Allergic rhinitis and asthma: A gene expression comparative analysis in mice

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Allergic rhinitis and asthma: A gene expression comparative analysis in mice

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ABSTRACT

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Allergic rhinitis (AR) and asthma are related diseases, however, recent reports suggest that the immune response to the same stimulus differs between the nose and the lungse. This study investigates the gene expression and related pathways associated with AR and allergic asthma in a mouse model using bulk RNA sequencing. We induced AR and allergic asthma simultaneously in mice, and the four samples of nose and the three samples of lung were analyzed. Histopathological examination confirmed successful induction of AR and asthma, with increased epithelial thickness, eosinophils, and goblet cells. Bulk RNA sequencing revealed not only common immune-related pathways but also different gene expression patterns in the nose and the lung. However, comparison between the two organs showed unique pathway activation, emphasizing the tissue-specific nature of allergic responses. Identified up-regulated genes, such as SLC26A4 and CXCL9 in asthma, and Ltf in AR, were validated through immunofluorescence (IF) staining. The study showed the potential for tailored therapeutic approaches considering the distinct characteristics of upper and lower airways. There are differences in highly expressed genes and enriched pathways between AR and allergic asthma. AR and allergic asthma can be said to be un-unified airways with different pathogenesis, and this aspect should be considered in the study of pathophysiology or developing therapeutic agents for these un-unified airways.

Key words : allergic rhinitis, asthma, differential gene expression

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I. INTRODUCTION

Allergic rhinitis (AR) is characterized as an immunoglobulin (Ig) E-mediated inflammatory disorder of the upper airway in response to airborne allergens at the nasal epithelium. Clinical symptoms include sneezing, rhinorrhea, nasal obstruction and itching[1]. The pathogenesis of AR involves both Th2 and Th17 cell immunity marked by the infiltration of T cell, mast cell, and eosinophil.[2] These cells release inflammatory mediators, cytokines, and chemokines, initiating a systematic inflammatory response.[3] Asthma is a chronic inflammatory disorder of the lower airway. This chronic inflammation often manifests as reversible airflow restriction, resulting in respiratory symptoms such as dyspnea, chest tightness, coughing, and wheezing.[4] Allergic asthma is associated with allergen-specific IgE and Th2 cells indicating a distinctive immunological profile. Eosinophil infiltration of the lung tissue often causes airway remodeling and mucus over-secretion. The recruitment of mast cell, basophil, and T cell also contributes to the pathogenesis of asthma.[5] The relationship between AR and asthma has been previously studied, with AR reported in 28% to 78% of asthma patients, and 19% to 38% of AR patients also having asthma.[6] Furthermore, AR has been identified as an independent risk factor for asthma development. Patients with both AR and asthma tend to have more severe clinical symptoms.[7] To explain this correlation between AR and asthma, “United airway disease” or “Combined Allergic Rhinitis and Asthma Syndrome (CARAS)” were suggested as a manifestation of one syndrome in the upper and lower airway tract.[8, 9] The presence of asthma should be considered in patients with moderate to severe AR according

to Allergic Rhinitis and its Impact on Asthma (ARIA) 2019 guidelines for the management of AR and asthma comorbidities.[10]

However, there are different anatomical changes in AR and asthma. AR did not cause significant changes in epithelium, basement membrane, and submucosa in an inflammatory environment. While this limited remodeling of the airway is observed in AR, the remodeling of allergic asthma is extensive to the lower airway.[11, 12] This difference is likely due to differences in epithelial cell composition. In the subtypes of the epithelial cells of the nose and lung, the tuft cell and alveolar cells types 1 and 2 were found only in the lung but were not found in the nose.[13] Depending on the epithelial cells, the alarming cytokines (IL-25, IL-33, TSLP, IL-33)[14] involved in type 2 inflammatory response are originated from different pathways even though they play the same role. For example, the tuft cells derived from the basal cells of the lung produce IL-25, which is involved in leukotriene synthesis and plays a role in asthma pathogenesis.[15] On the other hand, solitary chemosensory cells (SCC) were seen only in the nasal polyps and mainly involved in producing IL-25, which induces a type 2 immune response.[16] In addition, Immune cell composition analyzed by single-cell RNA sequencing showed less than 10% composition of B cells in the lung of asthma patients,[17] but showed about 20-30% composition of B cells in the nose of patients with nasal polyps.[18] It can be inferred that the role of B cells is different in nasal polyp and asthma patients.

Previous studies demonstrated differences between AR and asthma in T cell activation[19] or classification method of AR and asthma by using a circulating microRNA as a biomarker.[20] However, there have been no studies of gene expression or pathways yet comparing AR and allergic asthma to each other when they occur simultaneously in mouse models. In this study, we induced AR and allergic asthma simultaneously in mice, after that, we analyzed which genes and pathways were different in the lungs and nose by bulk RNA sequencing.

II. MATERIALS AND METHODS

1. Allergic rhinitis and asthma mouse model

The animals were housed in compliance with the environmental guidelines by the Department of Laboratory Animal Resources, Yonsei Biomedical Research Institute, Yonsei University College of Medicine. In 8-week-old male C57BL/6 mice, AR and asthma were induced together by allergen sensitization and challenges under sedation. For allergic sensitization, a house dust mite (HDM) extract (Stallergenes greer, Lenoir, NC, USA) and Alum (Thermo Scientific, Waltham, MA, USA) combination in phosphate-buffered saline (PBS) was administered intraperitoneally on days 0, 7, and 14. A week after sensitization, the total of four challenging shots were injected every day from days 21 to 24. Anesthesia using isoflurane (Hana Pharm, Gyeonggi, Korea) was perfomed to induce AR and asthma together. Intranasal challenges with PBS or HDM extract were performed on anesthetized mice. Following euthanasia via inhalation of CO2 gas, nasal and lung tissues were harvested for further analysis.

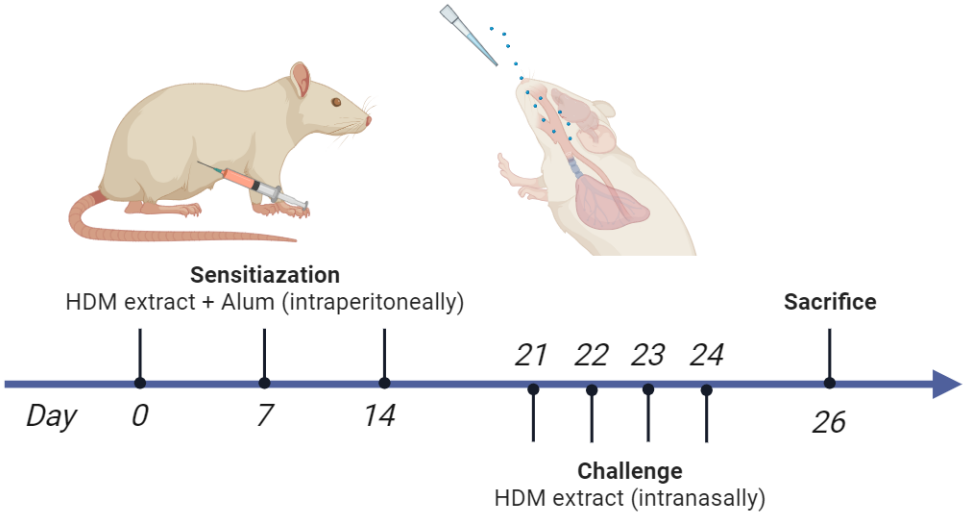


Figure 1. Mouse modeling of AR and asthma. Mice were sensitized to HDM on days 0, 7, 14, and challenged from days 21 to 24. Mice were sacrificed on day 26. The nose and lung of mice were harvested for further analyses (Figure was illustrated by using Biorender (https://biorender.com))

