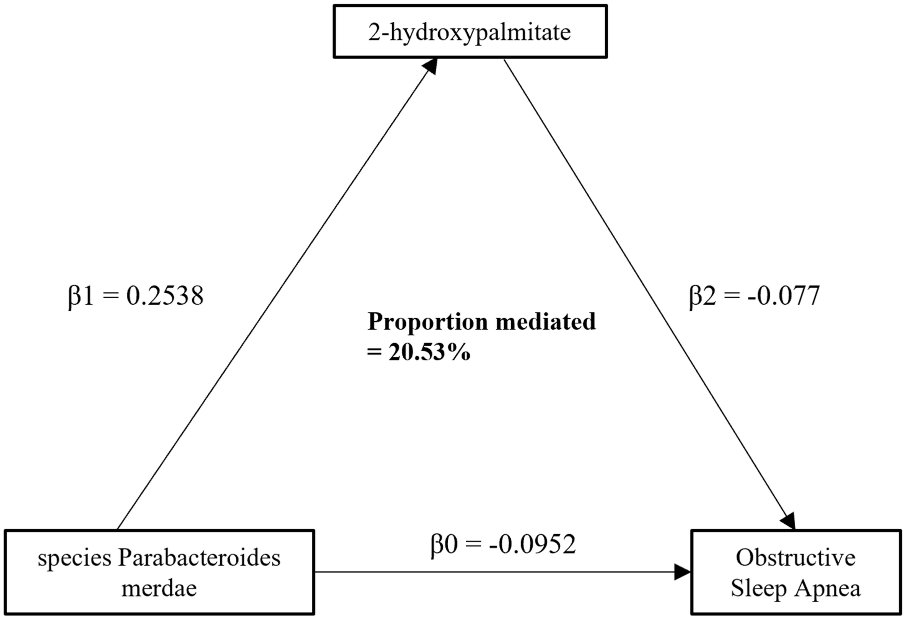


Figure 2 The 2-hydroxypalmitate mediated the causal effect of species Parabacteroides merdae on OSA. Two-step MR was used to evaluate the mediating role of 2-hydroxypalmitate in the causal pathway linking species Parabacteroides merdae to OSA. The causal effect of species Parabacteroides merdae on 2-hydroxypalmitate, 2-hydroxypalmitate on OSA and species Parabacteroides merdae on OSA were assumed to be β1, β2 and β0, respectively. The proportion of the mediated effect (bold text) was determined by dividing β1× β2 by the total effect (β0). MR estimates were derived from the IVW method in two-sample MR.
Abbreviations: OSA, obstructive sleep apnea; MR, mendelian randomization; IVW, inverse variance weighted.

one-out analysis suggested that certain SNPs may have disproportionately influenced the causal estimates. Based on the overall findings from our sensitivity analyses, the observed associations appear to be relatively robust. Nonetheless, these results should be interpreted with caution.

Discussion

In this study, we performed a comprehensive MR analysis utilizing large-scale GWAS summary data to explore the causal relationships between gut microbiota, plasma metabolites, and OSA. In the Dutch Microbiome Project, species Parabacteroides merdae, genus Faecalibacterium, species Faecalibacterium prausnitzii and species Bifidobacterium longum demonstrated a potential protective association with OSA. This study was exploratory in nature. We included the top 10 metabolites with potential biological significance as candidate mediators. Among them, only 2-hydroxypalmitate was found to mediate the association between species Parabacteroides merdae and OSA, with a mediation proportion of 20.53%.

