Metagenomic Anaysis of Nasal Microbiota-Derived Extracellular Vesicle in Patients with Allergic Rhinitis.

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Abstract

Nasal Microbiota is crucial for the pathogenesis of allergic rhinitis (AR). However, never study investigates the microbiota in nasal extracellular vesicles (EVs). **Objective:** We aim to compare the microbiome composition and diversity in EVs between AR and health controls (HCs), and reveal the potential metabolic mechanisms in AR. Eosinophil count and serum immunoglobulin E (IgE) were measured in AR patients (n=20) and HCs (n=19). Nasal EVs were identified by transmission electron microscopy and flow cytometry. 16S rRNA sequencing was used to profile microbial communities. Alpha and beta diversity were analyzed to reflect the microbial diversity. Taxonomic abundance was analyzed based on linear discriminant analysis effect size (LEfSe). Microbial metabolic pathways were characterized using PICRUSt and KEGG analyses. Eosinophils, total serum IgE, and specific IgE to *Dermatophagoides* were increased in AR patients. Alpha diversity in nasal EVs from AR patients were lower than that in HCs. Beta diversity showed the microbiome differences between AR and HCs. Microbial abundance was distinct between AR and HCs at different taxonomic levels. The significant higher level of genera *Acetobacter*, *Mycoplasma*, *Escherichia* and *Halomonas* in AR patients than those in HCs. Conversely, the genera *Zoogloea*, *Streptococcus*, *Burkholderia* as well as *Pseudomonas* were more abundant in the HCs group. Moreover, 35 microbial metabolic pathways were different between AR and HCs, and 25 pathways were more abundant in AR. AR patients had distinguished microbiota characteristics in nasal EVs compared with HCs. The metabolic mechanisms of microbiota regulating AR development also altered. The nasal fluid may reflect the specific pattern of microbiome EVs in patients with AR.

Introduction

Allergic rhinitis (AR) is a prevalent chronic allergic respiratory disease, and almost 10%-25% of population is affected by AR worldwide [1–3]. Inflammatory responses in the nasal mucosa are typic features of AR, which is frequently accompanied with the infiltration of immune cells, including T cells, eosinophils, and basophils [4, 5]. Previous study has confirmed that microbiome directly affects the inflammatory responses in allergic diseases, such as AR and asthma [6–8]. Therefore, the study of the composition of microbiota is of great importance for the understanding of the pathogenesis of AR.

Microbiome in humans is crucial for affecting health and diseases, which accounts for 90% of the cells by a ratio of 10:1[8]. Recent advances in microbiome research have shown that the exposure of environmental microbes is closely associated with the development of AR [9]. To our knowledge, gut and nasal microbiota are the two most frequently studied microbiota in AR. Several studies indicated that gut microbiota play essential roles in the course and symptoms of AR [10, 11]. For instance, adult AR patients had a unique gut microbiota with reduced microbial diversity and altered abundance of certain microbes compared to healthy subjects [10]. Gut microbiota were different between children with AR and health controls, and were associated with high serum immunoglobulin E (IgE) level [11]. Moreover, there is a stable microbial community in the nasal cavity of AR, and this microbiota could induce a cross-talking with immune system and eliminate pathogens [12]. Choi et al.[13] reported that there is a significant

increase in microbial species and bacterial diversity in the nasal tract of AR patients. Gan et al.[14] also indicated that nasal microbiota may exert pivotal effects on the pathogenesis of heterogeneous nasal mucosal inflammation.

Extracellular vesicles (EVs) are nanosized vesicles released from inflammatory and immune cells that are involved in allergic diseases. EVs are considered to be responsible for communication between cells and are frequently applied for investigating the pathogenesis of various diseases including AR[15]. The EVs secreted from gram-negative and gram-positive bacteria originally that called membrane vesicles (MVs) and outer membrane vesicles (OMVs) respectively. Microorganism-associated molecular patterns (MAMPs), such as nucleic acid, peptidoglycan, lipopolysaccharide (LPS) as well as toxins are contained in MVs or OMVs. Those MVs or OMVs encapsulated MAMPs triggered the intracellular immuno-modulatory signaling pathways via recognition of different pattern recognition receptor (PRRs) in host cells [16, 17]. Accumulating evidence indicated that the microbiota derived from EVs play key roles in immune function and disease development [18–20]. However, the microbiome alteration of nasal EVs in AR patients remains unknown.

In this article, the microbial composition of nasal EVs in AR patients was characterized via 16S rRNA sequencing. The potential metabolic pathways involving in the detected microbiome were determined as well. This study may clarify the microbiome difference in nasal EVs from AR patients and offers a new clue for investigating the pathogenesis of AR.

Materials And Methods

Human subjects sample collection

Patients were recruited from the outpatient clinic of the Department of Otorhinolaryngology of Xiamen Chang Gung Hospital, Xiamen, China from June 2020 to March 2021. A total of 39 subjects (aged 15-36 years) were recruited in the study, including 20 AR patients and 19 health controls (HCs). All patients with clinical symptoms suggestive of persistent AR were recruited. The inclusion criteria for AR patients were as follows: patients with at least one typical symptom associated with AR (runny nose, nasal obstruction, itchy nose, or sneezing) that persisted for at least four consecutive weeks. None of the patients with symptoms of chronic rhinosinusitis, nasal polyposis, immunological disease, neuro-developmental disabilities as well as respiratory infection. Age-and sex- matched healthy individuals without allergic disorders or chronic medical conditions were selected as HCs. In addition, none of the participants used probiotics, systemic antibiotics, or steroids within 14 days prior to the study. The study was approved by the Institutional Review Board of Xiamen Chang Gung Hospital (Approval number: XMCGIRB2020023) and conducted according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participates.

Eosinophil count and IgE evaluation

Eosinophil count was analyzed using ABX pentra 120 Retic (France). The serum samples collected from the enrolled participants were tested for total IgE levels using IMX Total IgE assay system (Abbott Laboratories, IL, USA) and specific IgE house dust mite (HDM) aeroallergens, such as Dermatophagoides pteronyssinus (d1), Dermatophagoides farinae (d2), using the Phadiatop P250 (ThermoFisher Scientific, Uppsala, Sweden) and . A cutoff of 0.7 KU/L was defined as positive result[42].