2. Histopathology

To confirm the results of the allergic reaction, histological sections were analyzed following hematoxylin and eosin (H&E), periodic acid Schiff (PAS), and Sirius red (SR) staining. After draining the blood with PBS, the head and lung tissue were fixed with 4% paraformaldehyde (PFA) (Duksan, Seoul, Korea) for 4 days. The tissues were decalcified with 10% ethylenediaminetetraacetic acid (EDTA) (Duksan, Seoul, Korea) for 3 weeks and fixed with 4% PFA once again. The nose of head and the lobe of lung were collected and stored in paraffin blocks. The tissues embedded in paraffin were cut at 4μm thickness by using a microtome, and placed on glass slides. The tissue was deparaffinzied with xylene (Duksan, Seoul, Korea) and dehydrated with descending grades of ethyl alcohol (EtOH) (Duksan, Seoul, Korea), 100% two times for 3 min, 95% two times for 3 min, and 70% for 1 min. Distilled water (DW) washing was performed for 3 min. The slides were stained with hematoxylin solution (Sigma Aldrich, Saint Louis, MO, USA) and washed again with DW. After that, eosin or PAS or SR solution (Sigma Aldrich) were used for each staining. Dehydration of the tissues proceeded conversely with 70%, 95%, and 100% EtOH. The slides were cleared three times with xylene for 2 min each and mounted on the glass. Histological photograph was imaged on Olympus BX43 microscope (Olympus, Tokyo, Japan) for group-wise comparisons.

3. Total IgE level measurement

Blood serum samples were obtained from the mice at the termination of the experiment on day 26. Quantification of serum IgE levels was performed by using a total IgE enzyme-linked immunosorbent assay (ELISA), following the manufacturer's protocol of the mouse IgE uncoated ELISA kit (Thermo Fisher Scientific). HDM specific IgE level was measured by HDM specific ELISA kit (Chondrex, Washington, WA, USA).

4. A gene expression comparative analysis in mice

A. Bulk RNA sequencing

Bulk RNA sequencing was analyzed with the nasal epithelium and lung lobe tissues of the mice. Blood was carefully removed from the tissue samples was using saline to ensure the accuracy of analysis. Subsequently, the de-blooded samples were preserved in the RNA later solution (Thermo Fisher Scientific) at -20˚C. The samples were then sent to Macrogen© (Seoul, Korea) for analysis.

B. Differential expression gene (DEG) analysis

Principal components analysis (PCA) was used for batch effect adjustment by the prcomp function and the corrplot function in R 4.2.2. Seven mouse batches were identified and eliminated using the ComBat algorithm.[21] DEGs were analyzed using DESeq2(ver 0.99.5) in HDM inhaled mice compared with controls. The p-value ≤0.05 was adjusted by Benjamin-Hochberg procedure. DEGs with p-value ≤0.05 and log2(fold change) ≥1 were considered statistically significant. The heatmap and volcano plot were generated by ComplexHeatmap package and ggplot2 in R4.2.2.

C. Gene set enrichment analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene ontology (GO) analysis were performed using ClusterProfiler4.2.2 from R. Genes were mapped to KEGG pathway entries using databases or tools like the KEGG Mapper. Genes were annotated with GO terms, categorizing them into biological processes (BP), molecular functions (MF), and cellular components (CC). Fisher's exact test was applied to assess whether the input genes were significantly enriched in specific KEGG or GO pathways. To control for false positives, p-values were adjusted using methods like the Benjamin-Hochberg procedure. The pathways with p-value ≤0.05 were considered as significant.

5. Identification of differential expression genes in the datasets

A. Gene expression datasets

Transcriptomic datasets were retrieved from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus database (GEO) as a publicly accessible database. The datasets included bulk RNA sequencing data for AR and allergic asthma of mus muculus. Specifically, the selected GEO dataset was GSE108417 available at http://www.ncbi.nlm.nih.gov/geo.

B. Identification of DEGs in the samples

Three series matrix files were obtained from the Gene Expression Omnibus (GEO), and each GEO matrix was stratified into two distinct cohorts: the AR group and the allergic asthma group (L-N). These datasets underwent screening utilizing the R package 'limma' for normalization and the identification of DEGs. Criteria for DEG selection included log(fold change) > 1 and P value ≤ 0.05. Following the derivation of six sets of highly expressed genes, the 'Venn' package in R was used to pinpoint genes shared across GEO datasets. A volcano plot was then generated using the 'ggplot2' package in R for providing a visual representation of DEGs and their distribution.

6. Immunofluorescence (IF) staining

The paraffin embedded slides were melted in an oven at 60℃ for 1 hr. The slides were deparaffinized by using xylene three times for 10 min. The slides were dehydrated in descending grades of EtOH, 100%, 95%, 80%, and 70% for 5 min in each solution; 100% EtOH was used two times while the rests were used only once. DW washing was performed twice for 3 minutes, followed by incubation in an antigen retrieval solution (IHC-Tek, USA) for 40 minutes, with subsequent cooling at room temperature (RT) for 20 minutes. Hydrogen peroxidase solution (Dako, Santa Clara, CA, USA) was used to block endogenous peroxidases in the tissue specimens at RT for 10 min. The slides were rinsed two times with tris buffered saline (TBS) (Amresco, Solon, Ohio, USA) for 5min and diluted with 5% bovine serum albumin (BSA) (Sigma Aldrich) for 1hr. The slides were then incubated overnight at 4℃ with primary antibodies; anti-Pendrin monoclonal antibody (G-11, Santacruz, CA, USA), anti-CXCL9 monoclonal antibody (11H1L14), and anti-Lactoferrin polyclonal antibody (BS-5810R). On the following day, the slides were washed in TBS three times for 5 minutes and incubated with Alexa Fluor 488-conjugated donkey anti-rat IgG (A21208), Alexa Fluor 568-conjugated donkey anti-rat IgG (A10037), and Alexa Fluor 647-conjugated donkey anti-rabbit IgG (A21208) (Thermo Fisher Scientific) at RT for 30 minutes in a dark room. After secondary antibody incubation, the slides were washed in 1% TBS three times for 5min and the nuclei were stained with DAPI (Thermo Fisher Scientific). The fluorescent images of the slides were acquired by a confocal microscope, 40X lens, LSM 780 (Zeiss, Oberkochen, Germany). Fluorescence intensities were quantified by ImageJ (imagej.nih.gov).