Species Parabacteroides Merdae and OSA

Species Parabacteroides merdae is a Gram-negative, anaerobic, rod-shaped bacterium commonly found in the human gut microbiota.[27] Consistent with our findings, species Parabacteroides merdae retained a strong negative association with OSA.[28] The potential protective effect of species Parabacteroides merdae against OSA may be related to its role in alleviating obesity.[29] Qiao et al reported that species Parabacteroides merdae significantly attenuated high-fat diet-induced weight gain in mice.[30] Given that obesity is a major risk factor for OSA, the beneficial effect of species Parabacteroides merdae on obesity may partially explain its protective role against OSA. Fecal microbiota transplantation has been shown to lower systolic blood pressure (SBP), with increased abundances of species Parabacteroides merdae associated with SBP reduction.[31] Given that OSA is a known risk factor for hypertension, it is plausible that the SBP-lowering effect of species Parabacteroides merdae may be related to improvements in nocturnal intermittent hypoxia. However, this hypothesis requires further investigation. Evidence linking species Parabacteroides merdae to OSA remains limited. However, further investigation into its potential protective role may help uncover novel therapeutic strategies. Given the complexity of the underlying mechanisms, further research is warranted to identify the key pathways and molecular targets through which species Parabacteroides merdae may influence OSA progression.

2-Hydroxypalmitate and OSA

2-hydroxypalmitate, also known as 2-hydroxyhexadecanoic acid, has emerged as a representative member of the 2-hydroxy fatty acid family.[32] These molecules constitute essential structural components of mammalian sphingolipids. Sphingolipids are essential components of cell membranes, particularly abundant in the brain, where they help maintain myelin integrity, facilitate neuronal signaling, and support intercellular communication.[33] Fatty acid 2-hydroxylase (FA2H) catalyzes the biosynthesis of hydroxylated sphingolipids by introducing a hydroxyl group at the α-carbon of long-chain fatty acids.[34] FA2H gene mutations are linked to leukodystrophy and spastic paraparesis, highlighting the importance of hydroxylated fatty acid-containing sphingolipids in nervous system function.[35,36] Increasing evidence suggests that alterations in sphingolipid pathways may contribute to the etiopathogenesis of neurodegenerative diseases.[37] Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders. Sphingolipids play a critical role in various cellular processes involved in the pathogenesis of PD, including mitochondrial function, autophagy, and endosomal trafficking.[38] Upper airway obstruction and dysfunction are observed in approximately 24% to 65% of PD patients, primarily attributed to laryngopharyngeal motor impairment.[39,40] This dysfunction reflects a broader impairment of neuromuscular control in the upper airway, characterized by reduced tone in the pharyngeal dilator muscles, impaired coordination of respiratory and swallowing reflexes, and delayed glottic opening.[17] These abnormalities may predispose individuals with PD to the development of OSA, particularly during sleep when compensatory mechanisms are further diminished. Given the essential role of sphingolipids in maintaining neuronal membrane stability and supporting axonal conduction, their dysregulation may plausibly impair the integrity of central or peripheral neural pathways involved in upper airway motor control. However, it must be acknowledged that current evidence directly linking 2-hydroxypalmitate to the regulation of upper airway motor function is limited. Further studies are warranted to clarify this potential association.

However, some studies have suggested that 2-hydroxypalmitate may contribute to the development of OSA. 2-hydroxypalmitate is associated with dyslipidemia, a condition commonly observed in obesity.[41,42] Given that obesity is a major risk factor for OSA, 2-hydroxypalmitate may contribute to the development of OSA through pathways related to fatty acid metabolism. Sullivan et al[43] found that vitamin D supplementation was associated with lower 2-hydroxypalmitate levels, while Ayyıldız et al[44] reported a potential beneficial effect of vitamin D supplementation on the prognosis of mild OSA. These findings are inconsistent with our results. Nonetheless, given the exploratory nature of this study, further research is needed to clarify the role of 2-hydroxypalmitate in OSA pathogenesis.

2-Hydroxypalmitate as a Putative Mediator: Implications and Future Directions

In this study, we identified 2-hydroxypalmitate as a potential mediator of the relationship between species Parabacteroides merdae and the reduced risk of OSA. The mediation analysis demonstrated that 2-hydroxypalmitate accounted for 20.53% of the total effect. In terms of effect size, this proportion represents a moderate mediation effect, comparable to those observed in other microbial-metabolite axes involved in disease progression. For example, prior studies have reported mediation proportions ranging from 6.5% to 25.1% in obesity,[45] 10.29% to 21.9% in type 2 diabetes,[46] 11.04% to 15.35% in PD,[47] 14.62% to 37.48% in chronic airway disease,[48,49] and 8.1% to 22.8% in various cancer types.[50] These findings suggest that the mediation effect observed in our study falls within a biologically meaningful range and supports the role of 2-hydroxypalmitate as a mediator within the causal pathway from species Parabacteroides merdae to OSA.