Nasal EVs Isolation

As previous study, nasal secretion was collected from AR patients and HCs. To collect the secretion, two small merocel (Ivalon, ThinPackTM) were inserted into the inferior meatus to each nostril for ten minutes. The merocel was weighed before and after application to calculate total secretion weights and the secretion was eluted by soaking in 3 mL (0.9% w/v) NaCl at 4°C for 1 hour and collected after centrifugation for 10 min at 3000g [43] . Reduced viscosity of nasal fluid samples by PBS dilution and removed debris by centrifugation at 2,000 × g for 30 min at 4 °C. The supernatant was collected into new falcon tubes and further centrifuged at 10,000 × g for 45 min at 4 °C. Subsequently, the supernatant was filtered with a 0.45 μm syringe filter, and then ultracentrifuged at 100,000 × g for 70 minutes at 4°C (Optima L-100XP, Beckman Coulter, Brea, CA, USA). Resuspend the exosomal pellet in 10 mL of cold PBS and repeat the ultracentrifugation step. The final exosomal pellet was resuspended in 50 μL of filtered PBS (0.22 μm) for subsequent analysis

EVs characterization

To confirm the morphology of exosomes isolated from the nasal fluid using TEM with negative staining. The absorbed exosomes were stained with 10 μL 2% uranyl acetate for 1 min, and the excess fluid was removed using filter paper. EVs were mounted on the grid and observed under TEM at 80 kV (HT7700, Hitachi High-Technologies Corporation, Minato, Tokyo, Japan). The purity of exosomes were analyzed using nFCM with nano-analyzer following the manufacturer's instructions. Briefly, 30 μL of each diluted exosome sample (1:4 dilution ratio by cold PBS) were stained with fluorescein isothiocyanate (FITC) mouse antihuman CD9 antibody (BD Biosciences, Franklin Lakes, NJ, USA) for 30 min at room temperature in the dark. The samples positive for CD9 was detected using nano-Analyzer (N30E, NanoFCM Inc., Xiamen, China)

DNA extraction from nasal EVs

Nasal EVs encapsulated DNA was extracted following a method described previously [44]. Briefly, the EV samples were boiled at 100 °C for 30 min and centrifuged at 10,000× g for 30 min. The quality and quantity of DNA were measured using a NanoDrop assay. DNA concentration and purity were con-firmed on a 1% agarose gel.

PCR amplification and purification

For the 16S rRNA gene sequencing, V3-V4 region was amplified by specific

primer set (319F: 5’-CCTACGGGNGGCWGCAG-3’, 806R: 5’-

GACTACHVGGGTAT CTAATCC -3’) according to the 16S metagenomic

sequencing library preparation procedure (Illumina). In brief, gDNA

was used for the PCR reaction carried out with KAPA HiFi HotStart ReadyMix

(Roche) under the PCR condition: 95°C for 3 minutes; 25 cycles of: 95°C for 30

seconds, 55°C for 30 seconds, 72°C for 30 seconds; 72°C for 5 minutes and hold

at 4°C. The PCR products were monitored on 1.5% agarose gel. Samples with

bright main strip around 500bp were chosen and purified by using the AMPure XP

beads for the following library preparation.

Library preparation and sequencing

Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA) was used to generate sequencing libraries following manufacturer’s recommendations. The PCR product quality was assessed on the Qubit 4.0 Fluorometer (Thermo Scientific) and Qsep100TM system. At last, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated

Microbiome analyses

For the ASVs (Amplicon Sequence Variants) construction, denoising pipeline was performed with QIIME2 DADA2 plugin (v2020.11) which implements quality filtering, dereplication, dataset-specific error model learning, denoising, paired-end reads joining and chimeras removing [45]. The feature-classifier [46] and algorithm in QIIME2 [47] were employed to annotate taxonomy classification for each representative sequence based on the information retrieved from the NCBI database. Alpha diversity was indicative of the species complexity within individual samples based on different criteria output from the QIIME pipeline. The weighted and unweighted UniFrac belong to beta- diversity were also calculated by using the QIIME pipeline. LEfSe applies LDA to those bacterial taxa was used to assesses the effect size of each differentially significant abundant taxon. In this study, taxa with LDA score (log 10) > 3 was considered significant. For functional analysis, functional abundances from 16S rRNA sequencing data were analyzed for the prediction of functional genes with PICRUSt (v1.1.1).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software , USA). Differences be-tween the two groups were assessed using an unpaired two-tailed Student’s t-test for EV size and alpha diversity. The Kruskal-Wallis test was used to assess the significance of other differences in the distributions. Statistical significance was accepted at a two-sided p value of <0.05.

Results

Clinical characteristics of participants

A total of 39 subjects (22 males and age 27 ± 8 years) participated in this study. Among them, 20 subjects were included in the AR group (10 males and 10 females, and age 30 ± 6 years), and 19 subjects were included in the HC group (12 males and 7 females, and age 25 ± 10 years). Eosinophils are the major effector cell of innate immunity, which are considered as a biomarker for AR [21]. As shown in Table 1, the percentage of eosinophils was significantly increased in AR patients than that in HCs ($P < 0.01$). Moreover, IgE is the molecular component of atopy and the evaluation of total serum IgE levels has become a diagnostic criterion for AR [22]. The result showed that patients with AR had higher total serum IgE levels as compared to HCs ($P < 0.01$). *Dermatophagoides* (D) allergen is a risk factor for the development of AR [23]. The levels of serum specific IgE to D (serum sIgE-D1 and D2) were also dramatically higher in the AR patients than that in the HCs ($P < 0.0001$) (Table 1).

Phenotypic characterization of nasal EVs

The presence of nasal EVs from AR patients and HCs was investigated using TEM and nano-flow cytometry. TEM showed that nasal EVs presented a clear spheroid morphology from AR patients and HCs, however, the size of most of nasal EVs from AR patients were slightly larger than that in HCs (Fig. 1a). CD9 is a tetraspanin commonly used as a exosomal specific marker for the identification of EVs[24]. Naon-flow cytometry showed an increase in CD9 from AR patients compared to HCs (Fig. 1b).

