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Gene Expression Analysis by Real-Time PCR in Nasal Brushings of Adult Patients with Allergic Rhinitis, Suspected Allergic Rhinitis, and Nonallergic Rhinitis

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Keywords
Allergic rhinitis · Nonallergic rhinitis · Epithelium · Real-time PCR

Abstract
Background: Epithelial gene expression in allergic rhinitis patients has been evaluated by microarray. However, gene expression in patients with nonallergic rhinitis and suspected allergic rhinitis who reported allergen-related nasal symptoms but presented a negative atopic test was unknown. ***Objectives:*** The aim of this study was to observe and compare epithelial gene expression in patients with allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis. ***Methods:*** Nasal brushings were collected from healthy controls and from patients with allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis. The expressions of 20 genes selected from a previous microarray study were measured by real-time PCR. Associations of these genes with allergen type, disease duration and severity, the grade of nasal smear eosinophilia, and serum total IgE were analyzed. ***Results:*** Twelve genes were confirmed to be upregulated in current adult allergic rhinitis patients allergic to multiple allergens, and 10 of them were also increased in the suspected allergic rhinitis and nonallergic rhinitis groups. TFF3 and ITLN1 expressions were increased in allergic rhinitis and suspected allergic rhinitis, but not nonallergic rhinitis. Different expressions between the allergic rhinitis and nonallergic rhinitis groups were found for 3 genes: CST1, TFF3, and ITLN1. In the allergic rhinitis patients, all 12 genes were upregulated in the seasonal and perennial groups; 9 of these 12 genes were also upregulated in the mixed group. In suspected allergic rhinitis patients, all 12 genes were upregulated in the perennial group; 8 of these 12 genes were also upregulated in the seasonal group and only 5 in the mixed group. No gene expression was associated with disease duration and serum total IgE. GCNT3 was positively correlated with the grade of nasal smear eosinophilia in the suspected allergic rhinitis group. Different genes were found to be associated with disease severity in different rhinitis groups. ***Conclusions:*** Patients with allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis showed much similarity with regard to epithelial gene expression; most genes were related to Th2 inflammation. CST1, TFF3, and ITLN1 might have the

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ability to differentiate allergic rhinitis from nonallergic rhinitis. Understanding the mechanisms underlying different types of rhinitis may be helpful for rhinitis diagnosis and treatment.

Introduction

Rhinitis is a common disease characterized by inflammation of the nasal mucosa. It could be roughly classified into allergic and nonallergic rhinitis based on allergen-related nasal symptoms and relevant sensitization detection by skin prick test (SPT)/serum-specific IgE (s-IgE) [1–3]. There are both differences and similarities between allergic rhinitis and nonallergic rhinitis. Some studies have shown different expressions of nasal nitric oxide [4], microRNAs, and Th2 cytokines [5] and different extent of epithelial impairment [6] between allergic rhinitis and nonallergic rhinitis. However, other studies have shown similar inflammatory infiltrates in allergic rhinitis and nonallergic rhinitis [7, 8].

A positive SPT/s-IgE in subjects reporting allergen-related nasal symptoms indicates allergic rhinitis, but a negative one does not preclude the possibility of allergic rhinitis. One explanation is that a patient may have been sensitized earlier, but atopy is relieved and no longer detectable by SPT/s-IgE, yet nasal symptoms remain [9]. Another possible explanation is that this population might be at the initial step in the natural history of allergic rhinitis and may develop sensitization later on. We presumed the mechanisms underlying this population might be similar to allergic rhinitis and defined it as suspected allergic rhinitis here in this study. Until now, less attention has been paid to the characteristics of this patient group.

Most studies on rhinitis have been based on peripheral blood samples, considering the invasiveness of typical nasal tissue collection. Nasal brushing provides a simple, noninvasive method of collecting local nasal samples [10], which makes it easier to investigate the local response of rhinitis. The main cellular component in nasal brushing is the epithelial cell [11], which plays an important role in allergic rhinitis development [12, 13]. One previous microarray-based study has reported some differentially expressed genes in nasal brushings of childhood allergic rhinitis patients allergic to house dust mites compared to healthy controls [14]. However, whether it is similar for adult allergic rhinitis patients allergic to other types of allergens and what is the difference or similarity between allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis were unknown.

In this study, the top 20 upregulated genes with the largest fold change and with a p value <0.05 in the previous study [14] were selected to be measured by real-time polymerase chain reaction (PCR) in nasal brushings of adult allergic rhinitis and suspected allergic rhinitis allergic to multiple allergens, and nonallergic rhinitis patients . We compared the expressions of these genes between groups and analyzed their association with allergen type, disease severity and duration, serum total IgE, and nasal smear eosinophilia grade.

Methods

Subjects

A total of 109 patients with rhinitis who came to the Rhinology Department of Beijing TongRen Hospital between 2015 and 2016 and 44 local healthy volunteers were enrolled in this study. Diagnosis of allergic rhinitis and nonallergic rhinitis was based on history, symptoms, clinical examination, and serum allergen-specific IgE test [1–3]. Patients with 1 or more typical rhinitis symptoms (nasal congestion, rhinorrhea, nasal itching, and sneezing) were first selected. Those with self-declared nasal response to at least 1 common allergen (house dust mites, animal dander, fungi, weed, grass, and tree pollen) and positive serum allergen-specific IgE were defined as allergic rhinitis; those with self-declared nasal response to at least 1 common allergen and negative serum allergen-specific IgE were defined as suspected allergic rhinitis; those with self-declared nasal response to no common allergens and at least 1 nonspecific stimulator (cigarette smoke, temperature changes, perfume, air-conditioning, exhaust gases, I do not know to what, and others) and with negative serum allergen-specific IgE were defined as nonallergic rhinitis. All patients had typical clinical manifestations at the time of visiting. Control subjects were confirmed to have negative serum s-IgE to common allergens and no history of allergic conditions, diagnosis, or symptoms. None of the subjects had received any antihistamine, leukotriene receptor antagonist, or topical steroid for at least 4 weeks at the time of recruitment. Individuals with chronic sinusitis, nasal polyps, or respiratory infection in the previous 4 weeks were excluded. Patients were given a diagnosis of asthma by a chest physician.

