Journal of Asthma and Allergy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Gut Microbiota Dysbiosis and Gut–Nasal Axis Alterations in Preschool Children with Allergic Rhinitis and Functional Constipation

Weikeng Yang[1,2,*], Xiaoli Li[3,*], Chunyan Wang[4], Xiuyun Li[3], Congfu Huang[3]

[1]Shantou University Medical College, Shantou, Guangdong, People's Republic of China; [2]Department of Pediatrics, The Second Affiliated Hospital, School of Medicine, The Chinese University of Hong Kong, Shenzhen & Longgang District People's Hospital of Shenzhen, Shenzhen, Guangdong, People's Republic of China; [3]Department of Pediatrics, Longgang District Maternity & Child Healthcare Hospital of Shenzhen City (Affiliated Shenzhen Women and Children's Hospital (Longgang) of Shantou University Medical College, Medical Research Institute of Maternal and Child, Shenzhen, Guangdong, People's Republic of China; [4]Department of Pediatrics, Shenzhen Fourth People's Hospital (Sami Medical Center), Shenzhen, Guangdong, People's Republic of China

*These authors contributed equally to this work

Correspondence: Congfu Huang, Email 78333755@qq.com

Background: The comorbidity of allergic rhinitis (AR) and functional constipation (FC) in preschool children represents an emerging clinical entity; yet its underlying microbial mechanisms remain inadequately elucidated. This study sought to comprehensively characterize the gut microbiota (GM) profile and functional alterations in children with concurrent AR and FC (ARFC), with specific emphasis on the gut-nasal axis.

Methods: In this cross-sectional analysis, fecal samples from 32 ARFC, 22 AR, and 21 healthy control (HC) children underwent *16S rRNA* gene sequencing (V3–V4 regions). Microbial α-and β diversity, taxonomic composition, functional pathways, and correlations with clinical parameters were systematically analyzed.

Results: The ARFC group exhibited significant GM dysbiosis, including elevated α-diversity (Shannon index: 5.2 ± 0.3 vs HC 4.5 ± 0.4; $P = 0.014$) and distinct β-diversity (PERMANOVA $P = 0.001$). Taxonomic analysis revealed elevated enrichment of Proteobacteria (7.92% vs HC 1.94%; $P = 0.001$) and depletion of Bacteroidetes (40.06% vs HC 50.72%; $P = 0.049$). Pathogen genera, including *Klebsiella* and *Escherichia*/*Shigella*, were elevated, while butyrate-producing genera (*Faecalibacterium* and *Ruminococcus*) were reduced. Paradoxically, *Bifidobacterium* abundance was higher in ARFC than AR (4.21% vs 1.80%; $P = 0.018$), suggesting a potential compensatory immunomodulatory response. Functional prediction indicated impaired carbohydrate and lipid metabolism alongside enhanced xenobiotic degradation. *Haemophilus* abundance positively correlated with constipation severity ($\rho = 0.52$ and $P = 0.008$) and rhinorrhea severity ($\rho = 0.56$ and $P = 0.003$).

Conclusion: ARFC comorbidity is characterized by a distinct GM signature featuring pathogenic expansion, metabolic dysfunction, and compensatory *Bifidobacterium* enrichment, which may modulate immune responses. Loss of butyrate-producing bacteria may disrupt the gut-nasal axis, highlighting potential microbial and therapeutic targets for integrated therapeutic strategies in this dual symptom condition. These findings provide a foundation for microbiota-based interventions targeting both allergic and gastrointestinal manifestations.

Keywords: allergic rhinitis, functional constipation, gut microbiota, gut-nasal axis, butyrate, preschool children, dysbiosis

Introduction

Allergic rhinitis (AR) is a Th2/Th17-mediated inflammatory disorder that affects 15.79% of Chinese children and is increasingly accompanied by gastrointestinal comorbidities such as functional constipation (FC).[1–3] Mounting evidence supports bidirectional interactions between the gut microbiota (GM) and the host immune system. In AR, GM dysbiosis has been implicated in aberrant IgE production and systemic immune activation;[4] whereas in FC, compositional shifts,

Received: 20 August 2025
Accepted: 1 December 2025
Published: 11 December 2025

typically characterized by a reduction in *Bacteroidetes* and an expansion of *Proteobacteria*, are associated with impaired gut motility and compromised barrier function.[5,6]

Specific commensal taxa, including *Bifidobacterium* and *Lactobacillus*, are known to modulate immune homeostasis by promoting regulatory T cell (Treg) induction and suppressing pro-inflammatory Th2/Th17 responses.[7,8] Conversely, enrichment of pathobionts like *Klebsiella* and *Escherichia/Shigella* can amplify systemic inflammation via Toll-like receptor signaling and NF-κB activation.[9] Epidemiological data indicate that up to 20% of preschoolers with AR also develop FC, leading to aggravated systemic inflammation and diminished quality of life.[10–12]

Short-chain fatty acids (SCFAs, eg, butyrate) have emerged as critical mediators of gut-immune crosstalk, modulating Treg/Th17 balance and intestinal motility.[13,14] As dietary fiber serves as the principal substrate for microbial SCFA production, diet exerts a pivotal influence on host-microbe homeostasis. Depletion of *Faecalibacterium prausnitzii*, a major butyrate producer, has been linked to Th2 hyperactivity in AR,[15] while FC-driven SCFAs deficiency disrupts serotonergic (5-HT) signaling, delaying colonic transit.[16] Collectively, these observations suggest that dietary patterns and metabolic outputs jointly influence disease susceptibility and progression in AR and FC. However, the manner in which SCFA-producing bacteria are perturbed in AR-FC comorbidity (ARFC), and whether such alterations contribute to immune dysregulation along the gut-nasal axis, remains to be elucidated.

Recent evidence underscores the gut-nasal axis as a critical mediator in the pathophysiology of upper airway allergic diseases.[14] However, the microbial and metabolic interplay underlying ARFC comorbidity remains unexplored, particularly in preschoolers. This study hypothesized that ARFC aggravates microbial dysbiosis through the depletion of butyrate-producing taxa, thereby disrupting gut-nasal immune homeostasis. Supporting this notion, a recent multi-omics study revealed that gut microbial alterations in children with AR-FC comorbidity enhance aromatic amino acid metabolism, further implicating microbial metabolic dysfunction in disease progression.[17] This study sought to address two fundamental questions: (1) Do children with comorbid allergic rhinitis and functional constipation (ARFC) exhibit distinct taxonomic and functional gut microbiota profiles? And (2) How are these microbial alterations associated with metabolic dysfunction?

To our knowledge, this is the first study to provide an integrated taxonomic and functional characterization of GM profiles in preschool children with ARFC, aimed at elucidating shared mechanisms underlying the gut-nasal axis. By systematically comparing GM across ARFC, AR, and healthy controls (HC), this work bridges a critical knowledge gap and lays the groundwork for understanding the microbial basis of this challenging comorbidity.[9,18,19] Nevertheless, the lack of a constipation-only comparative group constrains definitive attribution of dysbiosis to specific disease components, highlighting an essential direction for future studies.

Materials and Methods

Materials

Participants

A total of 63 preschool children (aged 3–6 years) were consecutively recruited from the Pediatric Allergy and Gastroenterology Clinics of Longgang District Maternity & Child Healthcare Hospital (Shenzhen, China) between January and December 2023. The inclusion criteria were as follows: 1) ARFC group: Children meeting both (i) the Chinese Guidelines for Allergic Rhinitis (2022)[2] and (ii) the Rome IV FC criteria;[20] 2) AR group: Diagnosed with AR without gastrointestinal symptoms (Rome IV questionnaire score <2); 3) Healthy controls (HC): Children with no history of allergic or chronic gastrointestinal diseases, as confirmed by pediatric examination.

Exclusion criteria (applied uniformly to all groups) were as follows: 1) Antibiotic/probiotic use within 1 month prior to sample collection; 2) Presence of organic constipation (eg, Hirschsprung disease) confirmed by a pediatric gastroenterologist; 3) Acute infections (fever >38°C or antibiotic therapy within the previous 2 weeks); 4) Chronic systemic diseases (eg, autoimmune disorders, cystic fibrosis).