Alpha diversity of microbiome in nasal EVs

Alpha diversity is a pivotal indicator in the abundance and diversity of observed microbes, which is assessed by Chao1, Shannon, PD_whole_tree, Pielou, and Simpson indices. The Chao1 index is a qualitatively measure for species richness[25]. As shown in Fig. 2a, there was no significant difference for the Chao1 index between AR patients and HCs ($P = 0.1107$). The Shannon index relates both species richness and evenness, and Pielou and Simpson give a greater weight to species evenness[26]. The Shannon diversity of AR patients and HCs also did not differ significantly ($P = 0.1354$), while Pielou and Simpson indices were dramatically decreased in AR patients compared to those in HCs ($P < 0.05$) (Fig. 2b-d). The metric PD_whole_tree is Faith's Phylogenetic Diversity, which is measured by adding up all the branch lengths based on the phylogenetic tree. There was no obvious difference in PD_whole_tree diversity between AR patients and HCs ($P = 0.1417$) (Fig. 2e).

Beta diversity of microbiome in nasal EVs

Beta diversity refers to microbial composition differences between different samples, presented by the principal coordinate analysis (PCoA) and hierarchical clustering based on UniFrac distance[27].According to the unweight UniFrac analysis, PCoA1 and PCoA2 accounted for 19.11% and 6.03% of total PCoA, respectively (Fig. 3a). For weighted UniFrac distances, PCoA1 and PCoA2 respectively accounted for 26.07% and 16.8% of total PCoA (Fig. 3b). Hierarchical clustering analysis showed that the microbial

composition of AR patients and HCs presented relative differences based on both unweighted and weighted UniFrac distances (Fig. 3c, d).

Bacterial composition at different taxonomic levels

At the phylum level, the top 10 bacterial phyla in nasal EVs from both AR patients and HCs were listed in Supplementary Table S1. Taxonomic classification at phylum level showed that *Proteobacteria* was the dominant bacterial phylum in both AR patients and HCs (Fig. 4a). However, the abundance of *Proteobacteria* was not significantly different between AR patients and HCs (90.564% in AR patients *vs.* 87.303% in HCs, $P = 0.888$). Among the top 10 bacterial phyla, the *Tenericutes* (0.314% in AR patients *vs.* 0.041% in HCs, $P = 0.002$, Kruskal-Wallis test) and *Verrucomicrobia* (0.067% in AR patients *vs.* 0.006% in HCs, $P = 0.012$, Kruskal-Wallis test) were significantly more abundant in AR patients than that in HCs (Supplementary Table S1, Fig. 4a). At the class level, the top 10 bacteria in nasal EVs from AR patients and HCs were listed in Supplementary Table S2. The dominant bacterial classes were *Alphaproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria* in nasal EVs from both AR patients and HCs (Fig. 4b). The abundance of these three bacterial classes all did not show significant differences between AR patients and HCs ($P = 0.273$ for *Alphaproteobacteria*, $P = 0.086$ for *Gammaproteobacteria*, and $P = 0.226$ for *Betaproteobacteria*). Among the top 10 bacterial classes, *Mollicutes* was significantly more abundant in AR patients than that in HCs (0.314% in AR patients *vs.* 0.041% in HCs, $P = 0.002$, Kruskal-Wallis test) (Supplementary Table S2, Fig. 4b). Within the order taxonomic rank, top 12 bacterial orders in nasal EVs from AR patients and HCs were listed in Supplementary Table S3. The most abundant orders presenting in both AR patients and HCs were *Pseudomonadales*, *Burkholderiales*, *Rhizobiales*, and *Sphingomonadales* (Fig. 4c). These four dominant orders all did not show significant differences between AR patients and HCs ($P = 0.123$ for *Pseudomonadales*, $P = 0.555$ for *Burkholderiales*, $P = 0.129$ for *Rhizobiales*, and $P = 0.778$ for *Sphingomonadales*). In other orders, *Rhodocyclales* was dramatically less abundant in AR patients than that in HCs (1.077% in AR patients *vs.* 5.147% in HCs, $P = 0.009$, Kruskal-Wallis test) (Supplementary Table S3, Fig. 4c). At the family level, the top 10 bacterial families in nasal EVs from AR patients and HCs were listed in Supplementary Table S4. The most abundant families in both AR patients and HCs were *Moraxellaceae*, *Burkholderiaceae*, *Methylobacteriaceae*, and *Sphingomonadaceae* (Fig. 4d). Among the top 10 families, *Zoogloeaceae* showed significantly less abundant in AR patients than that in HCs (1.060% in AR patients *vs.* 5.118% in HCs, $P = 0.028$, Kruskal-Wallis test) (Supplementary Table S4, Fig. 4d). Within the genus taxonomic rank, top 10 genera in nasal EVs from AR patients and HCs were listed in Supplementary Table S5. Among them, *Acinetobacter*, *Ralstonia*, *Methylobacterium*, and *Sphingomonas* were the dominant bacterial genera in both AR patients and HCs (Fig. 4e).The genus *Zoogloea* was dramatically less abundant in AR patients than that in HCs (1.054% in AR patients *vs.* 5.098% in HCs, $P = 0.002$, Kruskal-Wallis test) (Supplementary Table S5, Fig. 4e).

Differential bacterial communities in nasal EVs

To unveil the differential bacterial communities in nasal EVs from AR patients and HCs, LEfSe analysis was performed. The bacterial class that differed markedly between AR patients and HCs was *Mollicutes*. At the order level, *Mycoplasmatales* was increased in AR patients and *Rhodocyclales* was increased in HCs. Analysis at the family level showed ascending levels of *Halomonadaceae* and *Mycoplasmataceae* for AR patients, and ascending levels of *Streptococcaceae*, *Zoogloeaceae*, and *Pseudomonadaceae* for HCs. At the genus level, *Acetobacter*, *Escherichia*, *Halomonas*, and *Mycoplasma* were abundant in AR patients, and *Streptococcus*, *Burkholderia*, *Zoogloea*, and *Pseudomonas* were abundant in HCs (Fig. 5a). The 17 taxa mentioned above presented significant differences between AR patients and HCs (the logarithmic LDA score > 3.0) (Fig. 5b).