Previous studies suggest that species Parabacteroides merdae may reduce the risk of OSA through anti-obesity effects, while 2-hydroxypalmitate may confer protection via sphingolipid metabolism, which modulates upper airway motor function. In our analysis, species Parabacteroides merdae and 2-hydroxypalmitate were positively correlated and both linked to reduced OSA risk, supporting the plausibility of this mediation pathway. Although direct evidence for the regulatory relationship between species Parabacteroides merdae and 2-hydroxypalmitate is currently lacking, our findings suggest a biologically relevant connection that merits further investigation. Overall, this study reveals a gut microbiota-metabolite-OSA pathway and offers new evidence for microbial metabolic mediation in OSA. Given the exploratory nature of this study, future experimental and longitudinal studies are warranted to validate these results and elucidate the underlying mechanisms.

Limitations

This study has several limitations that should be acknowledged. First, the GWAS summary statistics used in our MR analyses were derived exclusively from individuals of European ancestry. As a result, our findings may not be fully generalizable to other populations, given known differences in genetic architecture, LD structure, allele frequencies, and environmental modifiers across ancestries. These population-specific factors could influence both the strength and direction of causal associations, potentially limiting the applicability of our results to non-European groups. To enhance the external validity of microbiota-metabolite-disease mediation frameworks, future studies should incorporate GWAS data from ancestrally diverse cohorts and perform replication analyses across multiple populations. Such efforts are essential to improve the global relevance and translational potential of microbiome-informed causal inference. Second, sleep apnea in the FinnGen study was defined using ICD codes (ICD-10: G47.3; ICD-9: 3472), and individual-level polysomnographic (PSG) data, such as the apnea-hypopnea index, oxygen desaturation index, and sleep architecture, were not available. This limitation precludes accurate confirmation of OSA diagnoses, assessment of disease severity, and identification of clinical subtypes (eg, rapid eye movement-predominant or positional OSA). Notably, ICD-10 code G47.3 includes both OSA and CSA. While Strausz et al[24] validated the registry-based OSA definition and reported high diagnostic accuracy, the potential for misclassification remains, particularly due to the inclusion of CSA cases. However, given the low prevalence of CSA[51] and the lack of evidence linking our exposures of interest to CSA risk, any such misclassification is likely nondifferential with respect to exposure. From a statistical perspective, this would tend to bias the effect estimates toward the null, resulting in a conservative estimate of the true causal effect. Future studies incorporating PSG-confirmed, individual-level OSA data are therefore warranted to validate and refine these findings. Third, a relaxed significance threshold ($P < 1e\text{-}5$) was used for SNP selection in the MR analyses to increase the number of valid IVs and improve statistical power. This approach has been adopted in previous MR studies involving complex traits and microbiota-related exposures,[50,52] and is considered acceptable in exploratory settings according to a strategy supported by previous methodological literature.[53–55] However, the use of a relaxed threshold may increase the risk of including weak or pleiotropic IVs. To mitigate this concern, we retained only SNPs with $F > 10$ to ensure sufficient IVs strength, and excluded those associated with known confounders whenever possible. In addition, the leave-one-out analysis suggested that one or more SNPs might disproportionately influence the causal estimates. Nonetheless, no evidence of heterogeneity (Cochran's Q statistic) or horizontal pleiotropy (MR-Egger intercept and MR-PRESSO global test) was observed, supporting the robustness of our results. These findings should be interpreted with caution. Future studies using larger GWAS datasets and more advanced MR methods are needed to validate our conclusions. For example, multivariable MR, summary-data-based MR to explore the relationships among genetic variants, exposures, and outcomes,[56,57] and Bayesian co-localization analysis to verify shared causal variants and strengthen causal inference[58] may offer further insights.

