

Microarray Assay Reveals Ciliary Abnormalities of the Allergic Nasal Mucosa

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Abstract

Background: Gene expression patterns (particularly, cilia-associated genes) of nasal mucosa, the first-line defense system, in allergic rhinitis (AR) are not well understood.

Objective: We sought to screen for AR-associated genes in inferior turbinate (IT) from patients with AR, and to validate the expression of common cilia-related genes and ciliary shedding.

Methods: Prime ViewTM Human Gene Expression Array, which consisted of more than 530 000 probes covering more than 36 000 transcripts and variants, was employed to compare individual gene expression of ITs from control subjects ($n = 11$) and patients with AR ($n = 19$). Gene ontology (GO) analysis was performed with Cytoscape software. Eight of the common cilia-related genes were validated with quantitative polymerase chain reaction. We applied a semiquantitative scoring system for immunofluorescence assay to demonstrate ciliary shedding in 5 areas per paraffin section, with individual sections being scored between 0 (normal ciliary distribution) and 1 (ciliary shedding).

Results: Compared with control subjects, 160 (38 upregulated and 122 downregulated) genes were differentially expressed for at least 2 folds (all $P < .05$) in AR. Seven GO categories were significantly enriched, 4 of which were related to cilium assembly and motility. Quantitative polymerase chain reaction validated the predicted direction of change for common cilia-related gene expression. The ciliary distribution score was significantly higher (more prominent ciliary shedding) in AR than in controls ($P < .05$).

Conclusion: The significant aberrant cilia-related gene expression, revealed by microarray assays, might be the critical driver of AR where ciliary shedding is prominent.

Keywords

allergic rhinitis, cilia, immunofluorescence, inferior turbinate, microarray

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Introduction

Allergic rhinitis (AR) is a type-2 airway inflammatory disorder of the nasal mucosa which arises from the exaggerated response of the allergen–IgE interaction in sensitized individuals and has conferred a significant health-care burden worldwide.¹ The diagnosis of AR has been clinically based upon the history of allergic symptoms and the diagnostic tests such as skin prick test and serum-specific IgE. However, in view of the united airways and personalized medicine concept, there is a need for better characterization of biomarkers for patient stratification to identify those who might benefit most from the targeted therapy.

The nasal mucosa constitutes the first-line defense system against the invading allergens and pathogens and is implicated in both innate and adaptive immune responses.^{2,3} The inferior turbinate (IT) is the major site of the nasal mucosa where the contact with the invading allergens and pathogens takes place. In patients with AR, total IgE levels and cedar pollen-specific IgE levels in IT tissue correlated significantly with the total and specific IgE levels in serum.⁴ The exaggerated response of the allergic nasal epithelium to aeroallergens may have also been responsible for nasal epithelial injury.⁵ However, the mechanisms underlying injury and changes to the nasal mucosa remain not fully explored. For instance, defective ciliary clearance, evidenced by impaired ultrastructure and/or function of respiratory cilia, has been implicated in chronic airway inflammatory diseases such as AR and warranted further investigations in their contribution to the pathogenesis of AR.⁶

To date, most studies of AR focused on genetic analysis and single genes or pathways which have limited ability to delineate the complex transcriptomic regulations and interactions from the panel of canonical genes that play critical roles in driving the pathogenesis of AR.^{7,8} By performing microarray analysis, various pathologic processes have been characterized in patients with other chronic inflammatory airway diseases. For instance, 143 upregulated and 3 downregulated genes have been identified as aspirin-intolerant asthma-specific genes that were enriched in the immune response according to gene ontology (GO) analysis in patients with nasal polyps.⁹

We hypothesized that there exist transcriptional changes related to the pathogenesis of AR. Here, we sought to investigate the global messenger ribonucleic acid expression in IT tissues derived from patients with AR and control subjects, which may help profile the gene expression patterns. We further conducted polymerase chain reaction and immunofluorescence staining to confirm the findings of microarray assays.

Methods

Patient Recruitment

This study was conducted based on the human research protocol approved by the institutional review boards of Guangdong General Hospital and the National Healthcare Group Domain Specific Review Board of Singapore. All patients gave written informed consent.