Enrichment of microbial metabolic pathways

To analyze the microbial metabolic function in nasal EVs of AR, the PICRUSt based on the KEGG database was conducted to predict corresponding microbial metabolic pathways. Total 35 metabolic pathways with significant differences between AR patients and HCs were identified ($P < 0.05$). Among them, 25 microbial metabolic pathways were unregulated in AR, including valine, leucine and isoleucine degradation, tryptophan metabolism, toluene degradation, propanoate metabolism, and primary bile acid biosynthesis, etc. The rest 10 pathways presented significantly more abundant in HCs than that in AR patients, including selenocompound metabolism, protein kinases, prenyltransferases, porphyrin and chlorophyll metabolism, nitrogen metabolism, glycosyltransferases, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, folate biosynthesis, D-arginine and D-ornithine metabolism, and C5-branched dibasic acid metabolism (Fig. 6). Accordingly, our data suggest that metabolism functions were changed by alteration of bacterial composition.

Discussion

AR is an inflammatory disorder of the upper airway, and EVs as inflammatory drivers are frequently used for investigating the pathogenesis of allergic diseases [28, 29]. This study analyzed the microbial community in nasal EVs from AR patients and HCs. The AR patients had a different microbiome profile, marked by a reduced microbial diversity and altered abundance of specific microbes in nasal EVs compared to HCs. Moreover, some different microbial metabolic pathways were revealed between AR cohorts and HCs.

Alpha diversity is a key quantity in microbiome research, which is defined as the mean diversity of species within a local scale [30]. Alpha diversity can be evaluated by diverse indices, including Chao1, Shannon, Pielou, Simpson, and PD_whole_tree. Among them, Pielou and Simpson indices represent microbial evenness. Our study found that these two indices presented a decreasing trend in nasal EVs of AR patients, indicating the evenness of microbes was reduced in AR. Other studies have also confirmed the reduced microbial evenness in AR. Watts et al. found that the evenness of gut microbiome was reduced in AR suffers [10]. Morin et al. also reported the lower evenness of airways microbiota in AR than that in control [31]. The lack of evenness in the microbial community may impair its capacity to resist

exogenous disturbances, thereby inducing AR [32]. In addition, beta diversity metric revealed relative differences in microbial community between AR patients and HCs. These results demonstrated that there were compositional differences in microbiota of nasal EVs between AR patients and HCs.

The differential abundance of specific microbial taxa is also a key point in microbiome analysis of AR. In this study, the differences on microbial composition and abundance were observed in nasal EVs between AR patients and HCs. A previous study found that *Tenericutes* is relatively more abundant in mice with allergic asthma [33]. Our study also found that *Tenericutes* at the phylum level also has a higher abundant in nasal EVs from AR patients than that in HCs. This result suggested that *Tenericutes* may be a harmful bacterium contributing to AR. In addition, LEfSe analysis found that *Zoogloea*, *Streptococcus*, *Burkholderia*, and *Pseudomonas* were enriched in HCs. A previous study suggested that *Streptococcus* reduces the nasal allergic reaction by producing H_2O_2 to inhibits IgE-stimulated degranulation[34]. *Pseudomonas* could ameliorate allergic sensitization in asthma mice via the regulation of T cell response [35]. Our results confirmed the potential beneficial role of *Streptococcus* and *Pseudomonas*, and indicated that *Zoogloea* and *Burkholderia* may also be the potential beneficial bacteria. Furthermore, relative high abundances of *Acetobacter*, *Mycoplasma*, *Escherichia*, and *Halomonas* were reveled in nasal EVs from AR patients at the genus level, indicating potential harmful roles of these bacteria in human.

Microbiota have the ability to influence various metabolic responses in host, thereby moderating growth process and chronic diseases occurrence [36]. According to the PICRUSt algorithm, we found that 35 microbial metabolic pathways were significantly different in AR patients and HCs. Among them, 25 pathways were relatively more abundant in AR patients. The majority of these metabolic pathways were found to be associated with allergic sensitization and inflammatory [37–41]. For instance, tryptophan metabolism can attenuate the airway inflammatory during immunotherapy in a rat asthma model [37]. Linoleic acid metabolism may be related to the increased asthma severity [40]. Geraniol has been proved to possess the anti-inflammatory properties in AR [41]. Our study further suggested that the microbiota in nasal EVs may influence these metabolic pathways, thereby affecting the development of AR.

In conclusion, the microbiome composition and diversity in nasal EVs had significantly differences between AR patients and HCs. Among bacteria in nasal EVs, *Zoogloea*, *Streptococcus*, *Burkholderia*, and *Pseudomonas* may be the beneficial bacteria, while *Acetobacter*, *Mycoplasma*, *Escherichia*, and *Halomonas* may be the harmful bacteria for AR. Moreover, 25 microbial metabolic pathways may be closely associated with AR. These findings will be useful for investigating the pathogenesis of AR and provide the basis for clarifying the potential mechanisms of microbial metabolism. However, there are still some limitations in this study. First, a larger sample size is needed to be utilized for confirming the differences of microbiome composition and diversity between AR patients and HCs. Second, it will be necessary to further study the mechanisms by which the core bacterial taxa regulating AR development.

Declarations

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Author contributions

Conceptualization, T.Y.C. and W.H.C.; methodology, C.H.F. and T.J.L.; formal analysis, T.Y.C; investigation, T.Y.C. and Y.R.Y.; resources, M.Y.Z, F.Y and Y.F.Z., data curation, T.Y.C, writing-original draft preparation, T.Y.C.; writing-review and editing, L.C. and C.J.C.; supervision, C.J.C.; project administration, C.J.C. All authors have read and approved the published version of the paper.

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Data Availability Statement: Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as online supplemental information. The data that support the findings of this study are available from the corresponding author, on reasonable request.

Conflicts of Interest disclosures: The authors declare that they have no conflict of interest.

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Tables

Table 1. The clinical characteristics of participants.

Variables	HC	AR	*P*-value
Subjects (N)	19	20	-
Gender (M/F)	12/7	10/10	NS
Age (years)	25 ± 10	30 ± 6	NS
Eosinophil (%)	1.90 (0.20-5.10)	3.30 (1.90-13.00)	< 0.01
Total serum IgE (IU/mL)	25.09 (4.23-92.20)	188.75 (91.42-1597.00)	< 0.01
Serum sIgE-D1 (kUA/L)	0.03 (0.01-0.55)	22.65 (0.72-76.60)	< 0.0001
Serum sIgE-D2 (kUA/L)	0.01 (0.00-0.64)	18.55 (0.33-88.20)	< 0.0001

HC, healthy control; AR, allergic rhinitis; N, number; M, male; F, female; NS, not significant; IgE, immunoglobulin E; sIgE, specific immunoglobulin E; D, dust mite. Data were shown as mean ± standard deviation (SD), or median (interquartile range). *P*-value < 0.05 was considered as statistical significance after performing t-test.