Conclusions

In summary, this study highlights the protective effect of species Parabacteroides merdae against OSA. 2-hydroxypalmitate may act as a partial mediator in the association between species Parabacteroides merdae and OSA. These findings provide novel insights into the mechanisms underlying OSA and suggest potential therapeutic targets, offering promising directions for future research and clinical interventions.

Abbreviations

OSA, Obstructive sleep apnea; MR, Mendelian randomization; IVs, instrumental variables; GWAS, genome-wide association studies; SNPs, single-nucleotide polymorphisms; FDR, false discovery rate; IVW, inverse variance weighted; P_{FDR}, P values adjusted for false discovery rate; HMDB, Human Metabolome Database; CSA, central sleep apnea; LD, linkage disequilibrium; FUMA, functional mapping and gene annotation; MR-PRESSO, the MR-Pleiotropy Residual Sum and Outlier; OR, odds ratios; CI, confidence intervals; SBP, systolic blood pressure; FA2H, Fatty acid 2-hydroxylase; PD, Parkinson's disease; PSG, polysomnographic.

Data Sharing Statement

The datasets supporting the conclusions of this article are available in IEU open GWAS project repository (https://www.ebi.ac.uk/gwas/). Details regarding the GWAS on OSA can be accessed through the following link: https://r10.risteys.finngen.fi/endpoints/G6_SLEEPAPNO/.

Ethics Approval and Consent to Participate

All data in this study were derived from publicly accessible GWAS using anonymized summary-level datasets. As this research exclusively analyzed de-identified aggregate data without involving individual participants, sensitive personal information, or commercial interests, ethical approval was formally waived by the Ethics Committee of Beijing Friendship Hospital, Capital Medical University (Approval No.: 2025-P2-115). This study was conducted in full compliance with the Declaration of Helsinki and relevant national ethical regulations.

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Author Contributions

Xiaona Wang: Conceptualization, Data Curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, and Writing - original draft. Ranran Zhao, Jia Guo, and Ke Yang: Data Curation, Formal analysis, Investigation, Methodology, Validation, and Writing - review & editing. Bo Xu: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, and Writing - review & editing. All authors made significant contributions to the study, approved the final manuscript for submission, agreed on the choice of journal, and take full responsibility for the integrity of the work.

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Disclosure

The authors declare that they have no competing interests.

References

1. Heinzer R, Vat S, Marques-Vidal P, et al. Prevalence of sleep-disordered breathing in the general population: the HypnoLaus study. *Lancet Respir Med*. 2015;3(4):310–318. doi:10.1016/s2213-2600(15)00043-0
2. Neelapu BC, Kharbanda OP, Sardana HK, et al. Craniofacial and upper airway morphology in adult obstructive sleep apnea patients: a systematic review and meta-analysis of cephalometric studies. *Sleep Med Rev*. 2017;31:79–90. doi:10.1016/j.smrv.2016.01.007
3. White DP. Pathogenesis of obstructive and central sleep apnea. *Am J Respir Crit Care Med*. 2005;172(11):1363–1370. doi:10.1164/rccm.200412-1631SO
4. Lv R, Liu X, Zhang Y, et al. Pathophysiological mechanisms and therapeutic approaches in obstructive sleep apnea syndrome. *Signal Transduct Target Ther*. 2023;8(1):218. doi:10.1038/s41392-023-01496-3
5. Cai Y, Juszczak HM, Cope EK, Goldberg AN. The microbiome in obstructive sleep apnea. *Sleep*. 2021;44(8). doi:10.1093/sleep/zsab061
6. Silva V, Palacios-Muñoz A, Okray Z, et al. The impact of the gut microbiome on memory and sleep in Drosophila. *J Exp Biol*. 2021;224(Pt 3). doi:10.1242/jeb.233619
7. Grosicki GJ, Riemann BL, Flatt AA, Valentino T, Lustgarten MS. Self-reported sleep quality is associated with gut microbiome composition in young, healthy individuals: a pilot study. *Sleep Med*. 2020;73:76–81. doi:10.1016/j.sleep.2020.04.013
8. Fei N, Choo-Kang C, Reutrakul S, et al. Gut microbiota alterations in response to sleep length among African-origin adults. *PLoS One*. 2021;16(9): e0255323. doi:10.1371/journal.pone.0255323
9. O'Connor KM, Lucking EF, Golubeva AV, et al. Manipulation of gut microbiota blunts the ventilatory response to hypercapnia in adult rats. *EBioMedicine*. 2019;44:618–638. doi:10.1016/j.ebiom.2019.03.029
10. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*. 2013;341 (6150):1241214. doi:10.1126/science.1241214
11. Andersen ML, Gozal D, Pires GN, Tufik S. Exploring the potential relationships among obstructive sleep apnea, erectile dysfunction, and gut microbiota: a narrative review. *Sex Med Rev*. 2023;12(1):76–86. doi:10.1093/sxmrev/qead026

