

## Original Article

# Upregulated TRPC5 plays an important role in development of nasal polyps by activating eosinophilic inflammation and NF- $\kappa$ B signaling pathways

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**Abstract:** The pathophysiology of nasal polyps (NP) remains unclear, however, several ion channels may participate. Whether transient receptor potential canonical (TRPC) channel play a role in NP remains unknown. We investigated expression of TRPC, eosinophil infiltration, IL-6, and NF- $\kappa$ B in 58 patients with NP and 35 control subjects using hematoxylin-eosin (HE) staining, immunohistochemistry, real-time fluorescence quantitative reverse transcription PCR (real-time RT-PCR), Western blotting, and calcium imaging. Compared with normal nasal mucosa, TRPC5 mRNA and protein expression increased in NP. Eosinophil counts, IL-6 expression, and phosphorylation levels of NF- $\kappa$ B were higher in NP than in normal mucosa. TRPC5 expression was positively correlated with eosinophils, IL-6, and phosphorylation levels of NF- $\kappa$ B. Blocking of TRPC5 channel decreased store-operated calcium influx, IL-6 expression, and phosphorylation levels of NF- $\kappa$ B in blood eosinophils from patients with NP. In conclusion, TRPC5 was upregulated in NP and played an important role in development of NP by activating eosinophilic inflammation and NF- $\kappa$ B signaling pathways.

**Keywords:** Nasal polyps, canonical transient receptor potential channel 5, eosinophil, NF- $\kappa$ B pathway

## Introduction

Nasal polyps (NP) are a subtype of chronic rhinosinusitis (CRS), a common otolaryngological disease characterized by chronic inflammation of nasal and/or paranasal sinus mucosa that persists for at least 3 months [1, 2]. CRS is typically classified into CRS with NP or CRS without NP [1-3]. Patients who have CRS without NP are more likely to manifest signs of bacterial infection and have been reported to have better response to medical treatment [1-3]. However, CRS with NP is typically characterized by infiltration of eosinophils or mixed immune cells in histopathology [4]. Previous studies have found that CRS with NP is more closely related with nasal obstruction and loss of smell [5]. Additionally, there is a higher recurrence rate in

CRS with NP after conventional surgical treatment [6]. Because the exact pathophysiology of nasal polyps remains unclear, it is important to identify new targets that play key roles in development of nasal polyps.

Recently, canonical transient receptor potential channels (TRPCs) have been indicated to mediate a number of diseases such as cancer [7], atherosclerosis [8], and airway diseases [9]. Studies have suggested that TRPCs mediate processing of store operated calcium influx into cells, resulting in longer-term intracellular calcium signaling [10, 11]. Calcium influx through TRPCs is believed to be an important event leading to airway disease [9]. Sel et al. found that TRPC6 deficiency reduced the allergic airway response as well as airway eosinophilia

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**Table 1.** Primers designed and used in real-time RT-PCR

Gene	Accession no.	Primers	Product size (bp)
TRPC 1	NM_003304	TTGTGGAGGTGGAATTCAGG forward CGTTTGTCAAGAGGCTCGTC reverse	148
TRPC 2	NR_002720	TCATGGTCATTGTGCTGCTC forward ACTCCACGTCAGCATCATCC reverse	85
TRPC 3	NM_016691	CAGCCAACACGTTATCAGCA forward CCTCAGTTGCTTGGCTCTTG reverse	172
TRPC 4	NM_016179	TAGCGAAGGTAATAGCAAGGACA forward TCTCGGATGAATCAGGGTGGT reverse	73
TRPC 5	NM_012471	ACAAAAGGTCAACTACTCACCG forward CAGTGGCATAGTCCCCCTTCT reverse	126
TRPC 6	NM_004621	CAGACAATGGCGGTCAAGTT forward TGGTCCACGCATTATCTTCC reverse	117
TRPC 7	NM_020389	AAGTTCGTTGCTCATCCTAACTG forward TGTTGACGTAAGCCTGAGAGATT reverse	77
IL-6	NM_000600	TTAGAGTCTCAACCCCAATAAAT forward GAGAAGGCAACTGGACCGAA reverse	156
GAPDH	NM_002046	AACTGCTTAGCACCCCTGGC forward ATGACCTTGCCACAGCCTT reverse	200

TRPC: Canonical transient receptor potential channel; IL-6, interleukin-6.

and blood IgE levels in methacholine-induced airway hyperresponsiveness [12]. Several studies have shown that other ion channels (CRAC, aquaporins, and chloride channels) are correlated with severity of CRS with NP [13-15]. However, whether TRPC channels participate in the disease state of nasal polyps remains unclear. In our present study, we investigated expression of TRPC channels in nasal samples of CRS with NP as well as potential roles of TRPC channels in NP, using NP tissue and blood eosinophils from NP patients.

### Materials and methods

#### Patients and samples

A total of 58 NP tissue samples were obtained from polypectomy or functional endoscopic sinus surgery in patients with CRS with NP at the Department of Otolaryngology, Qianfoshan Hospital, Shandong University, from 2009-2013. All of the patients met criteria for CRS as defined by the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force [16, 17]. Patients with established immunodeficiency, pregnancy, coagulation disorder, diagnosis of classic allergic fungal sinusitis, or cystic fibrosis were excluded from the study. Normal nasal inferior turbinate mucosal specimens were obtained

from inflammation-free control subjects (n=35) undergoing septoplasty surgery for nasal septum deviation. Our study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Qianfoshan Hospital, Shandong University. All of the subjects gave informed consent.

