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ORIGINAL RESEARCH

Assessing Gut Microbiome Alterations in Children With Allergic Rhinitis: Associations With Allergen-Specific IgE Levels and Sensitization Patterns

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Background: The relationship between gut microbiota composition and allergen exposure in children with allergic rhinitis (AR) remains insufficiently explored, particularly concerning variations in gut microbiota at different allergen-specific IgE (sIgE) levels and the distinction between monosensitization and polysensitization to allergens.

Methods: We employed metagenomic shotgun sequencing to compare the fecal microbiota of 50 healthy controls (HC) to 88 children with AR induced by house dust mites (HDM-AR). We further examined differences in gut microbiota among HDM-AR subgroups with extremely high house dust mite-sIgE (EH-HDM), high HDM-sIgE (H-HDM), as well as between monosensitized (mono-HDM) and polysensitized (poly-HDM) individuals.

Results: While no significant differences in overall gut microbiome diversity were observed between the HC and HDM-AR groups, a notable increase in the relative abundance of *Streptococcus sanguinis* within the genus was identified in children with AR. Further analysis revealed a significant enrichment of the *Streptococcus* genus in the EH-HDM group, particularly highlighting an increased relative abundance of the *Streptococcus salivarius*. Functional gene analysis via KEGG pathways indicated substantial enrichment in the salivary secretion pathway. Additionally, in comparisons among the HC, mono-HDM, and poly-HDM groups, *Streptococcus salivarius* emerged as the key differential species, showing a marked increase in the mono-HDM group.

Conclusion: Our study suggests that specific bacterial strains, particularly *Streptococcus salivarius*, may be potential biomarkers for assessing varying degrees and patterns of HDM sensitization. These findings open the avenues for developing targeted interventions aimed at mitigating the pathophysiology of AR.

Keywords: gut microbiome, allergic rhinitis, house dust mite, *Streptococcus salivarius*, allergen-specific IgE, sensitization patterns

Introduction

Allergic rhinitis (AR) is a chronic inflammatory condition of the nasal mucosa mediated by immunoglobulin E (IgE), triggered by exposure to allergens. It is characterized by symptoms such as nasal congestion, rhinorrhea, sneezing, and itching of the eyes, nose, and throat.[1–3] The prevalence of AR has been increasing globally, as shown by numerous epidemiological studies, and environmental factors are increasing recognized as significant contributors to this trend.[4,5]

The interaction between environmental factors and the human microbiome is pivotal, as it is believed that allergens can influence the gut microbiota through hematologic and intestinal pathways, significantly affecting the immune responses linked to allergic diseases.[6–8] Among various inhaled allergens, house dust mite (HDM) is the predominant trigger in many regions, including Shanghai, China.[9,10] It is particularly associated with high Th2-mediated airway

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inflammation in asthma, which is often co-morbid with AR.[11,12] In children, sensitization to HDM, characterized by elevated specific IgE (sIgE) levels, is strongly linked to both AR and asthma.[13] Notably, approximately half of the children sensitized to HDM are also responsive to other allergens, suggesting a complex interplay of sensitization patterns that influences disease phenotypes.[14,15]

Furthermore, the human microbiome plays a critical role in modulating immune responses and maintaining mucosal homeostasis,[16,17] with increasing evidence linking dysregulated gut microbiota to IgE-mediated allergic conditions such as AR and asthma in early childhood.[18] Specific interactions between dysbiotic gut microbiota and IgE-mediated pathways have been implicated in the pathogenesis of these conditions. For example, a 16S rRNA-based study demonstrated distinct gut microbial profiles in patients with atopic dermatitis (AD), revealing significant enrichment of *Klebsiella* and *Phascolarctobacterium*, which were associated with elevated total IgE levels.[19] These findings underline the potential influence of gut microbiota composition on systemic allergic responses and IgE dynamics.

Despite these insights, the relationship between specific IgE levels, allergen-specific sensitization patterns, and the gut microbiome remains poorly characterized. In particular, variations in gut microbiota composition associated with HDM-specific IgE (HDM-sIgE) levels and spectrum of allergen sensitization (monosensitization versus polysensitization) may contribute to distinct clinical phenotypes of AR. However, this critical area of research is underexplored, leaving a gap in understanding the interplay between gut microbial dysbiosis and allergic disease progression in children with AR.

This study employs metagenomic shotgun sequencing to examine the gut microbiota in healthy controls (HC) compared to children with AR induced by HDM (HDM-AR), focusing on variations associated with different HDM-sIgE levels and sensitization profiles. By delineating these associations, we aim to uncover microbial signatures that could inform new therapeutic strategies for allergy prevention and management.

Methods

Study Participants

Fifty HC and eighty-eight pediatric HDM-AR patients were recruited from the Department of Otorhinolaryngology at the Shanghai Children's Medical Center from August 2020 to August 2023. Detailed demographic information, including age, gender, mode of birth, feeding patterns, family history of allergies, and HDM-sIgE levels were recorded and presented in Table 1. The principal diagnostic classification of HDM-AR was made based on Allergic Rhinitis and its Impact on Asthma (ARIA) guideline.[20] Inclusion criteria for AR patients were as follows: (i) aged between 5 and 13 years old; (ii) children with typical nasal symptoms such as obstruction, itching, and sneezing; (iii) serological evidence of sensitization to at least one inhaled allergen; (iv) absence of other allergic conditions such as allergic asthma and atopic dermatitis. The HC group was selected based on no experience of nasal symptoms, allergic diseases, family history of allergic diseases, and serologically negative allergen-sIgE. Participants were excluded if they had received immunotherapy, consumed probiotics, prebiotics, or biostime in the past three months, or antibiotics therapy in the last month, or had severe conditions such as malignancies, autoimmune diseases, or immunodeficiencies. The flowchart of the present study was shown in Figure 1. All participants guardians signed written informed consent forms. The study was approved by Shanghai Children's Medical Center's Ethics Committee (SCMCIRB-YPDWJW2022001), and adhered to the principles of the Declaration of Helsinki.