Based on the self-reported allergen types, patients with allergic rhinitis and suspected allergic rhinitis were classified into seasonal (allergic to tree and/or grass pollen but not dust mite and/or animal dander), perennial (allergic to dust mite and/or animal dander but not tree and/or grass pollen), or mixed (allergic to both allergen types) allergic rhinitis. The severity of the rhinitis was classified into mild or moderate/severe based on the severity of the symptoms and the quality of life (sleep, daily activities, and work or school). Patients were categorized as "mild" if none of these items were affected and "moderate/severe" if at least one of these items were affected.

Detection of Serum Allergen-Specific IgE and Total IgE

Serum allergen-specific IgE was measured by immunoblot technique using inhaled and food allergen-specific IgE detection

Table 1. Primers used for real-time PCR

	Forward	Reverse
Actin	CTGGCCGGGACCTGACT	GCAGCCGTGGCCATCTC
CST1	CGGGTGGCATCTATAACGCA	GTCTGTTGCCTGGCTCTTAGT
POSTN	CGGGCAAATACTGGAAACCATC	ACCGTTTCTCCCTTGCTTACTCC
CPA3	QT00000336*	
ITLN1	AGAGGATGGAGTACAGATGAGG	TCAGTGCGGAGAAAATACAGG
CST2	AGGAGGACAGGATAATCGAGGG	CGCTGTACCCGCTCATCATT
FETUB	QT00014224*	
SERPINB2	CAGATCCAGAAGGGTAGTTA	CAGACTTCTCACCAAACAGCTT
DPP4	TACAAAAGTGACATGCCTCAGTT	TGTGTAGAGTATAGAGGGGCAGA
SH2D1B	CTGTGCCTCTGTGTCTCGTTT	CCACCATCCCCTGATTTGGT
CLC	TCAGAAGAGCCACCCAGAAG	GACCACACGACGACCAAAG
GCNT3	TCAAAGAGGCGGTCAAAGCAA	GCATAAACCACCCGAACCAG
CDH26	GATCTACCAGCCTCTACGGC	TGAACAGCTCACCAATGAGTTTG
CD200R1	ATGCTCTGCCCTTGGAGAAC	CTCCGCTTCGGCCACTAA
GCNT4	CTTTTGGGCTACCTTGATTC	CTGGGATAGAAAAAGCCTTC
TFF3	AATGCACCTTCTGAGGCACCT	CGTTAAGACATCAGGCTCCAGAT
TMEN16A	GATGAGGGTCAACGAGAAGTAC	GCATCAGGGTCCACAGATAAG
CD274	TCACTTGGTAATTCTGGGAGC	CTTTGAGTTTGTATCTTGGATGCC
NTS	TCTGTGCTCAGATTCAGAAGAGG	TTTCCTCAGCTGGGCTGTTC
SERPINB4	CCACGGTCTCTCGATATCTAA	GAAGGAGATGATAATTCGACTA
CA2	CTGGGGTTCACTTGATGGACA	GTTTAGCGCTGCCAACCTTC

* QuantiTect Primer Assay (Qiagen, Hilden, GmbH, Germany).

kit (EUROIMMUN Medizinische Labordiagnostika AG, Germany). Serum total IgE was measured by fluorescence immunoassay (Thermo Fisher, Uppsala, Sweden).

Nasal Brushings

Nasal epithelium sampling was performed by brushing the inferior turbinates and the adjacent nasal wall using a cytology brush. The cells adhering to the brush were removed by brisk agitation in 5 mL of sterile PBS and centrifuged to separate the supernatants from the cells. The cell pellet was immediately lysed with 1 mL of trizol and stored at −70°C.

Detection of Gene Expression by Real-Time PCR

Total RNA from nasal brushings was isolated with TRIzol reagent (Ambion-Life Technologies, Carlsbad, CA, USA). RNA was reverse transcribed into first-strand cDNA using SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed with the Applied Biosystems ViiA™ 7 Real-Time PCR System (Life Technologies).

Gene expression was determined using the Platinum SYBR Green qPCR SuperMix UDG (Invitrogen, Carlsbad, CA, USA). Primers used are shown in Table 1. The comparative Ct method (ΔΔCt) was used for relative gene expression analysis.

Nasal Smear Eosinophilia Grade

Part of the nasal brushings were smeared on a glass slide and stained by Wright's staining. Eosinophilia was evaluated semiquantitatively by scoring on a scale of 0–4, 0 eosinophils per high-power magnification fields were defined as "0," 1–5 as "1," 5–10 as "2," 10–20 as "3," and more than 20 as "4."

Statistical Analysis

SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were shown as the median and interquartile range. For qualitative data, the χ^2 or Fisher's exact test was used. For quantitative data, the Mann-Whitney U test was used for multiple comparisons among the different groups if the results of the Kruskal-Wallis test showed a significant difference. Relationships were evaluated by a Spearman correlation analysis. p values of 0.05 or less were considered statistically significant.

