

# Decreased Expression of Brg1 and $\beta$ -Catenin in Nasal Epithelial Cells in CRSwNPs

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## Research Article

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# Abstract

**Background:** Chronic rhinosinusitis with nasal polyps (CRSwNP) is a pathophysiologically complex disease process characterized by chronic inflammation of the nose and epithelial cell structure remodeling.

**Objectives:** Studies have shown that the chromatin remodeling complex subunit Brg1 can interact with  $\beta$ -catenin, which plays an important role in cell proliferation and differentiation. However, the role of Brg1 and  $\beta$ -catenin in epithelial cells of chronic rhinitis and sinusitis remains unclear.

**Material and Methods:** We detected the expression level of Brg1 in CRSwNP by qPCR, and observed the expression pattern of Brg1 in CRSwNP by immunohistochemistry and immunofluorescence. HNEpC cells were used to detect the expression changes of the two proteins after TNF- $\alpha$  stimulation. **Results:** In our study, the expression levels of Brg1 and  $\beta$ -catenin are significantly decreased in nasal mucosa of CRSwNP patients. To our interest, we also observed expression decrease and expression pattern changes of Brg1 and  $\beta$ -catenin by stimulating HNEpCs cells with TNF- $\alpha$ .

**Conclusions:** This study shows that the expression of Brg1 and  $\beta$ -catenin may be related to the development of CRSwNPs.

**Subject classification codes:** include these here if the journal requires them

## Introduction

Chronic rhinosinusitis (CRS) is characterized by mucosal inflammation involving both the nasal cavity and paranasal sinuses, with potentially diverse causes [1]. It affects approximately 15% of the general population, leading to an immense effect on the quality of life of patients, as well as creating a large financial burden on health care systems worldwide [2]. Nasal polyps are the consequence of persistent inflammatory and remodeling responses in several chronic inflammatory diseases. Current treatment can only alleviate the symptoms of chronic rhinitis and sinusitis, but it does not address the high incidence of recurrence [3], which underlines the need for specific research correlating novel molecular targets, inflammation and nasal mucosa function.

CRS is classified into CRS without nasal polyps (CRSsNP) and with nasal polyps (CRSwNP) according to whether it is accompanied by nasal polyps [4]. The symptoms of CRSwNP is more difficult to control and it shows worse surgical outcomes than CRSsNP. Abnormal proliferation of nasal epithelial cells is the main cause of structural remodeling of nasal epithelial cells in CRSwNP [5]. Human nasal epithelial cells are important in the tight junctional barrier and innate immune defense protecting against pathogens invading [6]. However, the influencing factors and regulatory mechanisms of the proliferation of nasal epithelial cells are still unclear.

Chromatin remodeling complex plays an extremely important role in mammalian embryonic development and postnatal growth. By regulating transcription and affecting cell proliferation and differentiation, it runs through single cell and postnatal developmental processes and has become one of the research hotspots of epigenetics in recent years [7]. Brg1 is a core hydrolase subunit of the mammalian chromatin recombination complex BAF (Brg1/Brm associated factor), which is located on human chromosome 19p. Brg1 consists of multiple functional domains, including the evolutionarily very conserved ATP hydrolase active region, the carboxy-terminal bromo region and the AT-hook motif, and the amino-terminal QLQ, HSA and BRK domains [8,9]. The SWI/SNF complex utilizes energy from ATP hydrolysis to disrupt or reposition nucleosomes and modify chromatin structure [10], thereby facilitating or inhibiting transcription of target genes or gene regions. Through these domains, Brg1 is able to specifically activate or inhibit the expression of thousands of genes by binding to different promoters, and participate in the pathophysiological processes of various diseases, in stem cell proliferation and differentiation, tumorigenesis, and neuronal development [11,12]. However, the role of Brg1 in chronic rhinitis and sinusitis, especially in the epithelial cells of the nasal mucosa, is still unclear.

