

Downregulation and Aberrant Localization of Forkhead Box J1 in Allergic Nasal Mucosa

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Keywords

Allergic rhinitis · Cilia · Forkhead box J1 · Microarray · Localization

Abstract

Background: Forkhead box J1 (FOXJ1) plays pivotal roles in motile cilia formation. However, it remains unclear whether abnormal expression or localization of FOXJ1 in nasal mucosa tissues is associated with allergic rhinitis (AR), in which impaired mucociliary clearance is implicated. **Objective:** We sought to investigate the expression and localization of FOXJ1 in inferior turbinate from patients with AR and controls. **Methods:** We assayed mRNA levels of FOXJ1, DNALI1, and DNAH9 by using whole-genome expression array and quantitative real-time polymerase chain reaction. We elucidated the localization of FOXJ1 by using immuno-

fluorescence assays in paraffin sections and primary single cells. Four patterns of FOXJ1 localization (normal, N; intermediate, I; mislocalization, M; absence, A) were defined. We developed a semiquantitative scoring system to elucidate their localization in 5 areas per paraffin section, with individual sections being assigned a score between 0 and 2. **Results:** The mRNA levels of FOXJ1, DNALI1, and DNAH9 were significantly reduced in patients with AR compared with controls (all $p < 0.05$). The median (1st and 3rd quartile) of the FOXJ1 score was 0.4 (0.0 and 0.85) in patients with AR, and 0.2 (0.0 and 0.4) in controls ($p < 0.05$). For primary cytospin samples, the mean percentages of FOXJ1 localization patterns N, I, M, and A were 46.7, 10.0, 30.0, and 26.7% in pa-

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tients with AR, and 82.5, 5.0, 5.0, and 7.5% in controls, respectively ($p < 0.05$). **Conclusion:** Downregulation and aberrant localization of FOXJ1 may be crucial characteristics of the allergic nasal mucosa.

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Introduction

Allergic rhinitis (AR) is characterized by T-helper type 2 (Th2)-skewed airway inflammation of the upper airway mucosa. AR represents a significant healthcare and socioeconomic burden worldwide [1], and has been associated with impaired mucociliary clearance of the nasal epithelium, the first-line of defense and critical physical barrier to invading pathogens and allergens [2, 3]. Normal nasal mucociliary clearance is essential to maintaining proper nasal epithelial host defense, and impaired mucociliary clearance has been a common cause of chronic airway diseases and may be implicated in the significant associations between AR and its comorbidities [4, 5]. In fact, abnormal expression or mislocalization of ciliary markers has been linked to defective host defenses of the epithelium that may be responsible for the pathogenesis of many chronic airway diseases [6, 7]. However, the molecular mechanisms underlying the impaired mucociliary clearance in allergic nasal mucosa remains unknown.

Forkhead transcription factor 1 (forkhead box J1; FOXJ1), previously known as hepatocyte nuclear factor-3 (HNF-3) or forkhead homologue 4 (HFH-4), is a member of the forkhead/winged-helix family of transcription factors, and is a critical regulator of mucociliary function and ciliogenesis [8]. Mice lacking *Foxj1* activity show a marked reduction or complete loss of motile cilia in airway epithelium, suggesting that the *Foxj1* gene is essential to motile cilia formation [9]. Polymorphisms of the *FOXJ1* gene (e.g., g.-460C>T, g.1805G>T, and g.3375G>C) have been associated with increased susceptibility to AR [10]. Therefore, *FOXJ1* might be implicated in the pathogenesis of AR.

Maintenance of the intact epithelial barrier depends on the normal ciliary ultrastructure and function. For instance, the presence of dynein arms is indispensable to the proper formation of microtubule-based axoneme [11]. Sugier et al. [12] and our study team [7] have consistently demonstrated that the abnormality of the dynein arm-related marker, dynein axonemal heavy chain 5 (DNAH5), might be implicated in chronic and allergic inflammation of the airway epithelium. However, no study has revealed whether the altered mRNA expression levels of dynein

axonemal intermediate chain 1 (DNAI1), dynein axonemal light intermediate chain 1 (DNALI1), and DNAH9, which are regulated by FOXJ1 [13], are also present in the allergic nasal mucosa.

We hypothesized that both the expression and localization of FOXJ1 are aberrant in allergic nasal mucosa. We sought to determine the mRNA expression of *FOXJ1* and ciliary structural markers (*DNAI1*, *DNALI1*, and *DNAH9*) in patients with AR and controls by using whole-genome expression array and quantitative real-time polymerase chain reaction (qRT-PCR). We also elucidated the localization patterns of FOXJ1 in paraffin specimens and primary dissociated ciliated cells by using immunofluorescence staining (IF), which allowed us to semiquantitatively evaluate the extent of FOXJ1 abnormalities.

Materials and Methods

Patient Recruitment

Study approval was obtained from the institutional review boards of Guangdong General Hospital and the National Healthcare Group Domain Specific Review Board of Singapore. AR was diagnosed according to the criteria of the Initiative on Allergic Rhinitis and Its Impact on Asthma (ARIA) [14], based on skin prick test (Allergopharma, Reinbek, Germany), ImmunoCAP test (Thermo Fisher Scientific, Waltham, MA, USA) for specific IgE against relevant aeroallergens, or serum total IgE detected using an Allergy Screen test (Mediawiss Analytic GmbH, Moers, Germany).

Controls ($n = 40$) and patients with AR ($n = 55$) were recruited from subjects undergoing septoplasty due to anatomic variations who did not have sinus disease. All subjects were free of clinical signs of rhinosinusitis and lower respiratory tract infection, and had no self-reported or physician-diagnosed asthma. Biopsies were taken from the inferior turbinate (IT) mucosa of the controls during septoplasty. Primary single-cell suspensions ($1-2 \times 10^5$ cells) were isolated from controls ($n = 4$) and patients with AR ($n = 3$) for cytospin preparations. The details are presented in Table 1.