Baseline and Clinical Characteristics

Demographic data (age and sex), anthropometric measurements (height, weight, and BMI z-score), dietary patterns (including breastfeeding history, and current dietary intake assessed by brief food frequency questionnaire or recall),

relevant environmental exposures (eg, pet ownership, household smoking), detailed clinical symptom scores for AR (eg, Total Nasal Symptom Score - TNSS, visual analogue scale - VAS) and FC (eg, Rome IV symptom frequency/severity sub-scores, Bristol Stool Scale), medication history (beyond exclusion criteria, eg, recent antihistamine/laxative use), and family history of atopy/functional GI disorders were systematically collected for all participants at enrollment. Dietary fiber intake (g/day) and recent medication use were included as covariates in DESeq2 models for downstream analyses.

Allergic Rhinitis (AR) Severity Assessment:

1. Total Nasal Symptom Score (TNSS): Sum of individual symptom scores (0–3 per symptom) for four rhinorrhea, sneezing, nasal itching, and nasal congestion, yielding a total range of 0–12.[2]
2. Visual Analogue Scale (VAS): Self-reported overall nasal symptom severity marked on a 10-cm horizontal line (0 = no discomfort; 10 = severe discomfort).[2]

Functional Constipation (FC) Severity Assessment:

1. Rome IV Criteria: Diagnosis required the presence of ≥2 of the following symptoms for ≥1 month: ① ≤2 defecations per week; ② ≥1 episode of fecal incontinence per week; ③ retentive posturing; ④ painful defecation; ⑤ large fecal mass in rectum; ⑥ large-diameter stools obstructing toilet.[20]
2. Bristol Stool Scale (BSS): Stool consistency classified from Type 1 (hard lumps) to Type 7 (watery). Constipation was defined as BSS Types 1–2.[20]

Correlation Analysis Parameters:

1. Allergy severity: Composite score calculated as (TNSS + VAS)/2.
2. Gastrointestinal symptoms: Rome IV severity score (range 0–6) and BSS classification (Types 1–2 = 1, Types 3–7 = 0).
3. Respiratory manifestations: Individual symptom scores for rhinorrhea, sneezing, and nasal pruritus (each rated 0–3).
4. Th2-mediated inflammation: Serum total IgE levels (kU/L) extracted from clinical records.

Medication Use:

1. Antihistamines: Systemic H1-receptor antagonists (eg, loratadine) administered within 7 days prior to sampling.
2. Laxatives: Osmotic agents (eg, polyethylene glycol) administered within 72 hours before sampling.

Sample Size Calculation

Based on previously reported effect sizes from pediatric microbiome studies (eg, Zhang et al,[9] which documented a Cohen's d of 0.8 for Shannon index differences between AR and HC groups), an a priori power analysis was performed using G*Power 3.1 software (*F*-tests, ANOVA: Fixed effects, omnibus, one-way). The parameters were set at a significance level (α) of 0.05 and a statistical power (1−β) of 0.8. The analysis indicated that a minimum total of 66 participants (ie, 22 per group) would be required to detect significant differences. The achieved sample sizes in the present study (ARFC=32, AR=22, HC=21) met or exceeded this requirement for the primary group comparisons (ARFC vs HC, and AR vs HC), thereby providing sufficient statistical power (>80%) to detect large effect sizes ($|d| > 0.8$). Nonetheless, it is acknowledged that the sample remains relatively modest for detecting subtler microbial associations or for robust post hoc comparisons among all three groups, particularly after stringent multiple testing corrections.

Sequencing and Bioinformatics

Fecal genomic DNA was extracted using the PowerSoil® Kit (MoBio, USA). The V3–V4 hypervariable regions of 16S rRNA were amplified using primers *341F* (5′-CCTACGGGNGGCWGCAG-3′) and *806R* (5′-GGACTACHVGGGTATCTAAT-3′), followed by paired-end sequencing (2 × 250 bp) on the Illumina NovaSeq 6000 platform. After quality control (Q30 >90%), a total of 3,125,439 high-quality reads (mean 49,610 reads/sample) were

retained. Amplicon sequence variants (ASVs) were inferred using the DADA2 algorithm (implemented in QIIME2 v2023.2) at 97% similarity, and taxonomic annotation was performed against the SILVA v138.1 reference database. Functional prediction was conducted using PICRUSt2 with reference to the KEGG database.

Sequence Processing Workflow:

1. Demultiplexing: Raw paired-end reads were demultiplexed using the demux plugin in QIIME2 (v2023.2).
2. Quality Trimming: Adapter sequences and low-quality bases (Q-score <20) were trimmed using cutadapt.
3. Denoising and Chimera removal: DADA2 was applied for denoising, chimera filtering, and ASV inference (error model: learnErrors; truncation length: forward=240 bp, reverse=200 bp).
4. Taxonomic Classification: ASVs were taxonomically assigned using the q2-feature-classifier plugin with SILVA v138.1 database (99% identity threshold).

Contamination Control: Sequences affiliated with non-bacterial sources (eg, chloroplast, mitochondrial) were excluded. Samples yielding fewer than 10,000 reads were removed from downstream analyses.

Functional Prediction: Functional pathways were inferred using PICRUSt2 (v2.5.0) with default parameters. KEGG Ortholog (KO) abundances were normalized to copies per million (CPM), and pathways with prevalence <10% across all samples were excluded. Functional predictions derived from 16S rRNA data were interpreted cautiously, recognizing the limited resolution relative to whole-metagenome sequencing.

Statistical Analysis

All statistical analyses were performed in R (v4.3.1) unless otherwise specified.

Diversity Analysis:

1. α-diversity: The Shannon diversity index was compared among groups using the Kruskal–Wallis test, followed by Dunn's post hoc test (FDR-adjusted);
2. β-diversity: Bray–Curtis dissimilarity matrices were generated and visualized using principal coordinates analysis (PCoA). Statistical significance of between-group differences was assessed by PERMANOVA (999 permutations; vegan::adonis2). PCoA confidence ellipses (95%) were calculated using the stat_ellipse function in R (ggplot2 v3.5.0). Effect sizes for β-diversity differences were reported as R^2 from PERMANOVA.

Differential Abundance Analyses:

1. Initial group-wise taxonomic differences were assessed using the Kruskal–Wallis test (FDR-adjusted $P<0.05$). For taxa showing significant global differences, pairwise comparisons (ARFC vs HC, ARFC vs AR, AR vs HC) were conducted using DESeq2 (negative binomial mo FDR-adjusted $P < 0.05$). The DESeq2 model included "group" as the primary fixed factor and adjusted for dietary fiber intake and recent medication use (antihistamines, laxatives) as covariates, where applicable. Relative abundance data (%) were used directly for non-parametric tests. For DESeq2, a variance-stabilizing transformation (VST) was applied to raw ASV counts to normalize variance prior to model fitting.
2. Associations between microbial genera and clinical parameters (eg, TNSS, VAS, Bristol Stool Scale, total IgE) were assessed using Spearman's rank correlation coefficient (ρ), with *P*-values adjusted by Benjamini–Hochberg FDR correction.

Multiple testing and Power Considerations: All *P*-values from diversity, taxonomic, and functional analyses were corrected using the Benjamini–Hochberg FDR method, with statistical significance defined as FDR-adjusted $P < 0.05$. Post hoc power analysis confirmed >80% statistical power to detect large effect sizes ($|d| > 0.8$) for primary group comparisons (ARFC vs HC, AR vs HC), consistent with the a priori calculation described in Sample Size Calculation.

Results

Participant Characteristics

Baseline demographic and clinical characteristics of participants are summarized in Table 1. All children were within the narrow age range of 3–6 years and exhibited BMI z-scores within the normal range (−1 to +1 SD). There were no significant group differences in age, sex distribution, BMI z-score, breastfeeding history, or pet exposure (all $P > 0.05$). As expected, both the ARFC and AR groups demonstrated significantly higher allergic rhinitis symptom scores (TNSS, VAS) compared to HC ($P < 0.001$). The ARFC group also exhibited significantly more severe constipation symptoms than both the AR and HC groups ($P < 0.001$). Group-wise differences in recent medication use are detailed in Table 1.