Results

Study Population

Characteristics of all subjects are described in Table 2. All groups were comparable with respect to age, gender, and disease duration. There was no difference in the proportion structure of seasonal/perennial/mixed between the allergic rhinitis group and the suspected allergic rhinitis group. The percentage of comorbid asthma, all well controlled, in the suspected allergic rhinitis group was higher than that in the control. As for disease severity, the percentage of moderate/severe type patients with suspected allergic rhinitis (76%) was higher than the percentage of moderate/severe type patients with allergic rhinitis (51%). Allergic rhinitis patients had higher levels

Table 2. Demographic and clinical characteristics of the subjects

	Control (*n* = 44)	AR (*n* = 43)	SAR (*n* = 34)	NAR (*n* = 28)	*p* value	
Age, median (IQR), year	38 (29.3–43.8)	36 (29–44)	37.5 (28.8–48.3)	34 (26–40)	ns	
Gender, female/male, *n*/*n*	22/22	22/21	16/18	12/16	ns	
Asthma, *n*	0	3	4	1	SAR versus control	0.032
Total IgE, median (IQR), kU/L	64.85 (40.7–73.5)	106 (56.9–450)	48.7 (25.4–90.8)	76.6 (34.5–148.5)	AR versus control AR versus SAR AR versus NAR	<0.001 <0.001 0.017
Nasal smear eosinophilia grade (±), median (IQR)	0 (0–1)	2 (1–3)	2 (1–2.3)	1 (1–2)	AR versus control SAR versus control NAR versus control	<0.001 <0.001 <0.001
Mild/moderate-severe, *n*/*n*	na	21/22	8/26	9/19	AR versus SAR	0.033
Duration, median (IQR), year	na	3 (2–7)	4.5 (1–8)	3 (1.3–7.8)	ns	
Seasonal/perennial/mixed, *n*/*n*/*n*	na	13/21/9	11/15/8	na	ns	

The ANOVA or Fisher's exact test was used for qualitative data, and the Mann-Whitney U test was performed for quantitative data. AR, allergic rhinitis; SAR, suspected allergic rhinitis; NAR, nonallergic rhinitis; na, not available/applicable; IQR, interquartile range; ns, not significant.

of serum total IgE than the control, suspected allergic rhinitis, and nonallergic rhinitis patients. The nasal smear eosinophilia grades in all 3 patient groups were higher compared to the control.

Gene Expression in Nasal Brushings of Patients with Allergic Rhinitis, Suspected Allergic Rhinitis, and Nonallergic Rhinitis

Of the 20 candidate genes, the expressions of 12 genes (POSTN, SERPINB2, CLC, CDH26, GCNT3, SH2B1D, CA2, SERPINB4, DPP4, CST1, TFF3, and ITLN1) in both the allergic rhinitis and suspected allergic rhinitis groups were significantly higher than those in the control; the former 10 genes were also increased in the nonallergic rhinitis group. Different expressions between the allergic rhinitis and nonallergic rhinitis groups were found for 3 genes (CST1, TFF3, and ITLN1) (Fig. 1). The expressions of 2 genes (CAP3 and CD274) were only mildly increased in the allergic rhinitis group with *p* values of 0.0115 and 0.336, respectively; and no difference among all groups was found for 6 genes (NTS, CD200R1, FETUB, CST2, GCNT4, and TMEM16A).

Gene Expression in Seasonal, Perennial, and Mixed Allergic Rhinitis

All the above 12 upregulated genes in the allergic rhinitis group showed significantly increased expressions in both seasonal and perennial allergic rhinitis groups compared to the control, and 9 genes (POSTN, SERPINB2, CLC, CDH26, CA2, SERPINB4, CST1, TFF3, and ITLN1) showed increased expressions also in the mixed allergic rhinitis group (Fig. 2).

Gene Expression in Seasonal, Perennial, and Mixed Suspected Allergic Rhinitis

The expressions of all the above 12 upregulated genes in the suspected allergic rhinitis group were increased in the perennial suspected allergic rhinitis group compared to the control; 8 of them (POSTN, SERPINB2, CDH26, GCNT3, SH2B1D, CA2, SERPINB4, and CST1) showed increased expressions also in the seasonal suspected allergic rhinitis group, and 5 of them (CDH26, GCNT3, SERPINB4, DPP4, and CST1) showed increased expressions also in the mixed suspected allergic rhinitis group. The TFF3 expression was higher in the perennial suspected allergic rhinitis group than that in the mixed suspected allergic rhinitis group (Fig. 3).

Association of Gene Expression with Disease Severity

In the allergic rhinitis group, GCNT3 expression was higher in the moderate/severe group than that in the mild group. In the suspected allergic rhinitis group, CLC expression was higher in the mild group than that in the moderate/severe group. In the nonallergic rhinitis group, CDH26 and CST1 expressions were higher in the moderate/severe group than those in the mild group (Fig. 4).