Figures

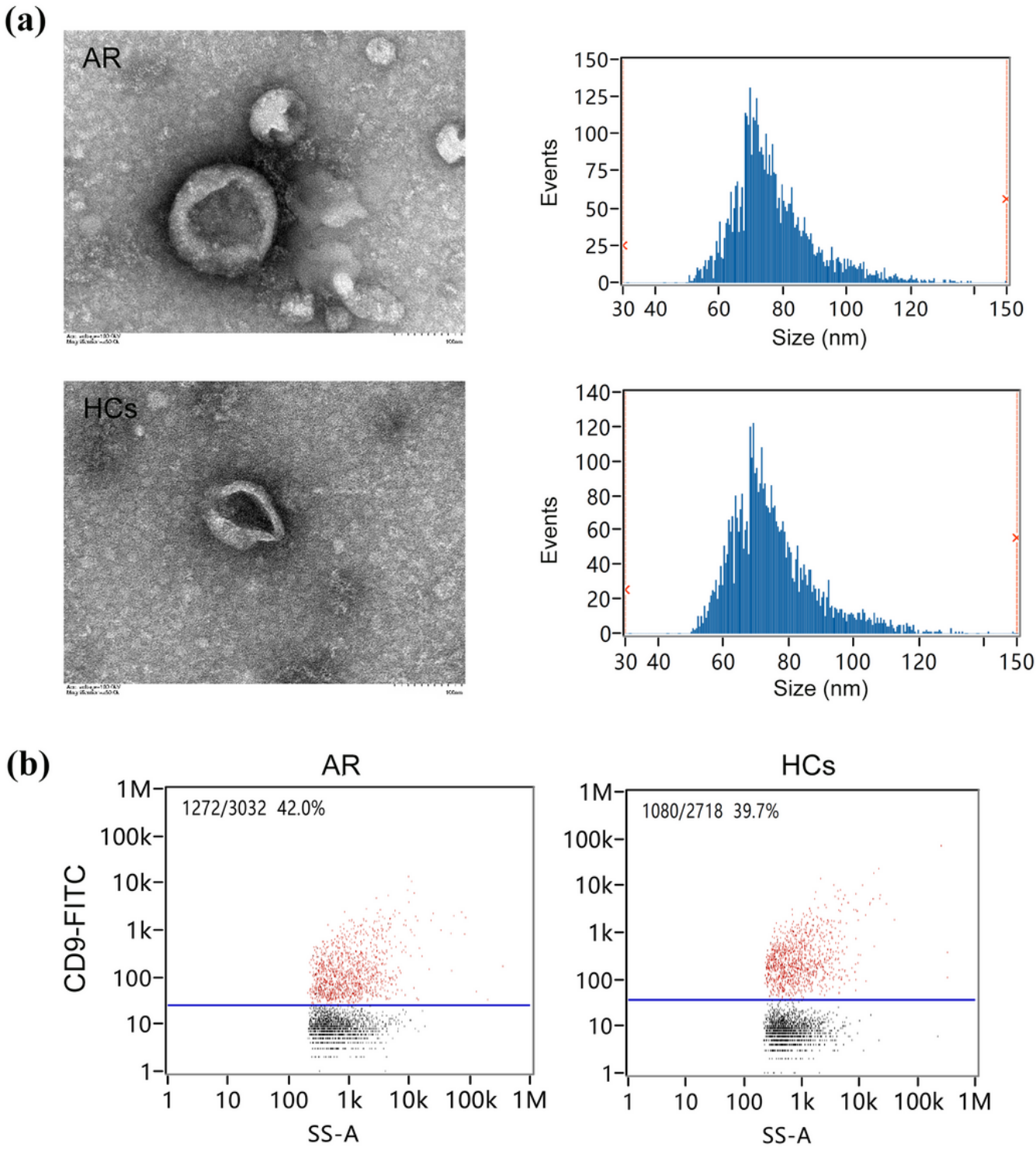

Figure 1

The characterization of nasal extracellular vesicles (EVs) from allergic rhinitis (AR) patients and health controls (HCs). (a) The morphology of nasal EVs was observed under transmission electron microscopy. Scale bar = 100 nm. (b) Nasal EVs positive for CD9 were measured using nano-flow cytometry.