Total Serum and Allergen-sIgE Levels Measurement

Serum samples were obtained from all participants to measure total IgE levels and allergen-sIgE levels using the Immuno CAP 1000 system (ThermoFisher Scientific, Uppsala, Sweden). The system assessed sIgE concentrations against seven common aeroallergens prevalent in Shanghai, including HDM, animal hair (cat hair and dog hair), molds, weed pollen and tree pollen. Results were generated automatically based on the reaction of fluorescence. For the total IgE level, a positive result was defined as a level exceeding 60 IU/mL in the pediatric population. The sIgE levels were categorized into six classes (class 1: 0.35–0.70 kU/L; class 2: 0.7–3.5 kU/L; class 3: 3.5–17.0 kU/L; class 4: 17.0–50.0 kU/L; class 5: 50.0–100.0 kU/L; class 6: >100.0 kU/L). Values below 0.35 kU/L were considered indicative of insensitivity to the tested allergens.

Table 1 Comparison of Basic Clinical Data of Multiple Groups of Children

	HC (n=50)	HDM-AR (n=88)	P_1 values	EH-HDM (n=31)	H-HDM (n=57)	P_2 values	Mono-HDM (n=46)	Poly-HDM (n=42)	P_3 values
Age (years) (Median, IQR)	7.8 (6.0~9.0)	7.2 (6.0~8.8)	0.38	7.3 (6.8~9.9)	7.0 (6.0~8.4)	0.53	7.3 (6.0~9.3)	6.8 (5.9~8.4)	0.52
BMI (kg/m^2)	15.7	16.0	0.88	15.7	16.0	0.93	16.4 (14.9~18.9)	15.3 (14.4~16.9)	0.25
(Median, IQR)	(14.5~17.8)	(14.6~18.0)		(14.8~17.9)	(14.6~18.0)				
Gender (n, %)			**0.04**			0.94			0.56
Male	27 (54%)	62 (70%)		22 (71%)	40 (70%)		34 (74%)	28 (67%)	
Female	23 (46%)	26 (30%)		9 (29%)	17 (30%)		12 (26%)	14 (33%)	
Birth mode (n, %)			**<0.01**			**<0.01**			0.75
Spontaneous delivery	24 (48%)	44 (50%)		9 (29%)	35 (61%)		24 (52%)	21 (50%)	
Cesarean section	26 (52%)	44 (50%)		22 (71%)	22 (39%)		22 (48%)	21 (50%)	
Feeding patterns in the first 6 months (n, %)			0.30			0.33			0.69
Breastfeeding	4 (8%)	8 (9%)		1 (3%)	7 (12%)		4 (9%)	4(10%)	
Formula feeding	34 (68%)	48 (55%)		19 (61%)	29 (51%)		22 (48%)	26 (62%)	
Mixed feeding	12 (24%)	32 (36%)		11 (36%)	21 (37%)		20 (43%)	12 (28%)	
Family history of allergic diseases (n, %)	0 (0%)	42 (48%)	**<0.01**	16 (52%)	26 (46%)	0.59	29 (63%)	12 (29%)	0.92
Total IgE (KU/L)	25.0	675.5	**<0.01**	1159.4	399.1	**<0.01**	592.6	752.3	0.41
(Median, IQR)	(5.9~89.0)	(211.0~863.5)		(722.8~4463.0)	(154.0~431.0)		(263.0~747.0)	(177.3~877.0)	
HDM-sIgE level									
I	0 (0%)	2 (2%)		0 (0%)	2 (4%)		0 (0%)	2 (5%)	
II	0 (0%)	3 (3%)		0 (0%)	3 (5%)		2 (4%)	2 (5%)	
III	0 (0%)	12 (12%)		0 (0%)	12 (21%)		6 (13%)	5 (12%)	
IV	0 (0%)	14 (14%)		0 (0%)	14 (25%)		9 (20%)	9 (21%)	
V	0 (0%)	27 (27%)		0 (0%)	26 (45%)		13 (28%)	8 (19%)	
VI	0 (0%)	30 (30%)		31 (100%)	0 (0%)		16 (35%)	18 (43%)	

Notes: Data are expressed as median (IQR) or number (percentage). *P* values calculated with independent-sample *t* test, $\chi 2$ or one-way analysis of variance (ANOVA) as appropriate. P_1-value, comparison between HC and HDM-AR; P_2-value, comparison between EH-HDM and H-HDM; P_3-value, comparison between mono-HDM and poly-HDM. *P* values <0.05 are indicated in bold.

Abbreviations: HC, healthy controls; HDM-AR, allergic rhinitis induced by house dust mite; EH-HDM, HDM-AR with extremely high HDM-sIgE (class 1 to 5); H-HDM, high HDM-sIgE (class 6); mono-HDM, sensitivity to only HDM; poly-HDM, sensitivity to HDM and at least one other allergen; BMI, Body Mass Index; sIgE, specific IgE; n, number.