Control subjects and patients with AR were recruited from individual sites. AR was diagnosed according to *Allergic Rhinitis and its Impact on Asthma*,¹ and the inclusion criteria were as follows: (1) Clinical history of persistent AR as evidenced by nose itching, sneezing, and clear rhinorrhea; (2) no history or symptoms of chronic rhinosinusitis, asthma, and suspicious of primary ciliary dyskinesia; (3) no respiratory tract infection and no use of glucocorticoids or antihistamines within the past 4 weeks; (4) positive skin prick test findings (Allergopharma, Reinbek, Germany) or serum total/specific IgE detected by using Allergy Screen test (Mediawiss Analytic GmbH, Moers, Germany). All control subjects were free from the clinical symptoms of AR and yielded negative reaction to skin prick test or serum-specific IgE assay. The exclusion of rhinosinusitis was made according to the *European Position Paper on Rhinosinusitis and Nasal Polyps 2012*.¹⁰ The exclusion of asthma was based on *Global Initiative for Asthma*,¹¹ the history inquiry and physician's diagnosis.

All biopsies were taken from the lower edge of the IT mucosa, approximately 2 cm posterior to the anterior margin, during septal plastic surgery by using the mucosal biopsy forceps for twice (one stored in 4% paraformaldehyde, and the other stored in RNAlater).

In our study, the ITs from patients with AR (n = 55) and control subjects (n = 40) who underwent septoplasty due to anatomic variations were eligible for analysis. The ITs from control subjects (n = 11) and patients with AR (n = 19) were used for microarray analysis. A separate set of samples from microarray, including the ITs from control subjects (n = 23) and patients with AR (n = 25), were used for validation with quantitative real-time polymerase chain reaction (qRT-PCR). Paraffin specimens from control subjects (n = 20) and patients with AR (n = 28), which were partly overlapped with the samples from microarray or qRT-PCR assays, were used for immunofluorescence staining (overlapped ITs from 10 control subjects and 17 patients with AR). The details are presented in Table 1 and Supplemental Table E1.

Microarray and qRT-PCR

RNA was extracted from frozen ITs in RNAlater[®] (Sigma-Aldrich, St. Louis, MO, USA) for Prime View[™] Human Gene Expression Array (Affymetrix,

Santa Clara, CA, USA), which consisted of more than 530 000 probes covering for more than 36 000 transcripts and variants, representing more than 20 000 genes mapped through UniGene or via RefSeq annotation. Data were normalized based on the quartile algorithm by using Genespring software (version 12.5; Agilent Technologies, Santa Clara, CA, USA). The Gene Cluster, Java TreeView, and Cytoscape software programs were used to perform the hierarchical cluster

analysis and GO analysis (biological process) of these differentially expressed mRNAs.¹²

According to the different functions or structural components of the respiratory cilia, the mRNA expression levels of 8 cilia-related genes [dynein axonemal assembly factor 3 (*DNAAF3*), forkhead box J1 (*FOXJ1*), dynein axonemal intermediate chain 1 (*DNAI1*), dynein axonemal light intermediate chain 1 (*DNALI1*), dynein axonemal heavy chain 9 (*DNAH9*), chromosome X open reading frame 41 (*CXorf41*), radial spoke head 1 (*RSPH1*), and radial spoke head 9 (*RSPH9*)] were validated with qRT-PCR (SYBR Green, Promega, Madison, WI, USA). The relative expression was calculated by using the $2^{-\Delta\Delta C_t}$ method normalized against the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase, in IT specimens. The details of chromosomal location, primer sequences, fold change, and *P* value are presented in Table 2.

Table 1. Summary Characteristics of Study Participants.

	Control Subjects	Patients With AR
Sample size (no.)	40	55
Age, years (mean $\pm$ SD)	33.3 $\pm$ 10.7	31.3 $\pm$ 11.8
Gender (M/F)	25/15	38/17
Perennial AR [no. (%)]	—	51 (92.7)
Allergen sensitization [no. (%)]		
House dust mites	—	47 (85.5)
Allergens other than house dust mites	—	8 (14.5)
Nationality, [no. (%)]		
Chinese	30 (75.0)	46 (83.6)
Singaporean	10 (25.0)	9 (16.4)
Methods		
Nasal tissues (no.)	40	55
Whole-genome expression array	11	19
qRT-PCR ^a	23	25
Paraffin specimens	20	28

Abbreviations: AR, allergic rhinitis; F, female; FOXJ1, forkhead box J1; M, male; qRT-PCR, quantitative real-time polymerase chain reaction; SD: standard deviation.

^aA completely separate set of inferior turbinate tissues from control subjects and patients with AR were selected for validation of the findings from microarray data by means of real-time qRT-PCR.