GM Diversity and Composition

The α-diversity of the GM, quantified using the Shannon index, was significantly higher in the ARFC group (mean=5.2 ± 0.3) compared with the HC (mean=4.5 ± 0.4) (FDR-adjusted P =0.014) (Figure 1A). No significant difference was detected between the ARFC and AR groups ($P = 0.77$) or between the AR and HC (FDR-adjusted $P = 0.063$). β-diversity analysis based on Bray-Curtis dissimilarity showed significant overall group differences (PERMANOVA: R^2=0.12, P=0.001). Pairwise comparisons indicated distinct clustering of ARFC relative to both HC (R^2=0.15, P=0.001) and AR (R^2=0.08, P=0.012), but no significant separation was detected between the AR and HC (R^2=0.04, P=0.102) (Supplementary Table S1). Visualization indicated partial clustering of ARFC (green) with limited overlap with HC (8.3%), whereas AR (pink) and HC (blue) groups showed substantial overlap (42.1%), consistent with the non-significant PERMANOVA result (Figure 1B).

Proteobacteria Enrichment in Disease Groups and Bacteroidetes Depletion in ARFC

At the phylum level, both ARFC and AR groups showed a marked enrichment of *Proteobacteria* compared to HC (ARFC: FDR-adjusted P = 0.001; AR: FDR-adjusted P = 0.005) (Figures 2 and 3, Table 2). In contrast, a significant depletion of *Bacteroidetes* was observed exclusively in ARFC children compared with HC (FDR-adjusted $P = 0.049$ vs HC) (Figure 2). No other significant differences occurred between ARFC and AR groups (Figure 4).

Pathogen Enrichment and Depletion of Butyrate Producers in ARFC

After FDR correction, the ARFC group exhibited significantly higher abundances of *Bifidobacterium, Phascolarctobacterium, Veillonella, Escherichia/Shigella, Klebsiella*, and *Streptococcus* compared with the HC group

Table 1 Demographic and Clinical Characteristics of Study Participants

Characteristic	ARFC (n=32)	AR (n=22)	HC (n=21)	*P* value
Age (years)	4.8 ± 0.9	4.6 ± 1.0	4.7 ± 0.8	0.712
Sex (Male/Female)	18/14	12/10	11/10	0.982
BMI z-score	0.32 ± 0.81	0.28 ± 0.75	0.35 ± 0.79	0.863
Breastfeeding >6mo (%)	65.6	68.2	71.4	0.802
Pet exposure (%)	28.1	31.8	33.3	0.877
TNSS score	8.2 ± 2.1*†	7.9 ± 1.8*	1.1 ± 0.9	<0.001
VAS score	6.5 ± 1.7*†	6.3 ± 1.5*	0.8 ± 0.6	<0.001
Rome IV severity	3.4 ± 0.8*†	0.4 ± 0.5	0.3 ± 0.4	<0.001
Bristol stool scale	2.1 ± 0.6*†	3.8 ± 0.7	4.0 ± 0.5	<0.001
Antihistamine use (%)	78.1*†	81.8*	0	<0.001
Laxative use (%)	18.8*†	0	0	<0.001

Notes: Data presented as mean ± SD or %. *P<0.05 vs HC; †P<0.05 vs AR (Kruskal–Wallis/Chi-square tests).

https://doi.org/10.2147/JAA.S561982

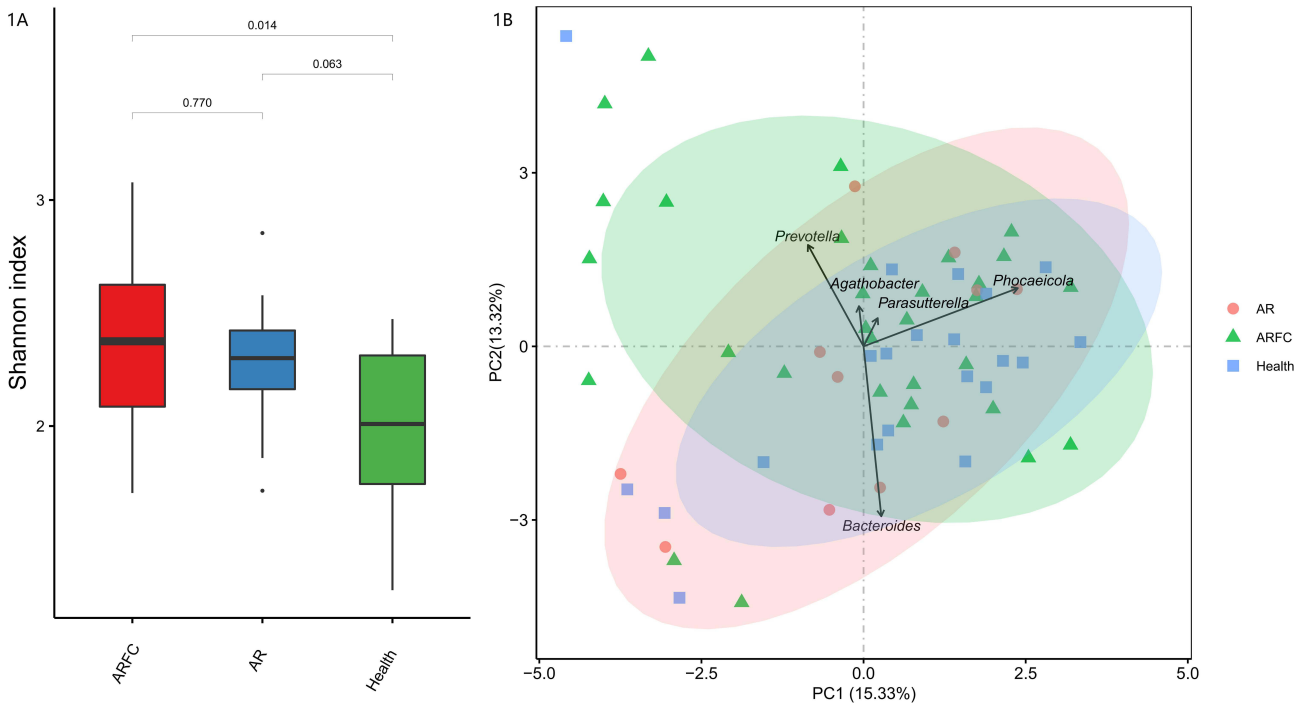

Figure 1 GM diversity and compositional differences across groups. (**A**) α diversity (Shannon index) comparison between the ARFC group (mean=5.2 ± 0.3) vs HC group (mean=4.5 ± 0.4) (*P* = 0.014). (**B**) Principal component analysis (PCA) based on Bray–Curtis distances (PERMANOVA, *P* = 0.001) revealed distinct clustering. ARFC clusters distinctly (green), while AR (pink) and healthy (blue) groups overlap partially. Ellipses denote 95% confidence intervals. AR-HC overlap: 42.1%; ARFC-HC overlap: 8.3%. Dominant genera driving separation include *Prevotella, Bacteroides*, and *Phocaeicola*.

