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# Urine Bacteria-Derived Extracellular Vesicles and Allergic Airway Diseases in Children

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**Keywords**
Asthma · Rhinitis · Microbiota · Extracellular vesicle · Metagenomic analysis

**Abstract**
***Background:*** Microbiota and human allergic airway diseases have been proven to be interrelated. Bacteria-derived extracellular vesicle (EV)s are known to play important roles in interbacterial and human-bacteria communications, but their relationship with allergies has not been examined yet. Urine EVs were investigated to determine whether they could be used as biomarkers for monitoring allergic airway diseases in children. ***Methods:*** Subjects were 4 groups of chronic rhinitis (CR), allergic rhinitis (AR), atopic asthma (AS) and healthy controls. Single voided urine samples were collected. Urine EVs were isolated and their DNA was extracted for 16S-rDNA pyrosequencing. ***Results:*** A total of 118 children participated in this study; 27, 39, 19, and 33 were in the CR, AR, AS, and control group, respectively. The AR had a significantly high Chao-1 index than that of controls. Principal component analysis revealed dysbiosis in the CR, AR, and AS compared to the controls. One phylum and 19 families and genera were significantly enriched or depleted in the disease groups compared to the controls; the Actinobacteria phylum and the Sphingomonadaceae family were more abundant in the AS and CR, the Comamonadaceae family, the Propionibacteraceae family, *Propionibacterium* and *Enhydrobacter* were more enriched in the CR, and the Methylobacteriaceae family and *Methylobacterium* were more abundant in each disease group, while the Enterobacteriaceae family was depleted in each disease group. ***Conclusions:*** CR, AR, and AS had a distinct composition of urine EVs. Urine EVs could be an indicator for assessing allergic airway diseases in children.
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## Introduction

Bacteria-derived extracellular vesicle (EV)s play crucial roles in the bacterium interactions with other bacterium and with the host. They act as a bridging factor in biofilms, play roles in the elimination of competing bacteria, modulate host immune responses, and transmit virulence factors into host cells [1]. Therefore, EVs were as-

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sumed to have important roles in the relation between microbiota and allergic diseases, which may be superior to those of the microbiota itself.

Recent airway studies have analyzed both microbiota and EVs in nasal lavage and lung tissue samples [2, 3]. EVs have distinct characteristics in relation to chronic rhinitis and chronic obstructive pulmonary diseases, and hence they have been proposed to play essential roles in immune dysfunction and inflammation in the airway diseases. However, the relation between EVs and allergic airway disease has not been examined yet.

EVs have been reported to move between body compartments; they have been found to be partially accumulated in the kidneys and excreted in urine after intraperitoneal and intramuscular injections in mice [4, 5]. In addition, EVs are highly stable and can be easily detected in biological fluids [1]. These characteristics support the possibility of using EVs as a biomarker if they are proven to have specific features relating to allergic airway diseases.

We hypothesized that urine EVs may have distinct patterns of bacterial diversity and abundance in allergic airway diseases that might be an indicator for allergic airway monitoring in children. Thus, we evaluated urine EVs in children with allergic airway diseases.

## Methods

*Participants and Sample Collection*

The subjects were children who visited the Inha University Hospital from 2013 to 2015. The inclusion criteria were: age 5–12 years, average BMI, and no concurrent major health concern. Exclusion criteria were: a history of antibiotic intake within 2 weeks before urine sampling and presence of urinary symptoms.

The controls were free of a history suggesting chronic rhinitis or allergic diseases and had negative skin prick tests. The chronic rhinitis group (CR) was defined by nasal symptoms for more than 12 weeks [6] and negative skin prick tests. Atopy was confirmed with sensitivity to one or more aeroallergens via skin prick tests and/or specific IgE. Among the atopic subjects, the allergic rhinitis group (AR) was characterized by the presence of one or more nasal symptoms without nasal polyps [7], and atopic asthma group (AS) was identified if the patient had suffered from at least one asthma attack in the previous 12 months with $PC_{20}$ (concentration of methacholine/provocholine causing a 20% decrease in $FEV_1$) of 16 mg/mL or less [8]. Two milliliters of voided clean catch midstream urine were collected and stored at –20 °C.

This study was approved by the Institutional Review Board of the Inha University Hospital (IRB No. 2017-05-008). Written informed consent was obtained from the patients and their parents/guardians.

