Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Mechanisms of Neuronal Differentiation and Notch Signaling as a Potential Therapeutic Target in Olfactory Dysfunction of Allergic Rhinitis

Chen Duan, Xinyi Ma, Fangying Liu, Xinyu Xie, Changhua Wu, Xiaojun Zhang, Ping Ye, Ye Yuan, Xin Feng

Department of Otorhinolaryngology, Qilu Hospital of Shandong University, National Health Commission Key Laboratory of Otorhinolaryngology (Shandong University), Shandong Provincial Key Medical and Health Discipline of Qilu Hospital of Shandong University, Jinan, People's Republic of China

Correspondence: Xin Feng; Ye Yuan, Department of Otorhinolaryngology, Qilu Hospital of Shandong University, National Health Commission Key Laboratory of Otorhinolaryngology (Shandong University), Shandong Provincial Key Medical and Health Discipline of Qilu Hospital of Shandong University, Jinan, People's Republic of China, Email drfengxin@sdu.edu.cn; 17863939378@163.com

Purpose: Olfactory dysfunction (OD) in allergic rhinitis (AR) significantly diminishes quality of life, yet its pathophysiology remains unclear. The Notch signaling pathway is known to regulate olfactory epithelium proliferation and differentiation in murine models, but its role in AR with OD (AR+OD) is yet to be elucidated. This study aims to explore neuronal expression patterns and the involvement of Notch signaling in AR+OD.

Patients and Methods: Symptom severity of 111 AR patients, 65 non-AR patients, and controls was evaluated according to the SNOT-22 criteria. Olfactory dysfunction in an AR mouse model was assessed via the Buried Food Pellet Test (BFPT). Immunofluorescence, H&E staining, ELISA, and PCR techniques were employed to detect inflammation and olfactory epithelium in AR+OD, AR without OD (AR-OD), and control groups. DAPT, a Notch signaling inhibitor, was administered to assess its therapeutic potential in OD of AR.

Results: AR patients had higher sneezing, rhinorrhea, nasal itching, nasal congestion and olfactory scores, but no correlation was found between nasal congestion and olfactory dysfunction. Significant increases in the symptom scores, eosinophil infiltration, OVA-specific IgE levels and worse olfactory function were observed in the AR mice model compared to the Control group. In particular, AR +OD mice group exhibited thinner olfactory epithelium, increased immature neuron expression, decreased mature neurons, and upregulated Notch expression as compared to AR-OD group. DAPT treatment significantly enhanced olfactory mature neuron expression and improved OD of AR mice.

Conclusion: Our research indicates that impaired differentiation of olfactory neurons may contribute to the underlying causes of olfactory dysfunction in AR. Additionally, inhibiting the Notch signaling pathway promotes the maturation of the olfactory epithelium and improves olfactory dysfunction in AR mice, offering potential therapeutic strategies for olfactory disorders in AR.

Keywords: olfactory dysfunction, allergic rhinitis, Notch signaling pathway, neuron

Introduction

Allergic rhinitis (AR) is a common chronic inflammatory disease of the nasal mucosa which affects approximately 30% of the population.[1] Olfactory dysfunction (OD) is one of the primary symptoms of AR, which ranged from 20–40% and increased with the duration and severity of this disease.[2,3] Moreover, The olfactory system is vital for identifying food, mates, and dangers, while olfactory dysfunction impairs the ability to perceive flavors, detect harmful odors, and diminishes overall quality of life.[4,5] However, the underlying mechanisms and therapeutic strategies of OD in AR remain poorly understood.

The olfactory epithelium (OE) is a pseudostratified structure composed primarily of apical sustentacular cells, mature and immature olfactory sensory neurons, globose basal cells (GBCs), and horizontal basal cells (HBCs).[6] Olfactory

Received: 19 November 2024
Accepted: 5 April 2025
Published: 16 April 2025

sensory neurons (OSNs), which are generated from basal cells, detect thousands of volatile environmental odors and contribute to the sense of smell.[7] Studies have indicated that cell damage and dysfunction in OE induced by inflammation plays a vital role in the development of OD.[8] In chronic rhinosinusitis (CRS) patients with OD, OSNs exhibit abnormal morphology, including a lack of discernible dendrites and/or axons, along with a reduced number of OSNs.[9,10]

The Notch signaling pathway is an important pathway of intercellular communication. There are four highly conserved transmembrane receptors, namely, Notch 1, Notch 2, Notch 3, and Notch 4, and five ligands, jagged 1, jagged 2, delta-like-1 (Dll1), delta-like-3 (Dll3), and delta-like-4 (Dll4), that participate in this pathway in mammalian cells.[11] The Notch signal transduction between cells is completed through the binding and activation of its ligand receptors, thereby activating downstream target genes.[12] It plays a critical role in regulating progenitor cell proliferation and differentiation across various tissues and organs.[13,14] Emerging evidence suggests that this pathway may similarly influence homeostasis in the olfactory epithelium.[15] In the OE, several Notch ligands, including Jagged1, Jagged2,[16] and delta-like ligands Dll1, Dll3, and Dll4,[17] as well as the receptors Notch 1−3,[17,18] have been identified. Notch2 is essential for maintaining sustentacular cell function[19], while Notch1 may play a distinct role in the commitment and differentiation of neuronal and glial lineages in the olfactory epithelium (OE).[18] Collectively, these findings underscore the crucial role of Notch signaling in regulating the differentiation of olfactory epithelial cells.

Our previous study[7] demonstrated that Notch activation in the OE promoted the retention of cells in a progenitor or immature state, leading to an increase in proliferative progenitor/stem cells and a decrease in mature neuronal cells. A limited number of studies have reported on the relationship between Notch signaling and the pathogenesis of AR.[20–22] These studies highlight the potential role of notch signaling in AR inflammation. Additionally, some studies[22] have reported that Notch signaling inhibitor (GSI-IX, DAPT) can reduce the inflammatory response in murine models of AR. However, the regulatory role of Notch signaling in AR with olfactory dysfunction (OD) remains poorly understood. Therefore, we hypothesize that the Notch signaling pathway plays a crucial role in AR with OD. We aim to explore the underlying mechanisms of OD in AR and investigate the therapeutic effects of blocking Notch signaling using a mouse model of AR with OD.

Materials and Methods

Sample Collection and Symptoms Assessment of Patients with Allergic Rhinitis

From January 2021 to December 2022, 111 AR patients, 65 non-AR patients and 21 control patients were enrolled in this study. The diagnosis of AR patients adhered to the 2015 Chinese guidelines for diagnosis and treatment of AR,[23] specifically excluding cases with significant nasal septum deviation, acute or chronic nasal infections, and severe systemic illnesses. The inclusion criteria consisted of six groups of 20 common inhalant allergens tested for sIgE and classified as grades 0–6 by concentration according to the determination method provided by the German allergen detection system: Patients exhibiting symptoms of rhinitis but having allergen-specific IgE negative were categorized into the non-AR group (comprising 65 cases), whereas those who display symptoms and allergen-specific IgE positive were assigned to the allergic rhinitis group (consisting of 111 cases).[24] In addition, we selected people who had neither nasal symptoms nor IgE positive as a control group. All experimental procedures received approval from the Ethics Committee of Qilu Hospital of Shandong University, and all patients provided informed consent. All subjects were evaluated for nasal symptoms (sneezing, rhinorrhea, itching, and congestion) and olfactory disturbances based on the Sino-Nasal Outcome Test (SNOT)-22 criteria. The SNOT-22 scoring system ranges from 0 to 5, corresponding to: No problem, Very mild problem, Mild or slight problem, Moderate problem, Severe problem, Problem as bad as it can be, respectively[25] Finally, three milliliters of peripheral blood were collected from the patients with 20 AR patients with OD, 10 AR patients without OD and 10 controls. It was left standing for 30–60 min and centrifuged at 600 g for 10 min. The serum was isolated and stored at −80°C for later use. The level of Notch1 expression in the serum was detected by ELISA. Notch1 ELISA kits were purchased from R&D Systems (USA).