#### Real-time RT-PCR analysis for mRNA expression of TRPC channels and IL-6

Total RNA was isolated from -80°C frozen nasal polyps or normal mucosal tissues or blood eosinophils using TRIzol Reagent (Invitrogen, USA). Primers for TRPC channels are listed in **Table 1**. Real-time RT-

PCR was performed, according to manufacturer protocol (Applied Biosystems, USA), using SYBR Green qPCR Mix (TaKaRa Biotechnology, Dalian, China). Primers for the TRPC channels are listed in **Table 1**. Specificity of PCR products for each primer was examined using the melting curve method and gel electrophoresis (data not shown). Relative expression levels of each channel gene were normalized to the mRNA of internal standard gene GAPDH. For each sample, the threshold cycle (Ct) was automatically calculated using ABI Prism SDS Software (Applied Biosystems, USA).  $\Delta Ct$  was used for analysis, with  $\Delta Ct = Ct(\text{test sample}) - Ct(\text{GAPDH})$ . Data were analyzed by the  $2^{-\Delta Ct}$  method.

#### Histopathological examination of nasal polyp tissue samples

HE-stained slides of tissues were examined for presence of eosinophils by two observers, randomly, without knowing any sample information in accordance with previous studies [18, 19]. The number of eosinophils was counted in 6 randomly selected high power fields (HPFs) with a magnification of 400 $\times$ , according to previous studies [19]. The average of number of eosinophils of the 6 HPFs was taken as the final count for one section.

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**Table 2.** Clinical characteristics of patients with nasal polyps and control subjects

	NP group (n=58)	Normal group (n=35)
Age (years)	45.3±10.3	41.8±13.6
Gender		
Male	38 (65.5%)	24 (68.6%)
Female	20 (34.5%)	24 (68.6%)
	23	12
BMI (kg.m <sup>2</sup> )	25.6±2.51	24.8±2.26
Systolic BP (mm Hg)	118±9.5	117.5±9.1

### *Immunohistochemistry for detection of TRPC5 channel and IL-6*

Immunostaining was performed on paraffin sections in all cases, according to a previous study [20]. On deparaffinized sections, endogenous peroxidase activity was quenched with 0.3% H<sub>2</sub>O<sub>2</sub> at room temperature for 5 minutes. Primary rabbit polyclonal anti-TRPC5 (1:500, ab63151, Abcam) or rabbit polyclonal anti-IL6 antibody (1:500, ab6672, Abcam) was applied overnight at 4°C. Slides were then washed in phosphate buffered saline (PBS) and exposed to biotinylated goat anti-rabbit IgG (H+L) secondary antibody (1:2000, Ab97080, Abcam) for 1 hour at room temperature. After additional PBS washings, the sections were developed with DAB Detection Kit (GK6005, GeneTech (Shanghai) Company Limited, China). Normal rabbit IgG at the same concentration as primary antibodies served as negative controls. Six HPFs were successively examined under standard light microscopy with a magnification of 400×. Membrane and/or cytoplasmic staining was considered for assessment of TRPC5 and IL-6 immunoreactivity. Immunostaining intensity was evaluated by intensive optical density (IOD) of immunostaining using ImagePro Plus 6.0 (IPP, Media Cybernetics).

### *Western blot analysis for protein expression of TRPC5, IL-6, and NF-κB*

Tissue samples or cell pellets were washed twice in 4°C PBS, cut into tiny pieces, and lysed in RIPA buffer containing 20 mM Tris-HCL, pH 6.8, 1 mM EDTA, 1% SDS, 1 mM PMSF, and 1× protease inhibitor cocktail (Roche, Germany). Lysates were centrifuged at 14,000 rpm for 10 minutes and a supernatant containing protein was obtained. After measuring concentration

by bicinchoninic acid (BCA) method, the protein solutions were denatured at 100°C for 10 minutes and stored at -40°C for Western blot analysis. Protein samples were separated by 10% SDS-PAGE gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA), in which nonspecific response was blocked by 5% skim milk at room temperature for 2 hours. Then, rabbit polyclonal anti-TRPC5 (1:1000, ab63151, Abcam), rabbit polyclonal anti-IL6 antibody (1:1000, ab6672, Abcam), anti-total-NF-κB p65 (1:1000, 8242S, Cell Signaling Technology, USA), and anti-phospho-NF-κB p65 (p-p65) (1:1000, 3033S, Cell Signaling Technology, USA) were added into the solution and incubated at 4°C overnight. Membrane was washed and incubated with goat anti-mouse (ab97040, Abcam) or anti-rabbit (ab97080, Abcam) IgG HRP preadsorbed secondary antibodies at 1:5000 dilution. Immunoreactive protein bands were visualized by ECL Western Blotting Detection Reagents (RPN2209, GE Healthcare, USA). Photographs were taken and band intensity was quantified using Alphamager HP system (ProteinSimple, USA).