*Urine EVs Isolation and DNA Extraction*

To isolate the EVs, the urine samples underwent differential centrifugation by a microcentrifuge (Labogene 1730R; BMS, Korea) at 10,000 *g* for 10 min at 4 °C [9]. The supernatant was filtered through a 0.22 μm filter to eliminate bacteria and foreign particles. For DNA extraction, the isolated EVs were boiled at 100 °C for 40 min and centrifuged at 13,000 rpm for 30 min at 4 °C to eliminate the remaining floating particles and waste. Subsequently, the supernatant was collected and subjected to DNA isolation using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories; Carlsbad, CA, USA) according to the standard protocol for DNA extraction. The DNA in each sample was quantified using a QIAxpert system (Qiagen, Hilden, Germany).

*16S rDNA Sequencing and Taxonomic Assignment*

Bacterial genomic DNA PCR amplification was executed with the primers 16S_V3_F (5′- TCGTCGGCAGCGTCAGATGTG-TATAAGAGACAGCCTACGGGNGGCWGCAG-3′) and 16S_V4_R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC-AGGACTACHVGGGTATCTAATCC-3′), which are specific for V3-V4 hypervariable regions of the 16S rDNA gene. The libraries were prepared using PCR products according to the MiSeq System User Guide (Illumina Inc., San Diego, CA, USA) and quantified using a QIAxpert system. Each amplicon was then pooled at the equimolar ratio and sequenced on MiSeq according to the manufacturer's recommendations.

The obtained raw pyrosequencing reads were filtered according to the barcode and primer sequences using MiSeq. Sequencing lengths ≥300 bp and average PHRED scores ≥20 (quality score) were used to select the high-quality sequencing reads. Operational taxonomy units (OTU) (>97% similarity) were clustered using the sequence-clustering algorithm CD-HIT [10]. Subsequently, taxonomy assignment was carried out by UCLUST and QIIME against the 16S rDNA sequence database in GreenGenes 8.15.13 [11, 12]. Based on sequence similarities, all 16S rDNA sequences were assigned to the following taxonomic levels. In cases in which the clusters could not be assigned at the genus level due to a lack of sequences or redundant sequences in the database, the taxon was assigned to the higher level, which was indicated in parenthesis. Genera that did not have a generic name were given the family name and annotated by (f). Taxonomic assignment was performed by the MDx-Pro profiling program (version 1; MD Healthcare Inc., Seoul, Republic of Korea).

*Statistical Analysis*

Biodiversity analyses were performed at the genus level. The α diversity of each group was assessed using the Chao-1 richness index at 14,000 reads. After rarefaction, the *t* test was used to assess the significance of differences between groups [13]. Principal component analysis based on the Euclidean distance was used to evaluate the β diversity [14]. Biodiversity analysis was performed using R version 3.4.1.

The nonparametric Wilcoxon rank-sum test was used to compare bacterial relative abundances between each disease group and the controls with the aid of MatLab 2015. Taxa that were detected in fewer than 50% of the samples or comprised less than 1% of the average composition of the enriched group were discarded. Bacterial genera that had a significant fold change against the controls were plotted in a heat map with hierarchical clustering using R version 3.4.1.

A *t* test was used to test the differences between each disease group and the controls for continuous variables. The presence of a correlation between either reads or OTU counts with age and



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**Table 1.** Demographics of the study subjects

| | Controls ($n$ = 33) | CR ($n$ = 27) | | AR ($n$ = 39) | | AS ($n$ = 19) | |
|---|---|---|---|---|---|---|---|
| | median | median | $p$ value | median | $p$ value | median | $p$ value |
| Age, years | 8 (5–12) | 7 (5–12) | 0.471* | 8 (5–11) | 0.305* | 9 (5–12) | 0.477* |
| Males | 19 (58) | 17 (63) | 0.607* | 28 (72) | 0.207* | 14 (74) | 0.254* |
| IgE | 77.01 (6.31–499.44) | 29.11 (5.06–287.26) | 0.582† | 176.84 (14.44–1,000) | <0.001† | 281.215 (26.67–1,000) | <0.001† |
| Eosinophil count, $\times 10^9$ cells/L | 175 (50–470) | 150 (30–460) | 0.383† | 460 (83–5,800) | 0.008† | 410.5 (59–1,103) | 0.006† |
| Eosinophils, % | 2.5 (0.8–5.6) | 2.5 (0.5–7.6) | 0.793† | 5.25 (1–15.8) | <0.001† | 7.3 (0.9–15.5) | <0.001† |
| Neutrophils, % | 53.4 (35.5–73.3) | 47.9 (37–64.1) | 0.016† | 45.45 (25.4–83.8) | 0.018† | 47.3 (39–65.3) | 0.050† |
| Positive sensitization to aeroallergens$ | 0 | 0 | – | 2 (1–4) | – | 2 (1–6) | – |