Microarray and qRT-PCR

RNA was extracted from frozen IT tissues in RNeasy[®] (Sigma-Aldrich, St. Louis, MO, USA) for Prime View[™] Human Gene Expression Array (Affymetrix, Santa Clara, CA, USA). Briefly, cDNA was conjugated to fluorescence dye and hybridized in 4 replicates. Data were normalized with the quartile algorithm using Genespring software (version 12.5; Agilent Technologies). Differentially expressed genes were identified based on the fold-changes (cut-off 2.0; ≥ 2.0 as upregulated and ≤ -2.0 as downregulated) and p values (cut-off 0.05) [15].

The mRNA expression levels of *FOXJ1*, *DNAI1*, *DNALI1*, and *DNAH9* were validated with qRT-PCR (SYBR Green, Promega, Madison, WI, USA). The relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method normalized against the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), in IT specimens. *FOXJ1*, *DNAI1*, *DNALI1*, *DNAH9*, and *GAPDH* ampli-

fication was performed with the following primers: *FOXJ1* forward (5'-GTGAAGCCTCCCTACTC-3'), *FOXJ1* reverse (5'-AATTCTGCCAGGTGGG-3'); *DNAI1* forward (5'-TCAGTGGGAGATC-TATGATG-3'), *DNAI1* reverse (5'-ACTCCATAGATGTCAG-CTTC-3'); *DNALI1* forward (5'-GGAGCAGTTAGACTTAAAGC-3'), *DNALI1* reverse (5'-ACTCATCAAAACACTGTGAG-3'); *DNAH9* forward (5'-AGCAGGCCTCTAATTATCTC-3'), *DNAH9* reverse (5'-CTCTTTGAAGTAAGTGTGGAG-3'); *GAPDH* forward (5'-ACAGTTGCCATGTAGACC-3'), *GAPDH* reverse (5'-TTTTTGGTTGAGCACAGG-3').

Cytospin Preparation

Single-cell suspensions were dissociated from fresh nasal biopsy specimens by incubation with 10 mg/mL of Dispase II (Sigma-Aldrich) at 4 °C overnight, followed by treatment with 1× Trypsin/Ethylene diamine tetra acetic acid at 37 °C for 15 min. Dissociated cells were fixed in 4% formaldehyde at room temperature for 10 min, followed by washing with 1× Dulbecco's phosphate-buffered saline and centrifugation. Cytospin (1–2 ×10⁴ cells/slide) preparations were prepared at 500 rpm for 5 min using a Shandon Cytospin 3 Cyto centrifuge (Thermo Fisher Scientific) [16].

IF Staining

Localization patterns of FOXJ1 and acetylated alpha-tubulin on paraffin sections and primary single-cell cytospin sections of nasal biopsies were examined using IF staining. The sections were incubated with primary polyclonal antibodies (rabbit anti-human FOXJ1, HPA005714, Sigma, Ronkonkoma, NY, USA, and mouse anti-human acetylated alpha-tubulin, clone ab24610, Abcam, Cambridge, MA, USA) overnight at 4 °C, followed by 1 h of incubation with Alexa Fluor 488- or Alexa Fluor 594-conjugated secondary antibodies (Life Technologies, Carlsbad, CA, USA) at 37 °C. Coverslips were mounted using SlowFade Gold anti-fade reagent with 4',6'-diamidino-2-phenylindole (DAPI; Life Technologies). For negative controls, primary antibodies were substituted with species- and subtype-matched antibodies of the same concentration. All slides were analyzed with fluorescence microscopy (Olympus IX51, Tokyo, Japan).

Evaluation of FOXJ1 Localization

Specific antibodies against FOXJ1 (red) and acetylated alpha-tubulin (green) were used in IF analyses to elucidate their differential localization. Nuclei were stained with DAPI (blue). In merged images, pink staining in the nuclei of ciliated cells suggested colocalization and the presence of FOXJ1 of interest, whereas yellow or green staining suggested mislocalization or the absence of FOXJ1. The localization of FOXJ1 expression was defined as follows: presence of FOXJ1 in the nuclei only (normal; N); presence of FOXJ1 in the nuclei and axoneme (intermediate; I); presence of FOXJ1 throughout the entire or part of the axoneme (mislocalization; M); absence of FOXJ1 in the nuclei and axoneme (absence; A).

Five areas of cilia staining were randomly selected from each sample in a blinded manner to elucidate the colocalization at ×400 magnification. We referred to the criterion from Shoemark et al. [17] and our previously published study [7]. That is, samples with more than 7 out of 10 ciliated cells (70%) clearly labeled with cilia markers would be considered normal. Our classification system was based on the same proportion (70%) for stained IF tissue sections. Each section was assigned a score between 0 and 2, with 0 points for the field containing >70% N localization; 1 point for

Table 1. Summary of patient characteristics and the methods

	Control subjects	Patients with AR
Sample size	40	55
Age, years	33.3±10.7	31.3±11.8
Male/female	25/15	38/17
Allergen sensitization		
Dust mites	0	47
Allergens other than dust mites	0	8
Methodology used		
Nasal tissues	40	55
Whole-genome expression array	11	19
qRT-PCR	34	44
Paraffin specimens	29	43
With cilia staining ¹	19	28
Cytospin preparations	4	3

Values are *n* or mean ± SD. None of the subjects had self-reported or physician-diagnosed asthma. AR, allergic rhinitis; FOXJ1, forkhead box J1; qRT-PCR, quantitative real-time polymerase chain reaction.

¹ The paraffin specimens with cilia staining (19 from controls and 28 from patients with AR) were confirmed by immunofluorescence (acetylated alpha-tubulin) and used for FOXJ1 score analysis in our study.