Immunofluorescence Staining and Assessment of Ciliary Distribution

All nasal biopsy specimens for paraffin sections were processed as previously reported.¹³ The sections were incubated with primary polyclonal antibodies (mouse anti-human acetylated alpha tubulin) (clone ab24610, Abcam, Cambridge, MA, USA) overnight at 4°C, followed by incubation with Alexa Fluor 488-conjugated secondary antibodies (Life Technologies, Carlsbad, CA, USA) at 37°C for 1 hour. Cellular nuclei were visualized by staining with 4'6-diamidino-2-phenylindole (Life Technologies). For negative controls, primary antibodies were substituted with species- and subtype-matched antibodies. Images were acquired with fluorescence microscopy (Olympus IX51, Tokyo, Japan).

Table 2. Chromosomal Locations, Primer Sequences, Fold Change, and *P* Value of the DEGs for qRT-PCR Validation.

Genes	Chromosomal Location	Microarray Analysis		qRT-PCR validation			
		Fold Change	<i>P</i>	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Fold Change	<i>P</i>
<i>CXorf41</i>	chrXq22.3	2.631	.047	CTCAAAGTCATCCCTGAAAC	CATACTCTGGGATTTCTCTAAC	1.986	.094
<i>DNAAF3</i>	chr19q13.4	3.086	.020	TTGCTTGATTCCTCGAAAAG	CACCCCTTTATCCTCCAAATATC	2.227	.035
<i>DNAH9</i>	chr17p12	2.262	.016	AGCAGGCCTCTAATTATCTC	CTCTTTGAAGTAAGTGTGGAG	3.008	.001
<i>DNAI1</i>	chr9p13.3	2.295	.019	TCAGTGGGAGATCTATGATG	ACTCCATAGATGTCAGCTTC	2.213	.034
<i>DNALI1</i>	chr1p35.1	2.386	.034	GGAGCAGTTAGACTTAAAGC	ACTCATCAAACACTGTGAG	2.321	.079
<i>FOXJ1</i>	chr17q25.1	2.613	.036	GTGAAGCCTCCCTACTC	AATTCTGCCAGGTGGG	2.224	.025
<i>RSPH1</i>	chr21q22.3	2.723	.038	GGAAAGAGGAGAAGAGGAAG	AATTCAGTGATTTGGGTAGC	2.501	.022
<i>RSPH9</i>	chr6p21.1	2.873	.021	GAATATGAACACACTGAGCTG	CTTGATCTGGACCACTATTTTC	1.996	.656
<i>GAPDH</i> ^a	chr12p13	1.121	.057	ACAGTTGCCATGTAGACC	TTTTTGGTTGAGCACAGG	—	—

Abbreviations: *CXorf41*, chromosome X open reading frame 41; DEGs, differentially expressed genes; *DNAAF3*, dynein axonemal assembly factor 3; *DNAH9*, dynein axonemal heavy chain 9; *DNAI1*, dynein axonemal intermediate chain 1; *DNALI1*, dynein axonemal light intermediate chain 1; *FOXJ1*, forkhead box J1; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; qRT-PCR, quantitative reverse transcription polymerase chain reaction; *RSPH1*, radial spoke head 1 homolog; *RSPH9*, radial spoke head 9 homolog.

^aHouse-keeping gene for qRT-PCR.

We randomly selected 5 areas of cilia staining from each paraffin section for assessment according to the semiquantitative scoring system as described previously. Based on the criterion (cutoff: 70% of the cellular staining of markers) proposed by Shoemark et al.,¹⁴ each area was assigned a score of 0 (normal ciliary distribution) or 1 (ciliary shedding), for which 0 point was assigned if the field contains >70% of epithelial areas with ciliary staining; 1 point was scored if the field contains $\leq$ 30% of epithelial areas with ciliary staining. Scoring of individual fields was undertaken by 2 independent reviewers blinded to the grouping, and the score of 5 areas was averaged. Discrepancy was harmonized by averaging the score of the 2 blinded observers.

Statistical Analysis

Statistical analyses were conducted with SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA). For microarray data, differentially expressed genes (DEGs) were identified based on the fold change (cutoff: 2.0; ≥ 2 as upregulated and ≤ 0.5 as downregulated) and P values (cutoff: 0.05). For GO analysis, the significant terms were retained if both the P value was less than 0.05 and the Benjamini–Hochberg false discovery rate (FDR) was less than 0.05. Mann–Whitney 2-sided nonparametric test was applied to compare the mRNA expression levels in qRT-PCR analysis and the ciliary distribution score. $P < .05$ was deemed statistically significant for all analyses.