Values are presented as medians (range) or numbers (%). CR, chronic rhinitis group; AR, allergic rhinitis group; AS, atopic asthma group. The $p$ values were calculated in comparison with the controls using the * $\chi^2$ or an † $t$ test. $ Count of positive specific SPT and/or serum-specific IgE to aeroallergens.

duration of sample preservation was tested via Pearson's correlation, while that with sex was tested via a $t$ test. A $\chi^2$ test was used to compare the study subjects' characteristics between each disease group and the controls. The $p < 0.05$ or fold changes of more than 2 were considered statistically significant. These statistical analyses were performed using MatLab 2015.

## Results

A total of 118 children were enrolled in this study, including the controls ($n$ = 33) and the CR ($n$ = 27), AR ($n$ = 39), and AS (n = 19). The AR and AS had significantly higher serum total IgE levels and blood eosinophils compared to the controls and were sensitized to 1–6 aeroallergens. All AS cases were well controlled (steps 1–3 by GINA guidelines) and associated with allergic rhinitis [8]. The characteristics of the study subjects are shown in Table 1.

The 16S-rRNA gene sequencing resulted in 1,287,892 reads and 8,069 OTU. The number of OTU and the number of reads did not differ with age, sex, or the duration of sample preservation (for OTU: age, $p$ = 0.162; duration of preservation, $p$ = 0.871; and sex, $p$ = 0.880) (for reads; age, $p$ = 0.936; duration of preservation, $p$ = 0.912; and sex, $p$ = 0.227).

The AR had a significantly higher Chao-1 richness index compared to the controls ($p$ = 0.049) (Fig. 1). In the principle component analysis, the samples of the CR, AR, and AS were clustered similarly with a degree of overlap with the controls; however, it denoted dysbiosis in the diseased groups (Fig. 2).

The major phyla detected in all samples were Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria, which together constituted 96.5–97% of the community composition. The phylum Actinobacteria was significantly abundant in the AS and CR compared to the controls (CR, $p$ = 0.017; AR, $p$ = 0.210; and AS, $p$ = 0.004) and members of the Propionibacteraceae, Corynebacteraceae, and Micrococcaceae families were major presenting taxa of the Actinobacteria; however, members of the Propionibacteraceae and Corynebacteraceae families were more frequently detected in the 3 disease group samples. The Micrococcaceae family and *Micrococcaceae*(f) were significantly more abundant in the CR, AR, and AS, whereas Propionibacteraceae family and the *Propionibacterium* genus were significantly more abundant in the CR compared to the controls. Neither the Corynebacteraceae family nor the *Corynebacterium* genus differed among the study groups.

Proteobacteria was the most abundant phylum in all groups and it was slightly more abundant in the controls compared to the disease groups, with no statistical significance. In comparison to the controls, the Methylobacteriaceae family, *Methylobacteriaceae*(f), the *Methylobacterium* genus, and *Sphingomonadaceae*(f) were significantly enriched in the CR, AR, and AS, and the Sphingomonadaceae family and *Comamonadaceae*(f) were significantly more abundant in the CR and AS, while the Comamonadaceae family and the *Enhydrobacter* genus were remarkably enriched in the CR (Table 2). The Enterobacteriaceae family, *Enterobacteriaceae*(f), the Rhizobiaceae family, the *Agrobacterium* genus, the Alcaligenaceae family, and the *Achromobacter* genus showed a greater prevalence in the controls compared to each disease group. Eleven of the control subjects had a history of eczema. The bacterial relative abundances in controls