7. Statistical analysis

Statistical analysis was performed using Graphpad Prism 10.1 software (GraphPad Software Inc., San Diego, CA, USA). Comparison of histopathology and ELISA were analyzed using t-test, and comparison of IF staining intensity was evaluated by one-way analysis of variance (ANOVA). A p-value ≤ 0.05 was considered statistically significant.

III. RESULTS

1. Histopathological changes of the nasal and lung tissue in mouse model

H&E staining was performed to identify the inflammatory reaction within the allergic group compared to control. The results showed the eosinophilic infiltration with increased epithelium thickness of the nose and lung tissue (Fig. 2A and B, H&E) in the inhaled HDM mice by H&E staining. Mucus hypersecretion is a notable feature of allergic reaction. PAS staining was used to measure the number of goblet cell and mucus hypersecretion. The goblet cells containing mucin were stained bright pink. PAS staining showed overproduced mucus of the inhaled HDM mice and the goblet cell hyperplasia (Fig. 2A and B, PAS). Eosinophils of the samples were detected by using SR staining, cytoplasmic granules of eosinophils were stained red (Fig. 2A and B, SR). Goblet cell and eosinophil counts were significantly increased in the inhaled HDM mice compare to control (Fig. 2C and 2D). IgE antibodies are well-known for triggering immediate hypersensitivity reactions in sensitized individuals.[22] The IgE levels in blood serum of the mice were evaluated by using total IgE ELISA to verify the allergic reaction in mouse model (Fig. 2E). Inhaled HDM exposure led to a significant increase in the total IgE levels in mice compared to control.

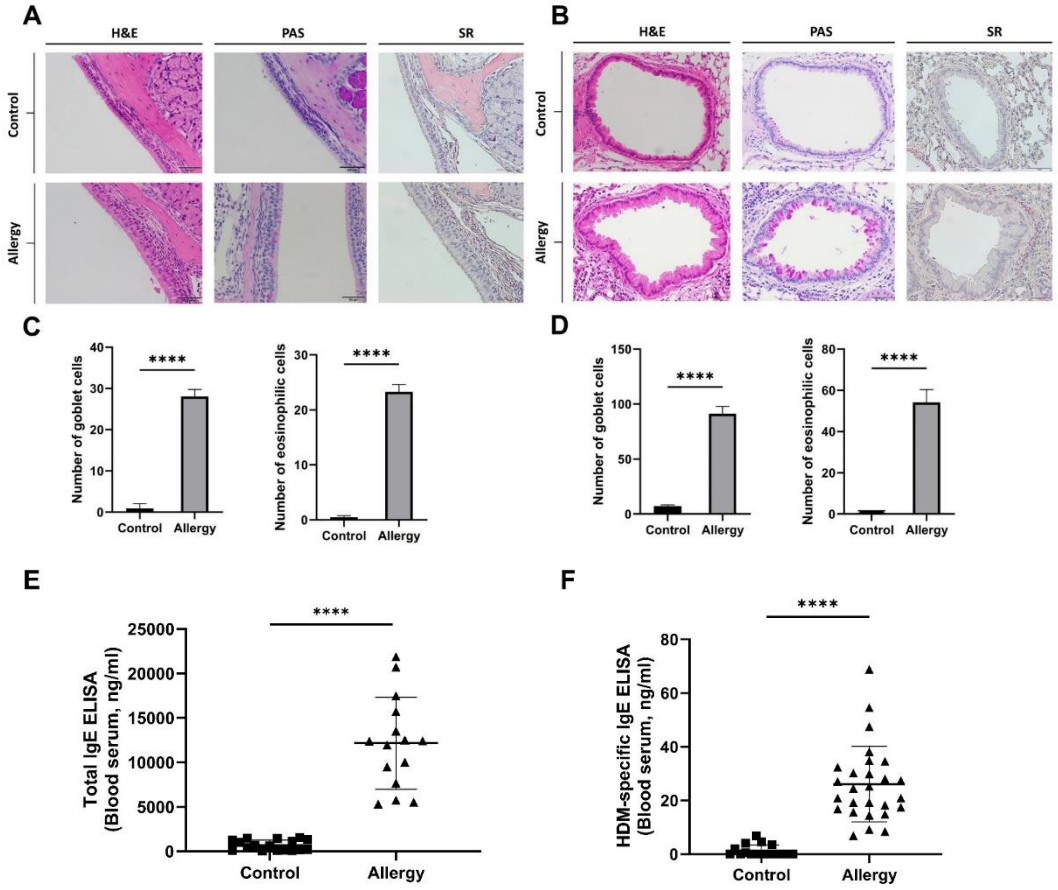


Figure 2. Histopathologic results of the epithelium of the nose and the lung in mouse model. The inhaled HDM mice showed thickened layer of the nasal epithelium (A) and the bronchial epithelium (B) compared to control. Histopathological features of AR (A) include increased numerous eosinophils, and monocyte recruitment in the nasal mucosa. In the bronchial epithelium of the inhaled HDM mice (B), there were the increased plicae mucosae, cellular swelling of airway epithelia, and many inflammatory cells infiltrated the bronchioles. PAS-positive goblet cells of the HDM inhaled mice were increased both in the nose (C) and the lung (D). The total IgE level (E) and HDM-specific IgE level (F) of the inhaled HDM mice were measured by ELISA and significantly increased compare to control. Original magnification X 400, **** means p-value < 0.001

2. Pathways of bulk RNA sequencing analysis in the nasal and lung tissues

A. Comparison between allergy and control groups in the nose and the lung

Total RNA was isolated from the nasal turbinate mucosa and lung tissues of mice and performed bulk RNA sequencing. Gene expression level of the samples were identified in general using heatmap of major genes related to allergic inflammation. All results show similar gene expression within group and differences in gene expression between each other groups.