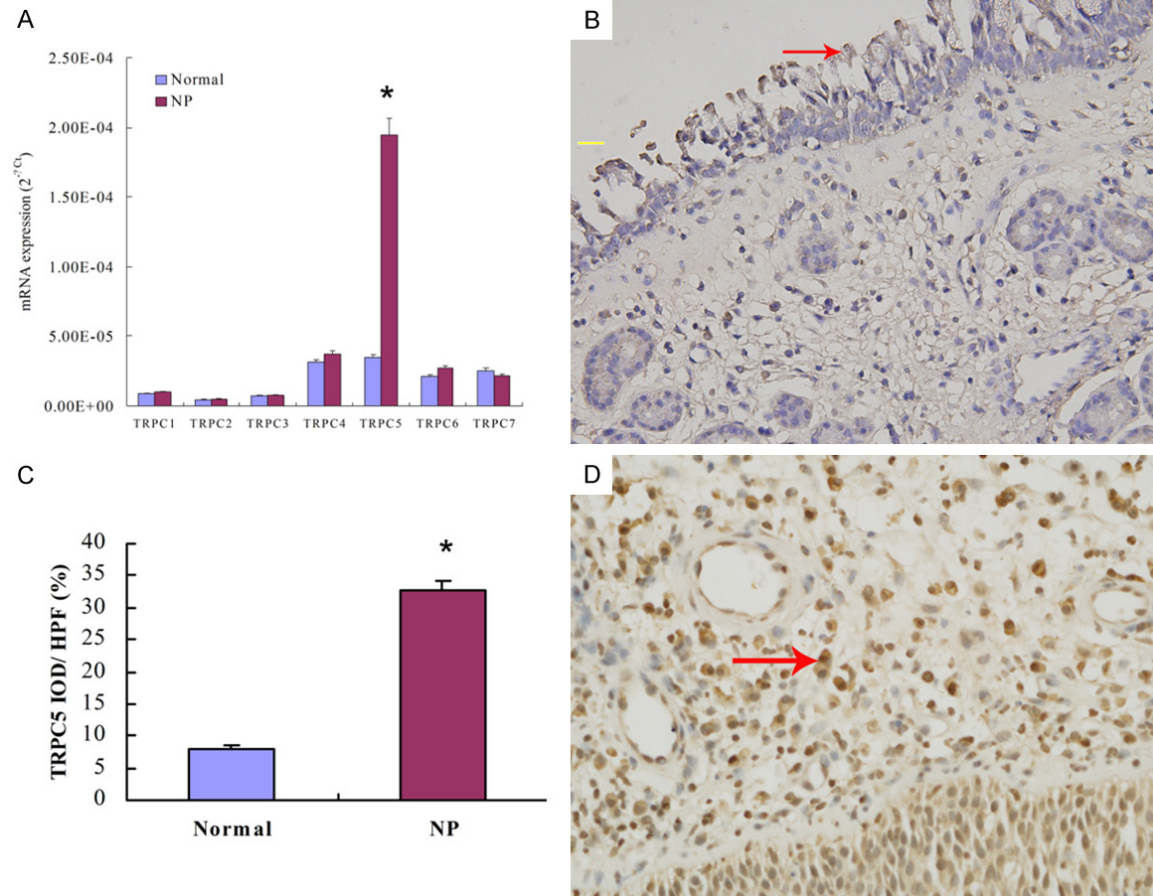
### *Periphery blood eosinophil isolation*

Untouched human blood eosinophils were isolated from 30 mL freshly drawn EDTA-anticoagulated whole blood using MACSxpress® Eosinophil Isolation Kit (Miltenyi Biotec GmbH, Germany), i.e., a method of immunomagnetic depletion. Briefly, 15 mL of MACSxpress Eosinophil Isolation Cocktail were added to 30 mL anticoagulated whole blood in a 50 mL tube. After incubating for 5 minutes at room temperature, the open tube was placed in the magnetic field of MACSxpress Separator for 15 minutes. Magnetically labeled non-target cells adhered to the wall of the tube whereas aggregated erythrocytes sedimented to the bottom. The supernatant, containing eosinophils, was carefully collected for subsequent experiments. Eosinophils were verified by Wright-Giemsa and HE staining and were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 µg/ml streptomycin, 100 U/ml penicillin, and 1.5% sodium bicarbonate at 37°C, 5% CO<sub>2</sub> and 95% air.

### *Intracellular Ca<sup>2+</sup> measurements*

Eosinophils were loaded with 2 µM Fura-2AM and plated onto Poly-L-Lysine coated cover-