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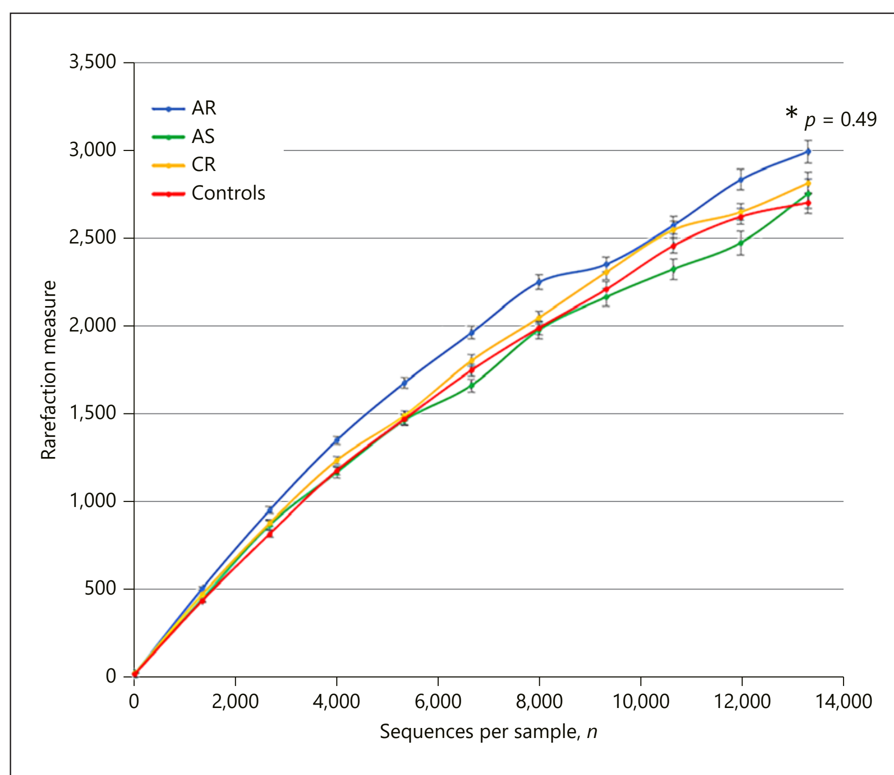


**Fig. 1.** The α diversity via the Chao-1 richness index is shown. The AR bacterial richness is significantly higher than that of the controls. * Statistically significant *p* value compared to the controls, determined by *t* tests. CR, chronic rhinitis group; AR, allergic rhinitis group; AS, atopic asthma group.

with a history of eczema ($n = 11$) and those without ($n = 22$) were compared, and no significant difference was found (data not shown).

The classification and abundance of families and genera that significantly differed between the controls and the disease groups are displayed in Table 2. The bacterial genera that had a significant fold-change in any disease group against the controls were plotted in a heat map to show the bacterial abundance in each sample (Fig. 3). Online supplementary Figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000492677) consists of taxonomic stack bars at the phylum, class, order, family, and genus levels showing the taxa that represented more than 1% of the bacterial composition of any group.

## Discussion

Recent evidence indicates that EVs have key roles in intercellular communication between the host and commensal microbes in terms of both physiologic and pathogenic processes [1]. In this study, we investigated, for the first time, whether urine EVs have a distinct pattern of bacterial abundance and diversity in children with allergic airway diseases and then we searched whether the alterations we detected had been correlated with CR, AR, or AS in previous airway microbiota studies.

Regarding bacterial diversity, the AR was the richest among the study groups, while, the CR and AS were very close to the controls. In previous airway studies, α diversity results were contradictory, which may be attributed to nonuniformity of the sampling sites and techniques [15, 16, 17, 18]. The CR, AR, and AS samples had similar principle component analysis clustering and, in spite of the presence of overlap between the controls and the disease groups, the results denoted the presence of dysbiosis in urine EVs of the CR, AR, and AS. The major phylum of urine EVs in all groups was Proteobacteria, followed by Firmicutes and Actinobacteria, which are consistent with a recent study on urine EVs in young adults [19].

In the current study, the phylum Actinobacteria notably occupied the urine EVs community in the CR and AS, the Micrococcaceae family and *Micrococcaceae*(f) were significantly more abundant in the CR, AR, and AS, and the Propionibacteraceae family and *Propionibacterium* were remarkably abundant in the CR, whereas *Corynebacterium* did not show a significant abundance or depletion in any disease group in comparison with the con-