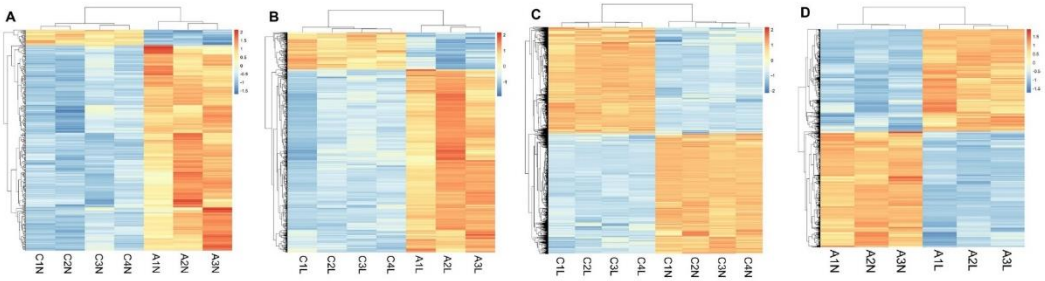
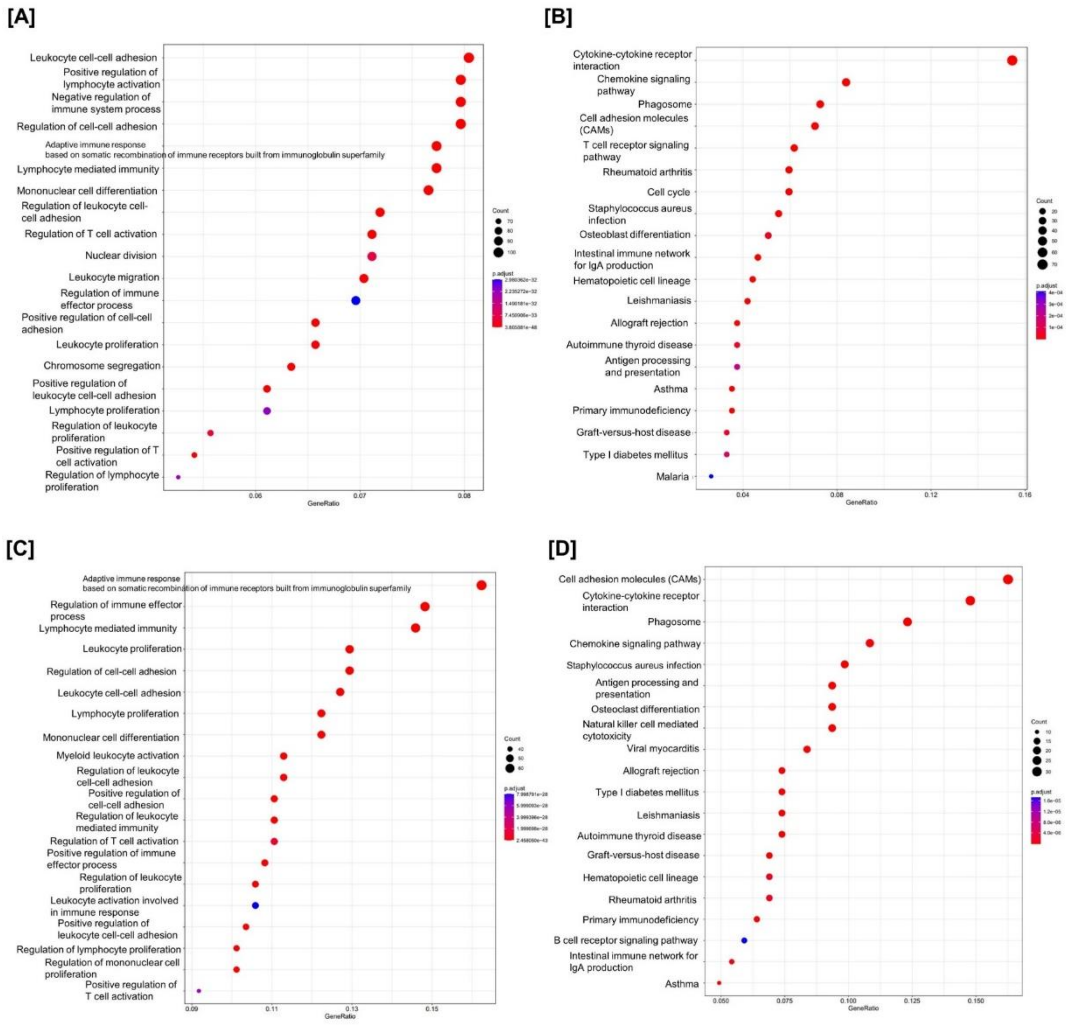


Figure 3. Bulk RNA sequencing in nasal and lung tissues: A heatmap analysis

Differential gene expression heatmaps ($P < 0.05$) compare control and allergy groups in the nose and lungs. Colors denote expression levels (blue to red). Subgroup analyses include nose (AR vs. control, Fig. 3A), lungs (allergy asthma vs. control, Fig. 3B), control group (nose and lungs, Fig. 3C), and allergic group (nose and lungs, Fig. 3D). (A: allergy, C: control, N: nose, L: lung, number: mice number)

Comparing the allergy and control groups in the lungs, the allergy group of the lung showed activation of GOBP such as leukocyte cell-cell adhesion, positive regulation of lymphocyte activation, negative regulation of immune system process, regulation of cell-cell adhesion, and adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains (Fig. 4A). Additionally, there was an activation of KEGG pathways, such as cytokine-cytokine receptor interaction, chemokine signaling pathway, phagosome, cell adhesion molecules (Fig. 4B). In comparing the allergy and control groups in the nose, the allergy group exhibited similar activation of GOBP (Fig. 4C) and KEGG (Fig. 4D) pathways as observed in the lung. Up-

regulation genes and down-regulation genes were identified by differential gene expression between the allergy groups and the control groups in lung (Fig. 4E) and nose (Fig. 4F). Despite similarities in pathways, no common genes were identified when focusing on the most expressed genes (Table 1, 2).



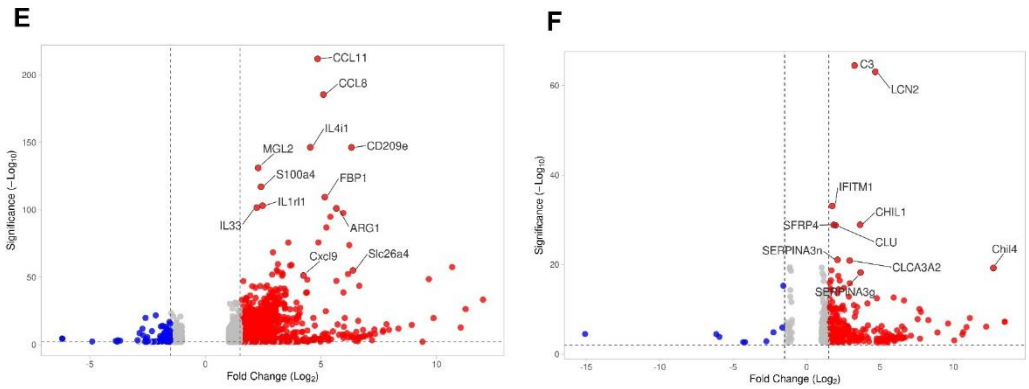


Figure 4. Pathways between allergy and control groups in the nose and the lung

(A) GOBP and (B) KEGG comparing allergy to control groups in the lung showed similarities to (C) GOBP and (D) KEGG in the nose. The volcano plots present the top 10 genes in each group based on significance (-log10pvalue). no common genes were identified among the top 10 genes with the highest expression levels in both the (E) lung and the (F) nose.