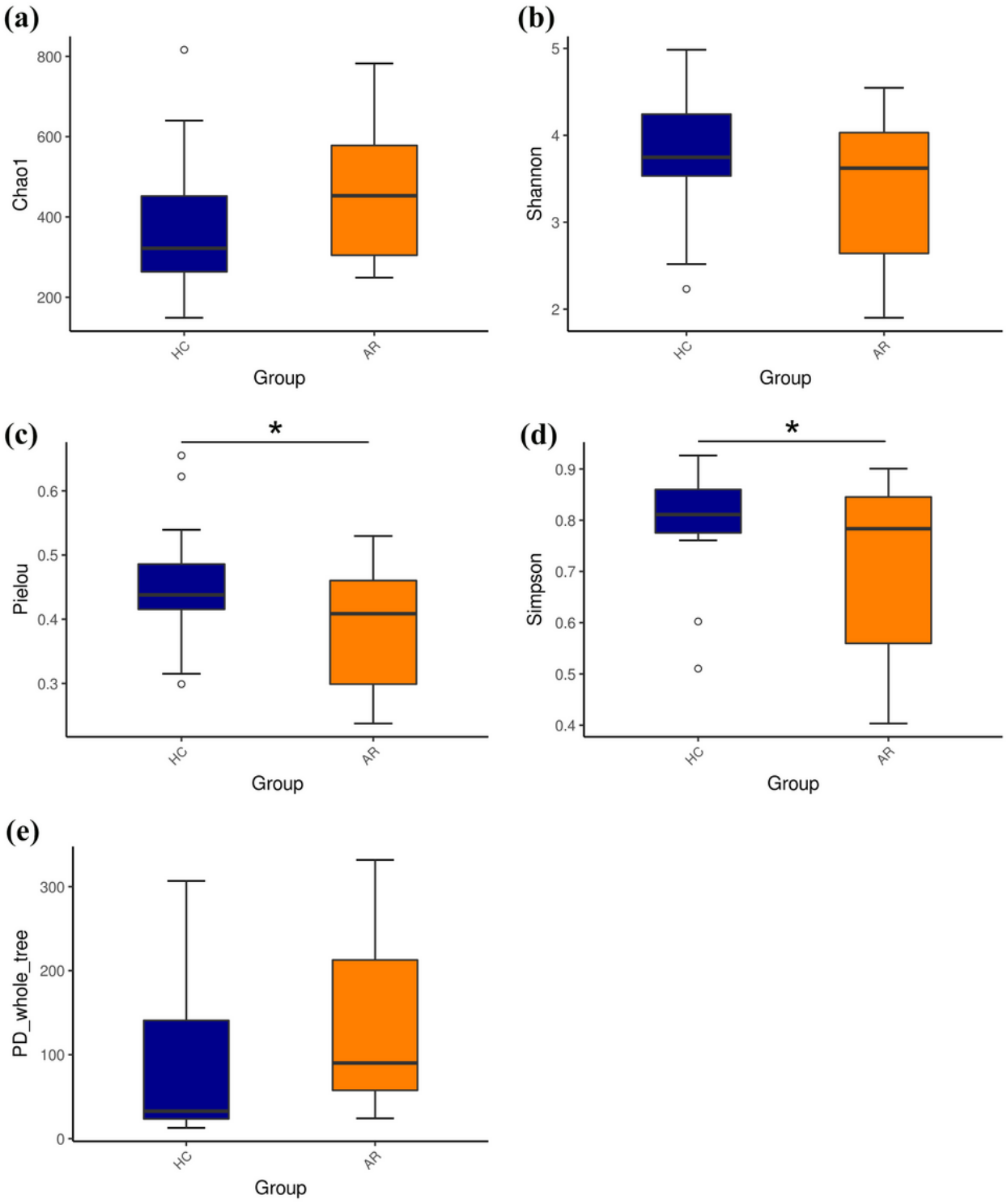

Figure 2

Alpha diversity metrics for AR and HCs. (a) Chao1 index. (b) Shannon index. (c) Pielou index. (d) Simpson index. (e) PD_whole_tree index. *P < 0.05.

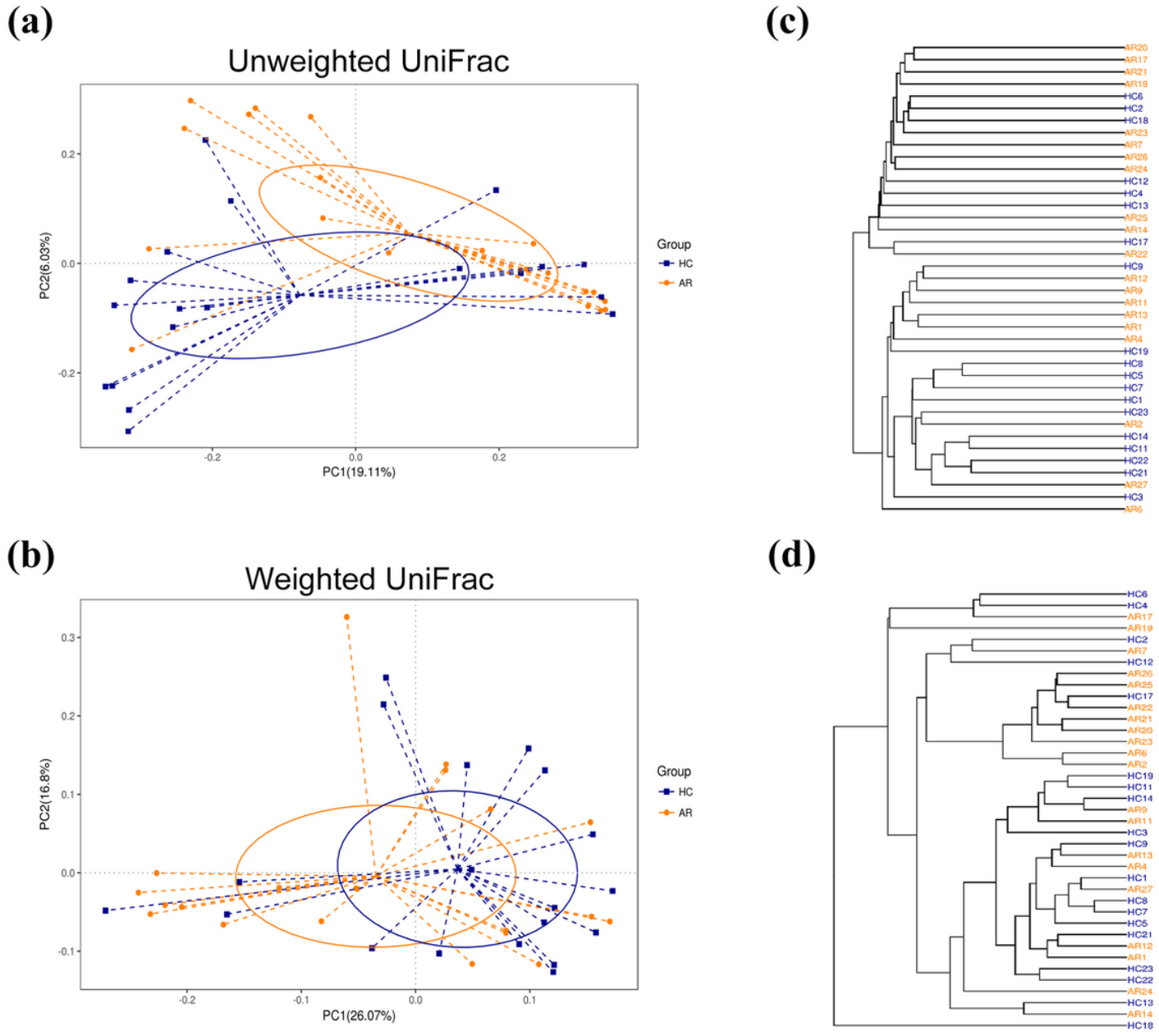

Figure 3

Beta diversity for AR and HCs. (a) Principal coordinates analysis (PCoA) of the unweighted UniFrac distance. (b) PCoA of the weighted UniFrac distance. (c) Hierarchical clustering of AR and HC samples based on the unweighted UniFrac distance. (d) Hierarchical clustering of AR and HC samples based on the weighted UniFrac distance.