>70% I+N localization, and 2 points for ≥30% with M or A localization (Fig. 1e–h). The scoring of individual fields was undertaken by 2 individuals blinded to the grouping, and the score of 5 areas was averaged. In cytospin preparations, 10 single ciliated cells from primary specimens were randomly selected, and the pattern of each single-cell was defined at ×1,000 magnification (oil immersion lens).

Statistical Analysis

Data were analyzed using SPSS software (version 18.0, SPSS, Chicago, IL, USA). The Mann-Whitney 2-tailed nonparametric test was used to compare FOXJ1, *DNAI1*, *DNALI1*, and *DNAH9* expression in different groups of IT tissues, and the FOXJ1 localization score in paraffin-embedded specimens. The Fisher exact test was employed to assess differences in FOXJ1 patterns between the groups from cytospin preparation specimens. The correlation of the mRNA levels between FOXJ1 and ciliary structure markers (*DNAI1*, *DNALI1*, and *DNAH9*) was evaluated with Spearman correlation analysis. *p* < 0.05 was considered statistically significant.

Results

Subject Characteristics

The clinical characteristics of controls and patients with AR are summarized Table 1; 85.5% (47/55) of patients with AR were sensitized to house dust mites. In our

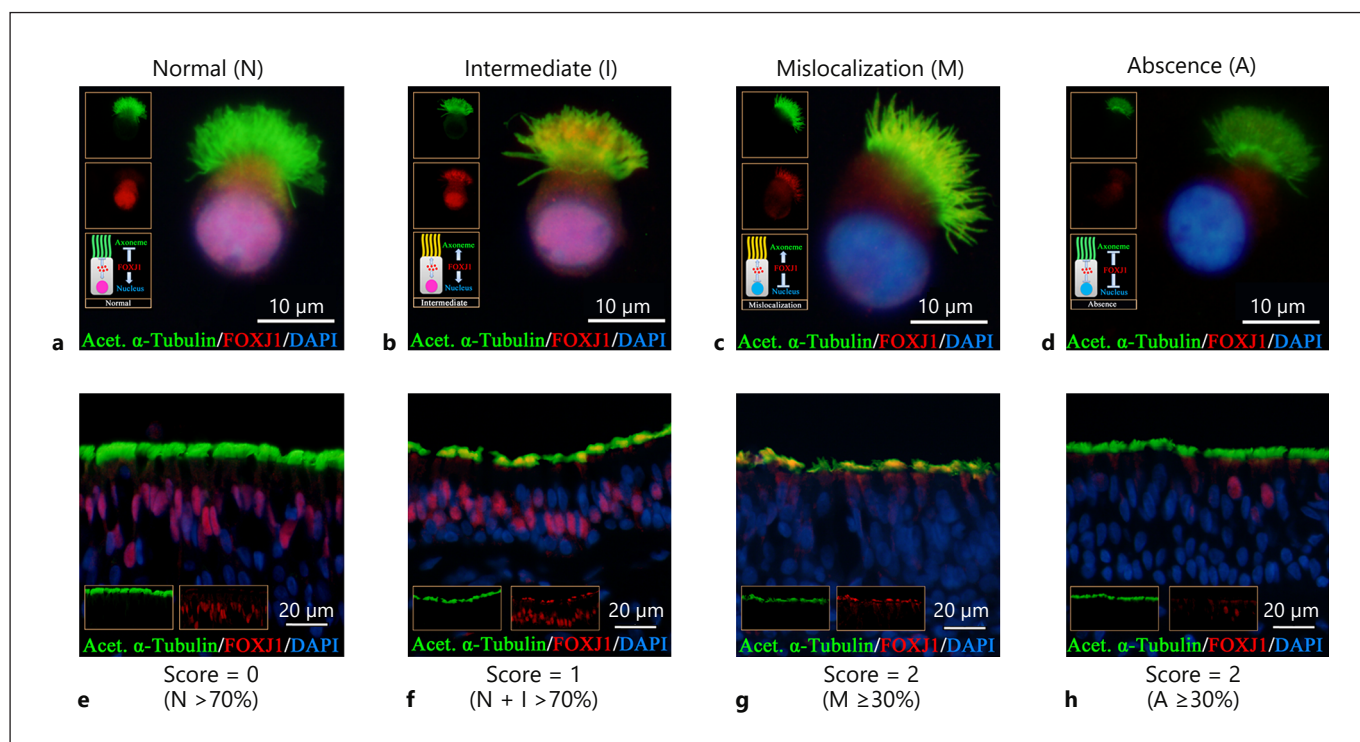


Fig. 1. Localization patterns of the FOXJ1 protein in single cells and paraffin specimens. Four distinct patterns were observed: N, normal; I, intermediate; M, mislocalization, and A, absence. These 4 localization patterns were observed in single cells (a–d) and paraffin specimens (e–h) of both patients with AR and healthy subjects, respectively. The semiquantitative FOXJ1 grading system is shown beneath the paraffin specimens (e–h).

study, IT tissues for IF analysis were initially obtained from controls ($n = 29$) and patients with AR ($n = 43$). However, due to the limitations of the quantity of epithelium, some paraffin specimens without cilia staining were excluded from our further analyses. Therefore, 65.5% (19/29) of controls and 65.1% (28/43) of patients with AR were included for FOXJ1 localization score analysis.