$\beta$ -catenin is a member of the catenin family. In cells, the physiological activities of cells are regulated mainly by binding to specific related proteins. Changes in the distribution and spatial location of  $\beta$ -catenin in the cell membrane, cytoplasm and nucleus can determine changes in cell function and affect stem cell differentiation [13,14]. Earlier reports indicate that the Brg1 complex contributes to the activity of the  $\beta$ -catenin transcription factor and affects Wnt signaling through  $\beta$ -catenin, which is involved in the regulation of proliferation and differentiation of stem cells [15,16]. Brg1 binds to  $\beta$ -catenin, thereby enhancing the transcriptional activity of the  $\beta$ -catenin-TCF complex against the gene of interest. However, Brg1, which has lost ATPase activity, has a significant mutation inhibition effect on Wnt signaling pathway, thereby destroying the normal development process of the organism and causing abnormal proliferation and differentiation of cells [15]. However, whether Brg1 and  $\beta$ -catenin are involved in the regulation of proliferation and differentiation of nasal epithelial cells in chronic rhinitis and sinusitis remains unclear. In the present study, our results show that the expression of Brg1 and  $\beta$ -catenin is significantly reduced in the nasal mucosa of CRSwNP. And  $\beta$ -catenin differs in the location of CRSwNP in healthy control nasal mucosal epithelial cells. With the stimulation of TNF- $\alpha$ , the localization and expression levels of Brg1 and  $\beta$ -catenin in HNEpCs changed significantly. Therefore, the study suggests that the expression of Brg1 and  $\beta$ -catenin is associated with the development of CRSwNP, which may provide new therapeutic targets and clinical ideas for the treatment of CRSwNP.

## Materials And Methods

### Sample collection

This study was approved by the ethical committee of local hospital, informed consent was obtained from each patient and all experiments were performed in accordance with relevant guidelines and regulations. Informed consent was obtained from all individual participants included in the study. 20 patients diagnosed with CRS and 14 patients diagnosed with simple nasal septum deviation were enrolled in our

study. All patients underwent routine medical history inquiry, physical examination and laboratory examination. Patients diagnosed with simple nasal septum deviation was regarded as normal control. The nasal polyp tissue from patients with CRS and inferior turbinate mucosa tissue from patients with nasal septum deviation was obtained during operation. Exclusion criteria included: (1) Patients who complicated with nasal polyps, fungal rhinosinusitis, cystic fibrosis, and rhinitis or/and asthma; (2) history of glucocorticoid usage in the past 1 month; (3) Patients who suffered from acute upper respiratory tract infection within 4 weeks. The diagnosis of allergic rhinitis is based on the diagnostic criteria of ARIA2008, which is based on GINA criteria. This study was approved by Medical Research Ethics Committee of Weifang People's Hospital and informed consent was obtained from each patient and all experiments were performed in accordance with relevant guidelines and regulations.

The nasal polyps and inferior turbinate tissue obtained during the operation are divided into three parts, one part immersed in Formalin for immunohistochemistry or immunofluorescence, the other part preserved in RNA later for RNA extraction, and the other part stored at - 80 °C for protein extraction.

## Cells culture

Human nasal epithelial cell (HNEpC) were purchased from Cellbio (Cat. No. CBR-130634) and maintained in DMEM/GLUCOSE medium supplemented with 10% FBS (Invitrogen, USA). HNEpC cells were plated at a density of  $3 \times 10^5$  cells per 60 mm dish and cultured for 24 hours. Then cells were stimulated with 2.5  $\mu$ l/ml TNF- $\alpha$ . The detection time was 0h, 6h, 12h, 24h, 48h, and 72h after stimulation. The RNA and protein were extracted for RT-PCR and WB experiments. The same method stimulated HNEpCs and collected cells for cellular immunofluorescence experiments.

## RNA extraction and quantitative real-time PCR

Total RNA was isolated from HNEpCs and nasal tissues using TRIzol (Invitrogen, USA) according to the manufacturer's instructions and reverse transcribed using PrimeScript<sup>TM</sup> RT reagent Kit (Takara, Tokyo, Japan). The primers were:

Brg1 forward, 5'-AATGCCAAGCAAGATGTCGAT-3', reverse, 5'-GTTTGAGGACACCATTGACCATA-3' (142bp);  $\beta$ -catenin forward, 5'-CATCTACACAGTTTGTATGATGCTGCT-3', reverse, 5'-GCAGTTTTTCTCAGTTCAGGGA-3' (150bp); GAPDH forward, 5'-GGGAAACTGTGGCGTGAT-3', reverse, 5'-GAGTGGGTGTCGCTGTTGA-3' (216bp). PCR amplification was performed in 20- $\mu$ l final volumes containing: 1  $\mu$ l of template cDNA, 0.6  $\mu$ l of each respective primer, 10  $\mu$ l SYBR green mix (Qiagen, Valencia, CA, USA), and 7.8  $\mu$ l DEPC water. All the amplifications were carried out in optical-grade 96-well plates on an ABI Prism 5700 sequence detection system (Applied Biosystems) with an initial step at 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, 56°C for 20 s and 68°C for 20 s. The  $C_T$  (cycle threshold) was determined automatically by the instrument. Relative gene expression normalized to GAPDH was determined via the  $2^{-\Delta\Delta CT}$  formula. All PCR reactions were performed in triplicate. The primer was synthesized by Invitrogen Company.

# Immunofluorescence

All nasal biopsy specimens were dissected, fixed in formalin and embedded in paraffin. Then, the tissue wax blocks were sectioned to a thickness of 4- $\mu$ m with a Leica microtome (Leica, Wetzlar, Germany). Sections were deparaffinized through xylene and an alcohol gradient. Antigen retrieval was carried out with EDTA. The sections were permeabilized in 0.1 % (w/v) triton-X 100, blocked with 10% goat serum (Cat. No.SL038, Solarbio), and incubated with anti- Brg1 or anti- $\beta$ -catenin antibody (Cell signaling, Beverly, MA, USA) overnight at 4°C followed by exposing to corresponding secondary antibodies for 1h at 37°C. After washed with PBS for three times, the slides were incubated with the. The slides were mounted with mounting medium containing DAPI (4, 6-diamidino-2-phenylindole; Vector Laboratories, Inc., Burlingame, CA, USA) and visualized under an Olympus fluorescence microscope (LSM 700, Zeiss).

# Immunohistochemistry (IHC)

Sections were deparaffinized through xylene and an alcohol gradient. The slides were then washed with PBS and endogenous peroxidase activity was quenched with 3% hydrogen peroxide methanol. Antigen retrieval was carried out with EDTA. After washed with PBS for three times (3 minutes per time), the sections were permeabilized in 0.1 % (w/v) TritonX-100, blocked with 10% goat blood for 30 minutes and incubated with anti- Brg1 or anti- $\beta$ -catenin antibody overnight at 4°C followed by exposing to corresponding secondary antibodies for 2h at 37°C. After washed with PBS for three times, the color reaction was developed with the DAB+ Chromogen for 10 min. The sections were counterstained with hematoxylin, dehydrated with gradient alcohol and mounted with neutral gum. Then expression of Brg1 and  $\beta$ -catenin on paraffin sections of nasal biopsies was analyzed with an Olympus microscope.

To compare the expression level of Brg1 and  $\beta$ -catenin more accurately, immunohistochemical average optical density analysis method was adapted. At least three 200-fold fields for each slice in each group were randomly selected for screenshots. Try to make the slice fill the entire field of view when making screenshots, while ensuring that the background light of each photo was consistent. Image-Pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) was used to select the same brown color as the uniform standard for judging all photos. Each photo was analyzed to obtain the cumulative optical density (IOD) and the area of the tissue (AREA). And the average optical density value (average optical, AO value) was calculated as:  $AO = IOD/AREA$ . Larger AO value represent higher expression level of target protein.

# Western blot

HNEpCs or frozen nasal tissues were homogenized in ice-cold RIPA lysis buffer (Beyotime, Shanghai, China) consisting of 1mM PMSF (Roche, Switzerland). After centrifugation at 4 °C, the supernatant was collected and the proteins were quantified with the BCA method. Samples containing 50 mg of protein were separated on a 12% SDS-PAGE gel and transferred to PVDF membrane. After blocking with 5% non-fat skim milk, the membrane was probed with antibodies. Following three washes with TBST, the

membranes were exposed to HRP-conjugated secondary anti-mouse or rabbit antibodies (1:5000; Abcam, USA) for 2h at 37°C. Protein expression was examined using a BioSpectrum Imaging System (UVP, USA).

## Statistical analysis

All data were presented as the mean  $\pm$  standard deviation (SD) of three replicates. Paired/unpaired Student's t-test was applied by using Graph Pad Prism Software 6.0.  $P < 0.05$  was considered statistically significant.