Establishment of the AR Model with OD

We chose C57BL/6 mice aged 6 to 8 weeks, supplied by the Shandong University Animal Experiment Center. Starting on day 0 and continuing on days 7 and 14, the mice were intraperitoneally injected with 200μL of phosphate-buffered

saline (PBS, Gibco 10010023) containing 100μg of ovalbumin (OVA, Sigma-Aldrich A5503) and 4 mg of aluminum hydroxide (AL(OH) 3, Sigma-Aldrich 21645–51-2). From day 21 to 28, the experimental group mice (n=36) were administered 20μL of PBS with 800μg OVA nasally once a day, and the control group mice (n=16) received the same volume of PBS nasally.[26] Then, on the evening of day 27, food was withheld and only water was given. After the final nasal challenge on day 28, behavioral observations for nasal symptoms and the Buried Food Pellet Test (BFPT) were conducted. The frequencies of rubbing and sneezing were recorded within 30 minutes, and scores were calculated as follows: 1, wipes the nose slight for several times or sneezes less than 3 times; 2, repeatedly wipes the nose or sneezes more than 3 times and less than 10 times; 3, keeps rubbing from nose to face or sneezes more than 11 times.[27] Additionally, runny nose was scored from 0 to 3 based on the location of nasal discharge: without discharge (0), to the anterior nostril (1), beyond the anterior nostril (2), or all over the face (3). The symptom score is obtained by adding three symptoms together. Animals not able to achieve to more than 4 scores are classified as the control group. The AR model with OD was established as described previously.[28] All experimental procedures were approved by the Animal Ethical Committee of Qilu Hospital of Shandong University.

Evaluation of Mouse Nasal Symptoms and the Buried Food Pellet Test (BFPT)

Two hours following the last nasal challenge (OVA or PBS), observers, unaware of the experiment details, recorded the frequency of sneezing, rhinorrhea, and nose rubbing in 30 min in each group of mice. If a total score above 5 was obtained, it was considered indicative of successful model creation. The scoring method of the mice was performed in accordance with the literature.[27] After a 12-hour fast, mice were introduced into a testing cage (45 × 24×20 cm) with a 3 cm thick layer of bedding, beneath which 2 grams of feed were buried 0.5 cm deep. The time taken by the mice to locate the feed was noted; if the search time exceeded 5 minutes, it was considered a failure to find the feed. For each test, both feed and bedding were refreshed. Mice that were unable to locate the food across three successive attempts were considered to exhibit olfactory dysfunction symptoms.

Hematoxylin and Eosin (H&E) Staining

Mouse heads were fixed for 48 hours in 4% paraformaldehyde (Biosharp BL539A), and afterward, they were decalcified in EDTA solution (BOSTER AR1071) until the skull could be penetrated by a large pin. Subsequent to paraffin embedding, sectioning (4 micrometers in thickness), xylene clearing, and water-based dewaxing, slides were stained with hematoxylin and eosin (H&E). After the paraffin sections were deparaffinized, hematoxylin was used to stain the nucleus and eosin was used to stain the cytoplasm. The morphology of the nasal mucosa was observed under a light microscope (×400). Five fields on each slice were randomly selected, in which the number of eosinophils was counted under the microscope and then averaged.

Preparation of Frozen Sections and Immunofluorescence Staining

After the decalcification process, the mouse heads were washed in PBS and then gradually dehydrated through 10%, 20%, and 30% sucrose solutions, before being embedded in OCT compound (Sakura 4583) and frozen with liquid nitrogen. Next, the frozen tissues were sectioned into 12 micrometer coronal sections using a cryostat (LECIA CM1900). Sections were first warmed for 15 minutes at 37°C, then treated with 0.3% Triton X-100 (Solarbio T8200) for membrane permeabilization for 30 minutes, followed by blocking with 5% bovine serum albumin (BSA) (Solarbio SW3015) for an hour, and finally, incubated with the primary antibody overnight at 4°C. On the following day, sections were washed thrice in PBS, each for 5 minutes, and subsequently incubated with the secondary antibody in the dark at 37°C for 1 hour. Lastly, after washing with PBS, sections were cover slipped using an anti-fade mounting medium containing DAPI (Solarbio S2110), and images were acquired using the SLIDEVIEW™ whole slide scanning system. The primary antibodies and dilutions used in the experiments were as follows: rabbit anti-Notch1 (1:200; Cell Signaling Technology, 4380); rabbit anti-GAP43 (1:200; Millipore, sc-17790); rabbit anti-Olfactory Marker Protein/OMP (1:400, abcam, ab183947); mouse anti- UCHL1/PGP9.5 (1:200, proteintech, 14730-1-AP). The secondary antibodies used in the experiments were as follows: Alexa Fluor 488 goat anti-Rabbit (ab150077), Alexa Fluor 594 goat anti-Rabbit (SA00013-4); Alexa Fluor 488 goat anti-Mouse (ab150113).

Real-Time Quantitative Polymerase Chain Reaction

To begin, total RNA was extracted from the mouse olfactory mucosa using Trizol (Sigma-Aldrich T9424), with its concentration measured using a NanoDrop™ One/OneC microvolume UV-Vis spectrophotometer. This was followed by rapid reverse transcription into cDNA, culminating in Real-time qPCR to evaluate gene expression levels within the samples using Vazyme HiScript III RT SuperMix for qPCR and Vazyme ChamQ SYBR qPCR Master Mix reagents. The necessary primers were sourced from Shanghai Biosheng Biotech. Primer sequences were: OMP Forward: 5′- TCCGTCTACCGCCTCGATTT-3′, Reverse: 5′-CGTCTGCCTCATTCCAATCCA-3′. GAP43 Forward: 5′-TGGTGTCAAGCCGGAAGATAA-3′, Reverse: 5′-ATGAGGTCCACCACCCTGT-3′. GAPDH Forward 5′- TGCGACTTCAACAGCAACTC −3′, Reverse 5′-ATGAGGTCCACCACCCTGT-3′.

Inhibiting the Notch Signaling Pathway with DAPT

We selected (n=7) AR+OD mice administered 5mg/kg DAPT (Sigma-Aldrich D5942) daily from day 29 to 35, alongside ongoing nasal provocations. AR+OD mice (n=5) and Control mice (n=5) were given the same amount of PBS. On the evening of day 34, fasting without water restriction was implemented, followed by behavioral evaluations for nasal symptoms and the BFPT conducted after the last nasal stimulation on day 35.[22,29]

Statistical Analysis

Data generated in this study were analyzed using GraphPad Prism 8 and SPSS version 26.0 (SPSS Inc., Chicago, IL). Symptom scores were represented as mean with standard deviation (SD). Spearman correlation analysis was used to evaluate the correlation between nasal congestion and olfactory dysfunction scores. In order to compare the mRNA expression and positive cell numbers among the different groups, one-way ANOVA followed by Tukey's multiple comparisons test was carried out to assess for the differences between groups. Statistical significance was determined by a p value of <0.05.