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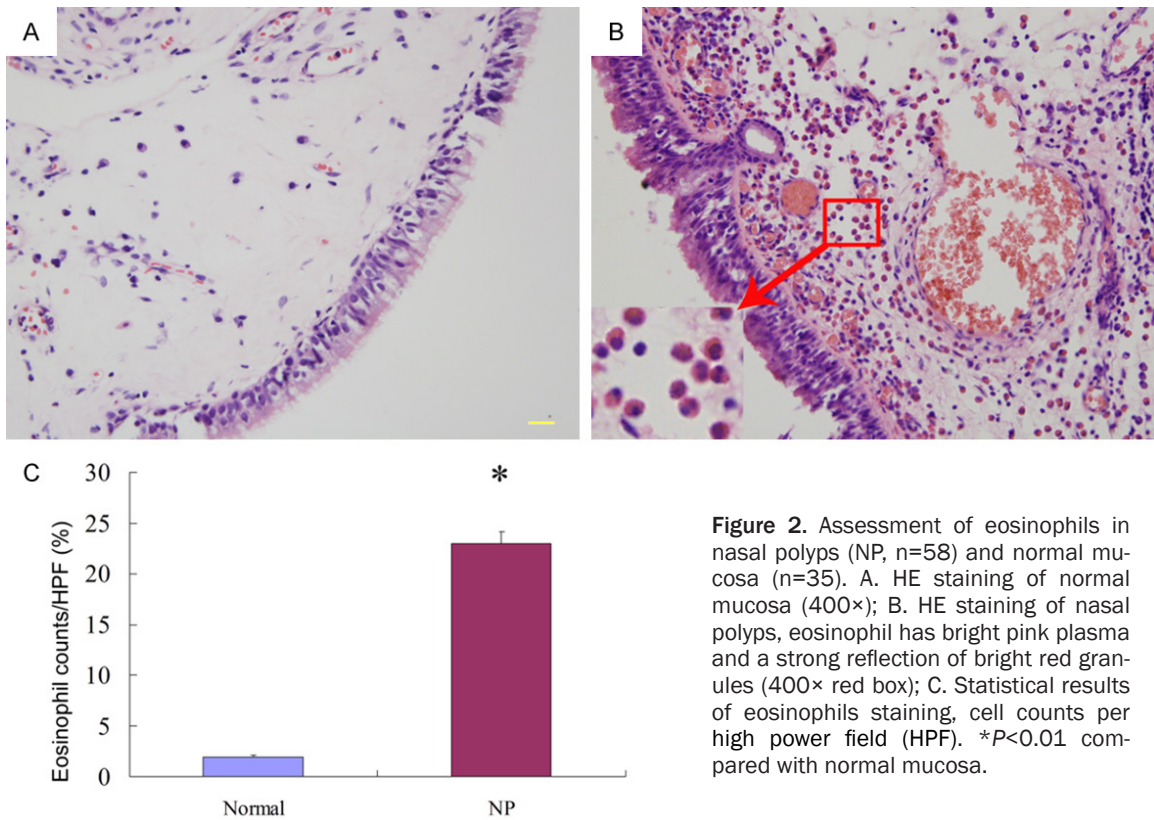
**Figure 1.** Real-time RT-PCR and immunohistochemistry assessment of TRPC channel expression in nasal polyps (NP, n=58) and normal mucosa (n=35). A. Statistical results of real-time RT-PCR for TRPC channels; B. Immunohistochemistry staining for TRPC5 channel in normal mucosa (400 $\times$ ), positive staining was mainly observed in epithelium (red arrow); C. TRPC5 staining in nasal polyps (400 $\times$ ), positive staining was in interstitial cells (red box); D. Statistical results of TRPC5 staining, intensive optical density (IOD) per high power field (HPF). \* $P < 0.05$  compared with normal mucosa.

slips. Intracellular  $Ca^{2+}$  measurements were made, as described in previously reported methods [21]. Store-operated calcium influx: After being loaded with Fura-2AM, eosinophils were treated with thapsigagin (1  $\mu$ M) in extracellular recording solution with EGTA (10 mM) and  $CaCl_2$  (0 mM) for 10 minutes to deplete intracellular calcium stores. Then, cells were superfused with extracellular recording solution with  $CaCl_2$  (2 mM) to recover calcium and induce calcium influx. Measurements were made using TILLvision digital imaging system (TILL Photonics GmbH, Munich, Germany).  $[Ca^{2+}]_i$  was indicated as the ratio of fluorescence intensity at excitation wavelengths of 340 and 380 nm (F ratio). Emission wavelength was 510 nm. Background intensity was subtracted from fluorescent intensity changes and

the resulting  $[Ca^{2+}]_i$  values were normalized as differences between fluorescence intensities and intensity at standard extracellular recording solution.

### Reagents and solutions

The standard extracellular recording solution contained (mmol/L): 140 NaCl, 5 KCl, 8 D-glucose, 10 HEPES, and 1.2  $MgCl_2$ , titrated to pH 7.4 with NaOH. When indicated, 2 mmol/L  $CaCl_2$  was added. TRPC5 specific blocker (T5E3-anti-TRPC5 blocking antibody) was a gift from Dr. Jing Li (lecturer in Cardiovascular Medicine Division of Cardiovascular and Diabetes Research LICAMM, Faculty of Medicine and Health, University of Leeds, UK) and was found to be a useful and specific functional tool in studying the TRPC5 channel [22, 23].



**Figure 2.** Assessment of eosinophils in nasal polyps (NP, n=58) and normal mucosa (n=35). A. HE staining of normal mucosa (400×); B. HE staining of nasal polyps, eosinophil has bright pink plasma and a strong reflection of bright red granules (400× red box); C. Statistical results of eosinophils staining, cell counts per high power field (HPF). \* $P < 0.01$  compared with normal mucosa.

*Statistical analysis*

Statistical analysis was performed with SPSS 13.0 for Windows. Continuous data are presented as means  $\pm$  SD and were compared using Student’s t-test or one-way ANOVA. Categorical data were presented as proportions and Chi-square test was used for comparison. Correlation between expressions of TRPC5, IL-6, and NF- $\kappa$ B were assessed using Spearman’s rank correlation analysis. Differences were considered statistically significant at  $P < 0.05$ .