## Results

### Patient characteristics

20 patients diagnosed with CRSwNPs and 14 patients diagnosed with simple nasal septum deviation were enrolled in our study. The basic characteristics of the two groups of patients were listed in **Table 1**. There were no significant differences in age and sex between the two groups, but Asthma, CT and endoscopy scores, and eosinophil infiltration were significantly different. 14 patients diagnosed with simple nasal septum deviation were regarded as normal controls. The nasal polyp tissue from patients with CRSwNPs and inferior turbinate mucosa tissue from patients with simple nasal septum deviation was obtained during operation.

### Expression of Brg1 and $\beta$ -catenin in CRSwNP tissues

To detect the expression of Brg1 and  $\beta$ -catenin in nasal mucosa, immunohistochemistry was performed. Immunohistochemical images showed that Brg1 was expressed in both CRSwNPs and healthy control nasal mucosa, and it was mainly localized to nasal mucosal epithelial cells (Figure 1A, 1B).  $\beta$ -catenin is similar to the localization pattern of Brg1 (Figure 1C, 1D). We examined the expression levels of Brg1 and  $\beta$ -catenin in CRSwNPs and healthy control nasal mucosa by AO analysis. The results showed that the expression of Brg1 and  $\beta$ -catenin in CRSwNPs tissues was lower than that in the control group (Fig. 1E, 1F). The above results have attracted our attention. We speculate that the expression levels of Brg1 and  $\beta$ -catenin may play an important role in the development of CRSwNPs.

### Brg1 and $\beta$ -catenin are significantly reduced in CRSwNPs

To examine the expression levels of Brg1 and  $\beta$ -catenin in CRSwNPs and healthy control tissues, we extracted RNA and protein from 20 nasal mucosa tissues from patients diagnosed with CRSwNPs and 14 patients diagnosed with simple nasal septum deviation. The expression levels of Brg1 and  $\beta$ -catenin in CRSwNPs and healthy control tissues were detected by RT-PCR and Western blot. The results showed that

the expression levels of Brg1 and  $\beta$ -catenin in CRSwNPs tissues were significantly lower than those in the control group at both RNA (Figure2A, 2B) and protein levels (Fig. 2C).

To further observe the localization pattern of Brg1 and  $\beta$ -catenin in nasal mucosa, we performed immunofluorescence staining. We are interested in the results showing that  $\beta$ -catenin is mainly localized in the nucleus of epithelial basal cells in CRSwNPs, but  $\beta$ -catenin is mainly localized in the cytoplasm in healthy controls; To our disappointment, there was no significant difference in the expression pattern of Brg1 between CRSwNPs and healthy controls (Figure 2D).

## **Stimulation of TNF- $\alpha$ causes changes in Brg1 and $\beta$ -catenin in nasal epithelial cells.**

In order to observe the expression changes of Brg1 and  $\beta$ -catenin in nasal epithelial cells in vitro, we used TNF- $\alpha$  to stimulate nasal mucosal epithelial cell line cells (HNEpC) to mimic the inflammatory stimulating environment of nasal mucosa epithelium. The RNA and protein of the cells were collected after different times of TNF- $\alpha$  stimulation, and the expression of Brg1 and  $\beta$ -catenin was detected. The results showed that the expression of RNA and protein of Brg1 and  $\beta$ -catenin decreased with the prolongation of TNF- $\alpha$  stimulation (Figure 3A~3C). We observed that the expression of Brg1 and  $\beta$ -catenin after TNF- $\alpha$  stimulated HNEpCs for 0h, 24h, 48h and 72h by immunofluorescence. The results showed that  $\beta$ -catenin changed from cytoplasmic localization to nuclear localization, but Brg1 was partially transferred from nucleus to cytoplasm with the prolongation of stimulation time of TNF- $\alpha$  (Figure3D), This is similar to the tissue staining results we observed earlier (Figure2D). Therefore, we believe that the inflammatory environment in which CRSwNP epithelial cells are located may be the main cause of changes in the localization of Brg1 and  $\beta$ -catenin.

## **Discussion**

Chronic rhinosinusitis is a pathophysiologically complex disease process characterized by chronic inflammation of the nose and paranasal sinuses. The inflammatory mechanisms underlying the disease process of CRS are complex and incompletely understood. Nasal polyps are the consequence of persistent inflammatory and remodeling responses in several chronic inflammatory diseases. Cardinal symptoms of CRS include chronic nasal congestion, nasal discharge, facial pressure, and hyposmia.