Results

The SNOT-22 Scale Scores of Patients with Allergic Rhinitis

The main symptoms including sneezing, nasal obstruction, rhinorrhea, nasal itching and olfactory disorder of 111 AR patients, 65 non-AR patients, and 21 healthy subjects (Ctrl group) were collected in the SNOT-22 scale (Table 1). Both the AR and non-AR group showed more severe allergy symptoms than controls (Figure 1A, Table 1). Moreover, The AR group displayed higher scores in nasal itching, nasal congestion and olfactory than that in non-AR group (Figure 1B–E). It emphasizes that AR exhibits more severe nasal symptoms and olfactory disorders compared to non-AR and control groups, and has a more profound impact on individuals' quality of life. In addition, no significant correlation was observed between nasal congestion and olfactory disorders (r=0.145, p=0.128), suggesting nasal obstruction may not be the main cause of olfactory dysfunction in AR. Therefore, we proposed using animal models to further investigate the pathogenesis.

Table 1 The SNOT-22 Scale Scores of Subjects Enrolled in the Study

The SNOT-22 scale scores	AR (n=111)	Non-AR (n=65)	Ctrl (n=21)
Age, mean ±SD	33.37±9.39	34.18±8.33	29.86±11.70
Female, (%)	89(64.86)	43(66.15)	15(71.43)
Sneezing, mean ±SD	1.78±0.95	1.63±0.91	0.14±0.36
Rhinorrhea, mean ±SD	2.87±0.48	2.68±0.69	0.10±0.30
Nasal itching, mean ±SD	1.23±0.99	0.85±0.83	0.19±0.40
Nasal congestion, mean ±SD	1.57±0.97	1.09±1.65	0.05±0.22
Olfactory disorder, mean ±SD	1.43±1.78	0.86±1.32	0.05±0.22

Abbreviations: SD, standard deviation; AR, allergic rhinitis; Ctrl, control.

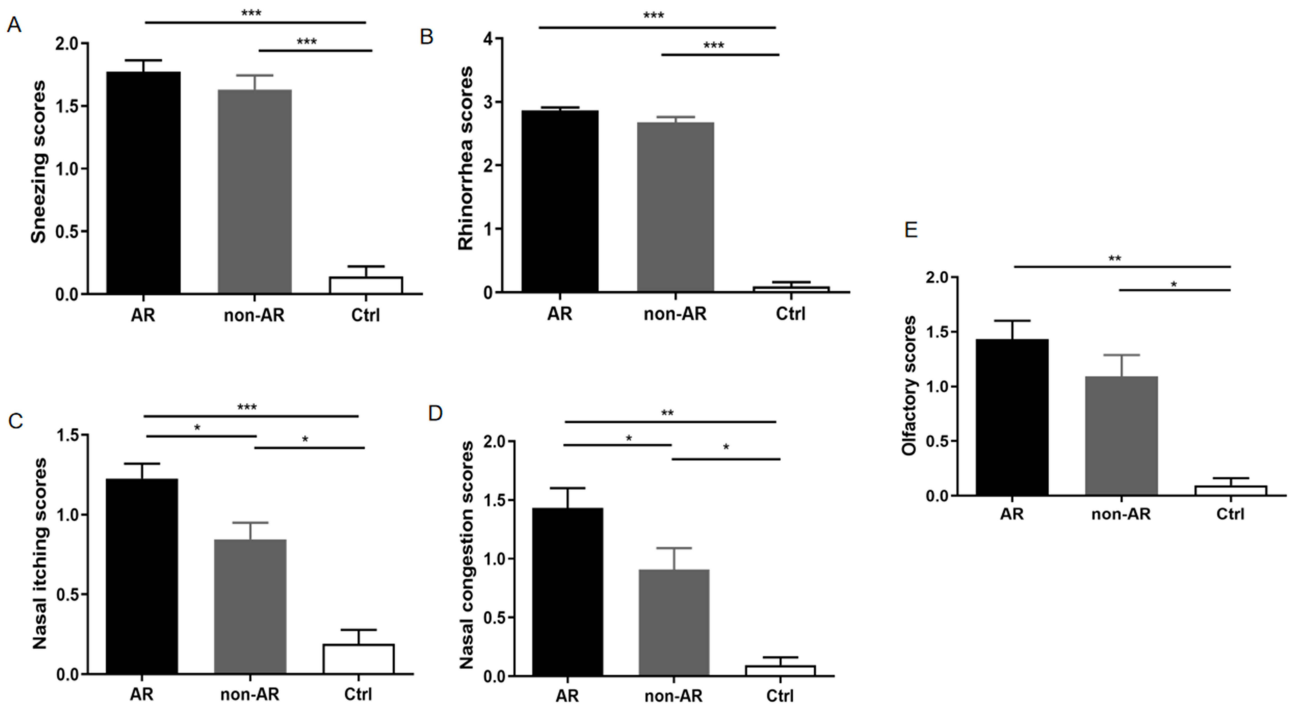

Figure 1 The SNOT-22 scale scores of 111 AR patients, 65 non-AR patients, and 21 healthy subjects. The sneezing scores, rhinorrhea scores, nasal itching scores and nasal congestion scores in these three groups (**A**–**D**). The olfactory scores in these three groups(**E**). All data are presented as mean ± SEM. *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$.
Abbreviations: AR, allergic rhinitis; Ctrl, control. SEM, standard error of the mean.

The OD Exhibited in the AR Mouse Model Was Independent of Inflammation

To further explore the OD in AR, we built OVA-induced AR mice models and measured their OD with BFPT (Figure 2A). Among the 36 AR mice, 55.56% (20/36) mice had OD (AR+OD), which exhibited a significantly prolonged latency in locating the pallet compared to the control group and AR-OD group (Figure 2B), further suggesting the widespread presence of OD in AR. Moreover, the frequency of sneezing, rubbing, symptom scores, eosinophil infiltration and OVA-specific IgE levels were significantly increased in the two AR mice groups compared with the Ctrl group (Figure 2C–H), but no difference were observed between the AR+OD and AR-OD groups in all these indicators, suggesting that the OD may be an independent impairment of AR that cannot be solely attributed to inflammation and nasal symptoms, other factors such as neuron, olfactory epithelium and olfactory bulb dysfunction may contribute to OD.

The Thickness of Olfactory Epithelium and the Subepithelial Eosinophils Infiltration in AR Mice

We stained the olfactory epithelium in the three groups and observed that the AR group had more eosinophils than the control group. However, no significant difference was observed in subepithelial eosinophil counts between the AR+OD group and the AR-OD group (Figure 3A and B), suggesting that eosinophil infiltration may not be a direct cause of olfactory dysfunction. Additionally, the olfactory epithelium in the AR+OD group was thinner than those in the AR-OD and Control group, whereas no significant difference was noted between the AR-OD and Control groups (Figure 3A–C), indicating alteration in the olfactory epithelium may serve as a potential factor contributing to OD in AR.