Downregulation of FOXJ1 in Allergic Nasal Mucosa

We performed a microarray analysis to compare the global gene expression in IT tissues from patients with AR ($n = 19$) and controls ($n = 11$). *FOXJ1* mRNA expression was substantially reduced in patients with AR compared with controls (fold-change = -2.613 , $p = 0.036$). Next, we confirmed the decreased expression of *FOXJ1* with qRT-PCR assay in tissues from patients with AR ($n = 44$) and controls ($n = 34$), the result of which was consistent with the microarray data (fold-change = -2.367 , $p = 0.004$). These 2 independent methods confirmed at least 2-fold alterations in the expression of *FOXJ1* in IT tissue from patients with AR versus controls (Fig. 2a; Table 2).

Downregulation of Ciliary Structural Markers in Allergic Nasal Mucosa

Next, we compared *DNAI1*, *DNALI1*, and *DNAH9* gene expression with microarray assays in IT tissues from patients with AR ($n = 19$) and controls ($n = 11$). Similarly, we further validated the findings with qRT-PCR from patients with AR ($n = 34$) and controls ($n = 44$). We found that the mRNA expression levels of these 3 genes were substantially reduced in patients with AR compared with controls, both in microarray and qRT-PCR ($p < 0.05$; Table 2).

Furthermore, the levels of *FOXJ1* mRNA expression positively correlated with the mRNA levels of *DNAI1* ($r = 0.827$, $p < 0.001$), *DNALI1* ($r = 0.832$, $p < 0.001$), and *DNAH9* ($r = 0.783$, $p < 0.001$; Fig. 3).

FOXJ1 Normal Localization Was Less Frequently Observed in Patients with AR

Primary single cells and tissue paraffin sections from patients with AR and controls were stained with FOXJ1 (red) and acetylated alpha-tubulin (green) for IF assays. As shown in Figure 1, 4 distinct localization patterns of

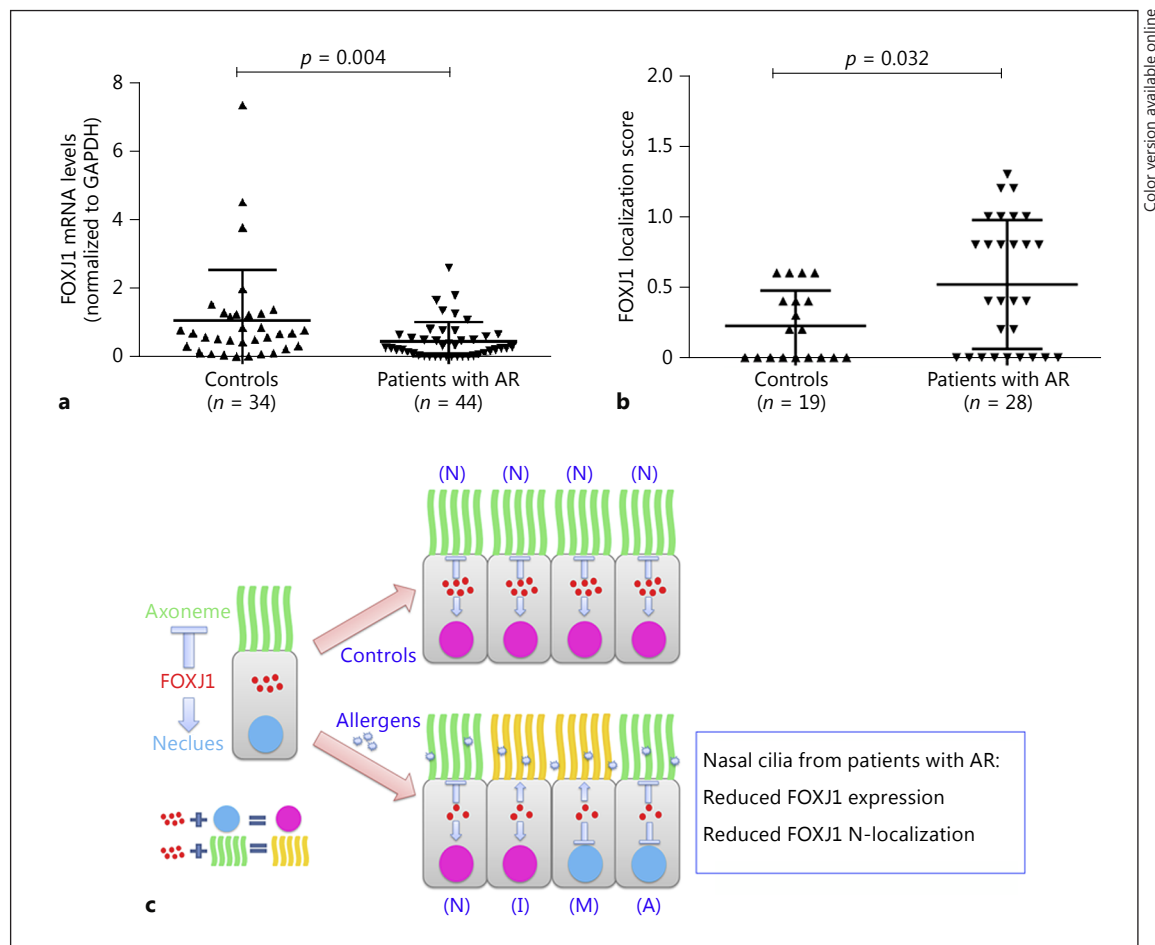


Fig. 2. The expression and localization of FOXJ1 between patients with AR and controls. Downregulation of FOXJ1 mRNA (**a**) and an increased FOXJ1 localization score (**b**) were more prominent in patients with AR compared with controls. **c** The 4 distinct localization patterns of FOXJ1: N, normal; I, intermediate; M, mislocalization, and A, absence.

FOXJ1 expression were observed in both the isolated primary single cells in cytospin preparations and paraffin specimens, respectively (Fig. 1a–d). These 4 patterns included: N, I, M, and A (Fig. 1a–d), which were observed in ITs from both patients with AR and controls. Based on our semiquantitative FOXJ1 scoring system (Fig. 1e–h), the median (the 1st and 3rd quartile) score was 0.40 (0, 0.85) for samples from patients with AR, and 0.20 (0, 0.40) for samples from controls ($p < 0.05$; Fig. 2b, see online suppl. Table S1–2; for all online suppl. material, see www.karger.com/doi/10.1159/000488014).