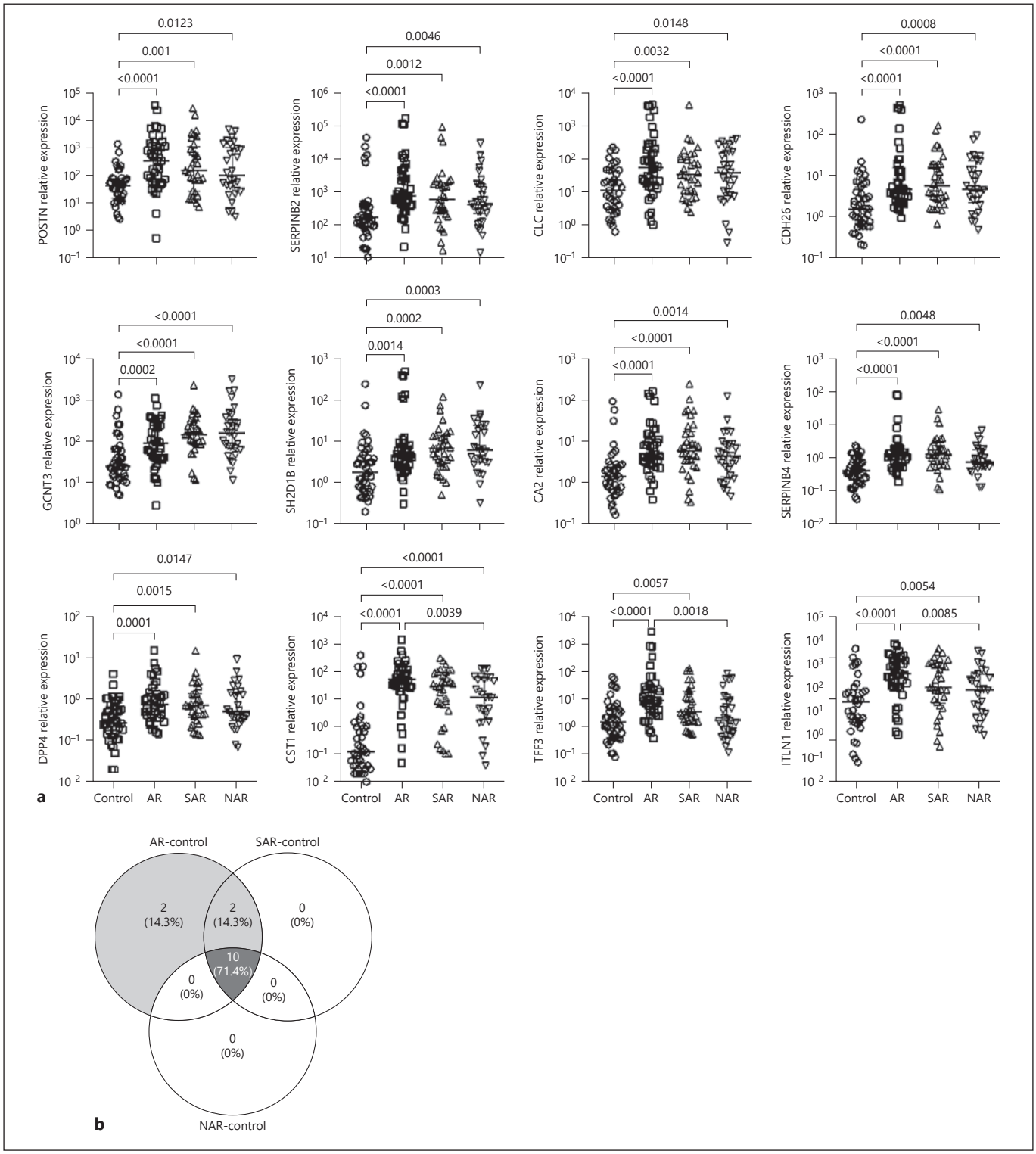

Fig. 1. a Gene expression in nasal brushings of the control, allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis patients by qPCR. **b** A Venn diagram showing the number of differentially expressed genes derived from the comparison of patient groups with the control group. s-IgE, serum-specific IgE; AR, allergic rhinitis; SAR, suspected allergic rhinitis patients who reported allergen-related nasal symptoms but had negative s-IgE; NAR, nonallergic rhinitis. The comparative Ct method (ΔΔCt) was used for relative gene expression analysis.