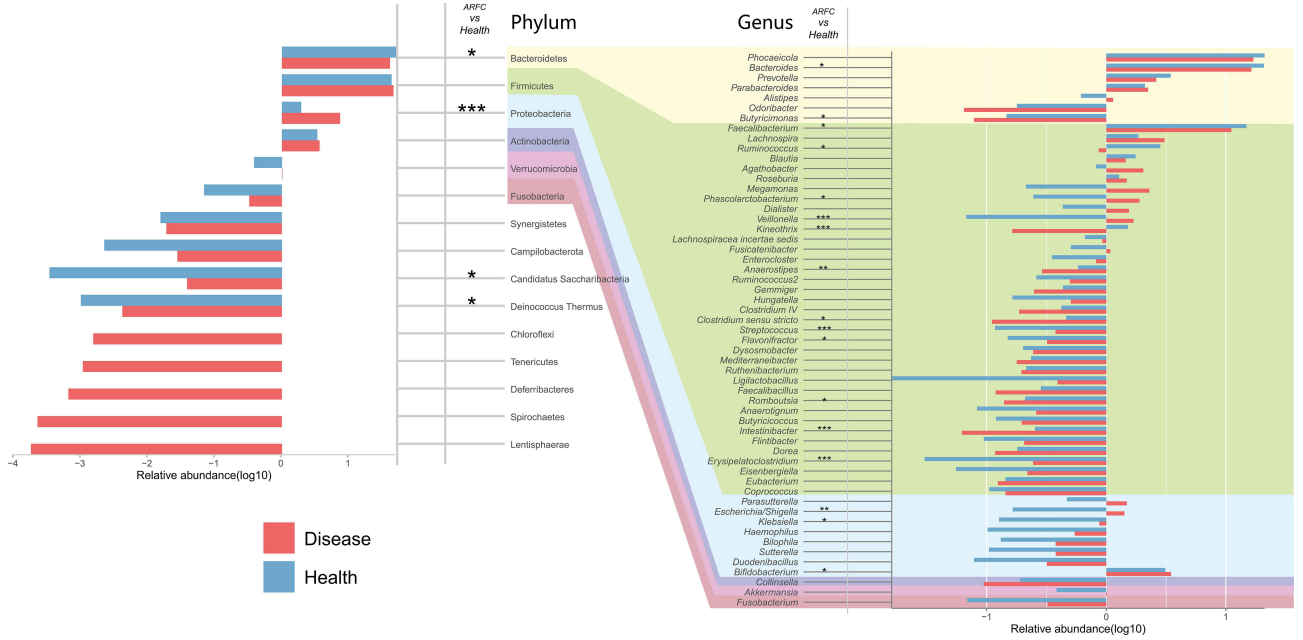

Figure 2 Differential abundance of gut microbiota at phylum and genus levels between the ARFC and HC groups. Wilcoxon rank-sum test was applied to compare the relative abundances of bacterial phyla and genera between the ARFC group (n = 32) and the HC group (n = 21). Statistically significant differences after false discovery rate (FDR) correction are denoted by asterisks: *FDR < 0.05, **FDR < 0.01, ***FDR < 0.001. A greater number of asterisks indicates a higher level of statistical significance.

Table 2 Analysis of the Three Groups of Children at the Phylum Levels (Top 5)

Top 5 Phyla	ARFC Group		AR Group		Healthy Group	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
Bacteroidetes	40.06	19.22	44.44	11.80	50.72[a]	12.21
Firmicutes	46.47	17.41	45.40	12.65	43.38	13.25
Proteobacteria	7.92	9.26	5.94	4.97	1.94[b,c]	1.33
Actinobacteria	4.39	6.32	1.48	1.78	3.38	8.85
Verrucomicrobia	0.85	1.99	1.50	4.27	0.38	1.02

Notes: [a]Comparison of *Bacteroidetes* abundance between ARFC and healthy groups (FDR-adjusted $P = 0.049$); [b]Comparison of *Proteobacteria* abundance between ARFC and healthy groups (FDR-adjusted $P = 0.005$); [c]Comparison of *Proteobacteria* abundance between AR and healthy groups (FDR-adjusted $P = 0.001$).

Table 3 Differentially Abundant Genus Between ARFC and HC Groups

Top 11 Dominant Genus	ARFC Group		HC Group		FDR-Adjusted *P*
	Mean (%)	SD (%)	Mean (%)	SD (%)	
Bacteroides	14.28	13.32	20.76	12.87	0.039
Faecalibacterium	10.5	9.5	14.84	8.00	0.039
Bifidobacterium	4.21	6.31	3.12	8.81	0.02
Ruminococcus	0.82	1.51	2.83	4.23	0.019
Phascolarctobacterium	1.79	2.88	0.25	0.50	0.045
Escherichia/Shigella	1.57	2.86	0.17	0.27	0.025
Veillonella	1.59	3.34	0.07	0.14	0.000
Klebsiella	1.13	3.03	0.13	0.43	0.035
Kineothrix	0.18	0.26	1.52	3.03	0.003
Anaerostipes	0.35	0.55	0.58	0.42	0.010
Streptococcus	0.38	0.56	0.12	0.21	0.002

Notes: Only genera showing statistically significant differences after Benjamini-Hochberg false discovery rate (FDR) correction (FDR-adjusted $P < 0.05$) are listed. Data are presented as mean relative abundance (%) ± standard deviation (SD).

(FDR-adjusted $P < 0.05$), whereas *Bacteroides, Faecalibacterium, Ruminococcus, Kineothrix*, and *Anaerostipes* were markedly depleted (Figure 2 and Table 3).

The AR group displayed similar enrichment patterns for *Veillonella, Escherichia/Shigella*, and *Streptococcus* but uniquely showed increased abundances of *Enterocloster* and *Haemophilus* (Figure 3 and Table 4).

When compared with the AR group, ARFC children exhibited a significantly higher relative abundance of *Bifidobacterium* (4.21% vs 1.80%, FDR-adjusted $P = 0.018$) and a lower abundance of *Veillonella* (1.59% vs 2.00%, FDR-adjusted $P = 0.042$) (Figure 4).

AR Group as an Intermediate Phenotype

The AR group demonstrated transitional GM features between HC and ARFC. Similar to ARFC, AR children showed enrichment of *Proteobacteria* (5.94% vs HC 1.94%, FDR-adjusted $P = 0.005$). However, AR uniquely exhibited increased relative abundances of *Enterocloster* (1.00% vs HC 0.35%, FDR-adjusted $P = 0.014$) and *Haemophilus* (0.60% vs HC 0.10%, FDR-adjusted $P = 0.024$) (Table 4). The relative abundance of *Bifidobacterium* in ARFC (4.21%) was significantly higher than in AR (1.80%, FDR-adjusted $P = 0.018$) and HC (3.12%, FDR-adjusted $P = 0.21$), indicating comorbidity-specific enrichment in ARFC children.

Functional Pathway Analysis

Functional prediction based on KEGG annotations revealed that the ARFC group presented downregulated pathways related to carbohydrate metabolism, lipid metabolism, the immune system, and the endocrine/nervous system (FDR-

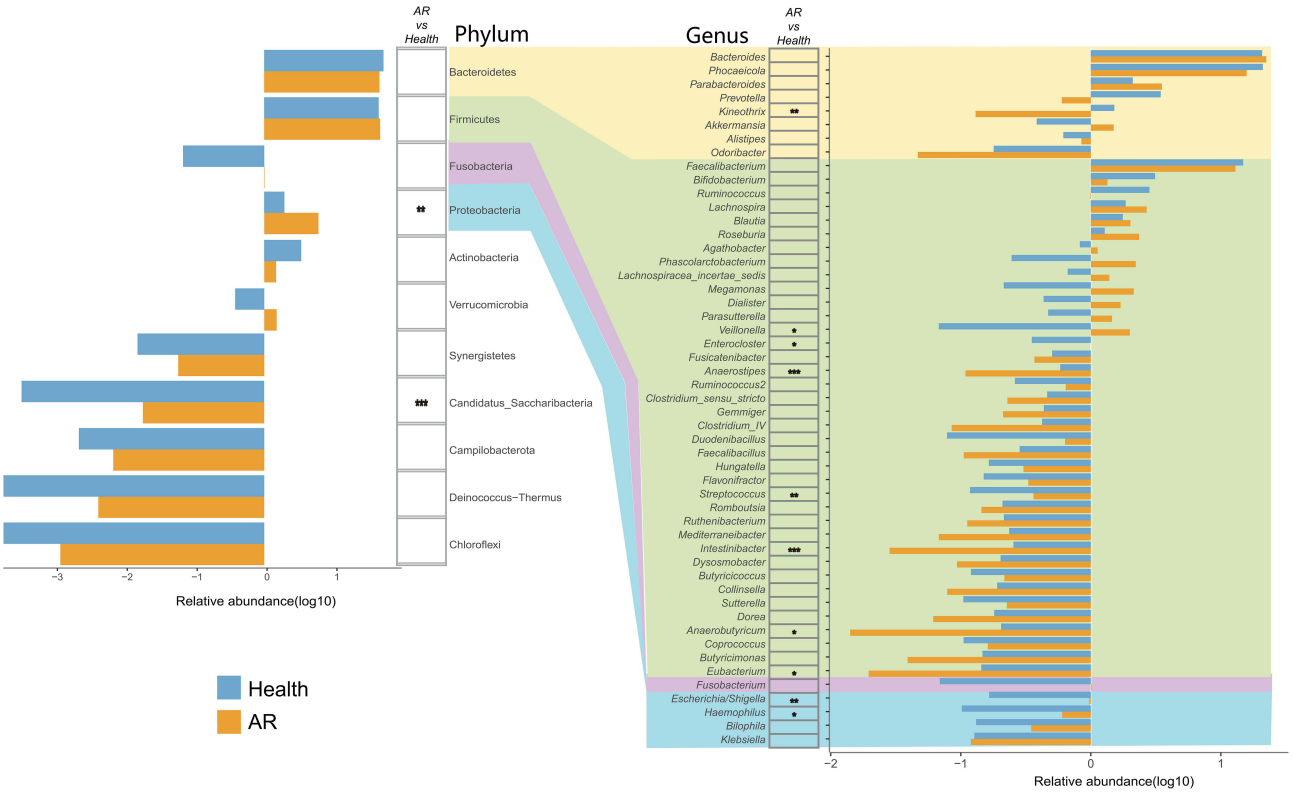

Figure 3 Differential abundance of gut microbiota at phylum and genus levels between the AR and HC groups. The Wilcoxon rank-sum test was applied to compare the relative abundances of bacterial phyla and genera between the AR group (n = 22) and the HC group (n = 21). Statistically significant differences after false discovery rate (FDR) correction are denoted by asterisks: *FDR < 0.05, **FDR < 0.01, ***FDR < 0.001. A greater number of asterisks indicates a higher level of statistical significance.