We also evaluated 10 single cilia cells from each primary sample from cytospin preparations. The N, I, M, and A pattern was observed in 46.7% (14/30), 10.0% (3/30), 16.7% (5/30), and 26.7% (8/30) of ciliated cells

Table 2. Comparison of fold difference in *FOXJ1*, *DNAI1*, *DNAL11*, and *DNAH9* in ITs from patients with AR and controls tested by gene array and qRT-PCR

Gene name	Whole-genome expression array	qRT-PCR
<i>FOXJ1</i>	–2.613 (0.036)	–2.367 (0.004)
<i>DNAI1</i>	–2.295 (0.019)	–2.410 (0.011)
<i>DNAL11</i>	–2.386 (0.034)	–2.582 (0.013)
<i>DNAH9</i>	–2.262 (0.016)	–3.677 (0.001)

Data are presented as fold-change (p value). AR, allergic rhinitis; DNAH9, dynein axonemal heavy chain 9; DNAI1, dynein intermediate chain 1; DNAL11, dynein axonemal light intermediate chain 1; FOXJ1, forkhead-box J1; IT, inferior turbinate; qRT-PCR, quantitative real-time polymerase chain reaction.

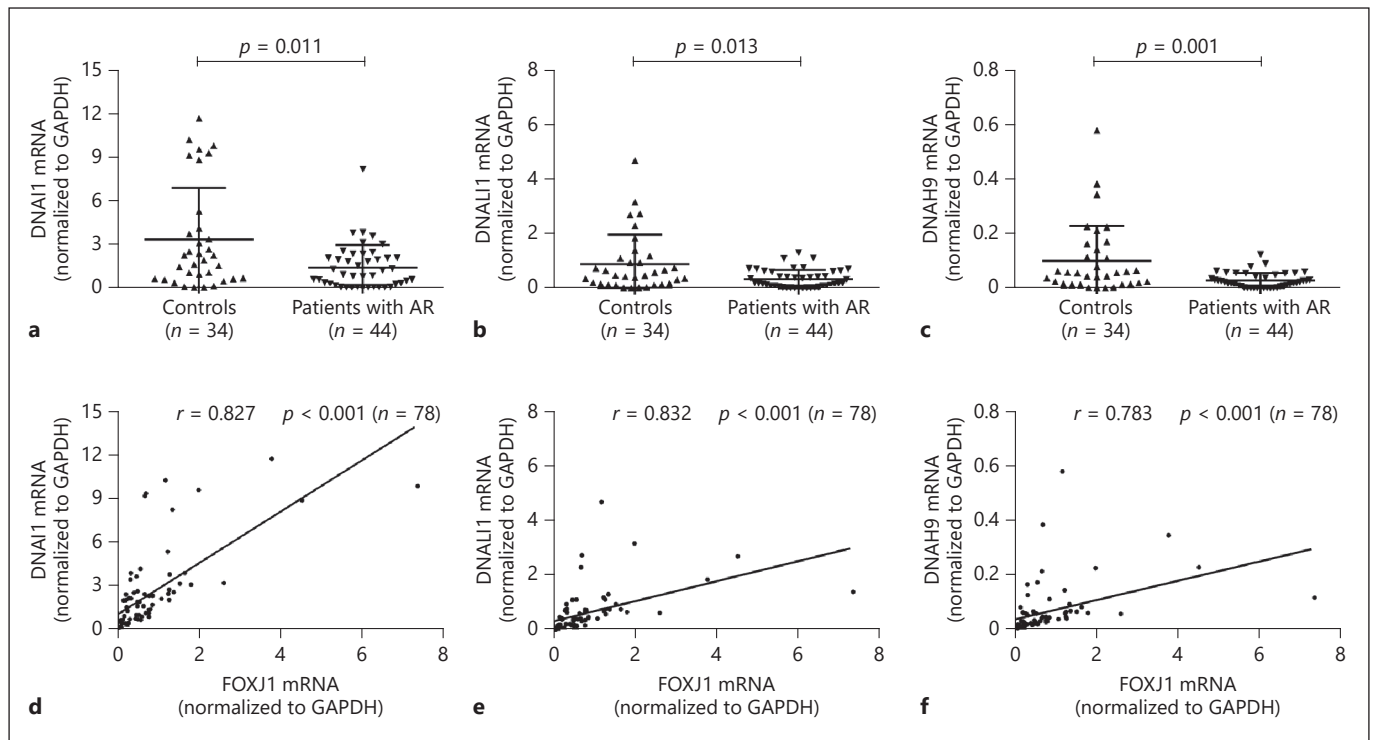


Fig. 3. The expression of DNAI1, DNALI1, and DNAH9 between patients with AR and controls, and their correlation with the expression of FOXJ1. Downregulation of the mRNA levels of DNAI1 (a), DNALI1 (b), and DNAH9 (c) in patients with AR compared with controls. The mRNA levels of FOXJ1 positively correlated with the DNAI1 (d), DNALI1 (e), and DNAH9 (f) expression.

Table 3. Summary of FOXJ1 localization in primary cells from cytospin preparations

	Controls	Patients with AR	<i>p</i> value
Primary cell specimens	4	3	
Single ciliated cells evaluated ¹	40	30	
FOXJ1 localization			0.012
Normal	33 (82.5)	14 (46.7)	
Intermediate	2 (5.0)	3 (10.0)	
Mislocalization	2 (5.0)	5 (30.0)	
Absence	3 (7.5)	8 (26.7)	

Values are *n* (%). AR, allergic rhinitis; FOXJ1, forkhead box J1.