**Results**

*Clinical characteristics of subjects*

Clinical data for patients with nasal polyps compared with control subjects are listed in **Table 2**. There were no differences between the groups in terms of age, sex, body mass index, and systolic blood pressure (all  $P > 0.05$ ).

*Expression of TRPC channels in nasal polyps*

Real-time quantitative RT-PCR showed that the main subtypes of normal nasal mucosa were

TRPC4 and TRPC5. However, only TRPC5 channel mRNA expression level of nasal polyps was significantly higher than that of the normal control group ( $P < 0.05$ , **Figure 1A**).

Immunohistochemical staining showed that TRPC5 positive staining was mainly located in the cell membrane (by buffy staining; **Figure 1B** and **1C**). In normal nasal mucosa, TRPC5 was expressed particularly in the epithelium (**Figure 1B**). However, in nasal polyps, TRPC5 staining was significantly higher than in the control group with distribution in interstitial cells rather than the epithelium ( $P < 0.05$ , **Figure 1C** and **1D**).

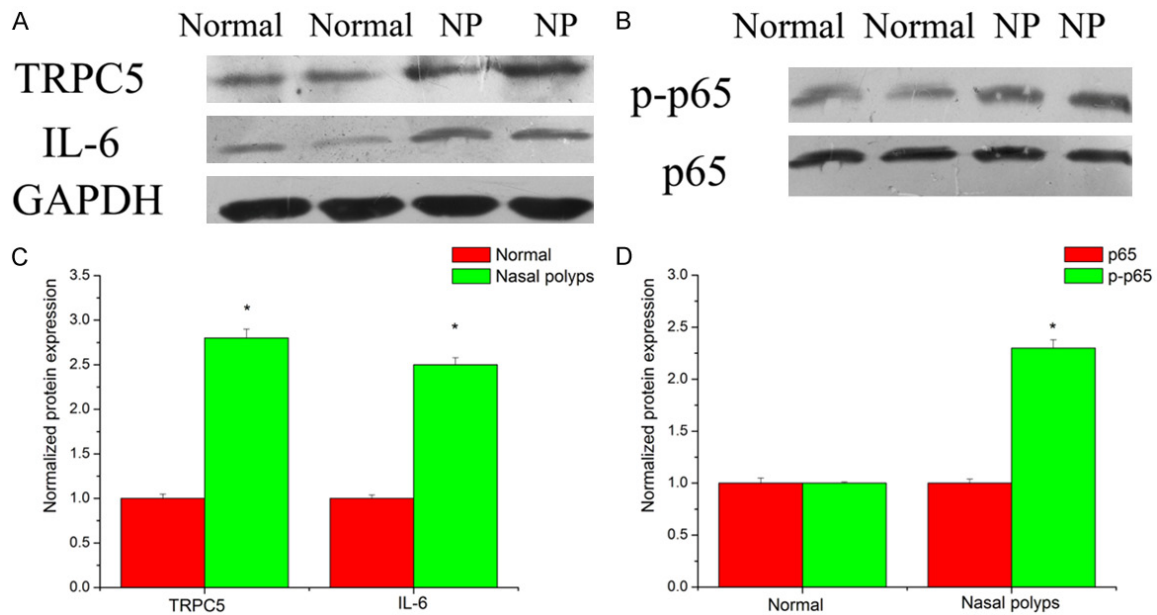
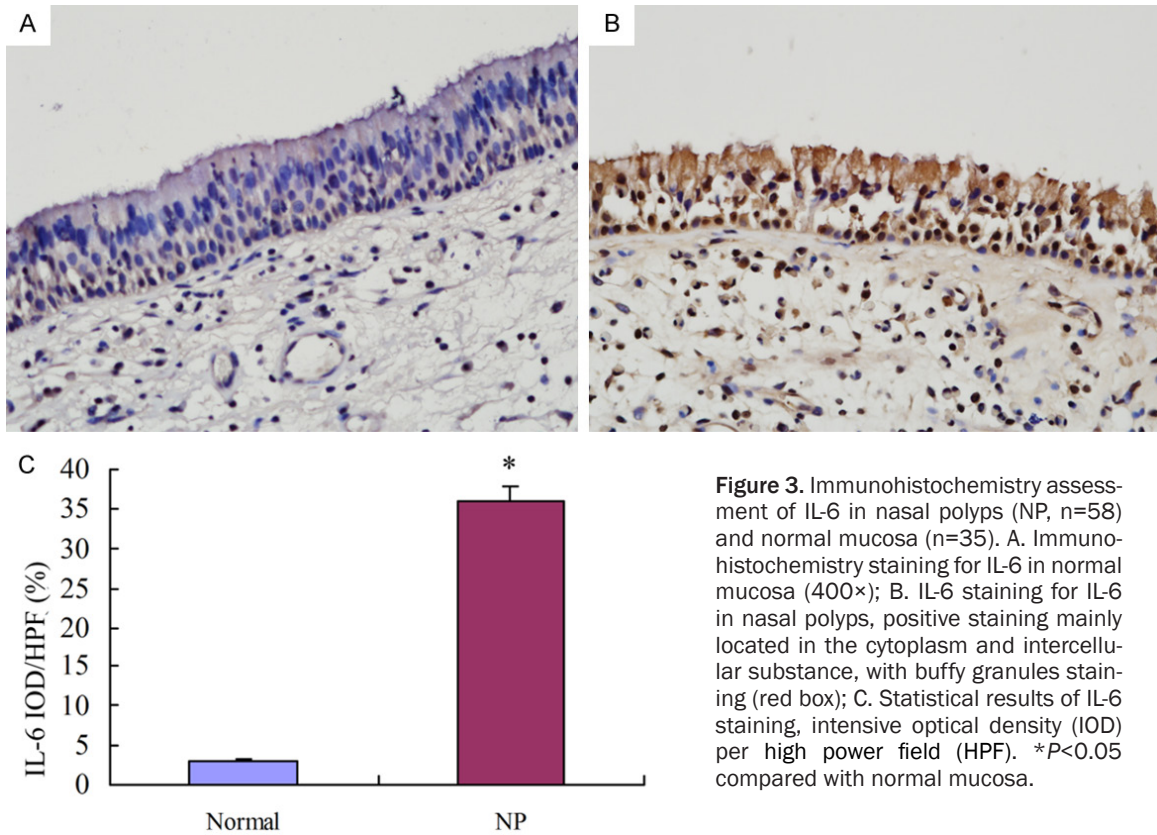
*Eosinophil infiltration analysis in nasal polyps*