Table 1. Top 10 up-regulated genes in allergic asthma compared to control in the lung based on –log(pvalue)

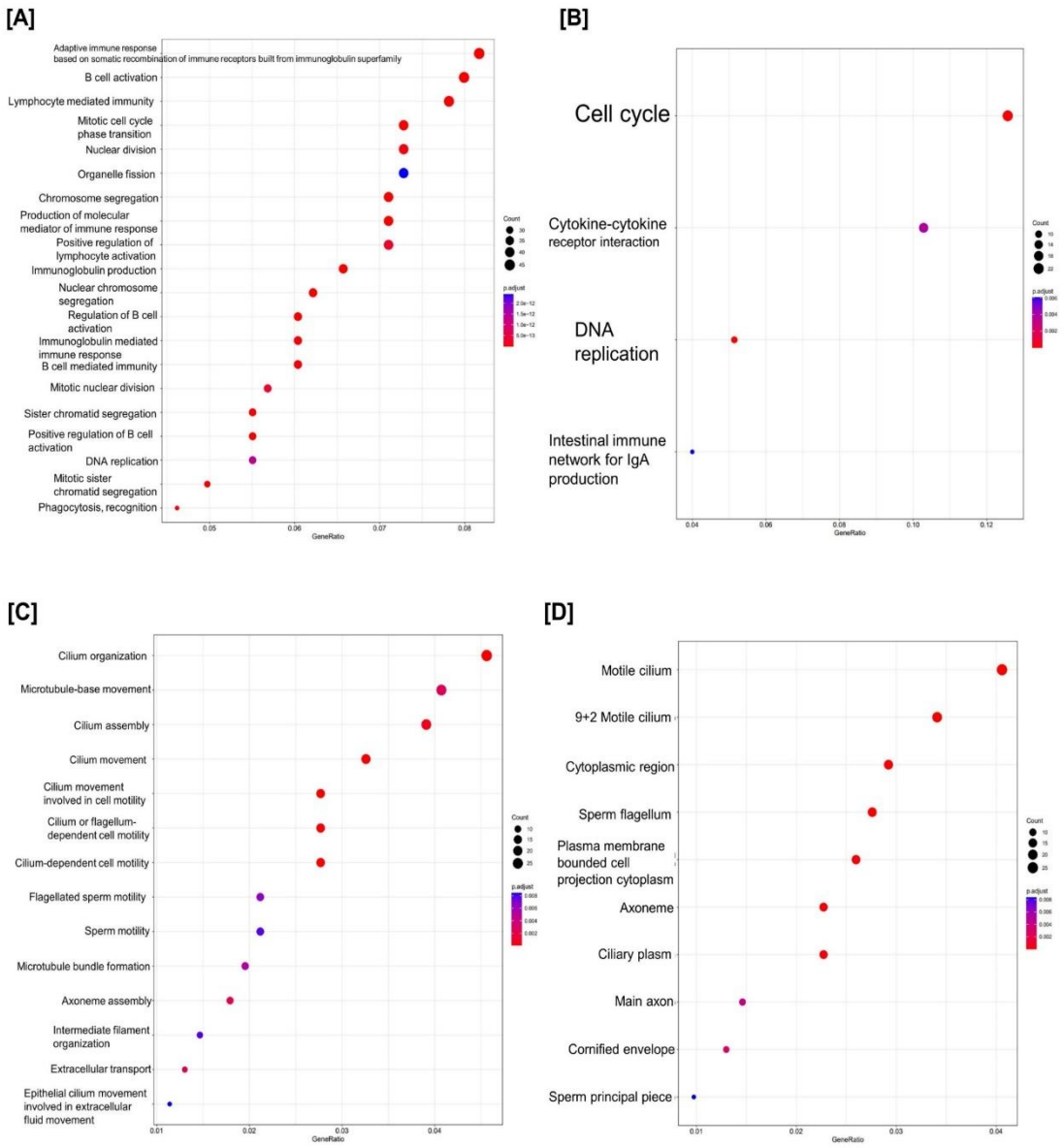
Gene	log2FC	-LOG(pvalue)
CCL11	4.85	212.08
CCL8	5.10	185.52
IL4i1	4.53	146.34
CD209e	6.31	146.30
MGL2	2.28	131.12
S100a4	2.40	117.08
FBP1	5.16	109.44
IL1rl1	2.47	103.05
IL33	2.22	101.65
ARG1	5.66	101.02

Table 2. Top 10 up-regulated genes in AR compared to control in the nose based on –log(pvalue)

Gene	log2FC	-LOG(pvalue)
C3	3.26	64.46
LCN2	4.67	63.04
IFITM1	1.74	33.09
CHIL1	3.64	28.87
SFRP4	1.84	28.85
CLU	1.97	28.75
SERPINA3n	2.10	21.05
CLCA3a2	2.93	20.88
SERPINA3g	3.67	18.21
KNG2	2.94	15.82

B. Comparison between the nose and the lung in the allergy groups

Comparing the allergy groups of the lung and nose, the lung showed activation of GOBP such as adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily, B cell activation, lymphocyte mediated immunity, mitotic cell cycle phase transition, nuclear division, and organelle fission pathway activation (Fig. 5A). Additionally, KEGG pathway analysis revealed activation of cell cycle, cytokine-cytokine receptor interaction pathway in the allergy group of the lung (Fig. 5B). The allergy group of the nose compared to the allergy group of the lung, showed activation of cilium related pathway in GOBP (Fig. 5C), GOCC (Fig. 5D) such as cilium organization, microtubule-base movement, cilium assembly, cilium movement, cilium movement involved in cell motility, cilium or flagellum-dependent cell motility, and cilium-dependent cell motility pathway. Differential gene expression analysis between the lung and nose in the allergy groups identified top 10 up-regulated genes based on significance (-log10pvalue). Notably, the lung exhibited up-regulation of genes such as SLC26A4, CXCL9, and GM43305, while the nose displayed up-regulation of genes including Ltf, Cyp2g1, S100A5, FSTL5, Sult1C1, UGT2A1, and GPX6 (Fig. 5E).