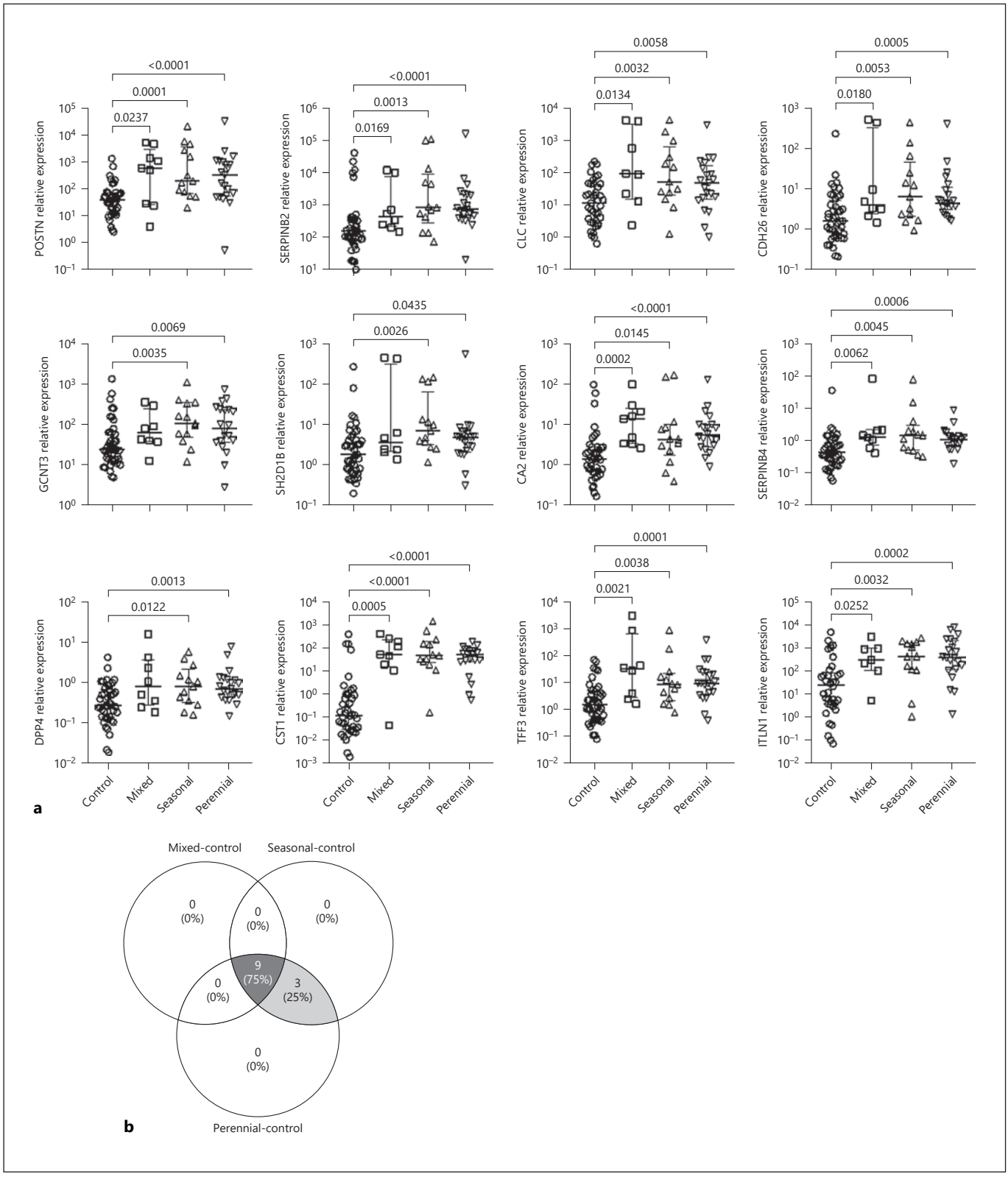

Fig. 2. a Gene expression in nasal brushings of the allergic rhinitis patients allergic to mixed, seasonal, and perennial allergens by qPCR. **b** A Venn diagram showing the number of differentially expressed genes derived from the comparison of patient groups with the control group. The comparative Ct method (ΔΔCt) was used for relative gene expression analysis.