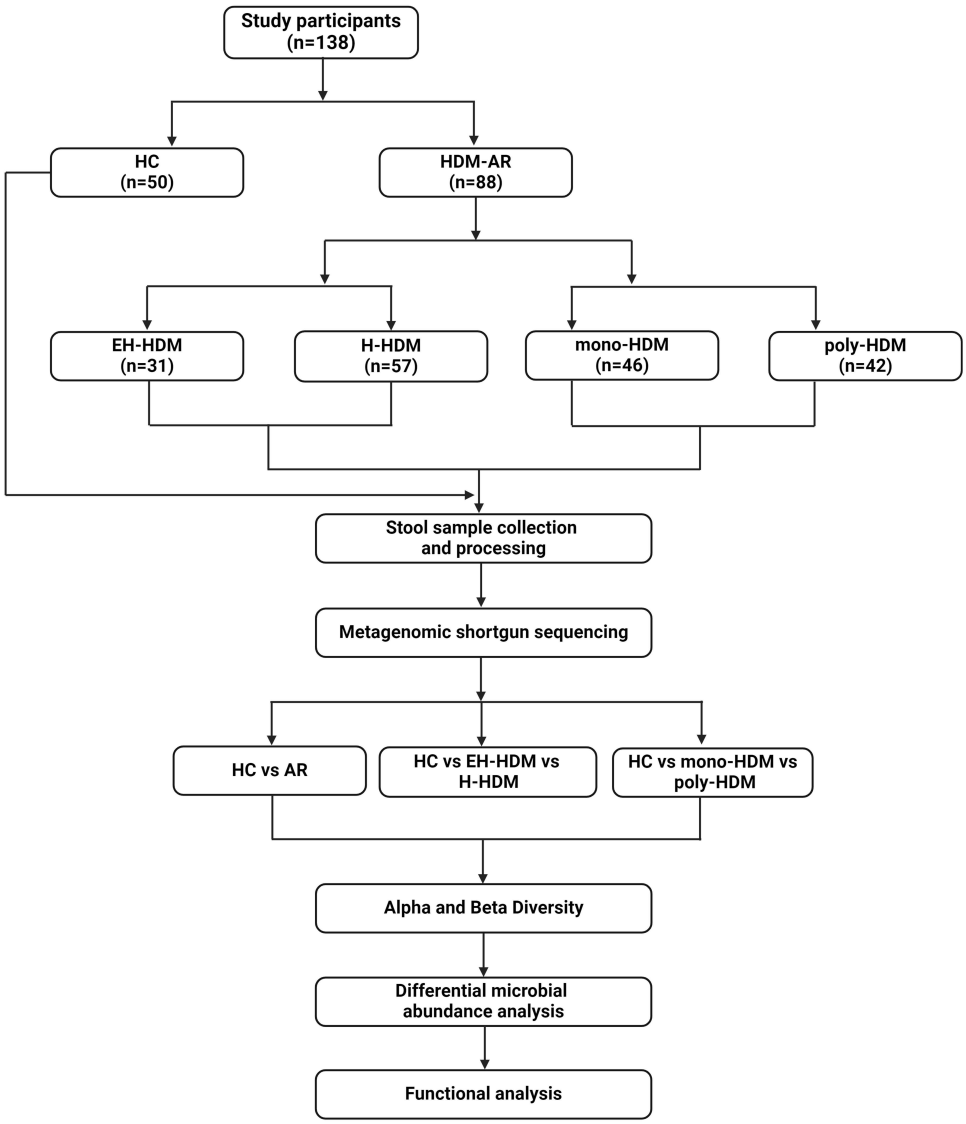

Figure 1 Flowchart of the present study.

Children were also categorized based on their sensitization profiles: HDM-AR with extremely high HDM-sIgE (EH-HDM, class 6) and high HDM-sIgE (H-HDM, class 1 to 5), monosensitization (mono-HDM, sensitivity to only HDM) and polysensitization (poly-HDM, sensitivity to HDM and at least one other allergen).

Sample Collection and Processing

Fresh fecal samples were collected from participants using sterile tubes pre-filled with fecal preservation solution (Shanghai Mobio Biomedical Technology Co., LTD). Immediately after collection, the samples were stored in household freezers. They were then transported to our laboratory under cold conditions to ensure sample integrity. Upon arrival, the samples were stored at −80°C until further analysis.

Metagenomic Shotgun Sequencing

Genomic DNA was extracted from fecal samples and mechanically fragmented to achieve an average size of approximately 400 base pairs (range: 200–600 base pairs). Fragmentation was performed using a Covaris ME220 (Covaris, USA) according to the manufacturer's recommended settings. Library preparation was conducted using the NEBNext® UltraTM DNA Libraries prep kit for Illumina (NEB, USA). Sequencing was carried out by Shanghai GeneSky

Biotechnologies Inc., utilizing the NovaSeq 6000 platform (Illumina) to generate 2×150 bp paired-end reads. Quality control of raw sequencing data was managed using Fastp v0.19.5.

Quality control of the raw sequencing data was conducted using Fastp software, which filtered out sequences containing more than three "N" bases, those with a Phred quality score of ≥20 less than 60% of the sequence, sequences that still had sequencing adapters or barcodes, and sequences shorter than 75 base pairs. Further cleaning involved the exclusion of human-derived sequences using Bowtie 2[21] with the –very-fast-end-to-end option against the human genome reference sequence (GRCh38) from the University of California, Santa Cruz.[22] The integrity and quality of the reads were assessed using FastQC v0.11.8.

Statistical Analysis

Normally distributed data were represented by the mean and standard deviation (SD), while non-normally distributed data were characterized by the median and interquartile range (IQR). All data analyses were conducted using GraphPad Prism 8.0 and SPSS 25.0 software. For statistical studies, one-way analysis of variance (ANOVA), Kruskal–Wallis rank-sum test, Dunnett's test, or Student's *t*-test were utilized. Multiple comparisons among multiple data sets were carried out using the ANOVA. In addition, False Discovery Rate (FDR) correction was applied to account for multiple comparisons in taxonomic and functional analyses. Effect sizes (eg Cohen's d, η^2) and confidence intervals were reported for analyses. *P*-value of less than 0.05 was considered statistically significant, while FDR-corrected *P* values less than 0.2 were considered statistically significant.[23,24]

The α-diversity and β-diversity were conducted and visualized using the vegan package (v2.5.6) and fossil packages in R (v0.3.7). The Kruskal–Wallis rank sum tests and Metastats analysis were used to assess differences in the relative abundance of taxa between the three or two groups. Linear discriminant analysis effect size (LEfSe) was used to analyze the abundance difference of bacterial taxa among all groups. Linear discriminant analysis (LDA) score > 2 and $p < 0.05$ were considered statistically significant. Differentially enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses was carried out through the Kruskal–Wallis rank-sum test by R software 3.6.3.