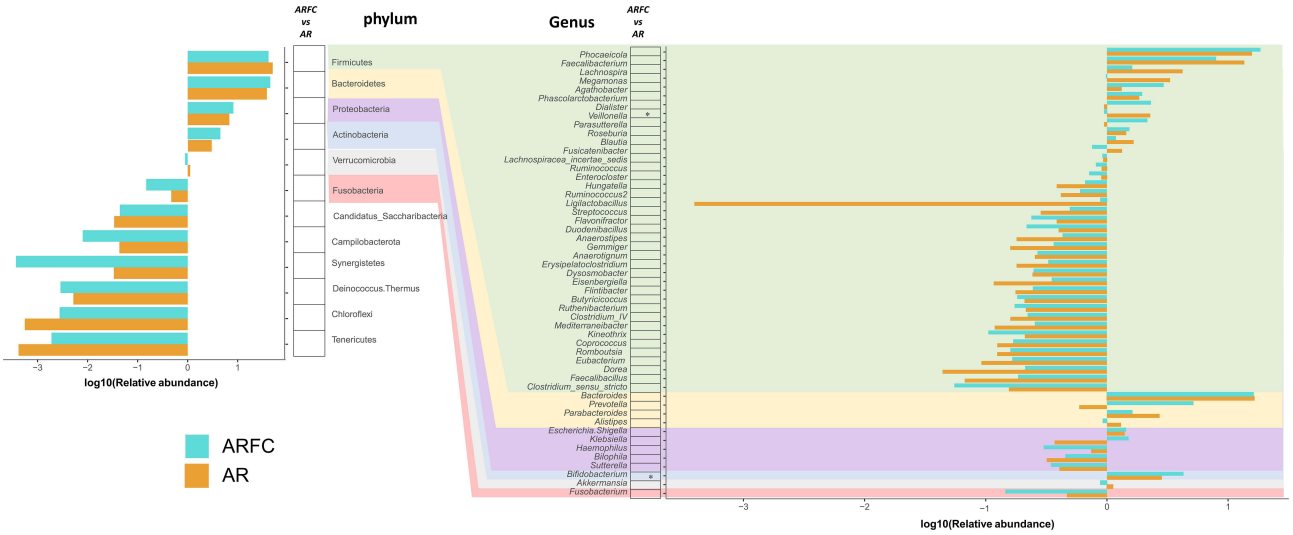

Figure 4 Differential abundance of gut microbiota at phylum and genus levels between the ARFC and AR groups. The Wilcoxon rank-sum test was applied to compare the relative abundances of bacterial phyla and genera between the ARFC group (n = 32) and the AR group (n = 22). Statistically significant differences after false discovery rate (FDR) correction are denoted by asterisks: *FDR < 0.05. A greater number of asterisks indicates a higher level of statistical significance.

adjusted $P < 0.05$). Conversely, pathways related to xenobiotic biodegradation and genetic information processing were upregulated (Figure 5A). The AR group showed a comparable reduction in endocrine-and nervous system-associated pathways but demonstrated increased activity in signal transduction pathways (Figure 5B). However, no significant

Table 4 Differentially Abundant Genus Between AR and HC Groups

Top 10 Dominant Genus	AR Group		HC Group		FDR-Adjusted *P*
	Mean (%)	SD (%)	Mean (%)	SD (%)	
Veillonella	2.00	2.94	0.07	0.14	0.012
Enterocloster	1.00	0.98	0.35	0.47	0.014
Escherichia/Shigella	0.97	1.56	0.17	0.27	0.003
Haemophilus	0.60	1.07	0.10	0.16	0.024
Streptococcus	0.36	0.35	0.12	0.21	0.009
Erysipelatoclostridium	0.20	0.24	0.03	0.06	0.001
Megasphaera	0.19	0.54	0.02	0.06	0.011
Negativibacillus	0.16	0.37	0.01	0.03	0.037
Kineothrix	0.13	0.206	1.52	3.03	0.008
Anaerostipes	0.11	0.16	0.58	0.42	0.001

Notes: Only genera showing statistically significant differences after Benjamini-Hochberg false discovery rate (FDR) correction (FDR-adjusted $P < 0.05$) are listed. Data are presented as mean relative abundance (%) ± standard deviation (SD).

differences in predicted functional pathways were observed between the ARFC and AR groups (Supplementary Table S2).

GM-Clinical Phenotype Correlations

Within the ARFC cohort (n=32), Spearman correlation analysis was performed to explore associations between bacterial genera (≥0.1% relative abundance) and clinical parameters (see Methods' Baseline and Clinical Characteristics):

1. Gastrointestinal Symptoms: ① *Haemophilus* abundance correlated positively with constipation severity (Rome IV score: $\rho = 0.52$, $P = 0.008$) and stool consistency (BSS: $\rho = 0.48$, $P = 0.012$); ② *Dysosmobacter* ($\rho = -0.45$, $P = 0.021$) and *Flintibacter* ($\rho = -0.43$, $P = 0.028$) were negatively correlated with constipation severity (Rome IV score).
2. Respiratory Symptoms: ① *Haemophilus* also correlated positively with rhinorrhea severity ($\rho = 0.56$, $P = 0.003$); ② *Streptococcus* ($\rho = 0.49$, $P = 0.010$) *and Anaerotignum* ($\rho = 0.46$, $P = 0.018$) correlated positively with sneezing and rhinorrhea, respectively. ③ *Megamonas* ($\rho = -0.44$, $P = 0.025$) and *Butyricimonas* ($\rho = -0.41$, $P = 0.038$) inversely correlated with rhinorrhea severity.
3. Th2 Inflammation: *Lachnospira* abundance correlated negatively with serum total IgE ($\rho = -0.47$, $P = 0.015$), whereas *Phocaeicola* showed a positive correlation ($\rho = 0.42$, $P = 0.032$).
4. Recurrent Cough Frequency: *Lachnospiraceae incertae sedis* ($\rho = -0.50$, $P = 0.009$), *Ligilactobacillus* ($\rho = -0.48$, $P = 0.011$), and *Erysipelatoclostridium* ($\rho = -0.46$, $P = 0.017$) abundances were inversely correlated with cough frequency.

Collectively, these findings reveal that specific GM taxa correlate with distinct clinical manifestations in ARFC. *Haemophilus* exhibited dual correlations, linking its enrichment to both gastrointestinal (constipation severity) and respiratory (rhinorrhea severity) symptoms (Figure 6), suggesting its potential as a comorbidity biomarker. Conversely, beneficial taxa, such as *Megamonas* and *Butyricimonas*, showed inverse associations with rhinorrhea severity, indicating protective effects against allergic inflammation.