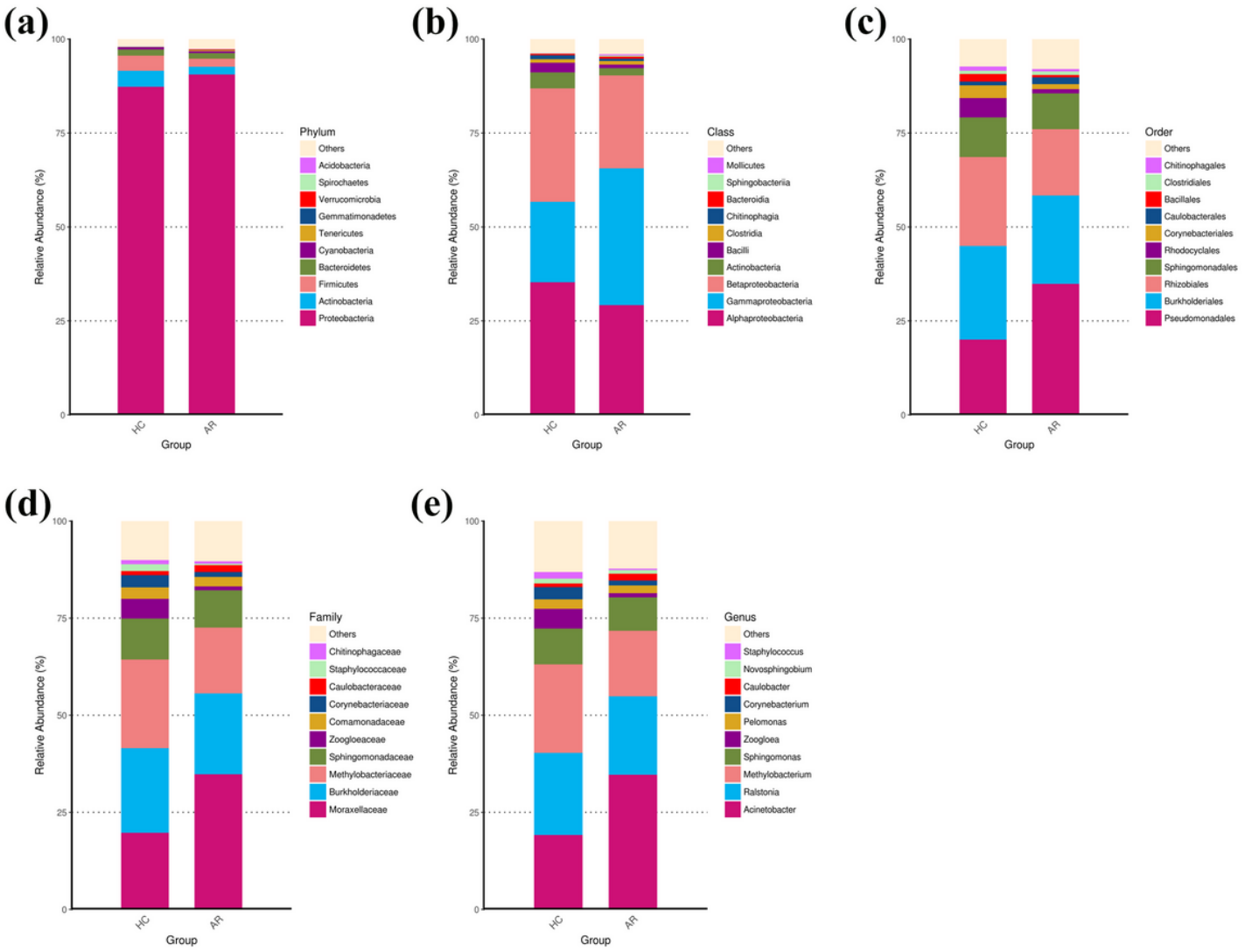

Figure 4

Relative abundance of bacteria at different taxonomic levels in nasal EVs from AR patients and HCs. (a) Phylum level. (b) Class level. (c) Order level. (d) Family level. (e) Genus level.