Expression of Neurons in AR Mice with OD

To explore the variations of OE, we stained neurons labeled by PGP9.5 and found the number of neurons was significantly decreased in the AR+OD group compared to the Ctrl and AR-OD group (Figure 4A and B). To clarify the changes in neurons, we subsequently stained both immature and mature neurons, labeled by GAP43 and OMP

https://doi.org/10.2147/JIR.S504050

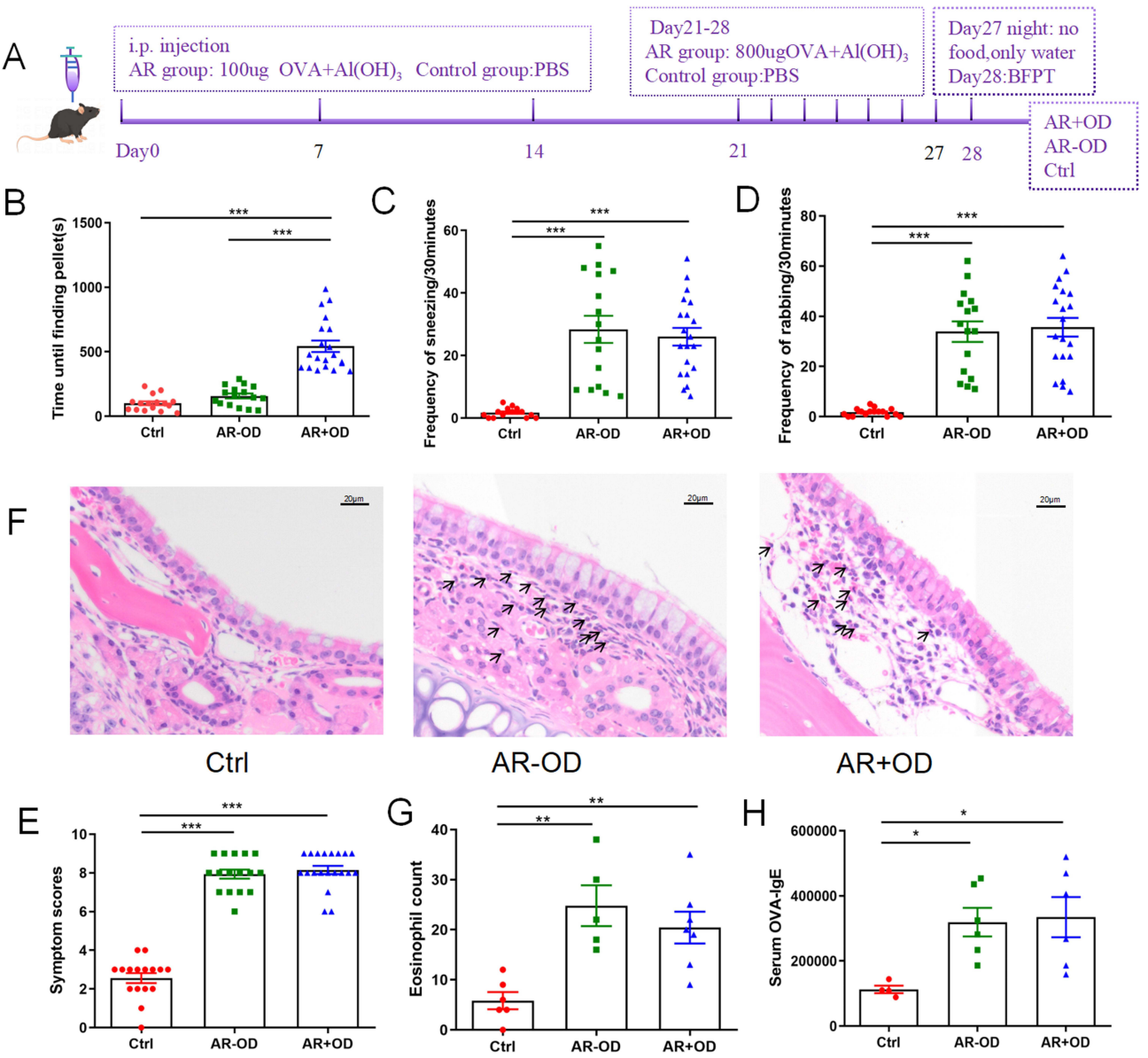

Figure 2 The AR mouse model with olfactory dysfunction (OD) was successfully established. The timeline of model establishment(**A**). The time taken to locate the pallet in the AR+OD, AR-OD, and control groups (**B**). The frequency of sneezing (**C**), rubbing (**D**), and overall symptom scores (**E**) were compared across the three groups. The number of eosinophils in the submucosa of these three groups (**F**–**G**). The levels of OVA-specific IgE in the three groups (**H**). All the pictures were taken under a light microscope at ×400 magnification (Scale bar = 20μm). All data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.
Abbreviations: AR, allergic rhinitis; OD, olfactory dysfunction; SEM, standard error of the mean; OVA, ovalbumin.

respectively and found the number of mature neurons and the mRNA expression of OMP was significantly decreased in the AR+OD group compared to the Ctrl and AR-OD group (Figure 4C–E), which may explain the loss of olfactory sensation. However, we further found that most of the neurons in olfactory epithelium of AR+OD group were immature neurons, which was significantly higher than those of control group in both protein and mRNA levels (Figure 4F–H). Collectively, these may suggest that the OD of AR may be attributed to the cessation of trans-differentiation of immature to mature olfactory neurons.

Our previous studies showed that Notch activation promoted progenitor cell proliferation while inhibited neuronal maturation in the olfactory epithelium, so we further performed Notch1 immunostaining and PCR analysis and observed a significant increase in Notch1 expression levels within the allergic group compared to the control group. Additionally, Notch1 was higher in the AR+OD group than in the AR-OD group (Figure 4I–K), indicating its crucial role in OD of AR.

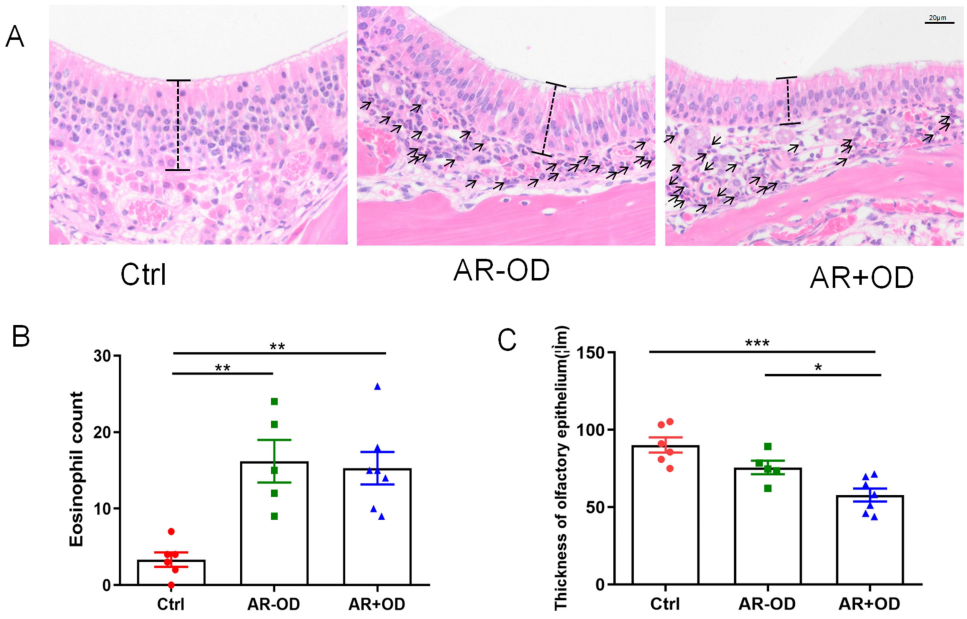

Figure 3 Comparison of the olfactory epithelium in the AR+OD, AR-OD, and control groups. The number of eosinophils in the olfactory subepithelial of the three groups, with black arrows indicating eosinophils (**A** and **B**). The thickness of olfactory epithelium across these groups (**A**–**C**). All the pictures were taken under a light microscope at ×400 magnification (Scale bar = 20μm). All data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.
Abbreviations: AR, allergic rhinitis; OD, olfactory dysfunction; SEM, standard error of the mean.