¹ Ten single cilia cells were taken randomly from each primary cell specimen.

from patients with AR (*n* = 3), respectively. Comparatively, the corresponding percentage was 82.5% (33/40), 5.0% (2/40), 5.0% (2/40), and 7.5% (3/40) in controls (*n* = 4), respectively (*p* < 0.05; Table 3).

Discussion

Although the mechanisms underlying the pathogenesis of chronic upper airway inflammatory diseases such as AR are not fully understood, impaired mucociliary clearance has been identified as an important feature contributing to the development of AR. Recently, we have identified mislocalization and altered expression of DNAH5, the critical cilia structural protein, in patients with chronic rhinosinusitis with nasal polyps, suggesting their possible contribution to impaired mucociliary clearance [7]. In this study, we expanded our investigation to the assessment of FOXJ1, a critical ciliogenesis marker, to determine whether the altered expression or localization of FOXJ1 was associated with AR. To the best of our knowledge, our study is the first report to indicate 4 distinct FOXJ1 localization patterns in ITs, both in paraffin specimens and isolated single cilia cells.

In this study, we observed an increase in the FOXJ1 localization score in allergic nasal mucosa compared with that in controls. Our findings also indicated certain in-

herent abnormalities in the localization of FOXJ1 in patients with AR. FOXJ1 polymorphisms may have affected nuclear localization signaling, thereby predisposing patients to the development of some comorbidities of AR [10]. Park et al. [18] recently reported that FOXJ1 mRNA levels were significantly lower in cultured allergic nasal epithelial cells at 14 days compared with cells derived from subjects with nonallergic disorders. Our study has extended this finding by demonstrating that FOXJ1 mRNA levels were reduced in IT tissues from patients with AR.

In this study, we also assayed 3 genes modulated by FOXJ1 (DNAI1, DNALI1, and DNAH9) which have been identified in *Xenopus*, zebrafish, and mice [13]. Apart from DNAH5 disorders, mutations of DNAI1, DNALI1, and DNAH9 have mostly been established as the characteristics associated with primary ciliary dyskinesia [19]. Marked suppression of DNAI1 expression was also reportedly induced by upregulation of transforming growth factor beta-1, which has been observed in patients with perennial and seasonal AR [2, 20]. Notably, the absence of DNALI1 protein frequently coexisted with the absence of DNAH5 protein in patients with primary ciliary dyskinesia [19]. In addition, single-nucleotide polymorphisms of DNAH9 reportedly correlated with early smoke exposure in patients with bronchial hyperresponsiveness, suggesting that changes in the ciliary ultrastructure may be particularly relevant for the development of chronic inflammatory diseases [21]. Further studies are needed to determine whether the localization of these ciliary structural markers is implicated in the pathogenesis of other allergic airway diseases, such as AR, asthma, and nasal polyps.

Findings from our previous study indicated that reduced FOXJ1 expression might represent a novel diagnostic and therapeutic target for AR management. Therefore, in patients with AR with reduced FOXJ1 expression, commercially available drugs such as Gelomyrtol Forte™, which are known to increase FOXJ1 expression in vitro, may potentially restore FOXJ1 expression [22]. Additionally, the reduced expression and aberrant localization of FOXJ1 may be further explored as possible mechanisms contributing to the polymorphic or other nongenetic differences. Further clinical trials are needed to explore whether treatment increasing FOXJ1 expression can reverse the aberrant localization back to normal in patients with AR.

To our knowledge, the imbalance between Th1 and Th2 cells in patients with AR might be responsible for the dysregulation of cytokine production that contributes to

allergic reactions [23]. The downregulation of the ciliary gene in patients with AR may further contribute to Th2-skewed airway inflammation [24]. Th2 cytokines interleukin (IL)-4 and IL-13 have been shown to directly impair ciliary function of the respiratory epithelium and contribute to impaired mucociliary clearance in asthmatic patients [25]. Further studies are needed to verify the relationship between ciliogenesis marker expression and Th2 inflammation.

Notably, the significance of our study was incremental because FOXJ1 polymorphism has been reported in patients with AR [10]. However, thus far there has not been any systematic investigation that directly linked abnormal FOXJ1 expression or localization to allergic nasal mucosa. Our study is the first to document the FOXJ1 expression levels and, more importantly, the localization patterns in patients with AR. The results have confirmed the aberrant low levels of FOXJ1 expression and FOXJ1 mislocalization in AR, indicating that FOXJ1 may be a critical and novel target for future clinical assessment and intervention.

In addition, FOXJ1 expression levels have not been validated with other approaches, including enzyme-linked immunosorbent assays. However, the expression characteristics of FOXJ1 protein can be assayed with immunofluorescence imaging [17], which has now been clinically validated. Importantly, compared immunofluorescence imaging can reveal the localization of markers of interest. Our results indicated that mRNA expression levels cannot reliably reflect ciliary defects associated with aberrant FOXJ1 localization. In some patients with AR, immunofluorescence imaging could also reveal FOXJ1 mislocalization that cannot be reliably measured based on the total amount of FOXJ1 proteins. Therefore, immunofluorescence imaging is more advantageous in terms of revealing the localization of FOXJ1 proteins and may help the early diagnosis of ciliary defects which cannot be readily detected with other validated methods.

There are some other limitations that deserve mention. First, we did not sequence DNA from tissue samples; therefore, we cannot confirm the causal link of FOXJ1 aberrant localization as the underlying mechanism of the FOXJ1 polymorphism. Second, we did not record the total symptom score or the visual analogue scale of rhinoconjunctivitis, which would be useful for assessing symptom severity in patients with AR. In addition, due to the limited availability of antibodies, we did not directly analyze the colocalization of ciliary structural markers and the association between the localization patterns of ciliary structural markers and FOXJ1 expression.