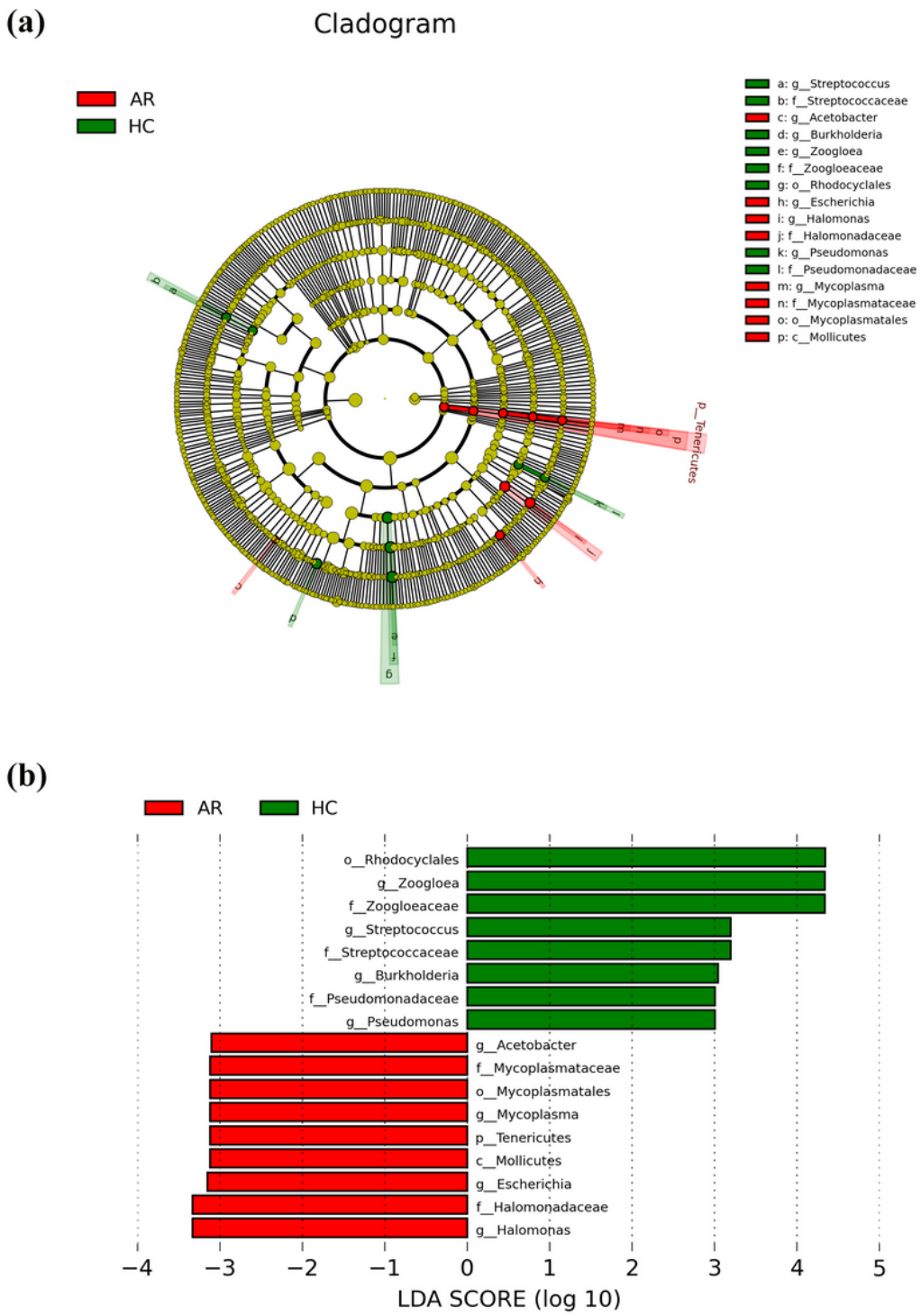

Figure 5

Compositional difference of microbiota in nasal EVs from AR patients and HCs. (a) A linear discriminant analysis (LDA) effect size (LEfSe) analysis for AR and HCs. (b) The enriched bacteria in AR (red) and HCs (green) with the logarithmic LDA scores > 3.0.

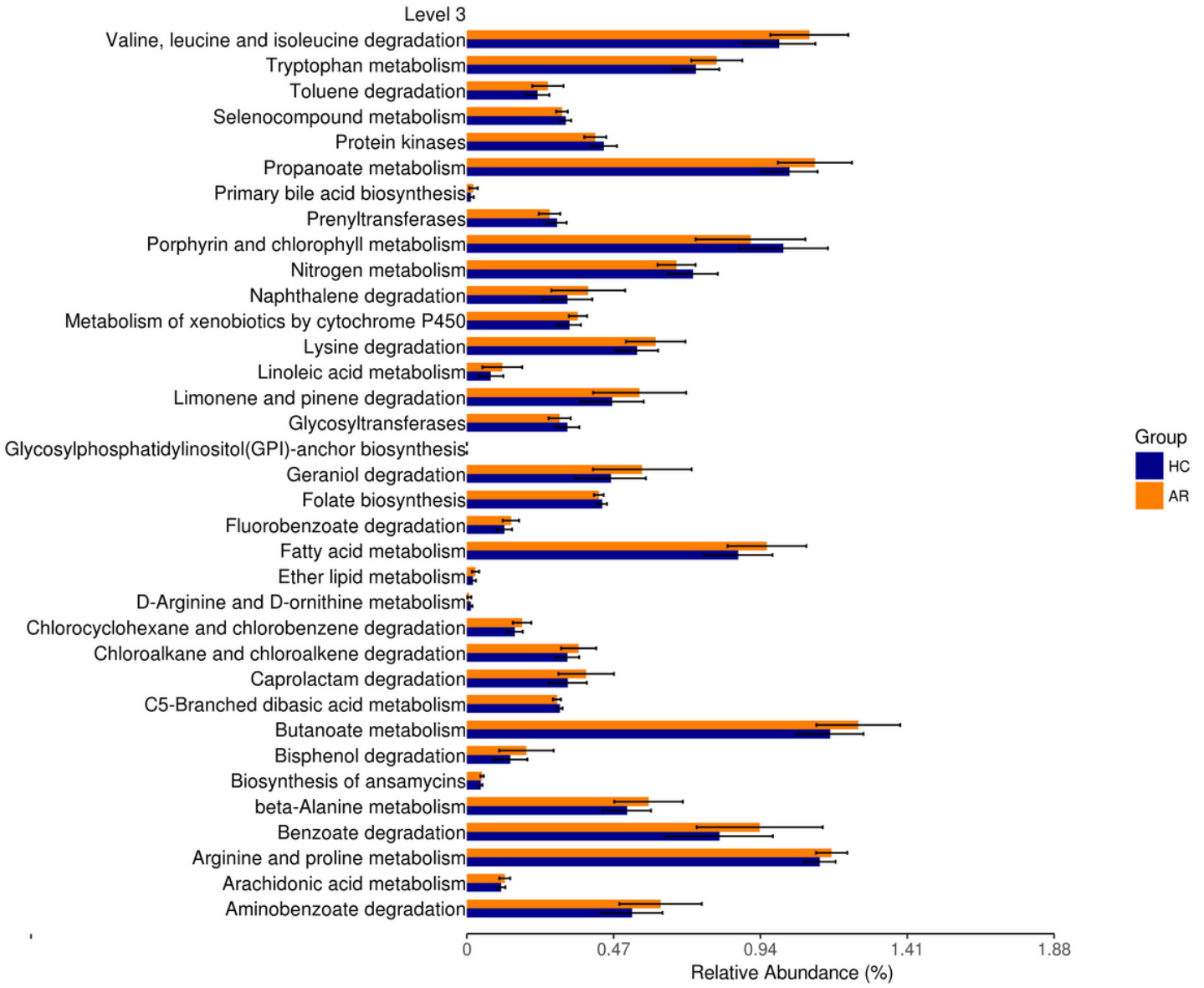

Figure 6

Microbial metabolic pathways relevant to AR and HCs. These pathways all differed significantly between AR and HCs ($P < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.xlsx
- TableS3.xlsx
- TableS4.xlsx
- TableS5.xlsx