Patients suffering from CRS with nasal polyps can benefit from topical and oral steroids, as well as from functional endoscopic sinus surgery, but even with the highest current standard of medical care and surgical treatments, the recurrence of upper airway inflammation and nasal polyposis remains high [17]. Current treatments relieve symptoms, but do not resolve the high incidence of recurrences [3,4], which underlines the need for specific research correlating novel molecular targets, inflammation and nasal mucosa function. Several studies have investigated the role of innate and adaptive immune responses, as well as the epithelial barrier function in patients with CRS. Cytokines are central mediators in these

processes, and numerous cytokines have been studied in CRS with differences in types depending on disease severity, therapy response, recurrent polyps, and microorganism colonization [18].

Nasal mucosal epithelial stem cells are subjected to excessive or repeated damage/inflammatory signal stimulation, and the dryness is reduced. The cells are in an abnormally differentiated state, thereby initiating the nasal mucosa [1-3]. At present, the mechanism of epithelial remodeling in CRS is still lacking. It is accepted that Wnt ligands can act through a  $\beta$ -catenin-dependent (canonical) or -independent (non-canonical) downstream signaling cascade, although some overlap exists between the two processes, and many regulatory aspects remain elusive [19,20]. Brg1 plays an important role in stem cell proliferation and differentiation, tumorigenesis, neuronal development, heart development and inner ear development [15-17].  $\beta$ -catenin promotes the differentiation of stem cells through the Wnt classical pathway. Therefore, we suspect that Brg1 and  $\beta$ -catenin may be involved in the structural remodeling of nasal epithelial cells.

Thus, total RNA and protein was extracted and expression level of Brg1 and  $\beta$ -catenin in CRS patients and normal controls were detected by RT-PCR and western blot. Results showed that the expression of Brg1 and  $\beta$ -catenin in CRS group was lower than that in the normal control group. On the basis of the above results, we further verified our assumptions by using in vitro test and a similar experimental result was obtained. The above results indicated that Brg1 and  $\beta$ -catenin were involved in the pathological process of CRS.

Previous study has concluded that  $\beta$ -catenin recruits SWI/SNF or Rsc-like complexes via interaction with Brg1 to Tcf target gene promoters, facilitating chromatin remodelling as a prerequisite for efficient transcriptional activation [15]. To explore whether there is interaction between Brg1 and  $\beta$ -catenin during CRS. Immunofluorescence and immunohistochemistry were performed to detect the subcellular localization of Brg1 and  $\beta$ -catenin. The ectopic expression of Brg1 and  $\beta$ -catenin in CRSwNPs tissues was noted. The expression of a small amount of Brg1 changed from nuclear to cytoplasm, while part of  $\beta$ -catenin changed from cytoplasm to nucleus. The nasal mucosal epithelial cell line HNEpCs was used as a research object in vitro. With the stimulation of TNF- $\alpha$ , the expression of Brg1 and  $\beta$ -catenin was decreased in HNEpCs cells, which was similar to the expression of both in CRSwNPs. In addition, the localization pattern of Brg1 and  $\beta$ -catenin in HNEpCs cells also changed. A brief description is that after TNF- $\alpha$  stimulation, part of Brg1 changes from the original nuclear expression to cytoplasmic expression, while the part of  $\beta$ -catenin expressed in the cytoplasm becomes nuclear expression. This is similar to the expression pattern of both in CRSwNPs, so we believe that TNF- $\alpha$  stimulation can be used as a mucosal epithelial cell model for studying CRSwNPs in vitro.

The transcriptional regulation of genes is a complex process involving multiple regulatory mechanisms. As an important subunit of chromatin remodeling complex, Brg1 plays an important epigenetic regulation role in gene transcriptional regulation in cells. This study showed that the expression trends of Brg1 and  $\beta$ -catenin in CRSwNPs are similar, but whether Brg1 can affect the proliferation and differentiation of nasal epithelial cells by regulating  $\beta$ -catenin needs further verification, which is our future work plan.

# Methodological limitations

Brg1 and  $\beta$ -catenin are involved in the pathological process of CRS and their translocation may have a certain role in promoting the occurrence and development of CRS. However, our research is mainly focused on phenotypic research, we need to further clarify the role of Brg1 and  $\beta$ -catenin in the nasal epithelium, which will be the next research direction.