Conclusion

The downregulation of the mRNA levels of FOXJ1, DNAI1, DNAH9, and DNALI1 in nasal mucosa might be associated with AR, and the aberrant localization of the FOXJ1 protein may be an important pathological characteristic of AR, which may provide novel candidate targets for clinical management. A further challenge is to understand the etiology and molecular mechanisms underlying this pathological condition.

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Statement of Ethics

Approval to conduct this study was obtained from the Ethics Committee of the Guangdong General Hospital (China) and the Ethics Committee of the National Healthcare Group Domain-Specific Review Board of Singapore (Singapore).

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Disclosure Statement

The authors declare that no conflicts of interest exist.

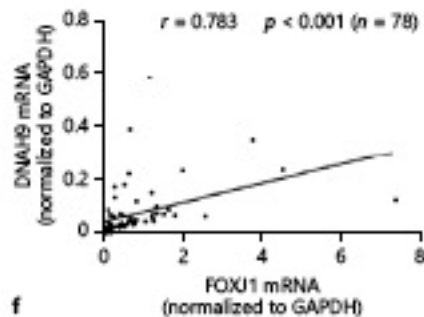
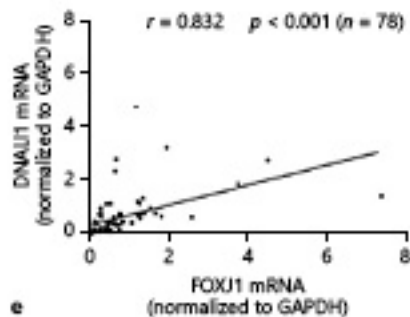
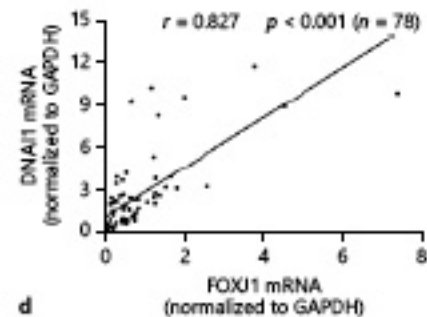
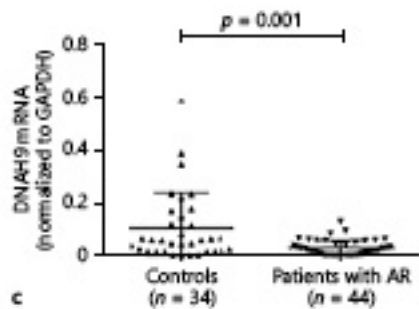
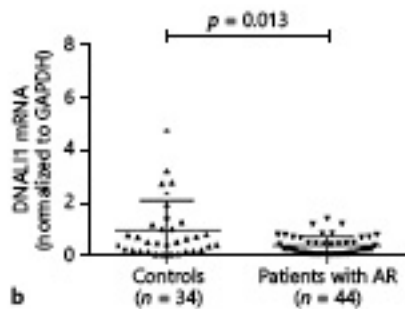
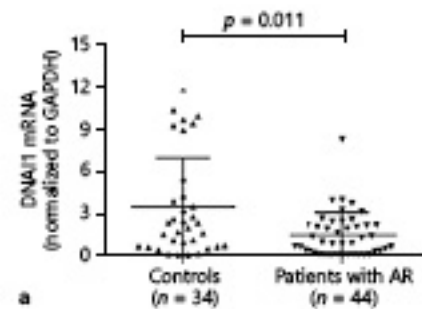
Funding Sources

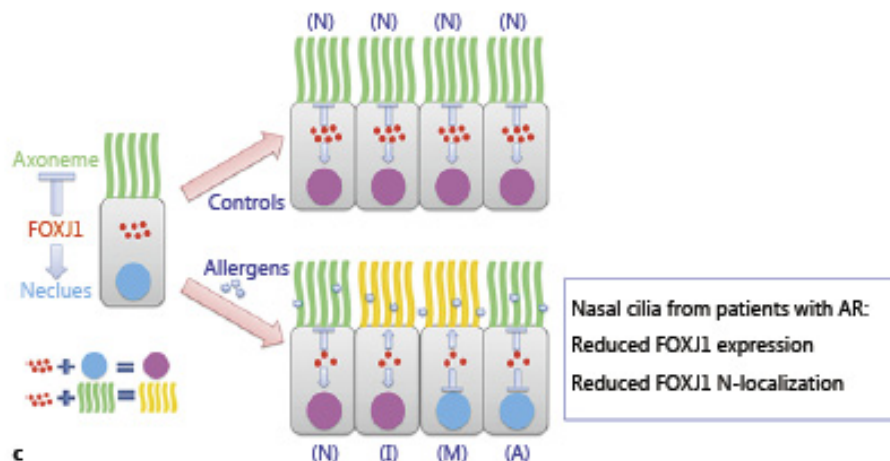
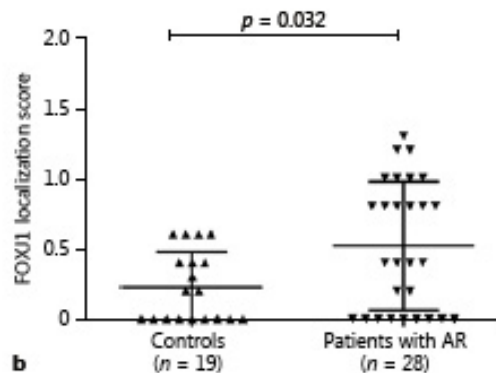
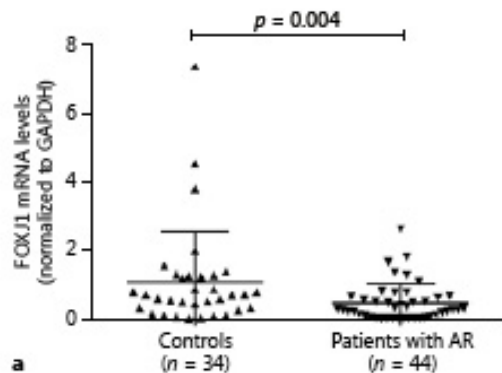
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Author Contributions