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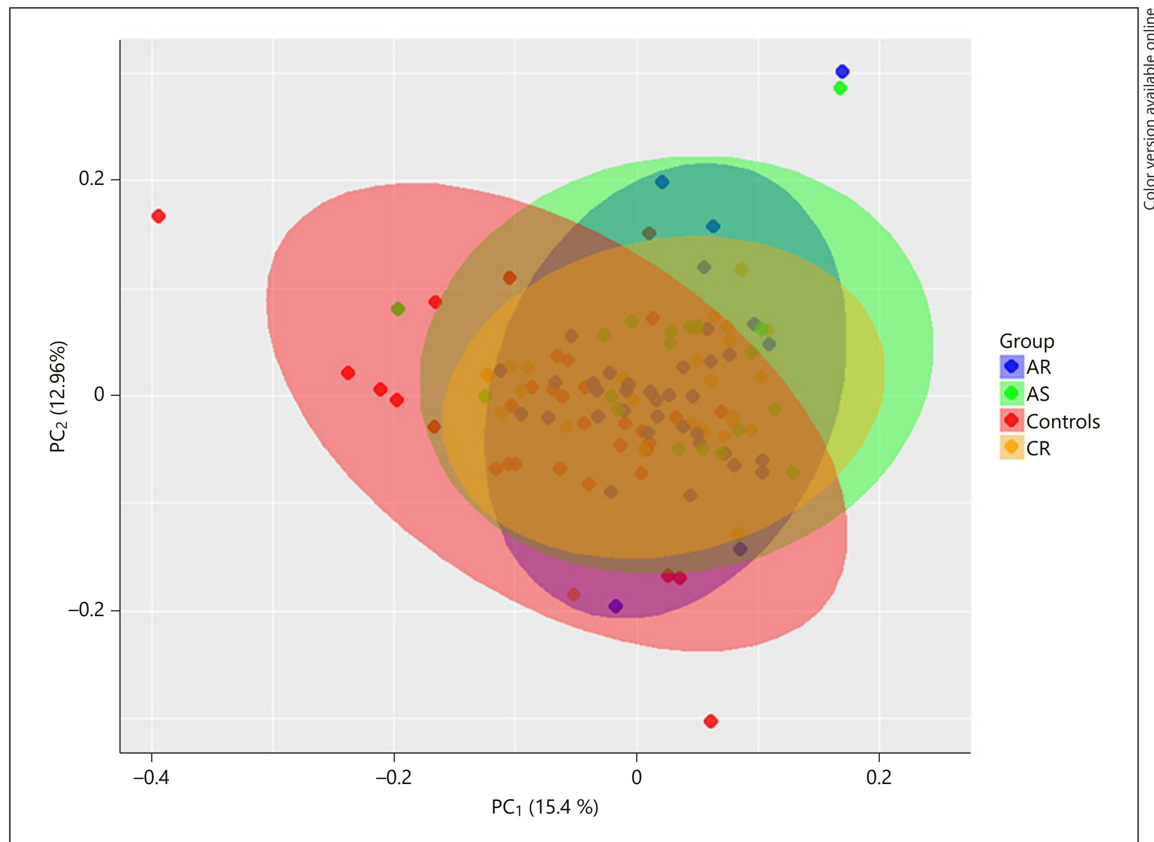


**Fig. 2.** Principal component analysis based on the Euclidean distance. Samples as represented by the first 2 principal components. Each point represents a single sample. The disease groups were clustered similarly with a degree of overlap with the control samples. CR, chronic rhinitis group; AR, allergic rhinitis group; AS, atopic asthma group.

trols. The Actinobacteria, *Propionibacterium*, and *Corynebacterium* were reported to be major members in airway microbiota; however, to the best of our knowledge, Micrococcaceae has not been reported in normal or atopic airway microbiota studies [20, 21]. Previous studies identified *Propionibacterium* and *Corynebacterium* as core taxa in nasal and sinus microbiota in CR patients and healthy subjects [22–24]. In a study on microbiota and EVs in nasal lavage, both *Propionibacterium* and *Corynebacterium* were significantly higher in chronic rhinitis patients compared to the controls [2]. Another study on sinus microbiota found that patients more enriched with Actinobacteria and *Corynebacterium* had better postoperative outcomes [17]. Airway microbiota studies in asthma have also commonly reported Actinobacteria in both asthma and healthy subjects [15, 21, 25]. Actinobacteria was found to be higher in bronchial brushing from patients with asthma compared to controls and in patients with severe asthma compared to those with mild-to-moderate asthma and controls [26, 27]. Taking the previous reports on airway microbiota together with our results in urine EVs, we suggest that Actinobacteria could have a role in the pathogenesis of asthma and chronic rhinitis. However, the pathologic mechanism has not been elucidated yet. Actinobacteria was only reported to be positively associated with eosinophil infiltration and elastin content in lung samples of chronic obstructive pulmonary diseases patients [28]. Reports on *Corynebacterium* appeared to be contradictory; therefore, further studies at the species level may clarify its role in chronic rhinitis.