Results

Subject Characteristics

The clinical characteristics of control subjects and patients with AR are summarized Table 1. All subjects for microarray assays (11 from control subjects and 19 from patients with AR) were recruited from Guangdong General Hospital, Guangdong province, China. Subjects from China and Singapore were included for validation with

qRT-PCR assay (23 from control subjects and 25 from patients with AR) and immunofluorescence staining (20 from control subjects and 28 from patients with AR).

Overview of the Expression Profile

Globally, microarray assay revealed 160 differentially expressed (38 upregulated and 122 downregulated) mRNAs in IT of patients with AR as compared with control subjects (fold change >2 or <-2 ; all $P < .05$) (Supplemental Tables E2 and E3). Figure 1(A) demonstrates the global expression patterns of genes between patients with AR and control subjects (with the top DEGs being highlighted). As further indicated in Figure 1(B), the global gene expression patterns differed considerably between patients with AR and control subjects. Despite a considerable heterogeneity in gene expression (variable expression levels of the 38 upregulated genes), 122 downregulated genes were significantly enriched in patients with AR (Figure 1).

The Cytoscape software was applied to explore for the DEGs involved in the regulatory networks that were annotated with GO items (Figure 2, Supplemental Table E4). At least 7 canonical GO categories were identified for all DEGs ($P < .05$ and FDR < 0.05). The biological processes of the canonical GO categories included motile cilium assembly, cilium movement, axonemal dynein complex assembly, cilium flagellum-dependent cell motility, negative regulation of transporter activity, response to zinc ion, and C-terminal protein lipidation. Of these biological processes, the former 4 were consistently related to cilium assembly and motility.

Validation With qRT-PCR Assay

To validate the above-mentioned findings regarding DEGs expression, we analyzed the expression levels of the 8 common cilia-related genes by performing qRT-PCR. A separate set of ITs were selected from control

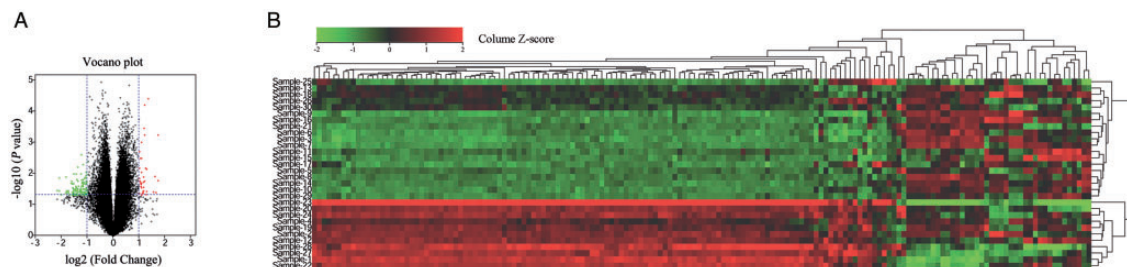


Figure 1. Alterations in mRNA expression profiles in the allergic nasal mucosa. Volcano plot (A) showing the DEGs in patients with AR as compared with control subjects. The horizontal line indicates the P value of .05 and the vertical line corresponds to the threshold of 2.0-fold change of either upregulation (red) and downregulation (green) (1.0 corresponds to the logarithm of 2 at the base of 2). The heat map (B) of expression of DEGs from inferior turbinate tissue in patients with AR ($n = 19$, Sample No. 1 to No. 19) and control subjects ($n = 11$, Sample No. 20 to No. 30). Increased expression is labeled with red grids and decreased expression with green grids. A total of 160 (38 upregulated and 122 downregulated) differentially expressed mRNAs are identified. AR, allergic rhinitis; DEGs, differentially expressed genes.