Results

Subjects' Characteristics

A total of 138 pediatric participants, comprising 50 hC and 88 patients with HDM-AR, were included in the present study. There was a significantly higher proportion of boys in the HDM-AR group compared to the HC group. Notable differences were observed, with the HDM-AR group showing significantly higher total IgE levels and family history of allergy disorders (both $P<0.05$). Furthermore, we divided the HDM-AR group into EH-HDM (31 cases) and H-HDM (57 cases), as well as mono-HDM (46 cases) and poly-HDM (42 cases). The baseline characteristics of children in all groups were compared in Table 1. No differences were found in terms of age, body mass index (BMI) and feeding pattern in all groups.

Microbiome Composition Analysis of HC and HDM-AR Groups

Fecal metagenomic sequencing was used to compare the gut microbiome composition and function between the HC and HDM-AR groups. The α-diversity, assessed by the observed number of amplicon sequence variants (ASVs), Chao1 index and Shannon index, demonstrated that the bacterial gut microbiota diversity of HDM-AR patients was not significantly different compared to that of HC (Figure 2A). The β-diversity of the gut microbiota in both groups was analyzed using Principal Coordinate Analysis (PCoA), which showed that the microbial community of HC and HDM-AR were not significantly different ($P=0.47$). The main coordinates 1 (PCoA1) and 2 (PCoA2) contributed 11.68% and 9.41% to the sample distribution, respectively, as shown in Figure 2B.

The relative abundances of taxonomic units from HC and HDM-AR were compared and the relative abundances of the bacterial species at the level of phylum, class, order, family and genus were constructed in Figure 2C, D and Figures S1-S3. The results showed that the composition of gut microbiota in the HDM-AR group varied at different levels. Analysis at the phylum level showed a significant increase of the *Bacillota* (*Firmicutes*) and *Pseudomonadota* phylum,