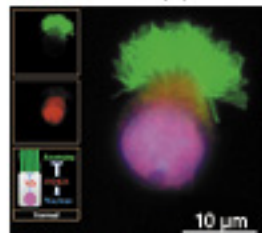
Qianhui Qiu and De-Yun Wang conceived and designed the experiments; Zhuo Chen, Zhenchao Zhu, Jie Zeng, Yixuan Li, Yew Kwang Ong, and Mark Thong collected the samples; Yang Peng, Xiaoxue Zi, Qintai Yang, and Li Shi performed the experiments; Yang Peng, Kai Sen Tan, and Wei-Jie Guan analyzed the data; Haiyu Hong contributed reagents/materials/analysis tools, and Yang Peng, Kai Sen Tan, Wei-Jie Guan, and De-Yun Wang wrote the paper.

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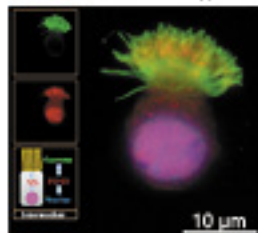
Normal (N)



a

Acet. α-Tubulin/FOXJ1/DAPI

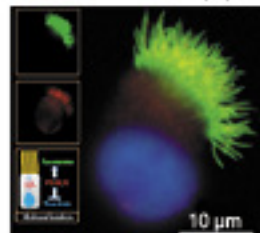
Intermediate (I)



b

Acet. α-Tubulin/FOXJ1/DAPI

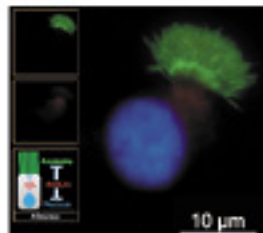
Mislocalization (M)



c

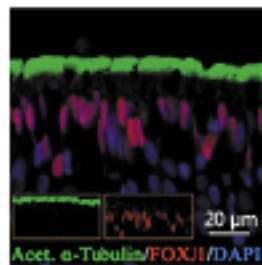
Acet. α-Tubulin/FOXJ1/DAPI

Absence (A)

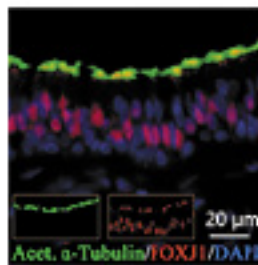


d

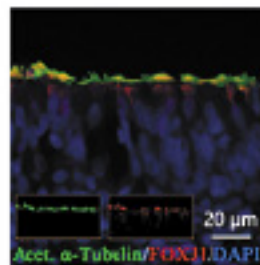
Acet. α-Tubulin/FOXJ1/DAPI



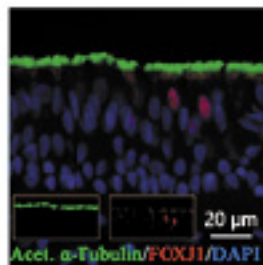
e

Score = 0
(N > 70%)

f

Score = 1
(N + I > 70%)

g

Score = 2
(M ≥ 30%)

h

Score = 2
(A ≥ 30%)

	Controls	Patients with AR	<i>p</i> value
Primary cell specimens	4	3	
Single ciliated cells evaluated ¹	40	30	
FOXJ1 localization			0.012
Normal	33 (82.5)	14 (46.7)	
Intermediate	2 (5.0)	3 (10.0)	
Mislocalization	2 (5.0)	5 (30.0)	
Absence	3 (7.5)	8 (26.7)	

Values are *n* (%). AR, allergic rhinitis; FOXJ1, forkhead box J1.

¹ Ten single cilia cells were taken randomly from each primary cell specimen.

Gene name	Whole-genome expression array	qRT-PCR
<i>FOXJ1</i>	-2.613 (0.036)	-2.367 (0.004)
<i>DNAI1</i>	-2.295 (0.019)	-2.410 (0.011)
<i>DNALI1</i>	-2.386 (0.034)	-2.582 (0.013)
<i>DNAH9</i>	-2.262 (0.016)	-3.677 (0.001)

Data are presented as fold-change (*p* value). AR, allergic rhinitis; DNAH9, dynein axonemal heavy chain 9; DNAI1, dynein intermediate chain 1; DNALI1, dynein axonemal light intermediate chain 1; FOXJ1, forkhead-box J1; IT, inferior turbinate; qRT-PCR, quantitative real-time polymerase chain reaction.

	Control subjects	Patients with AR
Sample size	40	55
Age, years	33.3±10.7	31.3±11.8
Male/female	25/15	38/17
Allergen sensitization		
Dust mites	0	47
Allergens other than dust mites	0	8
Methodology used		
Nasal tissues	40	55
Whole-genome expression array	11	19
qRT-PCR	34	44
Paraffin specimens	29	43
With cilia staining ¹	19	28
Cytospin preparations	4	3

Values are *n* or mean ± SD. None of the subjects had self-reported or physician-diagnosed asthma. AR, allergic rhinitis; FOXJ1, forkhead box J1; qRT-PCR, quantitative real-time polymerase chain reaction.

¹ The paraffin specimens with cilia staining (19 from controls and 28 from patients with AR) were confirmed by immunofluorescence (acetylated alpha-tubulin) and used for FOXJ1 score analysis in our study.