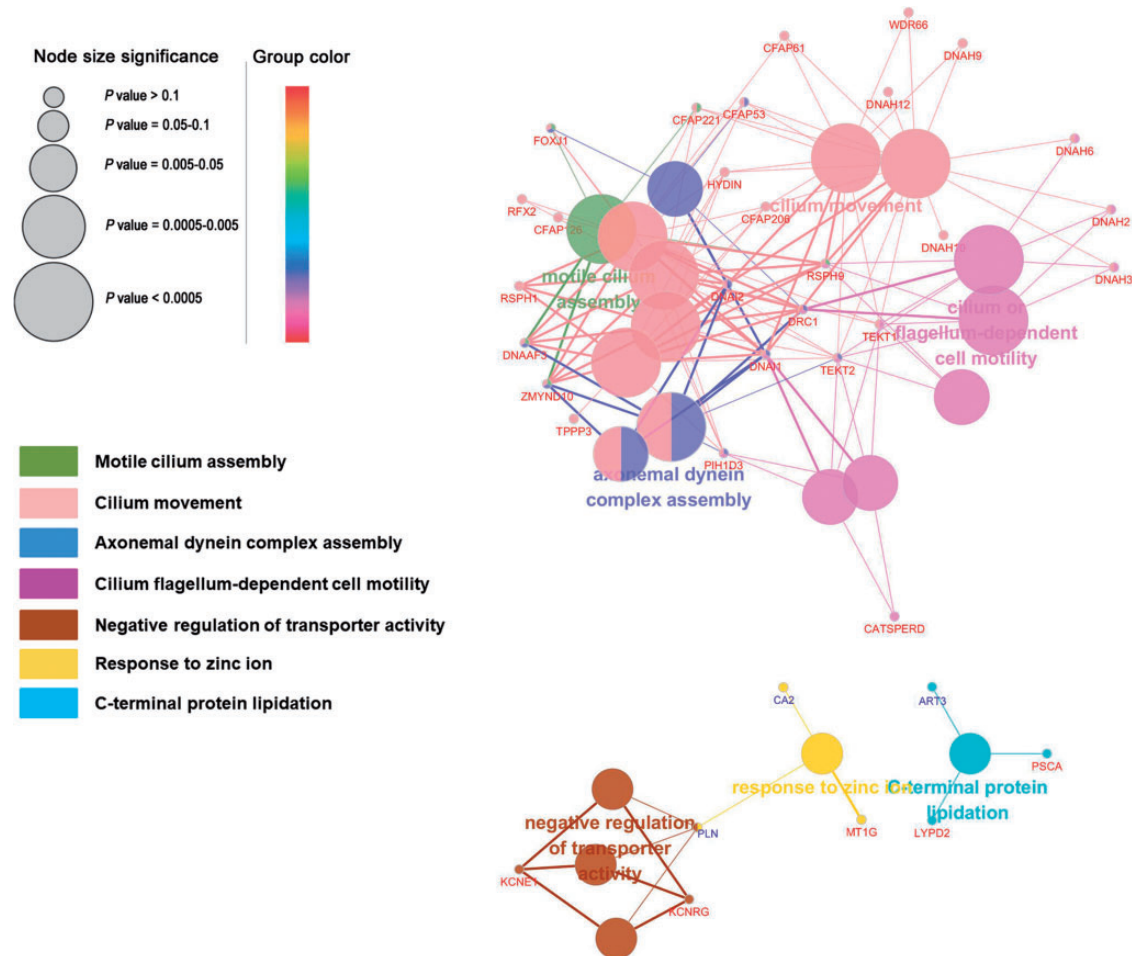


Figure 2. Gene ontology analysis showing the 160 DEGs in patients with allergic rhinitis. Seven clusters of the biological processes are found in the gene ontology analysis ($P < .05$ and the FDR < 0.05) for all the DEGs. The node sizes denote the P value, whereas the width of the lines connecting between the 2 nodes denotes the biological relevance. Compound colors indicate dual major biological processes that are involved. The 7 major biological processes (motile cilium assembly, cilium movement, axonemal dynein complex assembly, cilium flagellum-dependent cell motility, negative regulation of transporter activity, response to zinc ion, and C-terminal protein lipidation) are shown. For the individual gene, the upregulated genes (red) or downregulated genes (blue) were marked, respectively. FDR, false discovery rate; DEGs, differentially expressed genes.

subjects ($n = 23$) and patients with AR ($n = 25$) for qRT-PCR assays as a means of validation. The fold difference for individual DEGs as detected with qRT-PCR was calculated and compared with the data obtained from microarray analyses (Table 2). Except for *DNALI1* (mean fold change: 2.321, $P = .079$), *CXorf41* (mean fold change: 1.986, $P = .094$) and *RSPH9* (mean fold change: 1.996, $P = .656$), findings of qRT-PCR assay were globally in accordance with the microarray data (fold change > 2, $P < .05$, Figure 3).

Immunofluorescence Imaging

Upon confirming the reduced expression of ciliary genes, we further sought to determine whether the reduction in transcription of the ciliary function genes translated into reduced function and to ascertain the magnitude cilia loss

contributing to the possible pathogenesis of AR. Therefore, we further examined the distribution patterns of cilia in the nasal epithelium by performing immunofluorescence staining in IT tissues from control subjects and patients with AR. Two distinct distribution patterns were observed in paraffin sections (Figure 4(A) to (F)). Based on our semiquantitative scoring system, we scored 5 areas and obtained the average score of each sample. The median (the first and third quartile) score was 0.30 (0, 0.65) for IT tissues from patients with AR and 0 (0, 0.20) for IT tissues from control subjects ($P < .05$, Figure 4(G)).