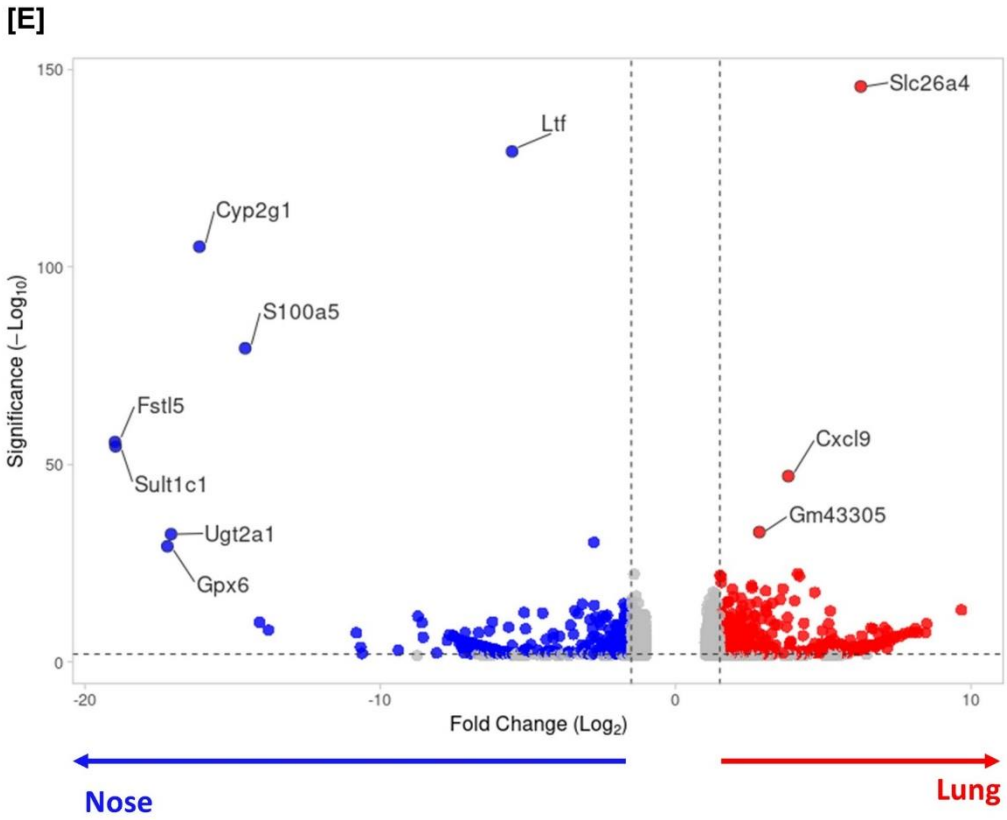


Figure 5. Pathways between the lung and nose in the allergy groups

(A) GOBP and (B) KEGG pathway analyses were performed to compare the allergy groups to the control groups in the lung. In parallel, (C) GOBP and (D) GOCC pathway analyses were conducted to compare the allergy groups to the control groups in the nose. (E) The volcano plot illustrates differential gene expression, with blue indicating higher gene expression in the lung and red indicating higher gene expression in the nose.

4. Identification of DEGs from NCBI GEO datasets

The dataset (GSE108417) was analyzed to identify genes that expressed differently in allergic asthma and AR. In total, 6 samples were obtained and analyzed. There were 476 DEGs in GSE108417, 283 of them are up-regulated genes and 193 of them are down-regulated genes. DEGs expressed between allergic asthma and AR are presented using volcano maps in Fig. 6. Table 3 shows Top 10 up-regulated genes of the nose and the lung.

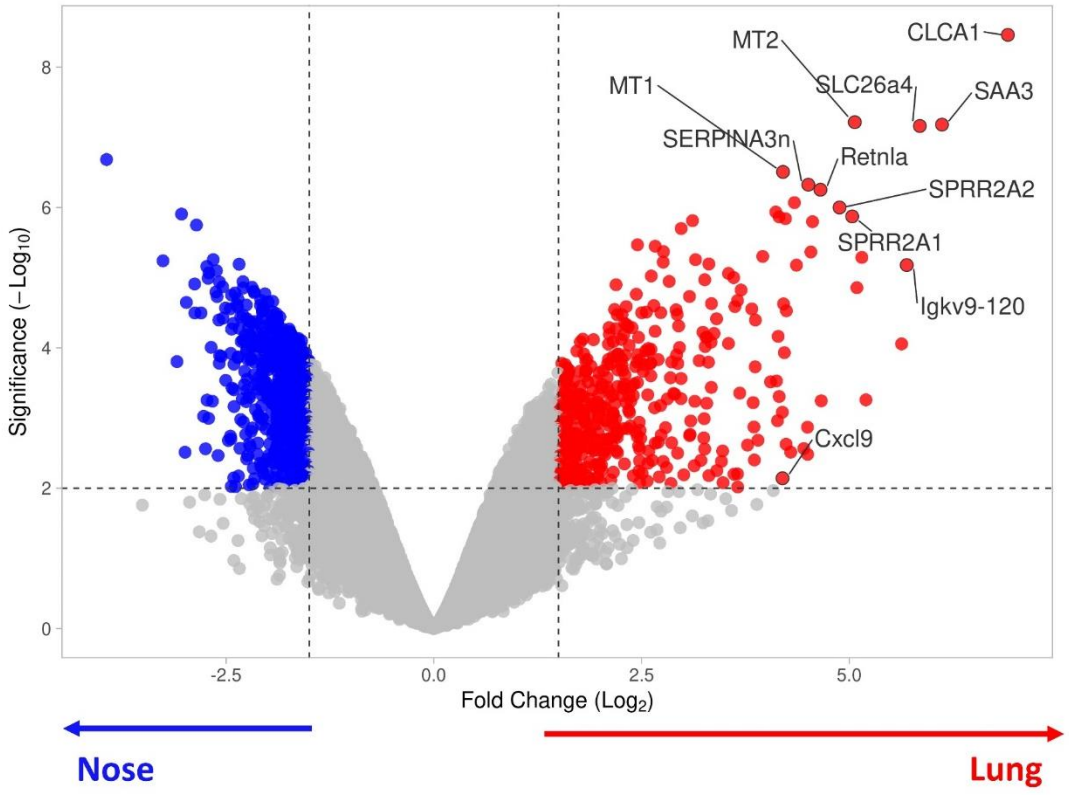


Figure 6. Volcano plot of DEGs expressed between allergic asthma and AR

Blue represents the higher gene expression of the lung, and red represents the higher gene expression of the nose.

Table 3 Top 10 up-regulated DEGs in allergic asthma compared to AR

Gene.symbol	log2FC	-Log(pvalue)
CLCA1	6.91	8.46
MT2	5.07	7.22
SAA3	6.12	7.18
SLC26a4	5.85	7.16
MT1	4.20	6.51
SERPINA3n	4.51	6.33
Retnla	4.65	6.25
SPRR1A	4.34	6.07
SPRR2A2	4.88	6.00
SERPINA3g	4.12	5.94

5. Immunofluorescence (IF) staining of pendrin, CXCL9, and lactoferrin

Immunofluorescence (IF) analysis was conducted to investigate the localization of pendrin, CXCL9 and lactoferrin in HDM inhaled mice. The nuclei of cell were stained blue with DAPI. Similar with the results of histopathology, it was observed that the epithelium thickened in both the nasal and lung as an allergic reaction following exposure to HDM. (Fig. 7 A-D) While an overall upregulation in pendrin and CXCL9 expression was observed in allergic asthmatic mice, this increase was not statistically significant in the nasal tissue following HDM inhalation. Pendrin and CXCL9 were notably pronounced in the bronchial epithelium, extending to the parenchyma in allergic asthmatic mice. In contrast, nasal tissues exhibited either no discernible changes or a gradual increase limited to the nasal epithelium. (Fig. 7 Pendrin and CXCL9). Consequently, the elevated expression of pendrin and CXCL9 in the bronchial epithelium was associated with the allergic response in the lung, in contrast to the nose. In the nasal epithelium of HDM inhaled mice, there was a notable increase in lactoferrin expression compared to control. Lactoferrin was not confined to the nasal epithelium but extended to the mucous glands of the nasal lamina propria (Fig.7 Lactoferrin). These IF staining results were consistent with the results of Bulk RNA sequencing.