12. Badran M, Khalyfa A, Ericsson AC, et al. Gut microbiota mediate vascular dysfunction in a murine model of sleep apnoea: effect of probiotics. *Eur Respir J*. 2023;61(1):2200002. doi:10.1183/13993003.00002-2022
13. Liu Q, Liu Y, Li F, et al. Probiotic culture supernatant improves metabolic function through FGF21-adiponectin pathway in mice. *J Nutr Biochem*. 2020;75:108256. doi:10.1016/j.jnutbio.2019.108256
14. Liu J, Li T, Wu H, et al. Lactobacillus rhamnosus GG strain mitigated the development of obstructive sleep apnea-induced hypertension in a high salt diet via regulating TMAO level and CD4(+) T cell induced-type I inflammation. *Biomed Pharmacother*. 2019;112:108580. doi:10.1016/j.biopha.2019.01.041
15. Xu H, Wang J, Cai J, et al. Protective Effect of Lactobacillus rhamnosus GG and its Supernatant against Myocardial Dysfunction in Obese Mice Exposed to Intermittent Hypoxia is Associated with the Activation of Nrf2 Pathway. *Int J Biol Sci*. 2019;15(11):2471–2483. doi:10.7150/ijbs.36465
16. Tomar MS, Araniti F, Sahai PK, et al. Urinary metabolite signatures reflect the altered host metabolism in severe obstructive sleep apnea. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2023;1231:123938. doi:10.1016/j.jchromb.2023.123938
17. Bahia C, Pereira JS. Obstructive sleep apnea and neurodegenerative diseases: a bidirectional relation. *Dement Neuropsychol*. 2015;9(1):9–15. doi:10.1590/s1980-57642015dn91000003
18. Loh JS, Mak WQ, Tan LKS, et al. Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct Target Ther*. 2024;9(1):37. doi:10.1038/s41392-024-01743-1
19. Xu H, Li X, Zheng X, et al. Pediatric Obstructive Sleep Apnea is Associated With Changes in the Oral Microbiome and Urinary Metabolomics Profile: a Pilot Study. *J Clin Sleep Med*. 2018;14(9):1559–1567. doi:10.5664/jcsm.7336
20. Yan W, Jiang M, Hu W, et al. Causality Investigation between Gut Microbiota, Derived Metabolites, and Obstructive Sleep Apnea: a Bidirectional Mendelian Randomization Study. *Nutrients*. 2023;15(21):4544. doi:10.3390/nu15214544
21. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. *JAMA*. 2017;318(19):1925–1926. doi:10.1001/jama.2017.17219
22. Lopera-Maya EA, Kurilshikov A, van der Graaf A, et al. Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. *Nat Genet*. 2022;54(2):143–151. doi:10.1038/s41588-021-00992-y
23. Chen Y, Lu T, Pettersson-Kymmer U, et al. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. *Nat Genet*. 2023;55(1):44–53. doi:10.1038/s41588-022-01270-1
24. Strausz S, Ruotsalainen S, Ollila HM, et al. Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health. *Eur Respir J*. 2021;57(5):2003091. doi:10.1183/13993003.03091-2020
25. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613(7944):508–518. doi:10.1038/s41586-022-05473-8
26. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun*. 2017;8(1):1826. doi:10.1038/s41467-017-01261-5
27. Sakamoto M, Benno Y. Reclassification of Bacteroides distasonis, Bacteroides goldsteinii and Bacteroides merdae as Parabacteroides distasonis gen. nov. comb. nov. Parabacteroides goldsteinii comb. nov. and Parabacteroides merdae comb. nov. *Int J Syst Evol Microbiol*. 2006;56(Pt 7):1599–1605. doi:10.1099/ijs.0.64192-0