Sphingomonadaceae was found to be more enriched in the bronchial brushings of atopic asthmatics than in those of atopic nonasthmatics and controls, and its level was strongly correlated with blood and sputum eosinophilia and serum IgE levels [27]. Another study on micro-



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**Table 2.** Families and genera that were significantly enriched or depleted in the CR, the AR, and the AS compared to the controls

| Phylum/family and | Control | CR | | AR | | AS | |
|---|---|---|---|---|---|---|---|
| genus | mean % | mean % | *p* value | mean % | *p* value | mean % | *p* value |
| **Actinobacteria** | | | | | | | |
| Micrococcaceae | 1.72 | 4.74* | 0.001 | 3.55* | 0.034 | 6.39* | 0.003 |
| *Micrococcaceae(f)* | 0.93 | 3.30* | <0.001 | 2.22* | 0.038 | 4.59* | 0.007 |
| Propionibacteriaceae | 0.87 | 2.25* | 0.036 | 1.05 | 0.718 | 1.70 | 0.106 |
| *Propionibacterium* | 0.86 | 2.24* | 0.046 | 0.98 | 0.91 | 1.70 | 0.094 |
| **Proteobacteria** | | | | | | | |
| Methylobacteriaceae | 1.12 | 8.26* | <0.001 | 7.78* | <0.001 | 9.59* | <0.001 |
| *Methylobacteriaceae(f)* | 0.45 | 1.92* | <0.001 | 1.54* | 0.002 | 1.74* | <0.001 |
| *Methylobacterium* | 0.67 | 6.34* | <0.001 | 6.23* | <0.001 | 7.85* | <0.001 |
| Rhizobiaceae | 9.36 | 5.56 | 0.019 | 4.44*,¯ | 0.003 | 4.98 | 0.015 |
| *Agrobacterium* | 8.52 | 5.10 | 0.025 | 3.80*,¯ | 0.001 | 4.69 | 0.017 |
| Sphingomonadaceae | 11.25 | 14.85 | 0.045 | 13.33 | 0.164 | 15.70 | 0.023 |
| *Sphingomonadaceae(f)* | 0.46 | 1.61* | <0.001 | 1.71 | <0.001 | 1.93* | <0.001 |
| Alcaligenaceae | 6.81 | 2.97*,¯ | 0.013 | 2.75*,¯ | 0.014 | 3.67 | 0.051 |
| *Achromobacter* | 6.68 | 2.89*,¯ | 0.011 | 2.70*,¯ | 0.014 | 3.60 | 0.043 |
| Comamonadaceae | 4.52 | 6.13 | 0.048 | 4.93 | 0.804 | 5.91 | 0.053 |
| *Comamonadaceae(f)* | 0.71 | 1.69* | 0.005 | 1.16 | 0.372 | 1.58* | 0.001 |
| Enterobacteriaceae | 8.98 | 4.16*,¯ | 0.003 | 5.94 | 0.016 | 3.36*,¯ | 0.003 |
| *Enterobacteriaceae(f)* | 8.72 | 3.43*,¯ | 0.002 | 4.06*,¯ | 0.007 | 2.84*,¯ | 0.001 |
| Moraxellaceae | 4.42 | 5.11 | 0.789 | 4.50 | 0.584 | 3.99 | 0.864 |
| *Enhydrobacter* | 0.45 | 1.75*,¯ | 0.024 | 0.52 | 0.214 | 0.39 | 0.918 |

The taxa that lacked a genus name are annotated by (f) (i.e., family name). The relative abundance of bacteria is represented as mean percents. CR, chronic rhinitis group; AR, allergic rhinitis group; AS, atopic asthma group. * Bacteria exhibited greater than 2-fold changes in abundance between the controls and the CR, the AR, or the AS groups. ¯ The taxa were more abundant in the controls. The *p* values were determined by comparing the disease groups to the controls using the Wilcoxon rank-sum test.

biome in bronchial brushing revealed that Sphingomonadaceae and Comamonadaceae were well correlated with the degree of bronchial hyperresponsiveness [16]. Notably, the present study reported that urine EVs of Sphingomonadaceae and the genera *Sphingomonadaceae*(f) and *Comamonadaceae*(f) were significantly higher in the AS compared to the controls. The cell wall glycosphingolipids of Sphingomonadaceae, which are present in the EVs wall as well, was found to have the ability to induce airway hyperreactivity via activation of natural killer T cells to produce TH2 cytokines [29], which may explain the relative enrichment of the AS with Sphingomonadaceae.