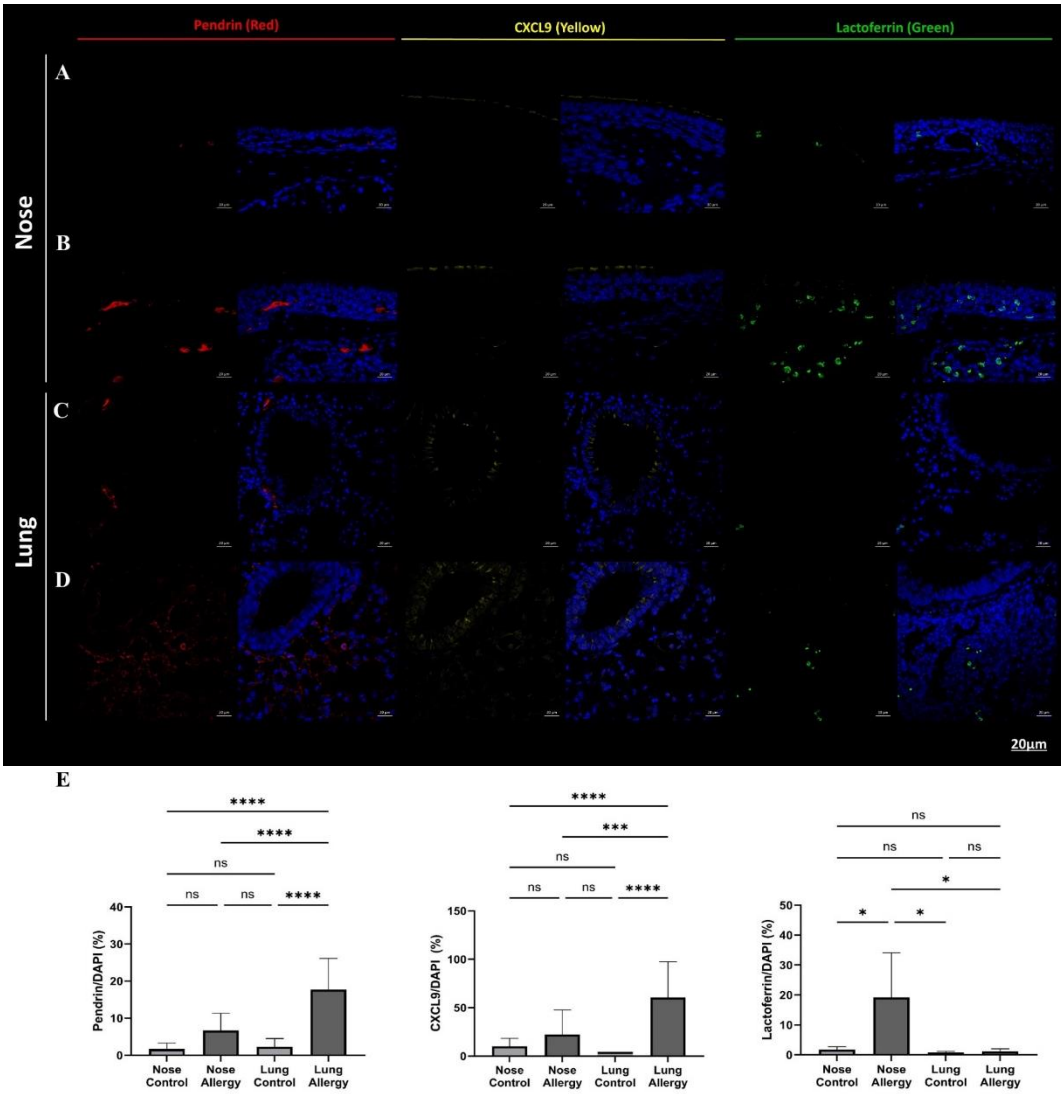


Figure 7. IF staining of pendrin, CXCL9 and lactoferrin in the nose and the lung

Representative images of pendrin (red), CXCL9 (yellow), and lactoferrin (green) were observed in confocal fluorescence microscopy. Nuclear acids were stained with DAPI (blue). There was no significant expression of pendrin, lactoferrin, and CXCL9 in the control groups of the (A) nasal and (C) lung without HDM inhalation. In HDM-exposed

(B) nasal and (D) lung, pendrin staining shows partial expression in the nasal epithelium, but a more pronounced expression extending to the parenchyma in the lung. Similarly, there's No change in CXCL9 for HDM-exposed (B) nose, but significant increase in (D) lung. In contrast, significant increased expression of lactoferrin is seen in (B) nasal tissues, but not in (D) lungs after HDM inhalation. (E) Quantified fluorescence intensity data from a fluorescence intensity of pendrin, CXCL9 and lactoferrin was measured with imageJ. Comparison of intensity of pendrin, lactoferrin, and CXCL9 in the nose and the lung.
**P<0.05; **P<0.01; ***P<0.001, ****P<0.001*

IV. DISCUSSION

This study aimed to explore the simultaneous induction of AR and allergic asthma in a mouse model. AR is hypersensitivity immune response induced by allergic antigen exposure of the nasal mucosa, and causes symptoms of nasal congestion, nasal itching, rhinorrhea, and sneezing.[23] Allergic asthma is a complex and chronic inflammatory disorder which is associated with airway hyper-responsiveness and tissue remodeling of the airway structure.[24] AR and asthma as allergic respiratory diseases are characterized by airway hyperresponsiveness and are related to each other.[25] Histopathology confirmed successful induction of AR and asthma in mice, shown by increased eosinophils, thicker epithelium, and more mucus in the nose and the lung. Elevated IgE levels in blood serum were measured by ELISA, consistent with previous reports associating IgE with immediate allergic reactions.[26]

To our knowledge, this study presents the first gene expression analysis using bulk RNA sequencing in mouse model to identify the differential expression between AR and allergic asthma. Bulk RNA sequencing analysis provided a comprehensive view of the gene expression profiles in the nasal and lung of mice with induced AR and allergic asthma. The heatmap analysis revealed distinct gene expression patterns within each group. The activation of immune-related pathways, such as leukocyte cell-cell adhesion, cytokine-cytokine receptor interaction, and adaptive immune response, was evident in both the nasal and lung tissues, highlighting the common nature of the allergic response.[27] Comparison of the allergy and control groups in the lung and nose revealed shared pathway activation, supporting the notion of common immunological features in upper and lower airways. The common enriched pathways have been reported in other studies such as cytokine-cytokine receptor interaction, chemokine signaling pathway, and cell adhesion molecules in AR[28] and allergic asthma.[29, 30]

However, comparison between the lung and nose in the allergy groups unveiled significant differences in pathway activation. The lung exhibited pathways related to adaptive immune responses and cell cycle regulation, emphasizing the extensive

remodeling and immune cell involvement in the lower airway of allergic asthma. In contrast, the nose showed activation of cilium-related pathways, indicating indistinct pattern of remodeling in the upper airway associated with AR. These findings align with previous studies highlighting anatomical differences in the airway remodeling process between AR and asthma.[11] emphasizing the tissue-specific nature of allergic responses. Previous studies suggested that the most significant pathway of the AR mice compared to the control mice was olfactory transduction.[31, 32] In this study, olfactory transduction was seen to be increased in AR when comparing AR and Asthma.