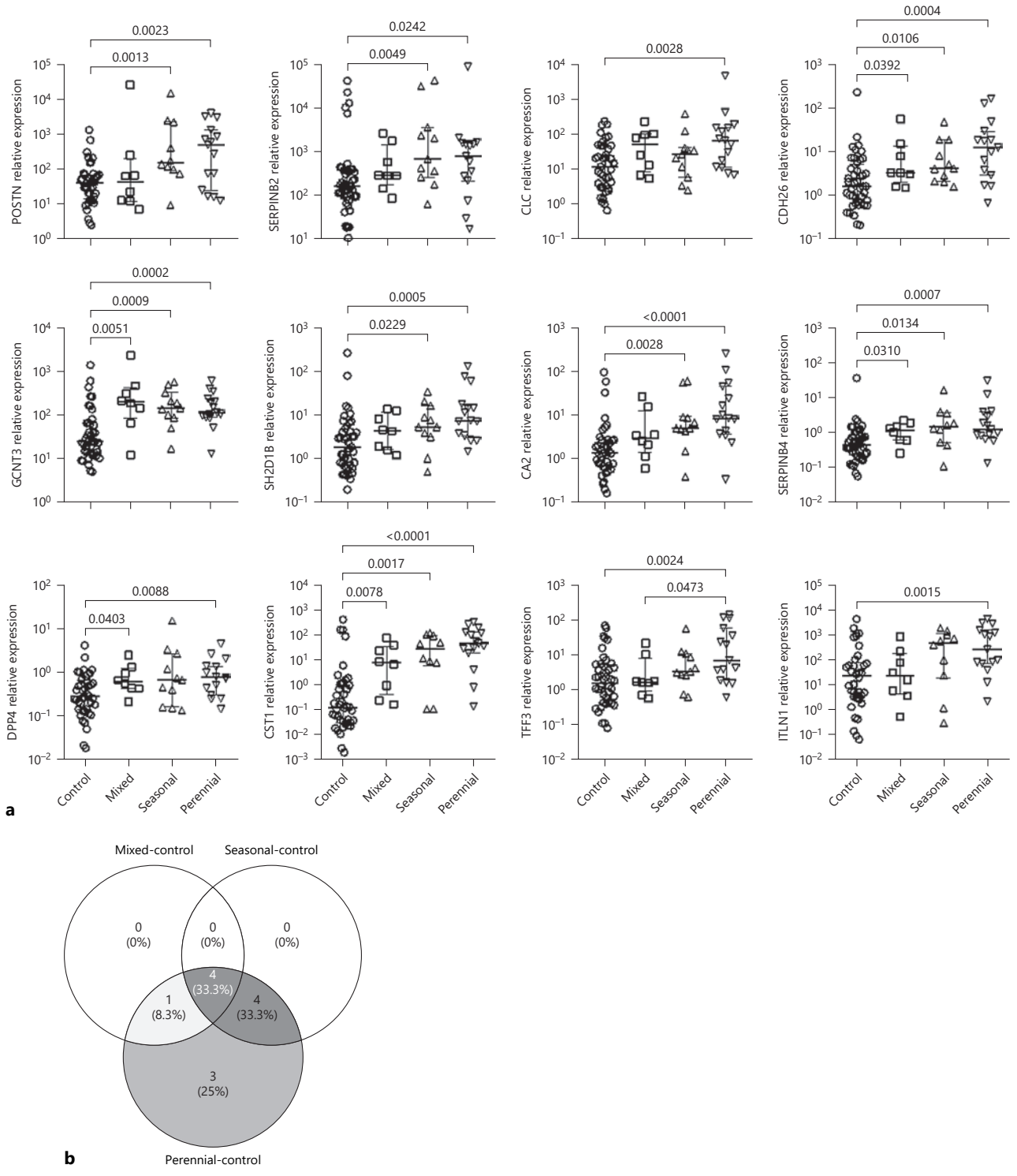

Fig. 3. a Gene expression in nasal brushings of suspected allergic rhinitis patients allergic to mixed, seasonal, and perennial allergens by qPCR. **b** A Venn diagram showing the number of differentially expressed genes derived from the comparison of patient groups with the control group. The comparative Ct method (ΔΔCt) was used for relative gene expression analysis.

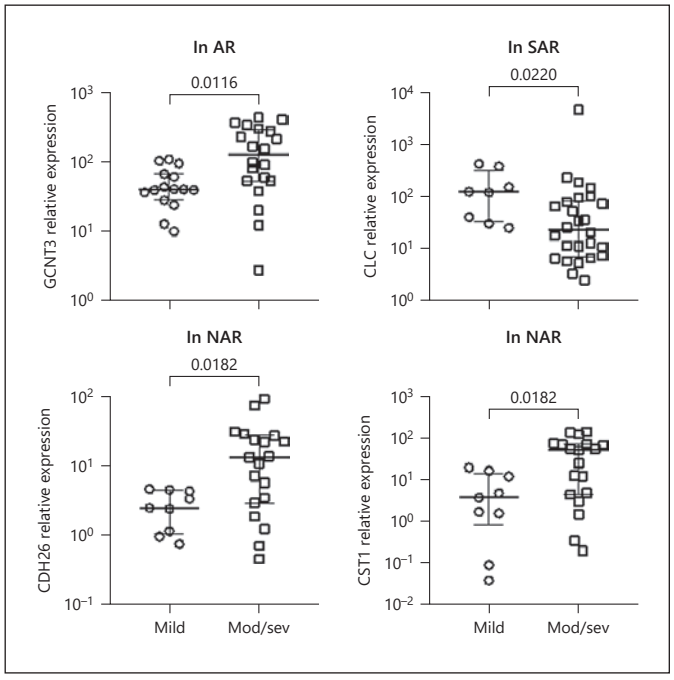

Fig. 4. Gene expression in nasal brushings of allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis patients with different grades of severity by qPCR. AR, allergic rhinitis; SAR, suspected allergic rhinitis patients who reported allergen-related nasal symptoms but had negative s-IgE; NAR, nonallergic rhinitis; Mod/sev, moderate/severe. The comparative Ct method (ΔΔCt) was used for relative gene expression analysis.

Correlation of Gene Expression with Disease Duration, Serum Total IgE, and Nasal Smear Eosinophilia Grade

No gene was correlated with years of disease and serum total IgE in the allergic rhinitis, suspected allergic rhinitis, or nonallergic rhinitis groups (data not shown). In the suspected allergic rhinitis group, GCNT3 expression was positively correlated with the eosinophilia grade ($r = 0.4359$, $p = 0.0160$), and POSTN had the trend to be positively correlated with the eosinophilia grade ($r = 0.3229$, $p = 0.0876$). In the allergic rhinitis group, SERPINB2 had the trend to be negatively correlated with the eosinophilia grade ($r = -0.2814$, $p = 0.0711$).

Discussion

Differential epithelial gene expression in childhood allergic rhinitis patients allergic to house dust mites compared to the control has been reported in a previous microarray-based study [14]. We further investigate whether it is similar in adult allergic rhinitis patients allergic to other types of allergens and the difference or similarity in patients with allergic rhinitis, suspected allergic rhinitis, and nonallergic rhinitis by real-time PCR in nasal brushing samples.

Similar to the previous study in childhood allergic rhinitis patients allergic to house dust mites [14], our findings showed most (60%, 12 out of 20) genes were greatly increased in adult allergic rhinitis patients allergic to various types of allergens, with all these genes increased in both seasonal and perennial allergic rhinitis patients and most in the mixed group. These findings indicate the upregulation of most epithelial genes in allergic rhinitis might be independent of age and allergen type. However, our result of more upregulated genes in the perennial group, fewer in the seasonal group, and fewest in the mixed group in the suspected allergic rhinitis patients indicates allergen type might influence the epithelial gene expression in suspected allergic rhinitis.

Though many differences in the inflammatory mechanisms between allergic rhinitis and nonallergic rhinitis have been identified [4–6], our study showed similar expression of most epithelial genes in allergic rhinitis and nonallergic rhinitis and only CST1, TFF3, and ITLN1 showed a significant difference between allergic rhinitis and nonallergic rhinitis. This similarity is consistent with the previous studies, which showed similar inflammatory cell infiltrates in allergic rhinitis and nonallergic rhinitis [7, 8], and indicates most genes do not have the potential to be used as genetic markers to differentiate allergic rhinitis from nonallergic rhinitis, except for CST1, TFF3, and ITLN1.