In the present study, Methylobacteriaceae and *Methylobacteriaceae*(f) and *Methylobacterium* were strongly enriched in the CR, AR, and AS in comparison with the controls. A study on lung tissue microbiota and EVs in chronic obstructive pulmonary disease patients, healthy smokers, and healthy nonsmokers found *Methylobacterium* to be one of the most predominant genera, indicating its importance in airway microbiota composition [3]. In addition, Methylobacterium was found to be abundant in bronchoalveolar lavage of clinically well infants with cystic fibrosis [30]. Furthermore, *Methylobacterium* species were reported to be a cause of healthcare-associated infection [31]. These findings may reveal the potential respiratory pathogenicity of *Methylobacterium*, which might explain its abundance in the disease groups. Methylobacteriaceae family members are methylotrophs; they use 1-carbon substrates, e.g. carbon monoxide, for nutrition [32]. Carbon monoxide is known to be elevated in exhaled air in chronic inflammatory airway diseases including asthma and rhinitis [33]; therefore, we assumed that it may make the chronically inflamed airways a suitable habitat for Methylobacteriaceae. Our findings coupled with previous findings denote that Methylobacteriaceae could have a role in chronic airway inflammation, but further studies are needed to clarify their relationship.

Our results detected a significant enrichment with *Enhydrobacter* in the CR. A study reported that *Enhydrobac-*



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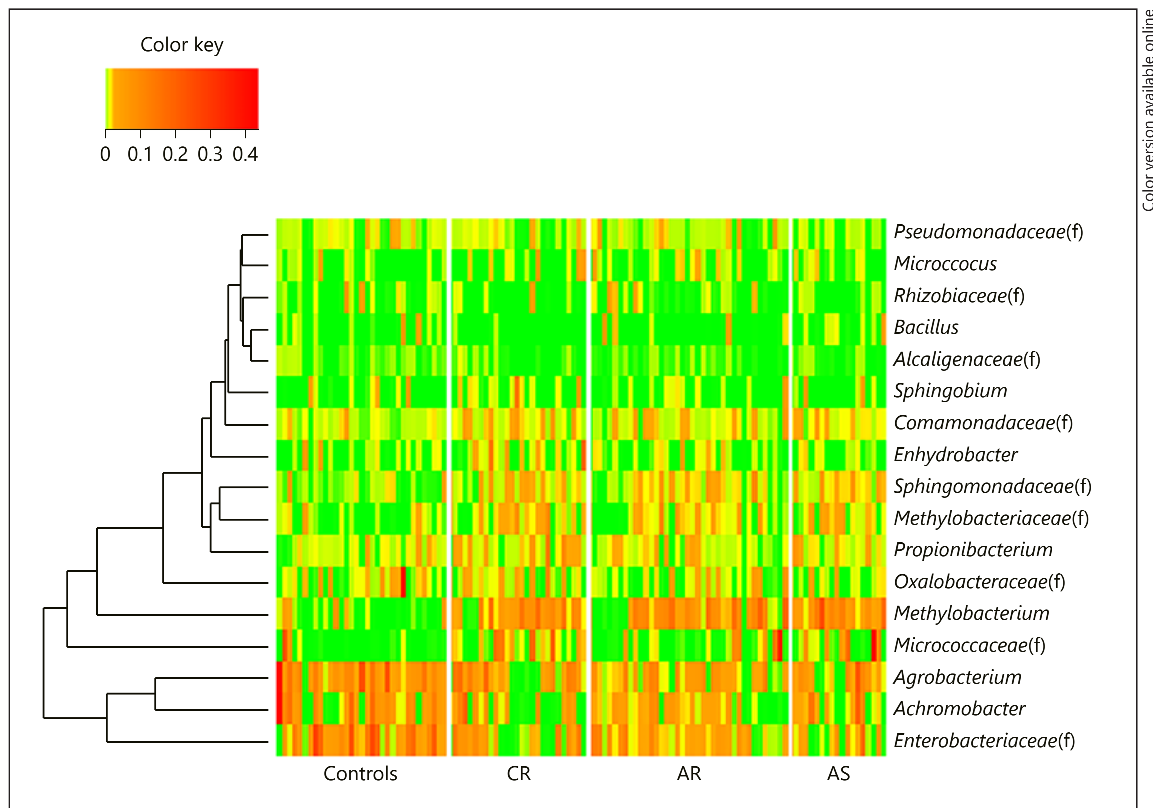