Blocking Notch Signaling Pathway Promoted Neuronal Differentiation and Improved OD of AR Mice

Subsequently, we inhibited the Notch signal with DAPT, which successfully decreased the high Notch expression in AR +OD group (Figure 5A and B). In the AR+OD+DAPT group, the expression of immature neurons decreased (Figure 5C and D), while the expression of mature neurons increased (Figure 5E and F). Additionally, we also found that the neuron expression was elevated, approaching that of the control group (Figure 5G and H). This suggests that inhibiting of Notch promote neuronal differentiation. It is noteworthy that Notch inhibition significantly improved OD in AR mice, as indicated by the shorter time until finding the pellet in AR+OD+DAPT group compared to the AR+OD group (Figure 5I). Finally, we measured the expression level of Notch1 in the serum of patients with allergic rhinitis using ELISA, specifically in the AR+OD group, the AR-OD group, and the Ctrl group. The results revealed that the expression of Notch1 in the AR+OD group was significantly higher than that in the other two groups, with no significant difference between the AR-OD and Ctrl groups (Figure 5J). This finding suggests that Notch1 plays a regulatory role both in humans and in mice with allergic rhinitis accompanied by olfactory dysfunction.

Discussion

The mechanism of OD in AR is not fully understood. Recent studies reported that the degree of nasal obstruction was not directly related to AR induced OD,[30] which is consistent with our finding that no correlation between olfactory disorders and nasal congestion was observed in AR patients. In our study, the olfactory scores of 111 AR patients, 65 non-AR and 21 control people were assessed using the SNOT-22 scale, in which both the AR and non-AR group showed more severe allergy symptoms than controls. Moreover, The AR group displayed higher scores in nasal itching, nasal congestion and olfactory disorders than that in non-AR group. These findings revealed that the occurrence of olfactory dysfunction in individuals with AR is notably high, and its etiology does not stem from nasal congestion. Consequently, we embarked on mouse modeling to delve deeper into its underlying mechanisms.

In this study, we evaluated whether the experimental animal model (AR+OD) is well established. In the AR group, the symptom scores, blood OVA specific IgE levels and eosinophils number in the subcutaneous layer of the nasal mucosa were significantly elevated compared to the control group. Kern et al[31,32] reported that chronic rhinosinusitis

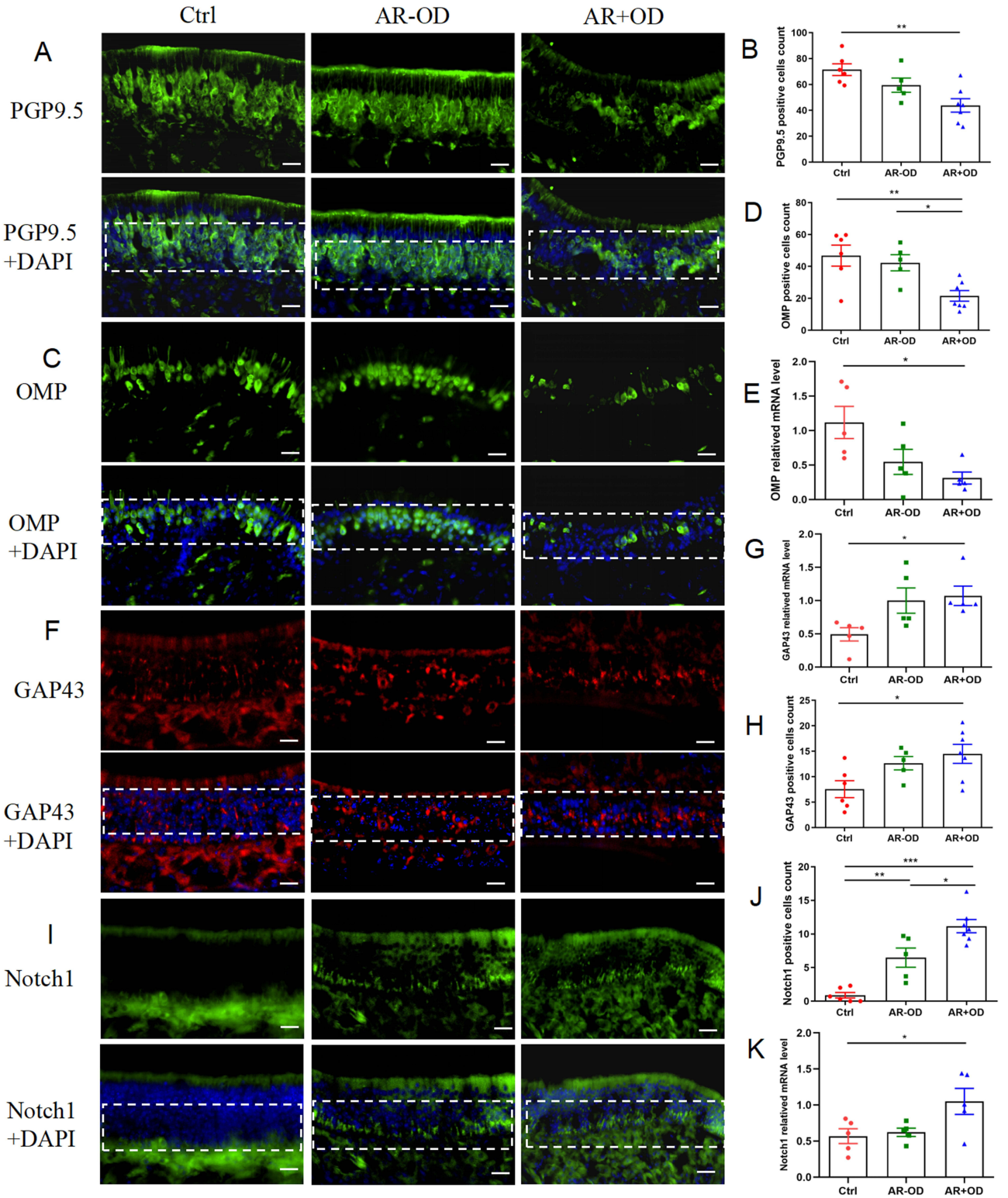

Figure 4 The expression of neurons and Notch1 in the mice models. The expression of neurons in the AR+OD, AR-OD and control groups (**A** and **B**). The protein and mRNA expression of mature neurons in the three groups (**C**–**E**). The protein and mRNA expression of immature neurons across these three groups. (**F**–**H**). Notch1 expression in these three groups (**I**–**K**). All the pictures were taken under a light microscope at ×400 magnification (Scale bar = 20μm). All data are presented as mean ± SEM. *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$.