Allergic rhinitis is known to be characterized by Th2-associated inflammation, while the existence of Th2-associated inflammation in nonallergic rhinitis was controversial [5–8]. Among the 10 genes upregulated in both allergic rhinitis and nonallergic rhinitis, all except CLC and GCNT3 were reported to be induced by Th2 cytokines [14–17]. Furthermore, some genes have been shown to promote the Th2 response, such as POSTN in asthma [18], SERPINB2 in enteric nematode infection [19], CLC in asthma [20], SERPINB4 in allergic airway disease [21], and ITLN1 in asthma and atopic dermatitis [22]. These evidences strongly support the existence of a Th2-related response in nonallergic rhinitis. GCNT3 and SERPINB4 may be involved in allergic rhinitis by regulating mucus production [23, 24]. Consistent with the increased expression of TFF3 in allergic rhinitis but not in nonallergic rhiintis in our study, the epithelial barrier showed impairment in allergic rhinitis but not in nonallergic rhinitis [6]. Together with the evidence that TFF3 plays a central role

in the maintenance of epithelial surface integrity [25, 26], we speculate TFF3 might regulate the re-epithelialization in allergic rhinitis. The increased expression of CST1 in our allergic rhinitis patients compared to the control subjects confirmed the results in the previous studies [27, 28] and it has recently been shown that cystatin SN (encoded by CST1) could suppress allergic rhinitis symptoms through inhibiting allergen protease and protecting the epithelial barrier [29].

We assume that suspected allergic rhinitis might be the initial or relieved phase in the natural history of allergic rhinitis and so mechanisms similar to those in allergic rhinitis instead of nonallergic rhinitis might exist. However, corresponding to the finding that most gene expressions increased in allergic rhinitis were similarly increased in nonallergic rhinitis except for TFF3 and ITLN1, most gene expressions increased in suspected allergic rhinitis were similarly increased in both allergic rhinitis and nonallergic rhinitis and only TFF3 and ITLN1 expressions were increased in both suspected allergic rhinitis and allergic rhinitis but not nonallergic rhinitis, which might support greater similarity of suspected allergic rhinitis to allergic rhinitis than to nonallergic rhinitis. To confirm the similarity of suspected allergic rhinitis to allergic rhinitis but not nonallergic rhinitis, more factors which are known to be differentially expressed between allergic rhinitis and nonallergic rhinitis need to be detected simultaneously in suspected allergic rhinitis, allergic rhinitis, and nonallergic rhinitis.

To evaluate the role of these genes in specific types of rhinitis, we analyzed the association of these genes with clinical features (disease severity and duration), serum total IgE, and nasal smear eosinophilia grade. The findings of no associations of the expression of these genes with disease duration and serum total IgE indicate these genes might not influence disease duration and IgE response. However, different genes were associated with disease severity and the nasal smear eosinophilia grade in different types of rhinitis, indicating different mechanisms might exist in different types of rhinitis.

There are several limitations to this study. First, the size of the cohort may be relatively small for genes with small but important differences and more genes of interest should be detected. Second, the findings were at the gene level and could not represent the protein level. Third, we did not evaluate the longitudinal changes in allergic rhinitis symptoms and sensitization in suspected allergic rhinitis, and thus, no direct evidence was provided for the assumption that suspected allergic rhinitis is related to allergic rhinitis.

In conclusion, epithelial gene expression in allergic rhinitis might be independent of age and allergen type and was very similar to those in patients with suspected allergic rhinitis, and nonallergic rhinitis, with most genes related to Th2 inflammation. CST1, TFF3, and ITLN1 expressions might have the ability to differentiate allergic rhinitis from nonallergic rhinitis. More studies are needed to elucidate the relationship between suspected allergic rhinitis and allergic rhinitis. The findings of this study might be helpful for the diagnosis and treatment of different types of rhinitis.

Statement of Ethics

This study was approved by the Ethics Committee of Beijing TongRen Hospital, and written informed consent was obtained from all subjects before enrollment in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.W. and W.Y.S. designed the study. W.Y.S., X.D.W., Y.Z., and X.Z. assisted with the sampling of nasal brushings. M.W. and J.Y. performed the experiments. M.W. analyzed and interpreted the data and wrote the manuscript. L. Z. supervised the study.

References

1 Bachert C, van Cauwenberge P, Olbrecht J, van Schoor J. Prevalence, classification and perception of allergic and nonallergic rhinitis in Belgium. Allergy. 2006;61(6):693–8.
2 Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group, World Health Organization. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001;108(5 Suppl):S147–334.
3 Mullol J, Valero A, Alobid I, Bartra J, Navarro AM, Chivato T, et al. Allergic rhinitis and its impact on asthma update (ARIA, 2008). The perspective from Spain. J Investig Allergol Clin Immunol. 2008;18:327–34.