## Declarations

### Acknowledgements

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### Conflict of interest

The authors declare that they have no conflict of interest.

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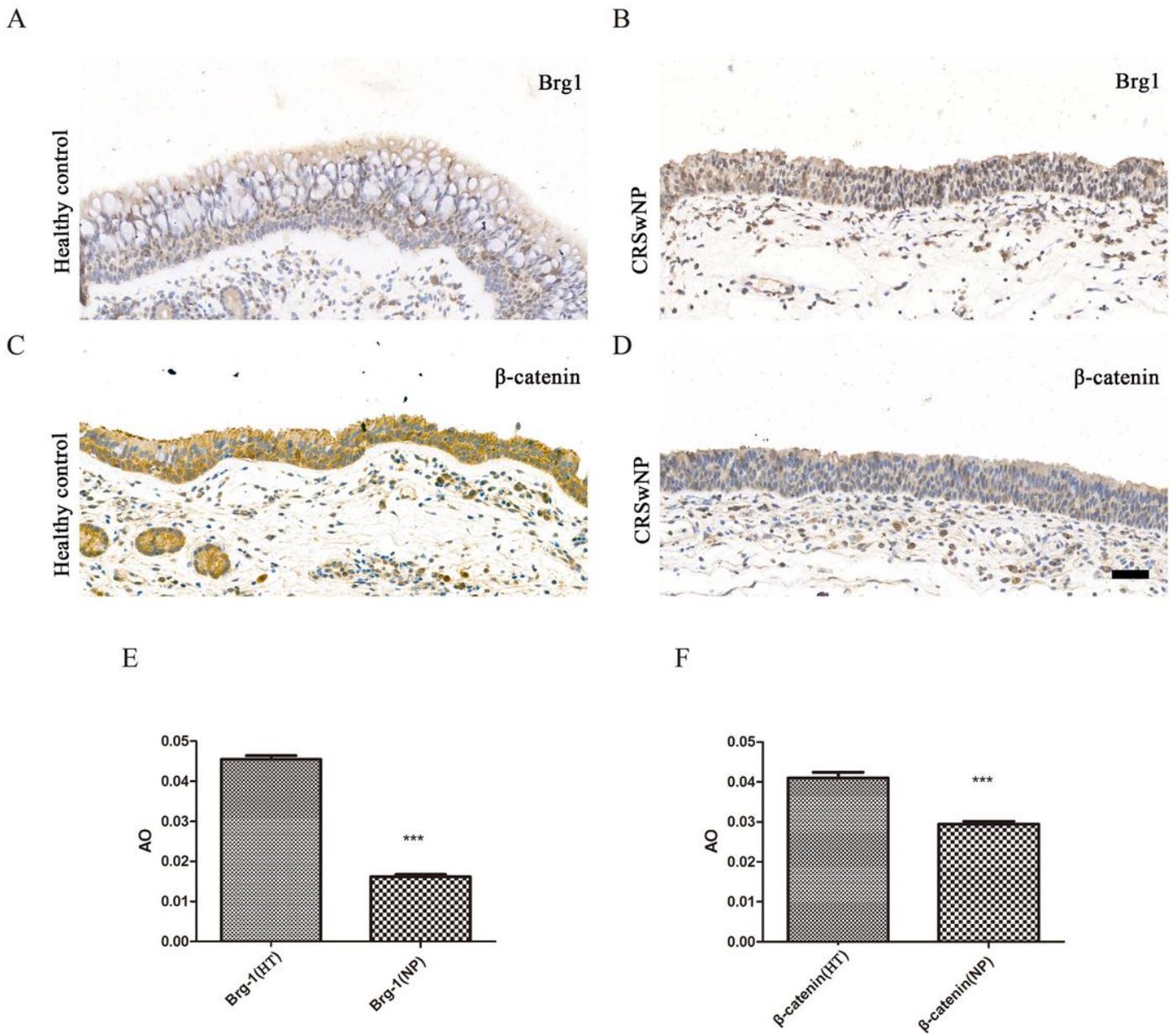
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## Tables