Discussion

AR is an allergic upper respiratory disease in which multiple pathogenic factors are implicated. Apart from

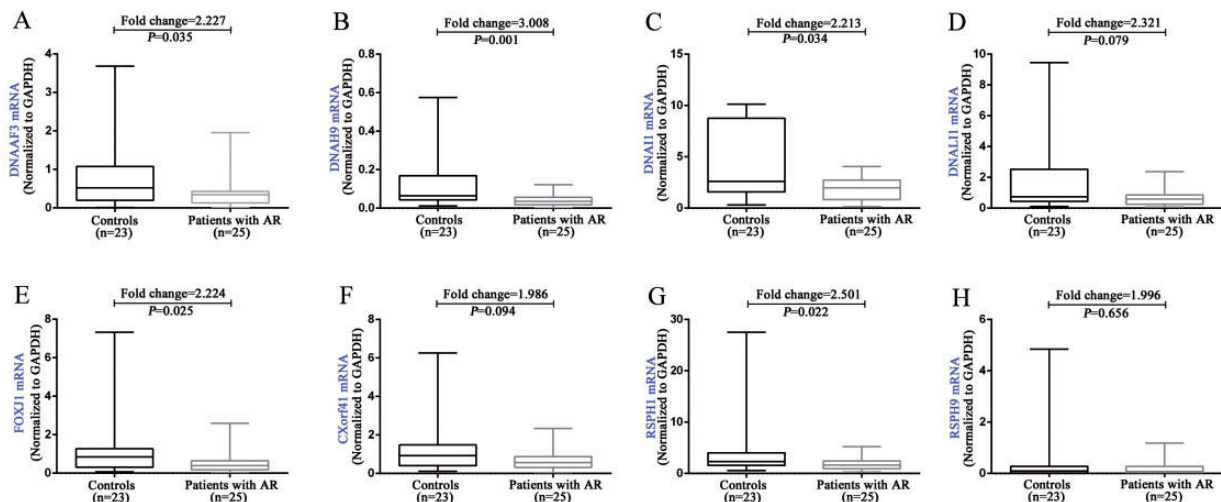


Figure 3. Validation of the expression levels of 8 common cilia-related genes in inferior turbinate between patients with AR and controls with quantitative reverse transcription polymerase chain reaction. AR, allergic rhinitis; *CXorf41*, chromosome X open reading frame 41; DEGs, differentially expressed genes; *DNAAF3*, dynein axonemal assembly factor 3; *DNAH9*, dynein axonemal heavy chain 9; *DNALI1*, dynein axonemal intermediate chain 1; *DNALI2*, dynein axonemal light intermediate chain 2; *FOXJ1*, forkhead box J1; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; qRT-PCR, quantitative reverse transcription polymerase chain reaction; *RSPH9*, radial spoke head 9 homolog; *RSPH10*, radial spoke head 10 homolog.