Discussion

GM Dysbiosis in AR and FC

Our findings corroborate previous evidence linking GM dysbiosis with both allergic and gastrointestinal disorders. The elevated *Proteobacteria* and reduced *Bacteroidetes* in ARFC children (Table 1) mirror alterations reported in constipation models, where such shifts impair intestinal motility and barrier integrity.[18] The increased *Veillonella* and *Streptococcus*

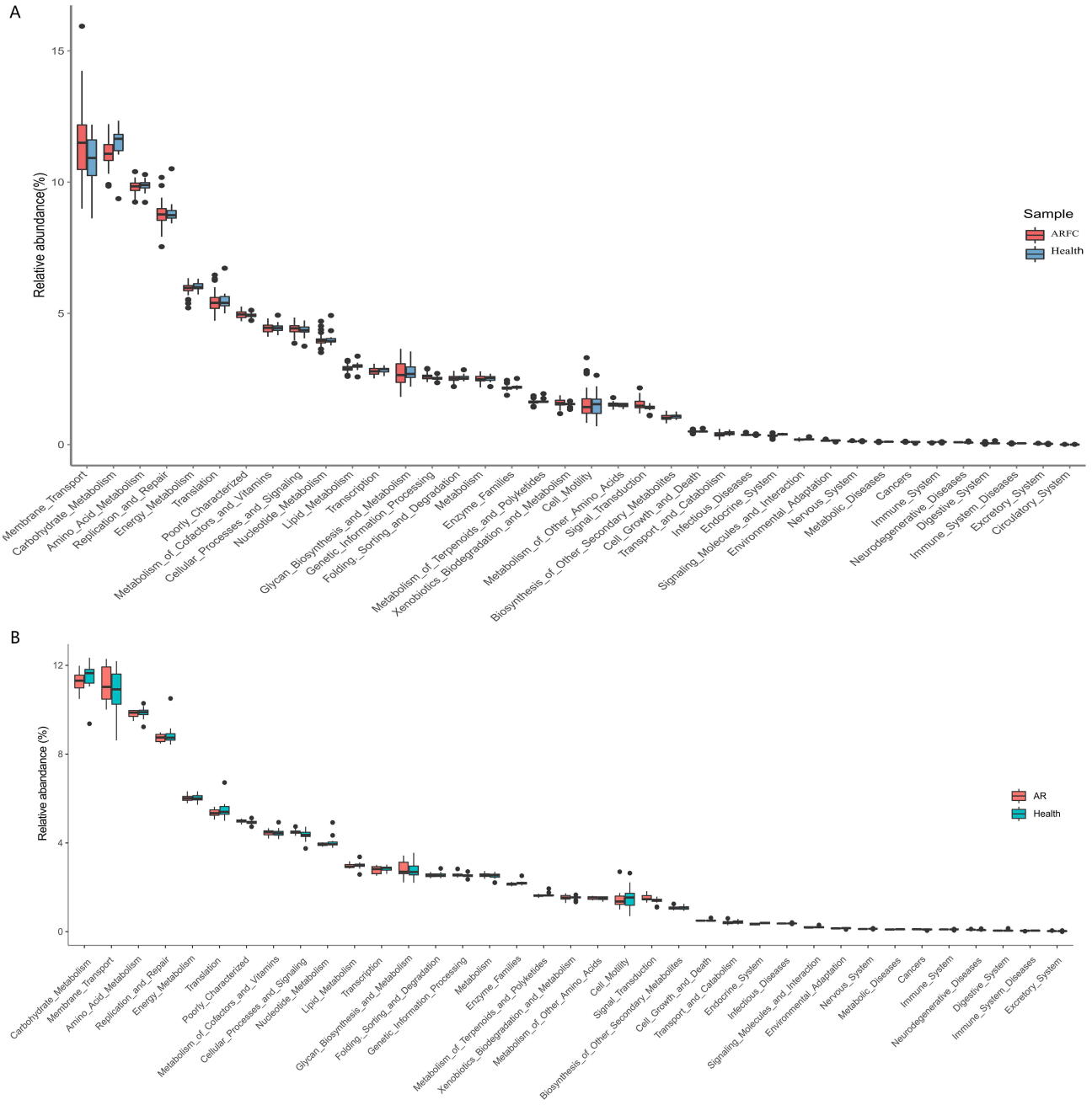

Figure 5 Comparison of GM function among the HC, AR, and ARFC groups. (**A**) *16S rRNA* sequencing data were utilized to assess variations in GM functions between the ARFC and HC groups of children through functional analysis conducted via the KEGG database. (**B**) *16S rRNA* sequencing data were utilized to assess variations in GM functions between the AR and HC groups of children through functional analysis conducted via the KEGG database.

abundances in AR and ARFC groups (Table 2 and Table 3) further support their reported roles in promoting Th2-mediated inflammation and IgE production.[13,21]

The enrichment of *Klebsiella* in children with ARFC may exacerbate inflammation through lipopolysaccharide (LPS)-mediated activation of the NF-κB pathway, leading to the release of pro-inflammatory cytokines such as TNF-α and IL-6.[9] Conversely, the depletion of key butyrate-producing genera, including *Faecalibacterium* and *Ruminococcus*, likely contributes to systemic immune dysregulation and delayed colonic transit. Short-chain fatty acids (SCFAs), particularly butyrate, are crucial for promoting regulatory T-cell (Treg) differentiation and maintaining gut motility.[16,22] Consequently, impaired SCFA signaling via receptors like GPR43 may disrupt the Treg/Th17 balance in both the gut

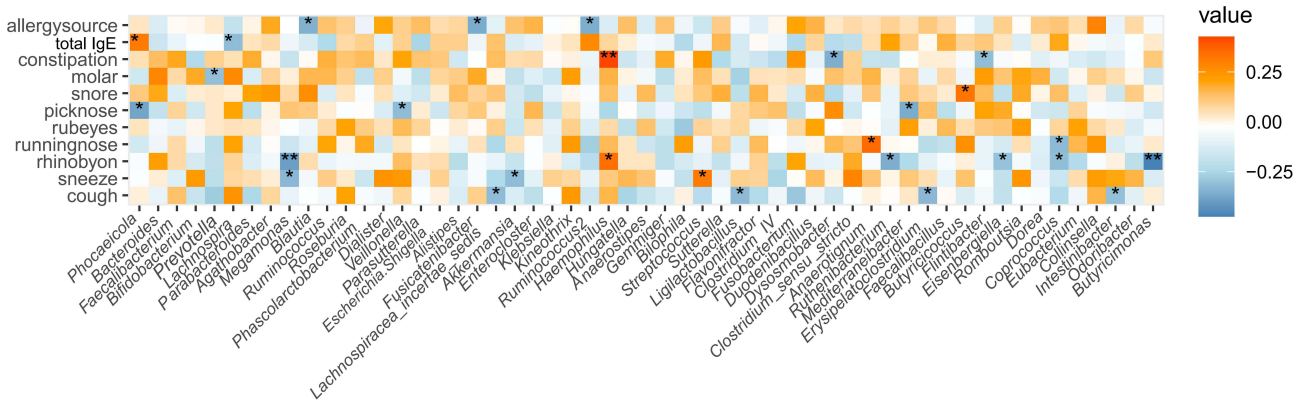

Figure 6 Spearman correlation analysis between GM genera (relative abundance ≥ 0.1%) and clinical symptom severity within the ARFC group (n = 32). A correlation analysis was performed with eleven clinical phenotypes and genera with a relative abundance of ≥ 0.1%. The results are shown above, where significance is expressed denoted as *FDR <0.05 and **FDR <0.01.

and nasal mucosa.[23] These observations collectively reinforce the concept of gut-airway crosstalk in comorbid conditions. Our data are consistent with the work of Kaczynska et al,[18] who demonstrated that FC-induced GM dysbiosis can amplify airway inflammation via a bidirectional mechanism, a process that may be intensified in ARFC comorbidity. Similarly, the *Proteobacteria* overgrowth observed here aligns with findings by Acevedo-Román et al[15] linking this phylum to systemic inflammation in allergic and functional comorbidities.

A notable and paradoxical finding was the significant elevation of *Bifidobacterium* in ARFC children, which contrasts with its typical depletion in isolated functional constipation.[24] This suggests a distinct, comorbidity-specific microbial adaptation. While FC alone is often characterized by *Bifidobacterium* loss, its enrichment in ARFC may represent a compensatory immunomodulatory response to the combined Th2/Th17 inflammatory milieu—an observation previously unreported in this context. Specific species such as *B. longum* and *B. infantis* are known to promote Treg differentiation and IL-10 production, thereby mitigating Th2-driven responses.[7,8] Thus, the systemic immune activation in ARFC may trigger a homeostatic increase in *Bifidobacterium*, potentially mediated by IgA upregulation or alterations in bile acid metabolism.[25] The emerging literature further underscores the strain-dependent role of *Bifidobacterium* in regulating the gut-lung axis and its therapeutic potential for comorbid allergic-gastrointestinal disorders.[26] However, the absence of an FC-only control group limits direct comparison of *Bifidobacterium* abundance between isolated FC and ARFC. Therefore, while the compensatory enrichment hypothesis is plausible, it requires validation in future cohorts that include an FC control group to clarify whether this effect is driven by the AR component or the comorbidity interaction itself.