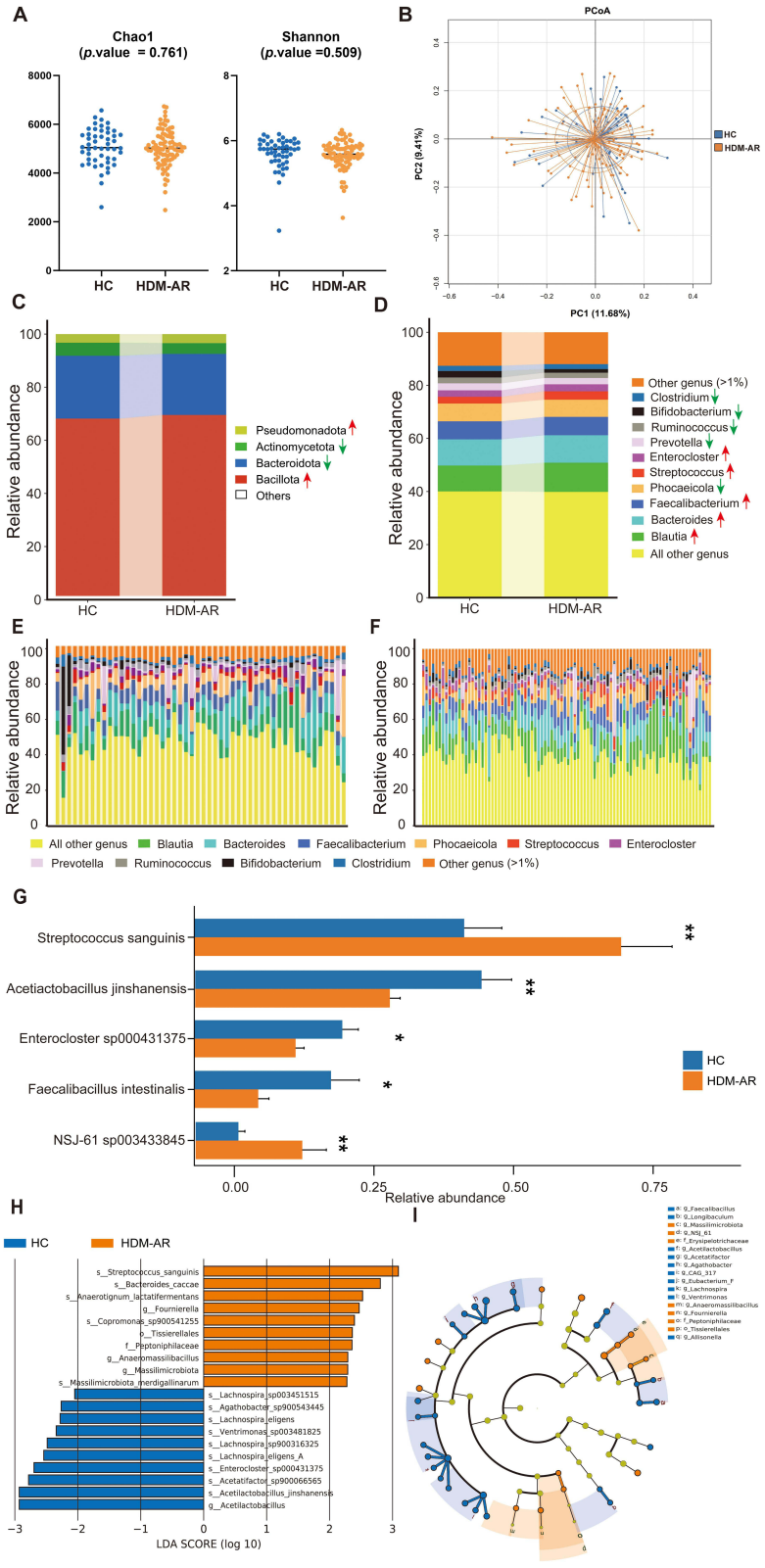

Figure 2 Microbial diversity analysis of gut microbiota in children with HC and HDM-AR. (**A**) Chao1 index and Shannon index were used to evaluate α-diversity of microbiota (**B**) Principal co-ordinates analysis (PCoA) was used to show β-diversity between the groups. (**C**) Relative abundance of bacteria at the phylum level in HC and HDM-AR groups. (**D**) Relative abundance of bacteria at the genus level in HDM-AR and HC groups. (**E**) Relative abundance of genus level bacteria by individual in the HC group. (**F**) Relative abundance of genus level bacteria by individual in the HDM-AR group. (**G**) Metastats analysis of bacteria in species level differences in HC and HDM-AR (Top 5). (**H**) The histogram of the LEfSe analysis of the LDA values in HC and HDM-AR groups. (**I**) A cladogram based on the LEfSe method for the children with HC and HDM-AR. *P < 0.05, **P < 0.01.

while the relative abundance of *Actinomycetota* and *Bacteroidota* phylum decreased significantly (Figure 2C). At the genus level (top 10), we observed a significant increase in the relative abundance of *Enterocloster, Streptococcus (S)., Faecalibacterium, Bacteroides* and *Blautia* in the HDM-AR group (Figure 2D). Figure 2E and F revealed the relative abundance of gut microbiota in single individuals of HC and HDM-AR at the genus level.

By Metastats difference analysis, species with significant differences in relative abundance between groups were screened. At the species level, we found that strains with significant differences (top5) in the HC and HDM-AR groups were *Streptococcus sanguinis, Acetilactobacillus jinshanensis, Enterocloster sp000431375, Faecalibacillus intestinalis* and *NSJ-61 sp003433845*. Among the differential strains, the relative abundance of *Streptococcus sanguinis* accounted for the highest proportion with a significant increase in the relative abundance of *Streptococcus sanguinis* in the HDM-AR group (Figure 2G and Table S1).

In order to assess the differences in gut microbiome associated with HDM-AR, linear discriminant analysis Effect Size (LEfSe) analysis was used to determine differences in microbial clade. The present results suggested that a total of 10 bacterial taxa, such as *Acetilactobacillus jinshanensis, Acetatifactor sp900066565, Enterocloster sp000431375* and *Lachnospira eligens_A*, had a higher abundance in the HC group compared with HDM-AR. However, the relative abundance of 10 taxa of bacteria, such as *Streptococcus sanguinis, Bacteroides caccae* and *Anaerotignum lactatifermentans* in HDM-AR group was higher compared with HC (Figure 2H). A cladogram based on the LEfSe method is shown and highlights the taxonomic groups in the HC and children with HDM-AR (Figure 2I). Based on the KEGG database, the functional diversity of samples was analyzed in terms of gene function in Figure S4.

Differential Abundance and Functional Analysis of Gut Microbiota in Children With Varying Degrees of HDM Sensitization

Although no significant differences were observed in overall gut microbiome diversity between the HC and HDM-AR groups, notable variances were detected at the species level. Metagenomic sequencing revealed 11,283 ASVs shared among the groups, with 335, 946, and 687 unique ASVs identified in the HC group, EH-HDM group and H-HDM group, respectively (Figure 3A).

Neither the α-diversity (Chao1 index and Shannon index) nor the β-diversity exhibited significant differences among the groups (Figure 3B and C). However, differential abundance analysis at the genus level, displayed in the polar circle bar chart (Figure 3D), highlighted the predominant enrichment of *Streptococcus* genus in the EH-HDM group compared to the HC and H-HDM groups (Figure 3D and E).

At the species level, seven types of *Streptococcus* species showed significant overrepresentation in the EH-HDM group, including *Streptococcus salivarius, Streptococcus parasanguinis, Streptococcus mitis, Streptococcus oralis, Streptococcus australis, Streptococcus infantis* and *Streptococcus xiaochunlingii*. Notably, *Streptococcus salivarius* was the most significantly enriched, suggesting its role as a potential biomarker for EH-HDM sensitization (Figure 3F and Table S2).

Further analysis using Linear Discriminant Analysis Effect Size (LEfSe) indicated significant enrichment of several bacterial genera in the EH-HDM group, including *Acetatifactor* genus, *Fusicatenibacter* genus, *Streptococcus salivarius, Streptococcus sp001556435* and *Streptococcus oralis*, etc. Conversely, the H-HDM group exhibited a higher abundance of *Enterocloster bolteae, Enterocloster lavalensis* and *Emergencia* genus, etc. The HC group showed a predominance of *Faecalibacillus* genus, *Faecalibacillus intestinalis* and *Enterocloster sp000431375*, etc (Figure 3G). A cladogram representation of the data for HC group, EH-HDM group and H-HDM group enriched taxa was generated (Figure 3H).

Gene function analysis based on the KEGG database revealed the enrichment of functional genes associated with pancreatic secretion, salivary secretion, secondary bile acid biosynthesis, and polycyclic aromatic hydrocarbon degradation pathways, further delineating the metabolic landscape associated with different levels of HDM sensitization (Figure 3I).