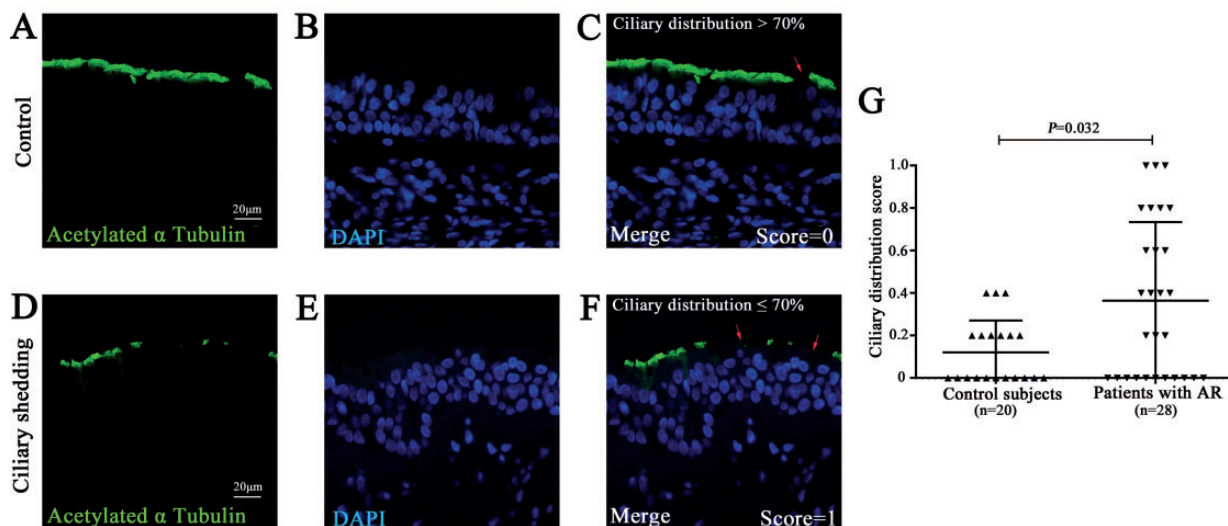


Figure 4. Two distinct ciliary distribution patterns and the score for paraffin specimens. Each area is assigned a score of either 0 or 1, for which score = 0 (normal ciliary distribution) was assigned if the field contains >70% of epithelial areas with ciliary staining (A–C); score = 1 (ciliary shedding) is assigned if the field that contains ≤30% of epithelial areas with ciliary staining (D–F). The median (the first and third quartile) score is 0.30 (0 and 0.65) for samples from patients with AR and 0 (0 and 0.20) for samples from control subjects ($P = .032$) (G). AR, allergic rhinitis; DAPI, 4',6-diamidino-2-phenylindole.

allergen sensitization, impaired ciliary clearance has recently been identified as a driver of AR.¹⁵ Nonetheless, based on genome-wide association study and epistasis analysis, ciliary abnormalities might represent a novel mechanism of atopy.¹⁶ However, most of the previous reports focused on the analysis of individual or a limited number of markers reflecting the ciliary

clearance.^{17,18} In this study, microarray assays were applied to test for more than 20 000 human genes to screen for those in which the expression was significantly altered for at least 2-fold in allergic ITs as compared with control ITs. Our analysis has revealed 38 upregulated and 122 downregulated genes that were significantly enriched in patients with AR despite that the

heterogeneity of gene expression in individual patients has also been observed.

Based on GO analysis, most DEGs were associated with the abnormality of ciliary assembly, ultrastructure, and motility, suggesting the critical roles of ciliary abnormalities in driving the development of AR (Figure 2). Previous literature reports have documented that ciliary abnormalities could be either primary or secondary or both.^{19,20} Primary ciliary dyskinesia is a genetically heterogeneous, autosomal-recessive disorder that is characterized by structural and/or functional ciliary abnormalities that contribute to impaired ciliary clearance.²¹ In the present study, to minimize the risks of misclassification, we have excluded patients who were suspected a shaving primary ciliary dyskinesia based on the past and present history (ie, recurrent lower respiratory tract infections, chronic cough, production of mucopurulent or purulent sputum, and a family history of congenital diseases indicating immotile cilia) and the clinician's diagnosis. In light of the chronic airway inflammation, the ciliary abnormalities detected in patients with AR were likely to have arisen from repetitive inflammatory insults.⁶

In addition, there have been a considerable number of literature reports demonstrating the pathogenic roles of chronic allergic airway inflammation in impairing the nasal ciliary clearance. Ciliary abnormalities and ultrastructural abnormalities, as revealed by transmission electron microscopy, significantly correlated with the severity of asthma and might have stemmed from the insults of chronic airway inflammation or infection in patients with severe asthma.²² Recently, our study group has also reported that aberrant localization of *DNAH5* and *FOXJ1* may be crucial characteristics of the chronic airway inflammatory diseases such as nasal polyps and AR (a part of the microarray results in this study).^{13,23} In addition, aberrant epithelial remodeling has been associated with significant impairment of cilia architecture and function in the chronically inflamed nasal mucosa of patients with nasal polyps.²⁴ However, thus far, there has not been a systematic histopathological investigation directly linking to ciliary shedding in airway inflammation. Consistent with the cardinal findings of these studies that demonstrated ciliary abnormalities in the milieu of airway inflammation, our study has extended the validity of these findings in the allergic nasal mucosa. More importantly, we have simultaneously characterized the greater magnitude of ciliary shedding that may collectively contribute to the significantly impaired ciliary clearance through the induction of defective ciliogenesis and ciliary ultrastructural defects.