Functional Implications of GM Changes

Functional predictions revealed marked alterations in KEGG pathways between ARFC and HC groups (Figure 5A). Downregulation of carbohydrate metabolism (ko00010) and lipid biosynthesis (ko00061) may reflect impaired energy extraction from dietary substrates, consistent with the depletion of SCFA-producing taxa, notably *Faecalibacterium*. In contrast, upregulation of xenobiotic biodegradation pathways (ko00980) likely represents microbial adaptation to altered luminal environments, although the precise mechanistic drivers remain to be elucidated.

These predictions are based on PICRUSt2, which infers function from phylogenetic marker genes and lacks resolution for strain-level activities or direct metabolite quantification.[27] Consequently, the observed functional shifts should be interpreted as hypothesis-generating and warrant validation via shotgun metagenomics and targeted metabolomics, including SCFA quantification. Such integrative approaches are essential to confirm[27] the functional consequences of GM dysbiosis in ARFC. Future studies integrating metabolomic profiling (eg, SCFAs quantification) are critical to validate these pathway predictions.[28] Supporting this perspective, recent multi-omics analyses of children with AR–FC comorbidity demonstrated gut microbial contributions to altered aromatic amino acid metabolism, highlighting

the importance of integrated functional profiling for deciphering microbial metabolic influences on disease pathophysiology.[17]

Clinical Relevance and Clinical Translation

The observed correlation between gut-enriched *Haemophilus* and rhinorrhea severity extends beyond the conventional paradigm of localized nasopharyngeal dysbiosis.[29,30] Its association with both gastrointestinal (constipation severity) and respiratory (rhinorrhea severity) manifestations provides novel evidence for a functional gut–nasal axis in ARFC comorbidity, suggesting that gut-resident *Haemophilus* may exert systemic effects influencing distal mucosal sites. The inverse association of *Megamonas/Butyricimonas* with allergy severity highlights the therapeutic relevance of butyrate-producing taxa in ARFC comorbidity. Although this study primarily addresses the gut-nasal axis, the gut-lung axis in lower airway diseases (eg, asthma) remains insufficiently explored and warrants independent investigation. Notably, the negative correlations of *Megamonas* and *Butyricimonas* with rhinorrhea severity ($\rho = -0.44$, $P=0.025$; $\rho = -0.41$, $P = 0.038$) along with the depletion of key butyrate producers like *Faecalibacterium* (10.5% vs HC 14.84%, FDR-adjusted $P = 0.039$) underscore butyrate metabolism as a promising therapeutic target. Restorating *Faecalibacterium* abundance and enhancing butyrate signaling offer a dual benefit by attenuating Th2/Th17-driven inflammation through Treg differentiation induction[16,23] and by enhancing gut motility.[16,19] Such an integrated approach could simultaneously alleviate allergic and gastrointestinal symptoms, addressing the shared pathophysiology mechanisms underlying ARFC comorbidity.

Mechanistically, these effects are likely mediated via the SCFAs–GPR43 axis, which promotes Treg differentiation,[16,23] suppresses Th2/Th17 responses,[23] and supports intestinal transit.[16,19] Dietary strategies aimed at augmenting butyrate production represent a rational translational approach; clinical evidence indicates that such interventions can improve gut motility and reduce IgE levels in allergic pediatric populations.[30,31] Furthermore, supplementation with *Faecalibacterium* has been shown to restore mucosal integrity and Treg/Th17 balance in children with allergic disorders.[32] These findings converge with recent literature advocating personalized probiotic and prebiotic interventions to modulate the gut–airway axis, reinforcing their potential applicability in comorbid allergic and gastrointestinal conditions.[33]

Limitations and Future Directions

This study has several important limitations. First, the absence of a constipation-only group precludes definitive attribution of observed gut microbiota (GM) alterations to the AR component, the FC component, or their interactive effect in comorbidity. For example, Proteobacteria enrichment in ARFC aligns with prior observations in isolated constipation,[5,19] suggesting it may be primarily driven by the FC component. Conversely, the paradoxical elevation of *Bifidobacterium*, which contrasts with its typical depletion in isolated constipation,[24] may represent a compensatory immunomodulatory response specific to AR or to the comorbid state. Inclusion of an FC-only group in future studies is essential to disentangle these effects.

Second, functional insights are derived from 16S rRNA gene sequencing and predictive metagenomics (PICRUSt2). While these approaches generate valuable functional hypotheses, they infer potential rather than directly measuring gene content, transcription, or metabolite production, and they lack species- or strain-level resolution.[27] Accordingly, predicted pathway alterations—such as impaired carbohydrate metabolism and upregulated xenobiotic degradation—require validation through shotgun metagenomics, metatranscriptomics, and targeted metabolomic analyses (eg, SCFA quantification).[28]

Third, although the sample size was sufficient to detect large effect sizes for primary comparisons (ARFC vs HC), it remains modest for identifying more subtle microbial differences between ARFC and AR, particularly after stringent multiple testing corrections. Correlation analyses within the ARFC cohort (n=32) may also have limited power to detect modest associations. Larger, multi-center cohorts are warranted to validate the ARFC-specific microbial signature and to robustly examine GM-clinical relationships.

Fourth, despite comprehensive baseline data collection and statistical adjustment for recent medication use (antihistamines, laxatives) and dietary fiber intake, residual confounding cannot be fully excluded. Medications can significantly influence GM composition, and although this study adjusted for recent use, detailed pharmacokinetic data or

controlled washout periods were not feasible in this clinical cohort. Future studies should incorporate granular tracking of medication history, dosage, and duration, alongside detailed dietary and environmental assessments, to minimize confounding.

To address these limitations, future research should: (i) Include well-characterized FC-only cohorts alongside AR, ARFC, and HC groups in larger, preferably multi-center studies; (ii) employ multi-omics approaches (shotgun metagenomics, metabolomics) to validate functional predictions, identify strain-level variations, and quantify key metabolites like SCFAs,[27,28] as demonstrated in a recent multi-omics study of AR-FC comorbidity;[17] (iii) conduct longitudinal studies to establish temporal relationships between GM shifts and symptom onset/fluctuations; and (iv) integrate detailed, prospective monitoring of medication, diet, and environmental exposures to better control for potential confounders.

Conclusion

This study provides the first characterization of GM dysbiosis in preschool children with comorbid AR and FC. ARFC patients exhibited a distinct GM profile, characterized by Proteobacteria enrichment, Bacteroidetes depletion, and reduced abundance of butyrate-producing taxa, including *Faecalibacterium*. The observed increase in α-diversity in ARFC may reflect pathogenic expansion, such as *Klebsiella*, whereas the paradoxical elevation of *Bifidobacterium*, contrasting with typical findings in isolated FC, suggests a compensatory immunomodulatory response within the context of concurrent Th2/Th17 inflammation. This response may enhance Treg differentiation and IL-10 production, potentially modulating systemic Th2/Th17 immune activity.[7,8] Predictive functional profiling indicated impaired carbohydrate and lipid metabolism alongside upregulated xenobiotic degradation pathways, possibly reflecting constipation-associated luminal alterations. Clinically, gut *Haemophilus* abundance correlated with both gastrointestinal and respiratory symptom severity, supporting its potential utility as a biomarker along the gut–nasal axis in ARFC comorbidity. The absence of a constipation-only group limits definitive attribution of observed GM alterations to AR, FC, or their interaction. Consequently, the compensatory enrichment of *Bifidobacterium* and the functional consequences of butyrate deficiency remain speculative and require validation in future studies incorporating FC controls and multi-omics approaches, including metagenomics and metabolomics. Collectively, our findings delineate a distinct GM signature in ARFC comorbidity and identify microbial and metabolic targets with potential therapeutic relevance. Future research should validate these observations using direct functional assays and larger, well-controlled cohorts, with particular emphasis on elucidating the precise immunomodulatory roles of key taxa, such as *Bifidobacterium*, within the gut–nasal axis.

Data Sharing Statement

The dataset generated in this study can be accessed from the NCBI Sequence Archive (SRA) database via the following direct link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1103935?reviewer=8ia81sf9papv0kd3vt99i69ir6, its number is PRJNA1103935. The corresponding author should be contacted if someone wants to request the data from this study.