HE staining showed that eosinophils had bright pink plasma (**Figure 2B**). Eosinophil count in the nasal polyps was significantly higher than that of the normal control group ( $P < 0.05$ , **Figure 2**).

*Expression of inflammatory factor IL-6 in nasal polyps*

IL-6 positive products were primarily located in cytoplasm and intercellular substance, with

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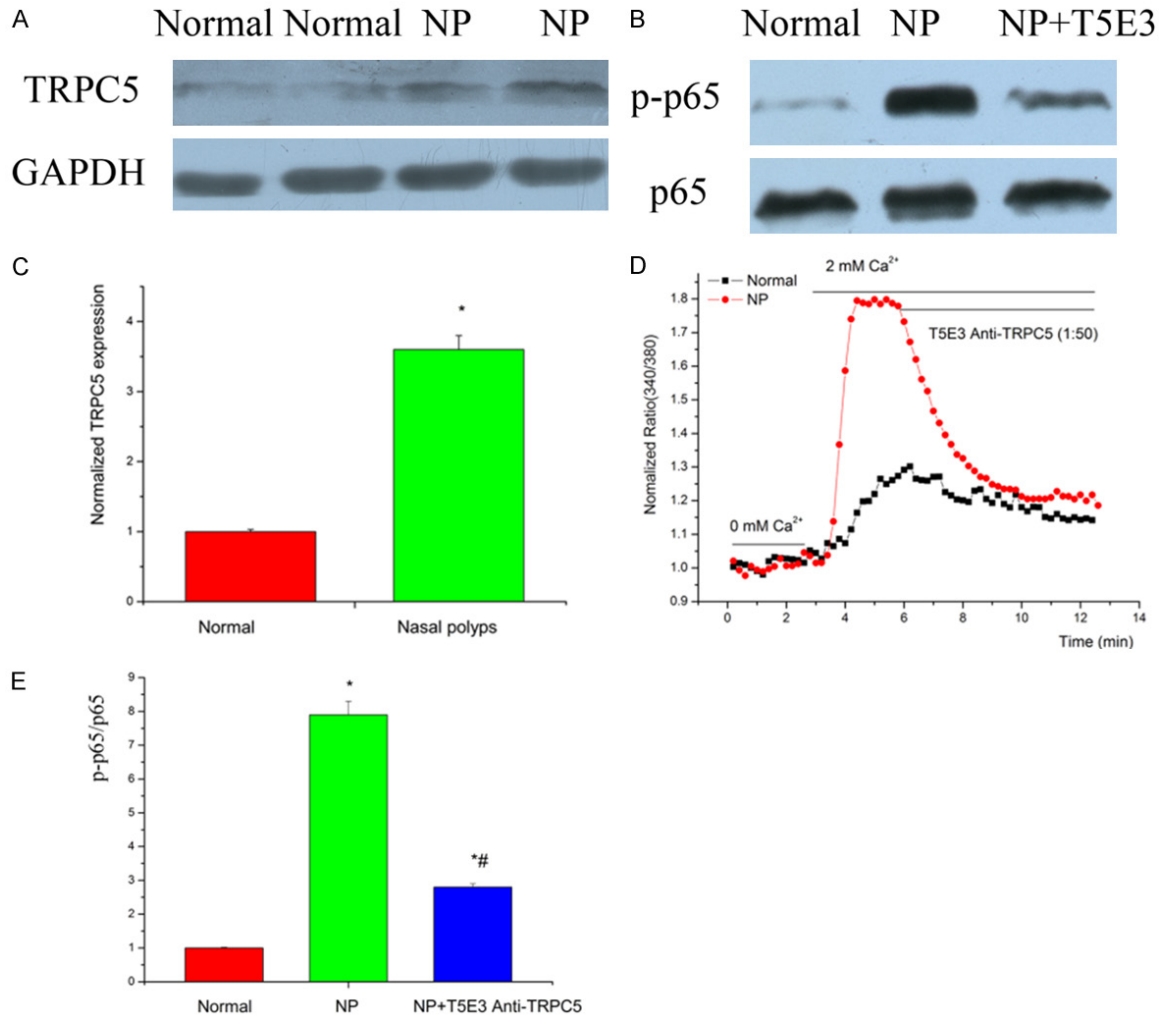