Abbreviations: AR, allergic rhinitis; OD, olfactory dysfunction; SEM, standard error of the mean.

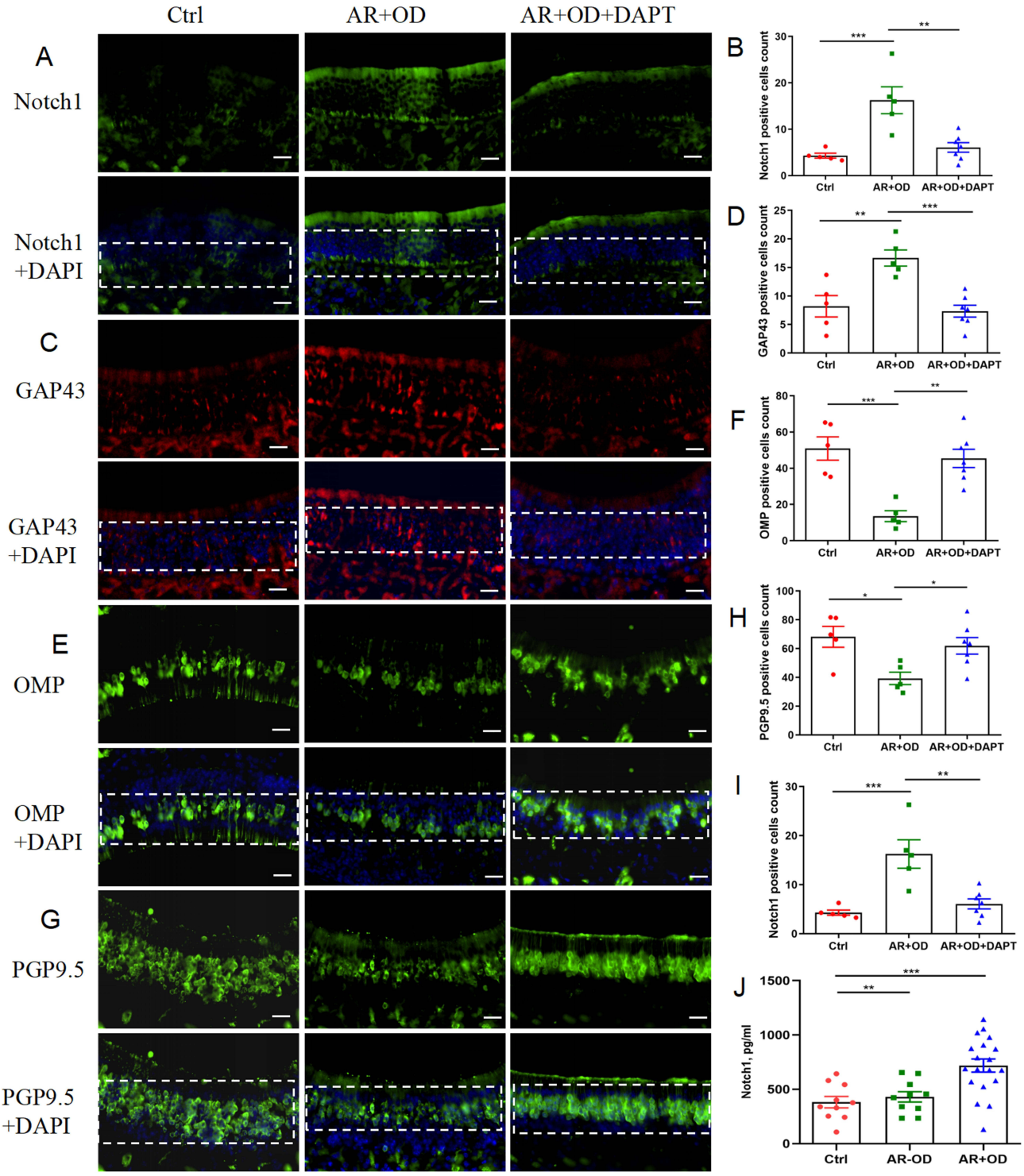

Figure 5 The expression of Notch1 in control, AR+OD, and Notch inhibited group (AR+OD+DAPT) (**A** and **B**). The expression of immature neurons across these three groups (**C** and **D**). The number of mature neurons in these three groups (**E** and **F**). The expression of neurons in the three groups (**G** and **H**). The results of the Buried Food Pellet Test for these three groups (**I**). The expression of Notch1 in AR patients (**J**). All the pictures were taken under a light microscope at ×400 magnification (Scale bar = 20μm). All data are presented as mean ± SEM. *:p<0.05, **:p<0.01, ***:p<0.001.

Abbreviations: AR, allergic rhinitis; OD, olfactory dysfunction; SEM, standard error of the mean.

https://doi.org/10.2147/JIR.S504050

(CRS) patients exhibit a high number of inflammatory cells in the olfactory mucosa, along with a thinning of the olfactory epithelium. Consistently, we observed that the olfactory epithelium in the AR+OD group was thinner than the AR-OD and control group, suggesting that thinning of the olfactory epithelium may contribute to the olfactory dysfunction in AR. Additionally, no significant difference was observed in subepithelial eosinophil infiltration between AR+OD and AR-OD group, indicating that subepithelial eosinophil infiltration may not be the causes of the olfactory dysfunction. However, we cannot exclude the potential role of other inflammatory cells. Human chronic olfactory inflammation was reported to be typically associated with CRS and characterized by local immune cell infiltration, the production of inflammatory mediators, loss of sensory neurons, and decreased olfactory function.[31,33] Chen et al[34] demonstrated that olfactory loss associated with chronic rhinosinusitis was linked to a functional shift in neuroepithelial stem cells from a regenerative role to one of immune defense, with T lymphocytes being the predominant immune cell population involved in this process. In light of these findings, our future research will focus on exploring the relationship between inflammatory cells and olfactory dysfunction in AR.

Wang et al[28] found that the inflammatory response of AR led to pathological changes in the olfactory mucosa, characterized by a decrease in the expression of olfactory marker protein and a reduction in the number of olfactory receptor neurons. Our research further found that the number of mature neurons reduced and the number of immature neurons increased in the AR mice with OD. Based on a comprehensive review of the literature, our study is the first to investigate the maturity of neurons in the olfactory epithelium of AR models. Given that previous research has indicated the influence of Notch activation in promoting the retention of cells in the olfactory epithelium (OE) at either a progenitor or immature state,[7] we sought to ascertain whether Notch plays a role. Consequently, we discovered that the Notch1 expression was elevated in the AR group compared to the control group, with even higher levels observed in the AR+OD group relative to the AR-OD group. This suggests that elevated Notch1 may contribute to the development of olfactory dysfunction, or conversely, that olfactory dysfunction leads to an increase in Notch1 expression, which fails to downregulate appropriately, resulting in neurons remaining in an immature state. Our findings further confirmed that inhibition of Notch signaling pathway enhanced