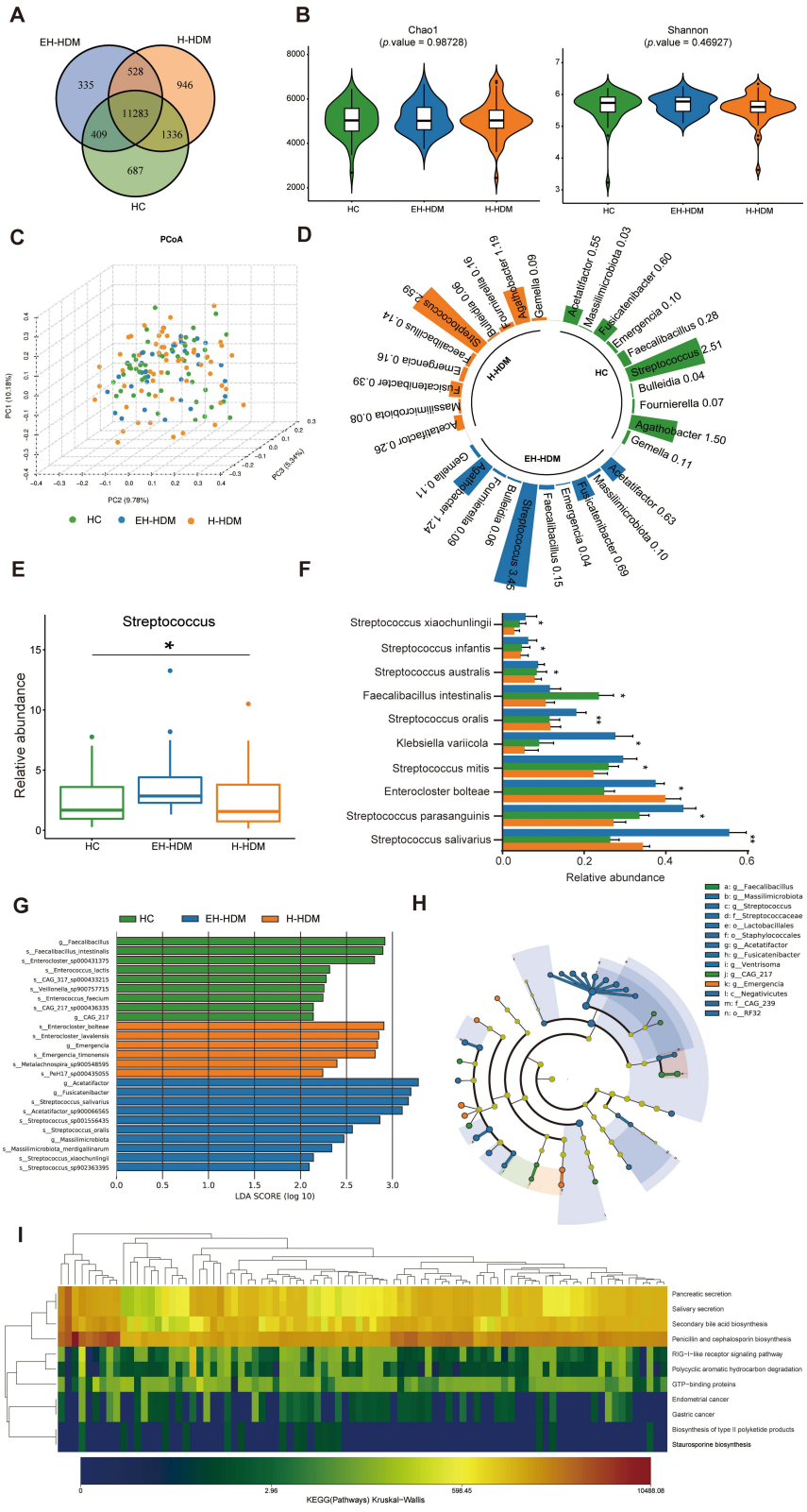

Figure 3 Microbial diversity analysis of gut microbiota in children with HC, EH-HDM and H-HDM. (**A**) Venn diagram illustrating the overlap of observed ASVs among groups. (**B**) Chao1 index and Shannon index were used to evaluate α-diversity of microbiota. (**C**) Principal co-ordinates analysis (PCoA) was used to show β-diversity among the groups. (**D**) Polar coordinate circular bar chart analysis of bacteria with genus levels differences among groups (Top 10). (**E**) Relative abundance of *S. genus* by individual in HC, EH-HDM and H-HDM groups. (**F**) Kruskal Wallis analysis of bacteria in species level differences among groups (Top 10). (**G**) The histogram of the LEfSe analysis of the LDA values among groups. (**H**) A cladogram based on the LEfSe method for the children with HC, EH-HDM and H-HDM. (**I**) The functional diversity among groups were analyzed in terms of gene function based on KEGG database. *$P < 0.05$, **$P < 0.01$.

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Differential Abundance and Functional Analysis of Gut Microbiota in Children With Varying Patterns of HDM Sensitization

To identify the difference in gut microbiota among HC, mono-HDM and poly-HDM groups, we performed a comparative analysis.

The Venn plot shows a total of 11501 ASVs intersecting across all groups, 687 unique to HC, 639 unique to mono-HDM and 521 unique to poly-HDM (Figure 4A). Similar to other comparisons, no significant differences were observed inα-diversity (Chao1 and Shannon indices) or β-diversity among these groups (Figure 4B and C).

The Kruskal–Wallis test was used to analyze the differential gut microbiota at the species level. *Streptococcus salivarius* showed a significant increase in relative abundance in the mono-HDM group, marking it as the predominant differential species among the groups (Figure 4D and Table S3). This suggests a potential microbiological marker for monosensitization to HDM.

LEfSe analysis highlighted that *Enterocloster bolteae, Bacteroides congonensis, Massilimicrobiota* genus, *Massilimicrobiota merdigallinarum* and *Merdimonas* genus, were significantly enriched in the poly-HDM group. In contrast, *Romboutsia* genus, *Streptococcus salivarius, Romboutsia timonensis, Clostridium sp900547735* and *Egerieimonas* genus, etc. were more abundant in mono-HDM. There was a higher LDA score of *Acetatifactor sp900066565, Faecalibacillus* genus, *Faecalibacillus intestinalis, Enterocloster sp000431375* and *Lachnospira sp900316325*, etc. in HC (Figure 4E). A cladogram visually represented these findings, highlighting the enriched taxa across the three groups (Figure 4F).