28. Liu L, He G, Yu R, et al. Causal relationships between gut microbiome and obstructive sleep apnea: a bi-directional Mendelian randomization. *Front Microbiol*. 2024;15:1410624. doi:10.3389/fmicb.2024.1410624
29. Sugawara Y, Kanazawa A, Aida M, Yoshida Y, Yamashiro Y, Watada H. Association of gut microbiota and inflammatory markers in obese patients with type 2 diabetes mellitus: post hoc analysis of a synbiotic interventional study. *Biosci Microbiota Food Health*. 2022;41(3):103–111. doi:10.12938/bmfh.2021-081
30. Qiao S, Liu C, Sun L, et al. Gut Parabacteroides merdae protects against cardiovascular damage by enhancing branched-chain amino acid catabolism. *Nat Metab*. 2022;4(10):1271–1286. doi:10.1038/s42255-022-00649-y
31. Fan L, Chen J, Zhang Q, et al. Fecal microbiota transplantation for hypertension: an exploratory, multicenter, randomized, blinded, placebo-controlled trial. *Microbiome*. 2025;13(1):133. doi:10.1186/s40168-025-02118-6
32. Hama H. Fatty acid 2-Hydroxylation in mammalian sphingolipid biology. *Biochim Biophys Acta*. 2010;1801(4):405–414. doi:10.1016/j.bbalip.2009.12.004
33. van Echten-Deckert G, Herget T. Sphingolipid metabolism in neural cells. *Biochim Biophys Acta*. 2006;1758(12):1978–1994. doi:10.1016/j.bbamem.2006.06.009
34. Guo L, Zhang X, Zhou D, Okunade AL, Su X. Stereospecificity of fatty acid 2-hydroxylase and differential functions of 2-hydroxy fatty acid enantiomers. *J Lipid Res*. 2012;53(7):1327–1335. doi:10.1194/jlr.M025742
35. Dick KJ, Eckhardt M, Paisán-Ruiz C, et al. Mutation of FA2H underlies a complicated form of hereditary spastic paraplegia (SPG35). *Hum Mutat*. 2010;31(4):E1251–60. doi:10.1002/humu.21205
36. Edvardson S, Hama H, Shaag A, et al. Mutations in the fatty acid 2-hydroxylase gene are associated with leukodystrophy with spastic paraparesis and dystonia. *Am J Hum Genet*. 2008;83(5):643–648. doi:10.1016/j.ajhg.2008.10.010
37. van Kruining D, Luo Q, van Echten-Deckert G, et al. Sphingolipids as prognostic biomarkers of neurodegeneration, neuroinflammation, and psychiatric diseases and their emerging role in lipidomic investigation methods. *Adv Drug Deliv Rev*. 2020;159:232–244. doi:10.1016/j.addr.2020.04.009
38. Vos M, Klein C, Hicks AA. Role of ceramides and sphingolipids in Parkinson's disease. *J Mol Biol*. 2023;435(12):168000. doi:10.1016/j.jmb.2023.168000
39. Herer B, Arnulf I, Housset B. Effects of levodopa on pulmonary function in Parkinson's disease. *Chest*. 2001;119(2):387–393. doi:10.1378/chest.119.2.387
40. Yu Q, Hu X, Zheng T, et al. Obstructive sleep apnea in Parkinson's disease: a prevalent, clinically relevant and treatable feature. *Parkinsonism Relat Disord*. 2023;115:105790. doi:10.1016/j.parkreldis.2023.105790
41. Gu X, Li C, He J, et al. Serum metabolites associate with lipid phenotypes among Bogalusa Heart Study participants. *Nutr Metab Cardiovasc Dis*. 2020;30(5):777–787. doi:10.1016/j.numecd.2020.01.004
42. Nussbaumerova B, Rosolova H. Obesity and Dyslipidemia. *Curr Atheroscler Rep*. 2023;25(12):947–955. doi:10.1007/s11883-023-01167-2
43. Sullivan VK, Chen J, Bernard L, et al. Serum and urine metabolite correlates of vitamin D supplementation in the Atherosclerosis Risk in Communities (ARIC) study. *Clin Nutr ESPEN*. 2025;67:523–532. doi:10.1016/j.clnesp.2025.03.172