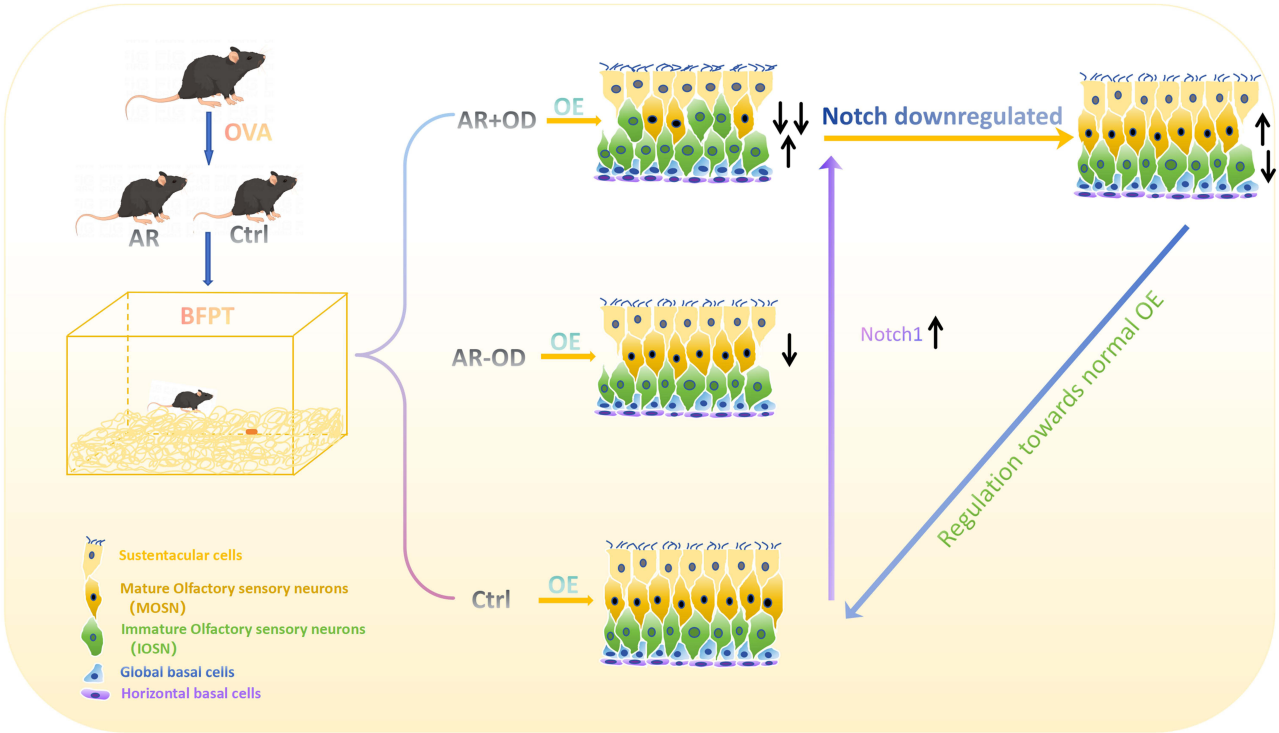

Figure 6 Schematic model of the expression of neurons in the olfactory epithelium and the role of Notch signaling pathway in allergic rhinitis with olfactory disorders. The cessation of differentiation from immature to mature olfactory neurons may lead to olfactory disorders in allergic rhinitis, whereas inhibiting Notch1 may facilitate neuronal differentiation and aid in the restoration of the olfactory epithelium.

neuronal differentiation and supported the recovery of olfactory function (Figure 6). In the final stage of our research, we confirmed that the expression of Notch1 was elevated in the AR+OD patients. Thus, targeting Notch signaling could be a promising therapeutic strategy for AR. However, further research is needed to determine the optimal timing for intervention and to develop effective strategies for implementing this approach.

There are several limitations to this study. First, the clinical applicability of our findings is constrained by the reliance on animal models, the relatively small sample size, and the absence of long-term or functional follow-up assessments. Additionally, our focus on the Notch signaling pathway, while providing valuable insights, may offer a somewhat narrow perspective on the complex mechanisms underlying the condition. To address these limitations, future research should prioritize larger-scale longitudinal studies in human populations, incorporating behavioral assessments and functional outcomes. Furthermore, exploring additional signaling pathways and molecular networks will be essential to achieve a more comprehensive understanding of the disease and to establish a stronger theoretical foundation for developing effective treatment strategies.

Conclusion

In conclusion, this study suggests that impaired differentiation of olfactory neurons may underlie olfactory dysfunction in AR. Additionally, inhibition of the Notch signaling pathway promotes the differentiation of olfactory epithelium in AR patients with OD, offering new insights into the pathogenesis of olfactory disorders in AR and presenting potential therapeutic avenues for treatment.

Abbreviations

AR, allergic rhinitis; OD, olfactory dysfunction; OE, olfactory epithelium; GBCs, globose basal cells; HBCs, horizontal basal cells; OSNs, Olfactory sensory neurons; CRS, chronic rhinosinusitis; SEM, standard error of the mean; OVA, ovalbumin, SD, standard deviation; Ctrl, control; BFPT, Buried Food Pellet Test; H&E, hematoxylin and eosin; Sino-Nasal Outcome Test-22, SNOT-22.

Data Sharing Statement

The datasets generated and analyzed during the current study are not publicly available but are available from Xin Feng on reasonable request.

Ethics Approval and Informed Consent

The studies involving human participants were reviewed and approved by The Medical Ethics Committee of Qilu Hospital of Shandong University (No. KYLL-202111-082-1). Our study complies with the Declaration of Helsinki. All procedures involving animals were approved by The Animal Ethical Committee of Qilu Hospital of Shandong University (No. DWLL-2022-023) and followed by the Guidelines for the Ethical Review of Laboratory Animal Welfare.

Acknowledgments

This study is supported by the National Natural Science Foundation of China (82171106, 82371120, 81700890), Taishan Scholar Program of Shandong Province (tsqn202103166), and Natural Science Foundation of Shandong Province (ZR2020QH151, ZR2023MH197).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Chen H, Cheng Y, Du H, et al. Shufeng Jiedu capsule ameliorates olfactory dysfunction via the AMPK/mTOR autophagy pathway in a mouse model of allergic rhinitis. *Phytomedicine*. 2022;107:154426. doi:10.1016/j.phymed.2022.154426
2. Stuck BA, Hummel T. Olfaction in allergic rhinitis: a systematic review. *J Allergy Clin Immunol*. 2015;136(6):1460–1470.