Functional genomic analysis using KEGG revealed distinct metabolic pathways enriched between mono-HDM and poly-HDM. Key pathways included Nitrotoluene degradation, D-arginine and D-ornithine metabolism, as well as pathways associated with rheumatoid arthritis, osteoclast differentiation, and neutrophil extracellular trap formation, indicating a diverse functional landscape influenced by allergen sensitization patterns (Figure 4G).

Discussion

Dysbiosis of the gut microbiota is increasingly recognized in allergic disease.[25,26] Yet, the impact of varying sIgE levels of specific allergens and differences between polysensitized and monosensitized on gut microbiota compositions, remains poorly understood. Our study aimed to address these gaps by examining the gut microbiota among children with HC and HDM-AR, and exploring the variations between groups with EH-HDM, H-HDM, mono-HDM, and poly-HDM sensitization. The key findings of this study include: (i) Children with HDM-AR exhibited altered gut microbiota compared to HC, notably with an increased relative abundance of *Streptococcus sanguinis*. This suggests a potential microbial signature linked to AR pathophysiology. (ii) The EH-HDM group displayed a marked increase in the relative abundance of the *Streptococcus* genus, with *Streptococcus salivarius* showed the most substantial elevation. This was further correlated with an enrichment of genes involved in the salivary secretion pathway, suggesting a functional adaptation of the microbiota in response to allergic sensitization. (iii) The *Streptococcus salivarius* was significantly more abundant in the mono-HDM group compared to the poly-HDM group. This finding is crucial as it underscores the distinct microbial landscapes associated with different sensitization patterns, which may influence the severity and management of AR.

As an important main colonizer of the oral cavity, the distribution of oral streptococcal species varies relative to the ecological niches of the oral cavity.[27,28] The major colonizers of the oral cavity, including *Streptococcus oralis, Streptococcus mitis, Streptococcus gordonii*, and *Streptococcus sanguinis*, are typically regarded as commensals but have been implicated in conditions like infective endocarditis.[29,30] In our study, the dysregulation observed in the microbiota of children with HDM-AR was characterized by shifts across various taxonomic classifications, suggesting a broader impact of allergen exposure on the microbial communities. Consistent with our findings, Ping M et al observed a significant increase in *Streptococcus* genus abundance in the nasal microbiome of adult AR patients, particularly *Streptococcus salivarius*.[31] Our results extend these observations to the gut microbiome, highlighting a consistent pattern of dysbiosis associated with AR across different body sites.