44. Ayyıldız F, Yıldıran H, Afandiyeva N, Gülbahar Ö, Köktürk O. The effects of vitamin D supplemantation on prognosis in patients with mild obstructive sleep apnea syndrome. *Turk J Med Sci*. 2021;51(5):2524–2533. doi:10.3906/sag-2101-83
45. Li X, Wu Q, Liu S. Identification of 1,400 metabolites as mediators of obesity in 473 gut microbiota taxa: a mediation Mendelian randomization study. *Microbiol Spectr*. 2025;13(6):e0189224. doi:10.1128/spectrum.01892-24
46. Zheng X, Chen M, Zhuang Y, et al. Genetic associations between gut microbiota and type 2 diabetes mediated by plasma metabolites: a Mendelian randomization study. *Front Endocrinol*. 2024;15:1430675. doi:10.3389/fendo.2024.1430675
47. Chen J, Zhu L, Wang F, et al. Plasma metabolites as mediators between gut microbiota and Parkinson's disease: insights from Mendelian randomization. *Mol Neurobiol*. 2025;62(6):7945–7956. doi:10.1007/s12035-025-04765-0
48. Du Y, Wang S, Zhou T, Zhao Z. Causal effects of gut microbiota and metabolites on chronic obstructive pulmonary disease: a bidirectional two sample Mendelian randomization study. *Int J Chron Obstruct Pulmon Dis*. 2024;19:2153–2167. doi:10.2147/copd.S472218
49. Zheng X, Yin J, Zhao L, Qian Y, Xu J. Mediation analysis of gut microbiota and plasma metabolites in asthma pathogenesis using Mendelian randomization. *J Asthma*. 2025;62(8):1351–1363. doi:10.1080/02770903.2025.2478504
50. Luan B, Yang Y, Yang Q, et al. Gut microbiota, blood metabolites, & pan-cancer: a bidirectional Mendelian randomization & mediation analysis. *AMB Express*. 2025;15(1):59. doi:10.1186/s13568-025-01866-w
51. Roberts EG, Raphelson JR, Orr JE, LaBuzetta JN, Malhotra A. The pathogenesis of central and complex sleep apnea. *Curr Neurol Neurosci Rep*. 2022;22(7):405–412. doi:10.1007/s11910-022-01199-2
52. Lv WQ, Lin X, Shen H, et al. Human gut microbiome impacts skeletal muscle mass via gut microbial synthesis of the short-chain fatty acid butyrate among healthy menopausal women. *J Cachexia Sarcopenia Muscle*. 2021;12(6):1860–1870. doi:10.1002/jcsm.12788
53. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol*. 2013;37(7):658–665. doi:10.1002/gepi.21758
54. Burgess S, Thompson SG. *Mendelian Randomization: Methods for Using Genetic Variants in Causal Estimation*. CRC Press; 2015.
55. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi:10.1136/bmj.k601
56. Zhu Z, Zhang F, Hu H, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat Genet*. 2016;48(5):481–487. doi:10.1038/ng.3538
57. Zhao C, Fan Z, Zhang R, Sun Y, Li WY. The role of the major histocompatibility complex region on chromosome 6 in skin atrophy: a Mendelian randomization study. *J Cosmet Dermatol*. 2025;24(3):e70040. doi:10.1111/jocd.70040
58. Mao R, Li J, Xiao W. Identification of prospective aging drug targets via Mendelian randomization analysis. *Aging Cell*. 2024;23(7):e14171. doi:10.1111/acel.14171