**Figure 4.** Western blot analyses of TRPC5, IL-6, and NF- $\kappa$ B protein expression in nasal polyps (NP, n=58) and normal mucosa (n=35). A. Representative Western blot of TRPC5 and IL-6; B. Representative Western blot of NF- $\kappa$ B p65 and phosphorylation-p65 (p-p65); C. Statistical results of TRPC5 and IL-6 relative expression; D. Statistical results of NF- $\kappa$ B p65 and p-p65 relative expression. \* $P < 0.05$  compared with normal mucosa.

buffy granule staining (**Figure 3**). IL-6 expression in patients with nasal polyps was signifi-

cantly higher than that in the normal control group ( $P < 0.05$ , **Figure 3**).

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**Figure 5.** Effects of TRPC5 on store-operated calcium influx and NF- $\kappa$ B protein expression of blood eosinophils from patients with nasal polyps (NP, n=5) and normal mucosa (n=5). A. Western blot of TRPC5 expression in blood eosinophils; B. Western blot of NF- $\kappa$ B p65 expression in blood eosinophils; C. Statistical results of TRPC5 protein expression, TRPC5 is upregulated in blood eosinophils from patients with nasal polyps; D. Store-operated calcium (SOC) influx of eosinophils, TRPC5 specific blocker (T5E3-anti-TRPC5 blocking antibody) can inhibit increased SOC of eosinophils from patients with nasal polyps; E. Statistical results of NF- $\kappa$ B p65 and p-p65 relative expression, T5E3-anti-TRPC5 blocking antibody can decrease phosphorylation level of NF- $\kappa$ B p65 in eosinophils from patients with nasal polyps. \* $P < 0.05$  compared with normal mucosa. # $P < 0.05$  compared with nasal polyps.

### Western blot analysis of TRPC5, IL-6, and NF- $\kappa$ B

Western blot results confirmed that protein expressions of TRPC5 and IL-6 were significantly higher in nasal polyps than in normal mucosa (all  $P < 0.05$ , **Figure 4A, 4C**).

Western blot analyses showed no differences in total NF- $\kappa$ B p65 protein expression between the two groups ( $P > 0.05$ , **Figure 4B, 4D**). However, phosphorylation levels of NF- $\kappa$ B p65 (p-p65, transcriptional response of NF- $\kappa$ B) were significantly higher in nasal polyps than in normal mucosa ( $P < 0.05$ , **Figure 4B, 4D**).

### Correlation between TRPC5 channel expression and various factors

Expression of TRPC5 in nasal polyps was positively correlated with number of eosinophils ( $r = 0.424$ ,  $P < 0.05$ ), IL-6 expression ( $r = 0.325$ ,  $P < 0.05$ ), and p65 phosphorylation ( $r = 0.739$ ,  $P < 0.05$ ).

### TRPC5 channel participates in store-operated calcium influx of eosinophils from patients with nasal polyps

To confirm the role of TRPC5 in nasal polyps, we isolated eosinophils from patients with nasal

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polyps to investigate the direct effect of TRPC5 on eosinophil function.

Western blot results showed that protein expressions of TRPC5 were significantly higher in blood eosinophils from patients with nasal polyps than in eosinophils from control subjects ( $P < 0.05$ , **Figure 5A, 5C**).

Calcium imaging experiments showed that store-operated calcium influx of eosinophils from patients with nasal polyps was significantly increased compared with eosinophils from control subjects ( $P < 0.05$ , **Figure 5D**). TRPC5-specific blocker (T5E3-anti-TRPC5 blocking antibody) was able to inhibit SOC influx of eosinophils from patients with nasal polyps ( $P < 0.05$ , **Figure 5A, 5C**), compared with normal eosinophils.

### *Effect of TRPC5 channel on IL-6 expression and NF- $\kappa$ B of eosinophils*

Compared with eosinophils from control subjects, IL-6 mRNA expression (normalized to control eosinophils,  $18.08 \pm 0.04$  vs. 1,  $P < 0.05$ ), and phosphorylation levels of NF- $\kappa$ B p65 were significantly higher ( $P < 0.05$ , **Figure 5B, 5E**) in eosinophils from patients with nasal polyps. However, total protein expression of NF- $\kappa$ B p65 between the two groups did not differ ( $P > 0.05$ , **Figure 5B**). TRPC5-specific blocker (T5E3-anti-TRPC5 blocking antibody) decreased IL-6 expression ( $18.08 \pm 0.11$  vs.  $7.06 \pm 0.03$ ,  $P < 0.05$ ), and phosphorylation levels of NF- $\kappa$ B p65 of eosinophils from patients with nasal polyps ( $P < 0.05$ , **Figure 5B, 5E**).

### **Discussion**

TRPC channels are recognized as one of the store-operated calcium channels involved in regulating functions of different mammalian cell types [7-11, 24]. Recently, TRPC channels have been found to play an important role in several diseases [7-9]. In our present study, TRPC5 channel mRNA and protein expression increased in nasal polyps when compared with normal nasal mucosa. Additionally, TRPC5 expression was positively correlated with eosinophil infiltration, inflammation, and NF- $\kappa$ B phosphorylation levels. Experiments in blood eosinophils confirmed that blocking TRPC5 decreased SOC influx, IL-6 expression, and NF- $\kappa$ B phosphorylation levels. These results suggest that TRPC5 might participate in development of

nasal polyps by activating eosinophilic inflammation and NF- $\kappa$ B pathways.