Ethical Approval

This study was approved by the Ethics Committee of Longgang District Maternity & Child Healthcare Hospital of Shenzhen City (Approval No. LGFYKYXMMLL-2023-1).

Informed Consent

The parents, as legal guardians, voluntarily accepted scientific research on their children's care, and the parents of the child signed a written sample submission and informed consent form. The procedures used in this study adhered to the tenets of the Declaration of Helsinki.

Acknowledgments

We thank all the participants for their support. We thank the doctors and nurses of Longgang District Maternity & Child Healthcare Hospital (Shenzhen, China) for assisting the research team in clinical examination and fecal sample collection. We also thank the authors who made their data publicly available. The authors would like to thank all the

reviewers who participated in the review, as well as MJEditor (www.mjeditor.com), for providing English editing services during the preparation of this manuscript.

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.

Funding

This work has been strongly supported by the Longgang District Science and Technology Innovation Bureau (LGWJ2023-038 and LGWJ2023-072), the Key Medical Discipline in Longgang District, and the Research Initiation Fund of Longgang District Maternity & Child Healthcare Hospital of Shenzhen City (Y2024011).

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

1. Chang MY, Kim JW, Rhee CS. The quality of health information on allergic rhinitis, rhinitis, and sinusitis available on the internet. *Allergy Asthma Immunol*. 2015;7(2):141–147. doi:10.4168/aair.2015.7.2.141
2. Gu YR, Li H. Interpretation of Chinese guideline for diagnosis and treatment of allergic rhinitis (2022, revision). *Chin J Ophthalmol Otorhinolaryngol*. 2022;22(2):209–211.
3. Yong L, Li J. The application of probiotics in children with allergic rhinitis. *Chin J Otorhinolaryngol Integ Med*. 2018;26(3):177–181.
4. Bradshaw O, Foy R, Seal AK, Darling JC. Childhood constipation. *BMJ*. 2021;375:e065046. doi:10.1136/bmj-2021-065046
5. Southwell BR. Treatment of childhood constipation: a synthesis of systematic reviews and meta-analyses. *Expert Rev Gastroenterol Hepatol*. 2020;14(3):163–174. doi:10.1080/17474124.2020.1733974
6. Zuar LR, Thompson LA. What parents should know about constipation in children. *JAMA Pediatr*. 2023;177(2):216. doi:10.1001/jamapediatrics.2022.5280
7. Fujimura KE, Sitarik AR, Havstad S, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med*. 2016;22(10):1187–1191. doi:10.1038/nm.4176
8. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504(7480):451–455. doi:10.1038/nature12726
9. Bai R, Guo J. Interactions and implications of *Klebsiella pneumoniae* with human immune responses and metabolic pathways: a comprehensive review. *Infect Drug Resist*. 2024;17:449–462. doi:10.2147/IDR.S451013
10. Wu MC, Jan MS, Chiou JY, et al. Constipation might be associated with risk of allergic rhinitis: a nationwide population-based cohort study. *PLoS One*. 2020;15(10):e0239723. doi:10.1371/journal.pone.0239723
11. Walker S, Khan-Wasti S, Fletcher M, et al. Seasonal allergic rhinitis is associated with a detrimental effect on examination performance in United Kingdom teenagers: case-control study. *J Allergy Clin Immunol*. 2007;120(2):381–387. doi:10.1016/j.jaci.2007.03.034
12. Vriesman MH, Rajindrajith S, Koppen IJN, et al. Quality of life in children with functional constipation: a systematic review and meta-analysis. *J Pediatr*. 2019;214:141–150. doi:10.1016/j.jpeds.2019.06.059
13. Chiu CY, Chan YL, Tsai MH, et al. Gut microbial dysbiosis is associated with allergen-specific IgE responses in young children with airway allergies. *World Allergy Organ J*. 2019;12(3):100021. doi:10.1016/j.waojou.2019.100021
14. Hu B, Kuang Y, Jing Y, et al. Pediatric allergic rhinitis with functional gastrointestinal disease: associations with the intestinal microbiota and gastrointestinal peptides and therapeutic effects of interventions. *Hum Exp Toxicol*. 2021;40(11):2012–2021. doi:10.1177/09603271211017325
15. Acevedo-Román A, Pagán-Zayas N, Velázquez-Rivera LI, et al. Insights into gut dysbiosis: inflammatory diseases, obesity, and restoration approaches. *Int J Mol Sci*. 2024;25(17):9715. doi:10.3390/ijms25179715
16. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504(7480):446–450. doi:10.1038/nature12721
17. Wang C, Liu H, Li X, et al. Multi-omics technology reveals the changes in gut microbiota to stimulate aromatic amino acid metabolism in children with allergic rhinitis and constipation. *Front Allergy*. 2025;6:1562832. Erratum in: Front Allergy. 2025;6:1655751. doi:10.3389/falgy.2025.1562832
18. Kaczynska A, Klosinska M, Chmiel P, et al. The crosstalk between the gut microbiota composition and the clinical course of allergic rhinitis: the use of probiotics, prebiotics and bacterial lysates in the treatment of allergic rhinitis. *Nutrients*. 2022;14(20):4328. doi:10.3390/nu14204328
19. Pan R, Wang L, Xu X, et al. Crosstalk between the gut microbiome and colonic motility in chronic constipation: potential mechanisms and microbiota modulation. *Nutrients*. 2022;14(18):3704. doi:10.3390/nu14183704
20. Vernon-Roberts A, Alexander I, Day AS. Systematic review of pediatric functional gastrointestinal disorders (Rome IV Criteria). *J Clin Med*. 2021;10(21):5087. doi:10.3390/jcm10215087

21. Yu Z, Yue L, Yang Z, et al. Impairment of intestinal barrier associated with the alternation of intestinal flora and its metabolites in cow's milk protein allergy. *Microb Pathog*. 2023;183:106329. doi:10.1016/j.micpath.2023.106329
22. Shetty SA, Boeren S, Bui TPN, et al. Unravelling lactate-acetate and sugar conversion into butyrate by intestinal Anaerobutyricum and Anaerostipes species by comparative proteogenomics. *Environ Microbiol*. 2020;22(11):4863–4875. doi:10.1111/1462-2920.15269
23. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341(6145):569–573. doi:10.1126/science.1241165
24. Khalif IL, Quigley EM, Konovitch EA, et al. Alterations in the colonic flora and intestinal permeability and evidence of immune activation in chronic constipation. *Dig Liver Dis*. 2005;37(11):838–849. doi:10.1016/j.dld.2005.06.008
25. Sinha SR, Haileselassie Y, Nguyen LP, et al. Dysbiosis-induced secondary bile acid deficiency promotes intestinal inflammation. *Cell Host Microbe*. 2020;27(4):659–670.e5. doi:10.1016/j.chom.2020.01.021
26. Vatanen T, Kostic AD, d'Hennezel E, et al. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell*. 2016;165(4):842–853. doi:10.1016/j.cell.2016.04.007
27. Douglas GM, Maffei VJ, Zaneveld JR, et al. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol*. 2020;38(6):685–688. doi:10.1038/s41587-020-0548-6
28. Vernocchi P, Del Chierico F, Putignani L. Gut microbiota profiling: metabolomics based approach to unravel compounds affecting human health. *Front Microbiol*. 2016;7:1144. doi:10.3389/fmicb.2016.01144
29. Essilfie AT, Simpson JL, Horvat JC, et al. Haemophilus influenzae infection drives IL-17-mediated neutrophilic allergic airways disease. *PLoS Pathog*. 2011;7(10):e1002244. doi:10.1371/journal.ppat.1002244
30. Lai H, Li Y, He Y, et al. Effects of dietary fibres or probiotics on functional constipation symptoms and roles of gut microbiota: a double-blinded randomized placebo trial. *Gut Microbes*. 2023;15(1):2197837. doi:10.1080/19490976.2023.2197837
31. Baxter NT, Schmidt AW, Venkataraman A, et al. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *mBio*. 2019;10(1):e02566–18. doi:10.1128/mBio.02566-18
32. Fiocchi A, Cabana MD, Mennini M. Current use of probiotics and prebiotics in allergy. *J Allergy Clin Immunol Pract*. 2022;10(9):2219–2242. doi:10.1016/j.jaip.2022.06.038
33. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol*. 2016;16(6):341–352. doi:10.1038/nri.2016.42