3. Liu P, Qin D, Lv H, et al. Activation of dopamine D2 receptor alleviates neuroinflammation in a mouse model of allergic rhinitis with olfactory dysfunction. *Allergy Asthma Immunol Res*. 2021;13(6):882–895. doi:10.4168/aair.2021.13.6.882
4. Liang C, Yang Z, Zou Q, Zhou M, Liu H, Fan J. Construction of an irreversible allergic rhinitis-induced olfactory loss mouse model. *Biochem Biophys Res Commun*. 2019;513(3):635–641. doi:10.1016/j.bbrc.2019.03.110
5. Croy I, Nordin S, Hummel T. Olfactory disorders and quality of life--an updated review. *Chem Senses*. 2014;39(3):185–194. doi:10.1093/chemse/bjt072
6. Ren W, Ma Z, Wang L, Feng X, Yu H, Yu Y. Lgr5(+) cells are required and dynamically participate in olfactory epithelium regeneration: a revisiting shows Lgr5 expression in multiple cell lineages. *Theranostics*. 2022;12(13):5631–5644. doi:10.7150/thno.60636
7. Dai Q, Duan C, Ren W, et al. Notch signaling regulates Lgr5(+) olfactory epithelium progenitor/stem cell turnover and mediates recovery of lesioned olfactory epithelium in mouse model. *Stem Cells*. 2018;36(8):1259–1272. doi:10.1002/stem.2837
8. Song J, Wang M, Wang C, Zhang L. Olfactory dysfunction in chronic rhinosinusitis: insights into the underlying mechanisms and treatments. *Expert Rev Clin Immunol*. 2023;19(8):993–1004. doi:10.1080/1744666X.2023.2235891
9. Yee KK, Pribitkin EA, Cowart BJ, et al. Neuropathology of the olfactory mucosa in chronic rhinosinusitis. *Am J Rhinol Allergy*. 2010;24 (2):110–120. doi:10.2500/ajra.2010.24.3435
10. Lee SH, Lim HH, Lee HM, Park HJ, Choi JO. Olfactory mucosal findings in patients with persistent anosmia after endoscopic sinus surgery. *Ann Otol Rhinol Laryngol*. 2000;109(8 Pt 1):720–725. doi:10.1177/000348940010900804
11. Zhong Z, Huang X, Zhang S, et al. Blocking Notch signalling reverses miR-155-mediated inflammation in allergic rhinitis. *Int Immunopharmacol*. 2023;116:109832.
12. Shen C, Detry B, Lecocq M, Pilette C. A novel IgA/Delta-like 4/Notch axis induces immunosuppressive activity in human dendritic cells. *Clin Immunol*. 2016;168:37–46. doi:10.1016/j.clim.2016.04.013
13. Fre S, Huyghe M, Mourikis P, Robine S, Louvard D, Artavanis-Tsakonas S. Notch signals control the fate of immature progenitor cells in the intestine. *Nature*. 2005;435(7044):964–968. doi:10.1038/nature03589
14. Stanger BZ, Datar R, Murtaugh LC, Melton DA. Direct regulation of intestinal fate by Notch. *Proc Natl Acad Sci USA*. 2005;102 (35):12443–12448. doi:10.1073/pnas.0505690102
15. Herrick DB, Lin B, Peterson J, Schnittke N, Schwob JE. Notch1 maintains dormancy of olfactory horizontal basal cells, a reserve neural stem cell. *Proc Natl Acad Sci USA*. 2017;114(28):E5589–E5598.
16. Ikeda K, Kageyama R, Suzuki Y, Kawakami K. Six1 is indispensable for production of functional progenitor cells during olfactory epithelial development. *Int J Dev Biol*. 2010;54(10):1453–1464. doi:10.1387/ijdb.093041ki
17. Schwarting GA, Gridley T, Henion TR. Notch1 expression and ligand interactions in progenitor cells of the mouse olfactory epithelium. *J mol Histol*. 2007;38(6):543–553. doi:10.1007/s10735-007-9110-9
18. Carson C, Murdoch B, Roskams AJ. Notch 2 and Notch 1/3 segregate to neuronal and glial lineages of the developing olfactory epithelium. *Dev Dyn*. 2006;235(6):1678–1688. doi:10.1002/dvdy.20733
19. Rodriguez S, Sickles HM, Deleonardis C, Alcaraz A, Gridley T, Lin DM. Notch2 is required for maintaining sustentacular cell function in the adult mouse main olfactory epithelium. *Dev Biol*. 2008;314(1):40–58. doi:10.1016/j.ydbio.2007.10.056
20. Jiao WE, Wei JF, Kong YG, Xu Y, Tao ZZ, Chen SM. Notch signaling promotes development of allergic rhinitis by suppressing foxp3 expression and treg cell differentiation. *Int Arch Allergy Immunol*. 2019;178(1):33–44. doi:10.1159/000493328
21. Hjalmarsson E, Petro M, Georén SK, Winqvist O, Cardell LO. Upregulated expression of Notch1/4 - JAG-1/DLL-1 detected in allergic rhinitis. *Allergy Asthma Clin Immunol*. 2023;19(1):41. doi:10.1186/s13223-023-00793-4
22. Shi L, Ma Y, Zheng C, Zhang Q. The effect of blocking Notch signaling by γ-secretase inhibitor on allergic rhinitis. *Int J Pediatr Otorhinolaryngol*. 2017;98:32–38. doi:10.1016/j.ijporl.2017.04.036
23. Chinese guidelines for diagnosis and treatment of allergic rhinitis. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2016;51(1):6–24. doi:10.3760/cma.j.issn.1673-0860.2016.01.004
24. Chen H, Li J, Cheng L, et al. China consensus document on allergy diagnostics. *Allergy Asthma Immunol Res*. 2021;13(2):177–205. doi:10.4168/aair.2021.13.2.177
25. Morawska J, Jeruzal-świątecka J, Politański P, Pietruszewska W. Sino-nasal outcome test-22: translation, cross-cultural adaptation, and validation in Polish-speaking patients. *Eur Arch Otorhinolaryngol*. 2024;281(12):6431–6443. doi:10.1007/s00405-024-08919-z
26. Yuan J, Liu Y, Yu J, et al. Gene knockdown of CCR3 reduces eosinophilic inflammation and the Th2 immune response by inhibiting the PI3K/AKT pathway in allergic rhinitis mice. *Sci Rep*. 2022;12(1):5411. doi:10.1038/s41598-022-09467-4
27. Xiao L, Jiang L, Hu Q, Li Y. MicroRNA-133b ameliorates allergic inflammation and symptom in murine model of allergic rhinitis by targeting Nlrp3. *Cell Physiol Biochem*. 2017;42(3):901–912. doi:10.1159/000478645
28. Wang X, Zhu Y, Ni D, Lv W, Gao Z, Qi F. Intranasal application of glucocorticoid alleviates olfactory dysfunction in mice with allergic rhinitis. *Exp Ther Med*. 2017;14(5):3971–3978. doi:10.3892/etm.2017.5042
29. Jiang S, Han S, Chen J, Li X, Che H. Inhibition effect of blunting Notch signaling on food allergy through improving T(H)1/T(H)2 balance in mice. *Ann Allergy Asthma Immunol*. 2017;118(1):94–102. doi:10.1016/j.anai.2016.10.024
30. Seiden AM, Duncan HJ. The diagnosis of a conductive olfactory loss. *Laryngoscope*. 2001;111(1):9–14. doi:10.1097/00005537-200101000-00002
31. Kern RC. Chronic sinusitis and anosmia: pathologic changes in the olfactory mucosa. *Laryngoscope*. 2000;110(7):1071–1077. doi:10.1097/00005537-200007000-00001
32. Jung AY, Kim YH. Reversal of olfactory disturbance in allergic rhinitis related to omp suppression by intranasal budesonide treatment. *Allergy Asthma Immunol Res*. 2020;12(1):110–124. doi:10.4168/aair.2020.12.1.110
33. Victores AJ, Chen M, Smith A, Lane AP. Olfactory loss in chronic rhinosinusitis is associated with neuronal activation of c-Jun N-terminal kinase. *Int Forum Allergy Rhinol*. 2018;8(3):415–420. doi:10.1002/alr.22053
34. Chen M, Reed RR, Lane AP. chronic inflammation directs an olfactory stem cell functional switch from neuroregeneration to immune defense. *Cell Stem Cell*. 2019;25(4):501–513.e5. doi:10.1016/j.stem.2019.08.011