TRPC channels include seven subtypes (i.e., TRPC1-7) and are divided into four subgroups, according to their structure and function: TRPC1, TRPC2, TRPC4/5, and TRPC3/6/7 [24]. In humans, TRPC2 is not expressed but is found in murine sperm and neurons [25]. TRPC1 channel is expressed in human vascular smooth muscle cells [26] and  $\beta$ -cells [27]. TRPC4/5 channels are known to be expressed in human monocytes, endothelial cells, and sensory neurons [28]. TRPC3/6/7 channels are expressed in the nervous and cardiovascular systems [29, 30]. For the first time, we showed that the main subtypes of normal human nasal mucosa are TRPC4 and TRPC5. TRPC5 is particularly expressed in the epithelium. Other reports in the literature have indicated similar findings on other TRP channels in the nasal mucosa [31, 32]. TRPM8 was found in nerve fibers of nasal mucosa, particularly around blood vessels [31]. TRPV4 was detected in normal sinus mucosa [32]. These results suggested that TRP channels are involved in maintenance of physiological function of nasal mucosa.

More interestingly, we found that only TRPC5 expression significantly increased in nasal polyps when compared with normal mucosa. Previously, Rehman et al. reported that inhibition of TRPV1 could reduce airway epithelial injury in IL-13-mediated mice asthma [33]. TRPV4 channel was found to be expressed in chronic rhinosinusitis and not in ciliated cells [34]. In intestinal epithelial cells, TRPC1 could potentially be a store-operated  $\text{Ca}^{2+}$  channel and regulate early mucosal restitution after wounding [35]. We found that TRPC5 distributed in interstitial cells of nasal polyps rather than in the epithelium. These studies provide evidence that TRPC channels may play a role in nasal epithelium function and nasal polyp diseases.

However, the mechanism of TRPC5 in nasal polyps remains unclear. We analyzed correlation between TRPC5 channel expression and eosinophil infiltration and inflammation factor (IL-6) and transcription factor (NF- $\kappa$ B) expression. In nasal polyps of our present study, a large number of eosinophils were observed underneath the epithelium. Eosinophils have been well-



established to be highly elevated in NP even without clear evidence for allergic disease [4, 36]. Eosinophils are important immune effector cells that produce a variety of pro-inflammatory factors such as IL-6, IL-13, and TGF- $\beta$  [37]. We found that IL-6 expression was significantly higher in nasal polyps than in normal mucosa, similar to previous studies [38, 39]. Peters et al. reported increased IL-6 activity in CRS with NP [38]. IL-6 was found to be essential for Staphylococcal exotoxin B-induced T regulatory cell insufficiency in nasal polyps [39]. In our sample of nasal polyps, TRPC5 channel expression was positively correlated with eosinophil number and IL-6 expression. There was a positive relationship between eosinophil number and IL-6 expression. Additionally, TRPC5 channel and IL-6 expression in blood eosinophils from patients with nasal polyps were upregulated. Blocking the TRPC5 channel decreased IL-6 expression in blood eosinophils from patients with nasal polyps. Thus, our findings suggest that TRPC5 channel can affect the pathogenesis of nasal polyps by elevating eosinophilic inflammation.

Because NF- $\kappa$ B pathways play a key role in regulating eosinophil function and inflammation [40], we further analyzed relationships among TRPC5 expression, eosinophil infiltration, and IL-6 in nasal polyps. Total NF- $\kappa$ B p65 protein expression was not different between nasal polyps and normal mucosa. However, the phosphorylation level of NF- $\kappa$ B p65 was significantly higher in nasal polyps, indicating an increased activation of NF- $\kappa$ B. Spearman's rank correlation analysis showed that TRPC5 expression, eosinophil count, and IL-6 were positively correlated with p65 phosphorylation, respectively. Moreover, p65 phosphorylation in blood eosinophils from patients with nasal polyps was higher than in normal controls. Blocking the TRPC5 channel could decrease p65 phosphorylation in blood eosinophils from patients with nasal polyps. Therefore, eosinophilic inflammation via the NF- $\kappa$ B signal pathway could be considered a mechanistic pathway for TRPC5 in nasal polyps. Several other studies seem to support this conclusion. First, NF- $\kappa$ B expression was reported to increase in patients with NP [41] and was used to predict clinical outcomes for nasal polyps [42]. Second, NF- $\kappa$ B has been shown to play important roles in recruitment, apoptosis, and inflammatory response in eosinophils [43]. Third, as a transcription factor, NF- $\kappa$ B has been shown to enhance

the transcription of a variety of genes including cytokines, growth factors, adhesion molecules, immunoreceptors, and acute-phase proteins [40].

### Conclusion

TRPC5 channel is upregulated in human nasal polyps and plays an important role in development of NP by activating eosinophilic inflammation and NF- $\kappa$ B signaling pathways. TRPC5 channel may be a useful new target in treatment of nasal polyps.

### Acknowledgements

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### Disclosure of conflict of interest

None.

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