4 Liu C, Zheng K, Liu X, Zheng M, Liu Z, Wang X, et al. Use of nasal nitric oxide in the diagnosis of allergic rhinitis and nonallergic rhinitis in patients with and without sinus inflammation. J Allergy Clin Immunol Pract. 2020;8(5):1574–81.e4.
5 Suojalehto H, Toskala E, Kilpeläinen M, Majuri ML, Mitts C, Lindström I, et al. MicroRNA profiles in nasal mucosa of patients with allergic and nonallergic rhinitis and asthma. Int Forum Allergy Rhinol. 2013;3(8):612–20.
6 Steelant B, Seys SF, Van Gerven L, Van Woensel M, Farré R, Wawrzyniak P, et al. Histamine and T helper cytokine-driven epithelial barrier dysfunction in allergic rhinitis. J Allergy Clin Immunol. 2018;141(3):951–63.e8.
7 Powe DG, Huskisson RS, Carney AS, Jenkins D, Jones NS. Evidence for an inflammatory pathophysiology in idiopathic rhinitis. Clin Exp Allergy. 2001;31(6):864–72.
8 Rondón C, Romero JJ, López S, Antúnez C, Martín-Casañez E, Torres MJ, et al. Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. J Allergy Clin Immunol. 2007;119(4):899–905.
9 Bodtger U, Linneberg A. Remission of allergic rhinitis: an 8-year observational study. J Allergy Clin Immunol. 2004;114(6):1384–8.
10 Mosler K, Coraux C, Fragaki K, Zahm JM, Bajolet O, Bessaci-Kabouya K, et al. Feasibility of nasal epithelial brushing for the study of airway epithelial functions in CF infants. J Cyst Fibros. 2008;7(1):44–53.
11 Guajardo JR, Schleifer KW, Daines MO, Ruddy RM, Aronow BJ, Wills-Karp M, et al. Altered gene expression profiles in nasal respiratory epithelium reflect stable versus acute childhood asthma. J Allergy Clin Immunol. 2005;115(2):243–51.
12 Lambrecht BN, Hammad H. The airway epithelium in asthma. Nat Med. 2012;18(5):684–92.
13 Toppila-Salmi S, van Drunen CM, Fokkens WJ, Golebski K, Mattila P, Joenvaara S, et al. Molecular mechanisms of nasal epithelium in rhinitis and rhinosinusitis. Curr Allergy Asthma Rep. 2015;15(2):495.
14 Giovannini-Chami L, Marcet B, Moreilhon C, Chevalier B, Illie MI, Lebrigand K, et al. Distinct epithelial gene expression phenotypes in childhood respiratory allergy. Eur Respir J. 2012;39(5):1197–205.
15 Blanchard C, Durual S, Estienne M, Bouzakri K, Heim MH, Blin N, et al. IL-4 and IL-13 upregulate intestinal trefoil factor expression: requirement for STAT6 and de novo protein synthesis. J Immunol. 2004;172(6):3775–83.
16 Kamsteeg M, Zeeuwen PL, de Jongh GJ, Rodijk-Olthuis D, Zeeuwen-Franssen ME, van Erp PE, et al. Increased expression of carbonic anhydrase II (CA II) in lesional skin of atopic dermatitis: regulation by Th2 cytokines. J Invest Dermatol. 2007;127(7):1786–9.
17 Watanabe T, Chibana K, Shiobara T, Tei R, Koike R, Nakamura Y, et al. Expression of intelectin-1 in bronchial epithelial cells of asthma is correlated with T-helper 2 (Type-2) related parameters and its function. Allergy Asthma Clin Immunol. 2017;13:35.
18 Bentley JK, Chen Q, Hong JY, Popova AP, Lei J, Moore BB, et al. Periostin is required for maximal airways inflammation and hyperresponsiveness in mice. J Allergy Clin Immunol. 2014;134(6):1433–42.
19 Zhao A, Yang Z, Sun R, Grinchuk V, Netzel-Arnett S, Anglin IE, et al. SerpinB2 is critical to Th2 immunity against enteric nematode infection. J Immunol. 2013;190(11):5779–87.
20 Persson EK, Verstraete K, Heyndrickx I, Gevaert E, Aegerter H, Percier JM, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. Science. 2019;364(6442):eaaw4295.
21 Shamji MH, Temblay JN, Cheng W, Byrne SM, Macfarlane E, Switzer AR, et al. Anti-apoptotic serine protease inhibitors contribute to survival of allergenic TH2 cells. J Allergy Clin Immunol. 2018;142(2):569–81.e5.
22 Yi L, Cheng D, Zhang K, Huo X, Mo Y, Shi H, et al. Intelectin contributes to allergen-induced IL-25, IL-33, and TSLP expression and type 2 response in asthma and atopic dermatitis. Mucosal Immunol. 2017;10(6):1491–503.
23 Li RW, Li C, Elsasser TH, Liu G, Garrett WM, Gasbarre LC. Mucin biosynthesis in the bovine goblet cell induced by Cooperia oncophora infection. Vet Parasitol. 2009;165(3–4):281–9.
24 Sivaprasad U, Askew DJ, Ericksen MB, Gibson AM, Stier MT, Brandt EB, et al. A nonredundant role for mouse Serpinb3a in the induction of mucus production in asthma. J Allergy Clin Immunol. 2011;127(1):254–6.
25 Mashimo H, Wu DC, Podolsky DK, Fishman MC. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science. 1996;274(5285):262–5.
26 Paulsen FP, Woon CW, Varoga D, Jansen A, Garreis F, Jäger K, et al. Intestinal trefoil factor/TFF3 promotes re-epithelialization of corneal wounds. J Biol Chem. 2008;283(19):13418–27.
27 Imoto Y, Tokunaga T, Matsumoto Y, Hamada Y, Ono M, Yamada T, et al. Cystatin SN upregulation in patients with seasonal allergic rhinitis. PLoS One. 2013;8(8):e67057.
28 Ndika J, Airaksinen L, Suojalehto H, Karisola P, Fyhrquist N, Puustinen A, et al. Epithelial proteome profiling suggests the essential role of interferon-inducible proteins in patients with allergic rhinitis. J Allergy Clin Immunol. 2017;140(5):1288–98.
29 Fukuoka A, Matsushita K, Morikawa T, Adachi T, Yasuda K, Kiyonari H, et al. Human cystatin SN is an endogenous protease inhibitor that prevents allergic rhinitis. J Allergy Clin Immunol. 2019;143(3):1153–62.e12.