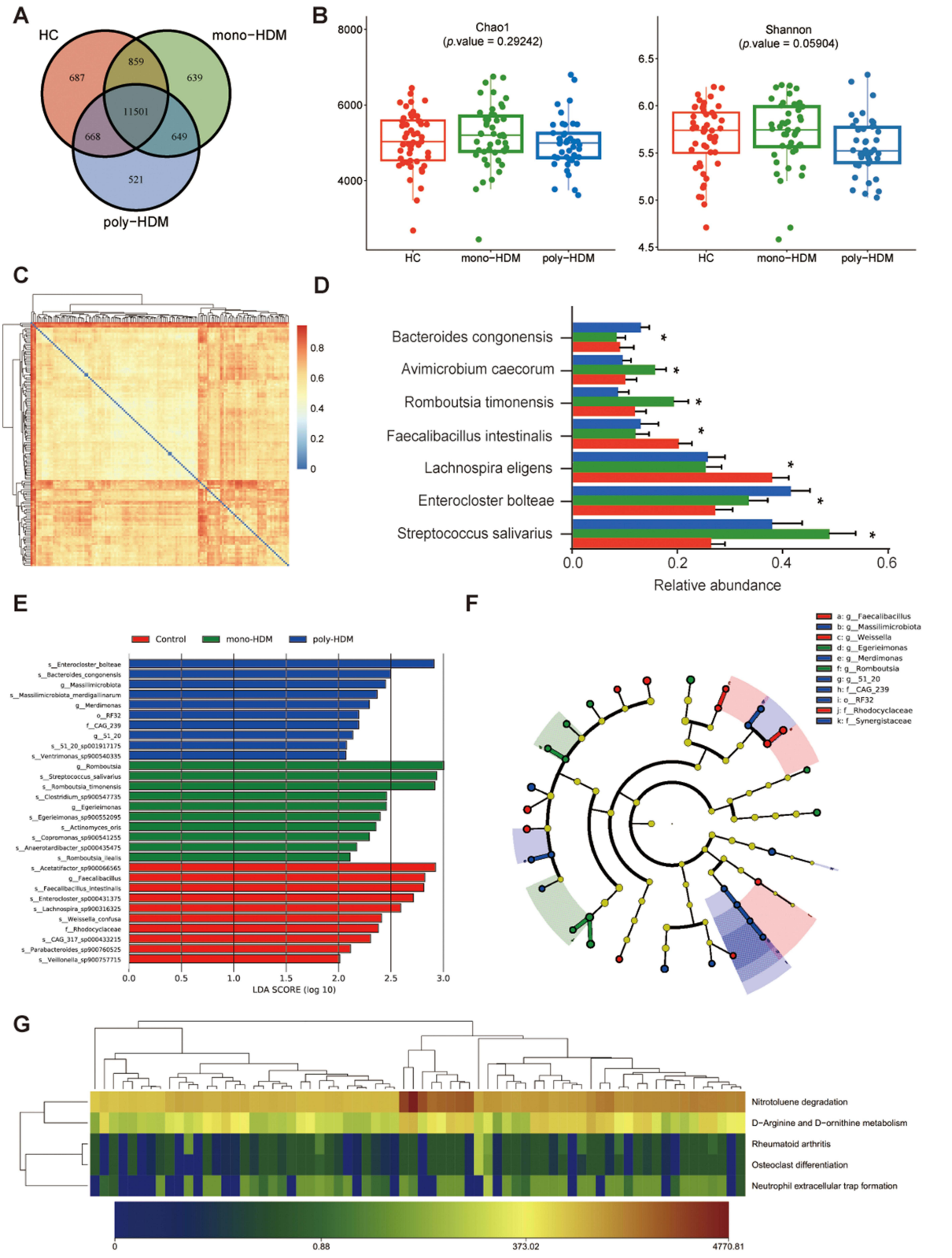

Figure 4 Microbial diversity analysis of gut microbiota in children with HC, mono-HDM and poly-HDM groups. (**A**) Venn diagram illustrating the overlap of observed ASVs among groups. (**B**) Chao1 index and Shannon index were used to evaluate α-diversity of microbiota. (**C**) Principal co-ordinates analysis (PCoA) was used to show β-diversity among the groups. (**D**) Kruskal Wallis analysis of bacteria in species level differences among groups (Top 7). (**E**) The histogram of the LEfSe analysis of the LDA values among groups. (**F**) A cladogram based on the LEfSe method for the children with HC, mono-HDM and poly-HDM. (**G**) The functional diversity among groups were analyzed in terms of gene function based on KEGG database. *$P < 0.05$.

In our study, we explored the variations in gut microbiota across different levels of HDM-sIgE in comparison to the HC group. Notably, we observed a marked increase in the relative abundance of *Streptococcus* genus within the EH-HDM group. At the species level, *Streptococcus salivarius* stood out significantly, representing 7 of the top 10 most differentiated species within this group. Furthermore, our KEGG pathway analysis reinforced that these microbial differences were related to the salivary secretion pathway, suggesting a possible interaction between oral and gut microbiomes in allergen response. Additionally, the interplay between allergen-sIgE and gut microbiome dysbiosis appears to play a crucial in modulating susceptibility to allergy diseases. Supporting our findings, Chiu C et al highlighted that total IgE level in stool was particularly linked to HDM-sIgE level, revealing a robust association between children with HDM-AR and a decline in *Dorea* and *Ruminococcus* spp.[18] This decline was inversely correlated with total IgE levels in stool, underscoring a potential mechanistic pathway in allergy susceptibility.

Furthermore, our comparative analyses distinguished between children sensitized to mono-HDM and those with poly-HDM sensitization. Interestingly, *Streptococcus salivarius*, identified as the most significant altered bacterium, showed a pronounced increase in relative abundance specifically in the mono-HDM. This finding is particularly relevant given the limited existing research on the differential impacts of mono and polysensitization on the gut microbiome. Francesca D et al conducted a related study assessing gut microbiome variations in children with food and respiratory allergies, differentiating between those sensitized to poly-allergens and those sensitized to a mono-allergen.[32] Their findings revealed no significant differences in the overall microbiome composition between these groups. However, children with poly-allergies exhibited a greater presence of *Ruminococcus gnavus* genes, specifically those associated with the production of a pro-inflammatory polysaccharide. This suggests that different strains of *Ruminococcus gnavus* may play distinct roles in the inflammatory responses observed in polysensitized individuals. Nomura A et al investigated the relationship between gut microbiome composition and sensitization to specific inhaled allergens.[33] The percentage composition of *Bacteroides* in participants aged 20–49 years was significantly higher among participants mono-sensitized to Japanese cedar pollen than in those unsensitized. The percentage composition of *Lactobacillales* in participants with mono-sensitization to house dust was significantly lower than in unsensitized participants. The presence of bacteria of the order *Lactobacillales, Bifidobacteriales*, and *Bacteroidales* in the gut microbiota may affect sensitization to inhaled allergens.

Our study benefits from a robust dataset with a large cohort, enhancing the reliability of our findings through matched factors comparison and comprehensive subgroup analyses. As metagenomic techniques evolve, they continue to refine our understanding of the gut microbiome's role in allergic diseases, offering new avenues for targeted interventions. However, further research, including in vivo and in vitro studies, is necessary to validate the role of *Streptococcus salivarius* in EH-HDM and mono-HDM sensitization and to explore its potential as a therapeutic target. While our findings are promising, the observational nature of this study limits our ability to establish causality. Additionally, potential confounding factors, such as unmeasured environmental exposures or host factors, may have influenced the observed associations. The cross-sectional design of the study prevents us from drawing conclusions about long-term effects or causality. Furthermore, in some subgroups, such as EH-HDM group, the limited sample size reduces the statistical power and generalizability of our findings. While *S. salivarius* shows promise as a potential biomarker, its functional role in salivary secretion pathways and immune modulation may be more relevant for understanding the pathophysiology of AR. Future studies should aim to delineate the mechanistic pathways linking gut microbiota alterations to specific allergen sensitization and clinical outcomes in AR.

Conclusion

Our study identified significant changes in the gut microbiota composition of children with HDM-AR, across various levels of sIgE and distinct sensitization patterns. Notably, we identified a pronounced increase in the relative abundance of *Streptococcus salivarius* in groups with EH-HDM and in those with mono-HDM, suggesting that *Streptococcus salivarius* could serve as a potential biomarker for evaluating the degree of sensitization in HDM-AR. These findings pave the way for future research focused on developing targeted interventions that leverage our understanding of the microbiome to mitigate the clinical impacts of AR.

Data Sharing Statement

Raw sequence data have been deposited in the China National Center for Bioinformation database under accession identification. The data that support the findings in this study are available from the corresponding author upon reasonable request.

Ethics Statement

The studies involving human participants were reviewed and approved by Institutional Review Board and the Ethics Committee of Shanghai Children's Medical Center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. This study adhered to the principles of the Declaration of Helsinki.

Author Contributions

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

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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