**Fig. 3.** Heat map with hierarchical clustering showing the relative abundance of bacteria at the genus level. The samples are listed horizontally on the *x*-axis. Genera that exhibited a significant fold change in any disease group against the controls were selected and are listed vertically along the y-axis on the right side of the plot. For each sample listed on the *x*-axis, the relative proportion of the genus on the *y*-axis is represented by a color; green corresponds to a low abundance, and red corresponds to a high abundance, see the color key. Genus names are used to identify the taxa. The taxa without generic names are annotated by (f) (i.e., family name). CR, chronic rhinitis group; AR, allergic rhinitis group; AS, atopic asthma group.

*ter* is one of the core microbiota in the nasopharynx of children [23]. Otherwise, it had not been previously correlated with CR.

Previous studies on airway microbiota linked enrichment of the Enterobacteriaceae family with an elevated risk of allergy development, which is warrantable as many of its members are known to be pathogenic [21, 26, 34]. In the current study, Enterobacteriaceae was significantly higher in the controls; however, an undefined genus, i.e., *Enterobacteriaceae*(f), occupied 97% of Enterobacteriaceae's total abundance, which points out a lower proportion of pathogenic genera in the controls. Enterobacteriaceae have common epitopes in their lipopolysaccharides which cause bovine and human sera to show an immune response against enterobacteria species they have not been exposed to before and they have been proposed to be capable of conferring protective immunity to enterobacterial pathogens in humans [35, 36]. Thus, we suggest that the undefined genus of Enterobacteriaceae might have a role in preventing the overgrowth of pathogenic genera, which may explain the relative enrichment of Enterobacteriaceae in the controls in our study.

In the present study, Rhizobiaceae, *Agrobacterium*, Alcaligenaceae, and *Achromobacter* were significantly more abundant in the controls compared to the disease groups. Meanwhile, they have not been reported in normal or allergic airway microbiota studies [20, 21]. Bacterial genera such as *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Haemophilus*, *Moraxella*, and *Prevotella* were commonly reported in the previous allergic airway microbiota studies [34, 37], while their urine EVs did not show any significant difference between the controls and the disease groups. This highlights that the EVs may not simply reflect the abundance of the micobiome in the body, but



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there is a possibility that individual bacterial species have a specific EVs secretion activity related to their virulence and function.

In the current study, the β diversity and bacterial relative abundances were quite similar in the 3 disease groups. From a pathological point of view, there is overlap among CR, AR, and AS, as predisposing epidemiologic and genetic factors are alike and excess mucosal inflammation with immune dysfunction is a common feature with an emerging important role in innate immunity in the 3 diseases [38]. Clinically, the AR and AS were closely related, as all cases of AS are atopic and have concomitant allergic rhinitis, whereas the CR, whose subjects were confirmed to be nonatopic, was uniquely enriched with Comamonadaceae, Propionibacteriaceae, *Propionibacterium,* and *Enhydrobacter*. These findings raise the possibility that the observed dysbiosis might be driven by the atopic syndrome. However, due to the cross-sectional nature of this study, we could not demonstrate the causal relationship to either support or deny this possibility.

This study has limitations; despite careful review of the patients' recordings, it was not possible to confirm that the patients were not taking antibiotics a few weeks before sampling. In addition, the effects of allergy medications on urine EVs could not be excluded. The cross-sectional design is not adequate to confirm the relationship between features of urine EVs and the disease; concomitant sampling from the site of pathology and urine at multiple time points would clarify that relation. Further studies are suggested to examine the correlation between urine EVs and atopy markers.

In conclusion, the results of this study suggest that urine EVs have distinct characteristics in chronic rhinitis, allergic rhinitis, and atopic asthma of children, which may open up a new perspective in the relationship between microbiota and allergic airway diseases. Specific bacterial taxa that have been correlated with features of chronic rhinitis, allergic rhinitis and asthma appeared to be well represented in urine EVs. Consequently, with the ease of urine sampling and the high stability of the EVs, urine EVs could be a useful indicator for assessing chronic rhinitis, allergic rhinitis, or asthma in children.

## Acknowledgement

This study was supported by the Korean Ministry of the Environment.

## Statement of Ethics

This study was approved by the Institutional Review Board of the Inha University Hospital (IRB No. 2017-05-008). Written informed consent was obtained from the patients and their parents/guardians.

## Disclosure Statement

All authors declare that they have no conflicts of interest regarding this study.

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