The differential gene expression between the two diseases emphasized the unique molecular signatures associated with each. Our study identified specific up-regulated genes in the lung (SLC26A4, CXCL9, GM43305) and nose (Ltf, Cyp2g1, S100A5, FSTL5, Sult1C1, UGT2A1, GPX6). Allergic asthma showed highly up-regulated expression of SLC26A4 gene, which is a gene that makes a protein called Pendrin, a transmembrane anion exchanger that exists on the surface of epithelial cells involved in mucin production and intraluminal acidification.[33] In a recent study, SLC26A4 gene was reported to be the most up-regulated gene in bronchi of the lung in allergic asthma, mainly expressed in alveolar type 2 cell that exist only in lungs.[34] Therefore, significant expression of the Pendrin in the lung may be possible due to differences in epithelial cell types. To confirm this, measuring protein level by cell type will be necessary in the future. CXCL9, one of the chemokines, is known for its role in immune cell recruitment and inflammation such as induce of chemotaxis, immune cell migration, and activation.[35] Up-regulated expression of the lactoferrin (LTF) gene and other genes (CYP2G1, S100A5, FSTL5, SULT1C1, UGT2A1, GPX6) known to be associated with the olfactory receptor was observed in AR compared with allergic asthma. Lactoferrin protein is commonly found in submucosal gland secretions of both lower and upper respiratory tracts in humans and is present in neutrophil granules.[36] This protein is known to have diverse biological functions, such as promoting iron absorption, antibacterial, antifungal, and antiviral activities, and is associated with immunomodulatory and anti-inflammatory activities. Lactoferrin is known

to have increased levels in the nasal lavage fluid of patients with AR.[37]

Publicly available datasets (GSE108417) from NCBI GEO further supported findings of the mouse model through revealing DEGs between allergic asthma and AR in mouse. SLC26A4, CXCL9 were some of the genes strongly expressed in allergic asthma in a comparative analysis of asthma and rhinitis using a gene dataset from NCBI GEO. Comparison with existing datasets (GSE108417) further identified DEGs between allergic asthma and AR. The top up-regulated genes in allergic asthma, including CLCA1, MT2, SAA3, and SLC26A4, have been implicated in airway inflammation and remodeling.[38]

IF staining for pendrin, CXCL9, and lactoferrin validated our gene expression findings. The localization of these markers in the bronchial epithelium and nasal mucosa further supports the tissue-specific nature of allergic responses. Our study not only adds to the understanding of the gene expression between AR and allergic asthma but also raises intriguing questions about the clinical implications of these findings. This identification of specific genes and pathways associated with the nose and the lung can give the tailored therapeutic approaches that consider the distinct characteristics of upper and lower airways.

However, our study has limitations. The use of a mouse model may not fully recapitulate the complexity of human AR and asthma. While the mouse model allows for controlled experiments, translating these findings to clinical applications requires careful consideration of interspecies differences. Moreover, the focus on gene expression highlights associations, but does not establish causation. Future research should explore the identified genes and pathways in human cohorts to validate their relevance to the clinical manifestations of AR and allergic asthma. The use of bulk RNA sequencing, though informative, limits the ability to identify cell-specific contributions to gene expression patterns. The use of single-cell RNA sequencing would have allowed for a more nuanced exploration of cellular heterogeneity in nasal and lung tissues, revealing specific cell types responsible for the observed gene expressions and pathways.

V. CONCLUSION

Our study contributes to the understanding that AR and allergic asthma possess different pathogenic mechanisms in a mouse model of co-occurring AR and asthma. Upon simultaneous induction of AR and asthma, we observed similar pathways compared to control, yet the top genes differed for each disease. When comparing AR between asthma, the allergic asthma was related to the cell cycle and cytokine-cytokine receptor interaction pathway, while AR was related to cilium-related pathways such as mobile cilium and cilium organization. There are differences in highly expressed genes and enriched pathways between AR and allergic asthma. In particular, SLC26A4 and CXCL9 genes were some of the highly expressed genes in allergic asthma, not only in the mouse model but also in the dataset. These findings provide valuable insights that may inform future clinical strategies, underscoring the significance of organ-specific approaches in the management of allergic respiratory diseases. Our study not only supports a shared immunological basis but also emphasizes the unique pathogenesis of AR and allergic asthma. These observed differences in gene expression between the lung and nose prompt a reevaluation of the "united airway disease" concept.

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알레르기 비염 및 천식 : 마우스 모델에서 유전자 발현 비교 연구

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오 장 원

알레르기 비염과 천식은 서로 연관된 것으로 알려져 있는 질병이지만 최근 연구에 따르면 동일한 자극에 대한 면역 반응이 코와 폐에서 다르게 나타날 수 있다고 보고되었다. 그러나 어떤 기전이 다른지는 아직 잘 알려져 있지 않다. 본 연구에서는 생쥐에서 알레르기성 천식과 알레르기성 비염을 동시에 유발한 후 Bulk RNA 시퀀싱을 통해 폐와 코에서 어떤 유전자와 경로가 다른지 분석했다. 실험은 총 7마리의 생쥐, 4개의 대조군 생쥐와 3개의 알레르기 생쥐에 대해 수행되었다. 병리조직학검사를 통해 알레르기 비염과 천식이 동시에 유발되었음을 확인하였으며, 조직 상피 두께, 호산구, 점액세포의 증가가 관찰되었다. Bulk RNA 시퀀싱 결과 코와 폐는 공통적인 면역 관련 경로를 보였지만 연관된 유전자 발현은 다른 것으로 나타났다. 또한 알레르기 반응에서 두 기관을 비교했을 때는 서로 다른 경로의 활성화가 나타나 알레르기 반응의 기관 특이성을 확인할 수 있었다. Bulk RNA 시퀀싱을 통해 유전자 발현을 비교했을 때 천식에서는 SLC26A4 및 CXCL9, 비염에서는 락토페린 유전자의 과발현이 보였고 이는 면역형광 염색에서도 관찰되었다. 알레르기성 호흡기 질환에서 폐와 코는 발현되는 유전자와 경로에서 많은 차이가 있었다. 그러므로 폐와 코는 서로 다른 병인을 갖는 비통합 기도라 할 수 있으며, 병태생리학이나 치료제에 있어서 이러한 측면이 고려되어야 할 것이다.

핵심되는 말 : 알레르기 비염, 알레르기 천식, Bulk RNA 시퀀싱