To validate the findings of microarray assay, we have performed qRT-PCR which is a well-recognized assay for detecting mRNA expression. With exception of *DNALII*, *CXorf41* and *RSPH9*, we did identify a

consistent trend of changes in gene expression levels between microarray assays and qRT-PCR assay. Furthermore, the difference in expression levels of *DNALII* and *CXorf41* for qRT-PCR assay trended toward statistical significance. The lack of statistical significance of most ciliary markers (except for *RSPH9*) might be partially related to the limited sample sizes. However, in light that altered cilia-related gene expression in the milieu of chronic airway inflammation might reflect motile ciliary disorders such as impaired ciliogenesis, the verification of the loss of cilia (ciliary shedding) through immunofluorescence assay can further ascertain whether the changes in ciliary transcriptomic regulation contributed to loss of ciliary function. Consistent with our hypothesis, more prominent ciliary shedding was observed in the allergic nasal mucosa. Nonetheless, the causality of ciliary shedding and altered gene expression cannot be ascertained with the current study design, as did the association between airway inflammation and altered cilia-related gene expression. Clarification of the role of chronic airway inflammation in inducing ciliary shedding and altered cilia-related gene expression must await further mechanistic investigation.²⁵ Ciliary shedding might be an important metric which should be incorporated in future clinical assessment of ciliary clearance and prediction of concomitant diseases complicating the management of airway disease. Moreover, it might represent a novel therapeutic target because the commercially available drugs such as Gelomyrtol ForteTM are capable of normalizing ciliary gene expression in vitro (eg, *FOXJ1*, a critical regulator in the maintenance of airway epithelial cell differentiation through the regulation of ciliogenesis).²⁶

The downregulation of cilia-related genes in patients with AR may have further contributed to T helper (Th) 2-skewed airway inflammation. Activation of Th2 cells and the subsequent release of the cytokines could have resulted in chemotaxis and migration of various immune cells to the nasal epithelium, contributing to AR via direct or indirect impairment of mucociliary biomarker expression and mucociliary function.^{27,28} However, we did not identify the GO enrichment of Th2-related markers, which might be because we mainly biopsied the epithelia but not subepithelial tissues (where inflammatory cells were mostly located in) for transcriptomic microarray assays. In addition, AR is characterized by episodes of hypersensitivity responses, which may remit during the period outside the sensitization episodes. In our study, most biopsies were taken outside the episodes of allergen exposure (stable state) in patients with AR when undergoing septal plastic surgery. This may have contributed to the lack of differentially expressed inflammatory markers. Further research elucidating the association between altered ciliary gene expression and Th2 inflammation is needed.

In our study, whole-tissue biopsies tend to overlook the characteristics of individual cells and could only indicate the mean expression levels among the whole cell populations. The application of single-cell sequencing technology has become increasingly mature.^{29,30} Hence, single-cell sequencing should be pursued further exploration of the characteristics of ciliated cells be investigated.

This study has some limitations. First, we did not perform scanning electron microscopy to confirm the magnitude of ciliary shedding and the defects of ultra-structural markers associated with motile ciliary disorders. Second, the nasal mucosa was sampled at a single time point both in patients with AR and controls. Therefore, we cannot comment on the stability of the present findings throughout the course of AR. Third, the potential impact of recent use of steroids or antihistamines on nasal mucosa has been eliminated because none of the patients with AR had received any antihistamine or nasal/systemic corticosteroids within 4 weeks before surgery (verified against the medical charts), further research on the effects of antihistamine or corticosteroids in allergic nasal mucosa would be needed. Moreover, whether ciliary shedding and the magnitude of cilia-related gene expression are more prominent during flare-up of nasal symptoms or viral infections warrants further investigations. In addition, we did not record the total symptom score or the visual analogue scale of rhinoconjunctivitis, which would be useful for assessing the symptom severity. Furthermore, we did not perform genotyping to exclude the genetic factor that could have been responsible for the pathogenesis of AR.

Conclusion

In conclusion, motile ciliary disorders as revealed with transcriptomic microarray assay might play a critical role in the pathogenesis of AR. Further functional validation of the cilia-associated genes is warranted to elucidate the mechanisms underlying AR.

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Authors' Contributions

Qian-hui Qiu and De-Yun Wang conceived and designed the experiments; Zhuo Chen and Zhen-chao Zhu collected the samples; Yang Peng, Xiao-xue Zi, Qin-tai Yang, and Li Shi performed the experiments; Yang Peng, Kai Sen Tan, and Wei-jie Guan analyzed the data. Hai-yu Hong contributed reagents/

materials/analysis tools; and Yang Peng, Wei-jie Guan, Kai Sen Tan, and De-Yun Wang wrote the paper.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

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