### Table 1 Patients' characteristics

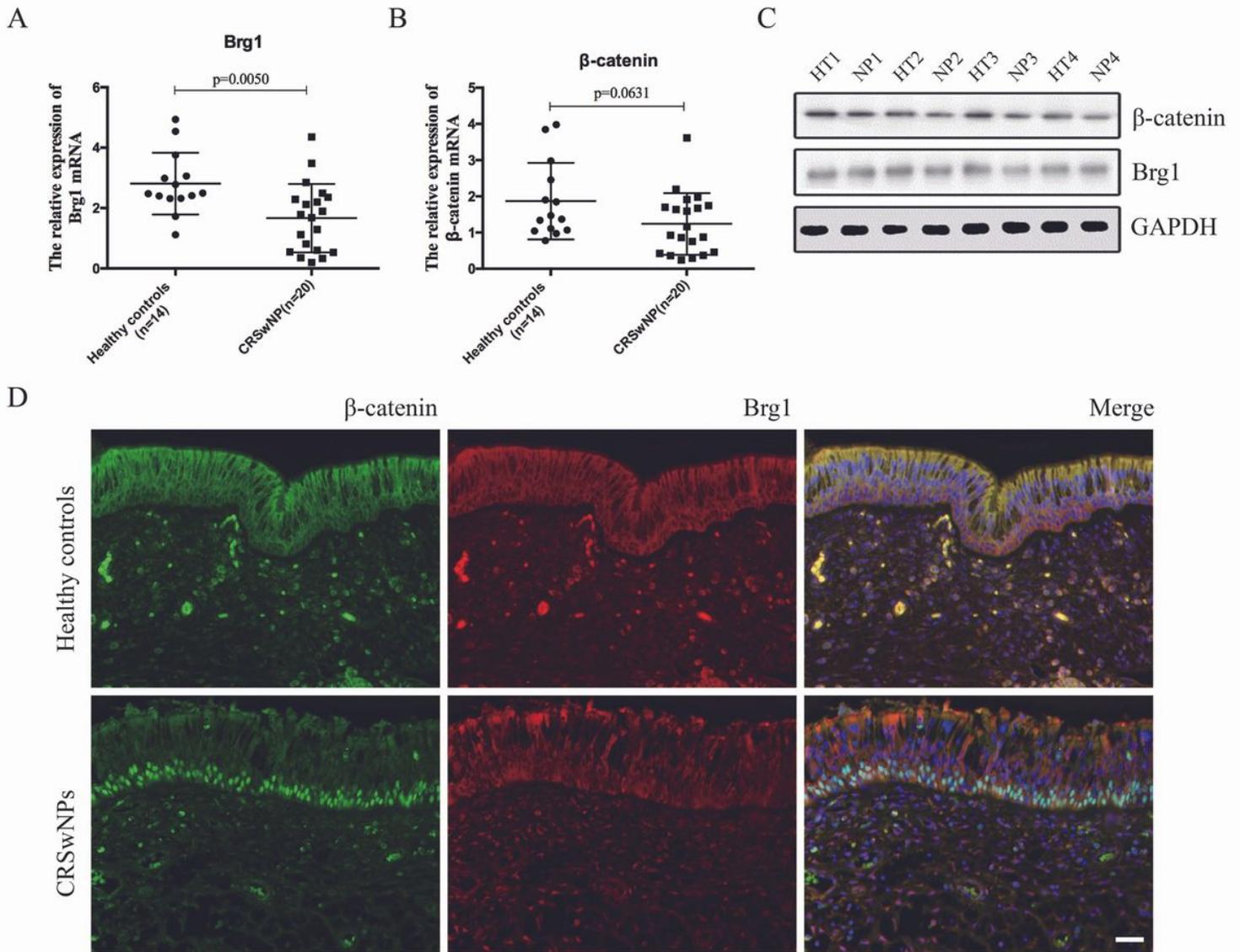
	Nasal mucosa Healthy control	CRSwNPs
Sample sizes	14	20
Median age, years (IQR)	37.0 (15-50)	39.5 (12-76)
Gender, male/female	9/5	12/8
Atopy, n/N	1/14	2/20
Asthma, n/N	0	1/20
Median CT score (IQR)	0	7.72 (5-12)***
Median endoscopy score (IQR)	0	6.50 (3-9)***
Eosinophilia‡	2/14	12/20***

## Figures



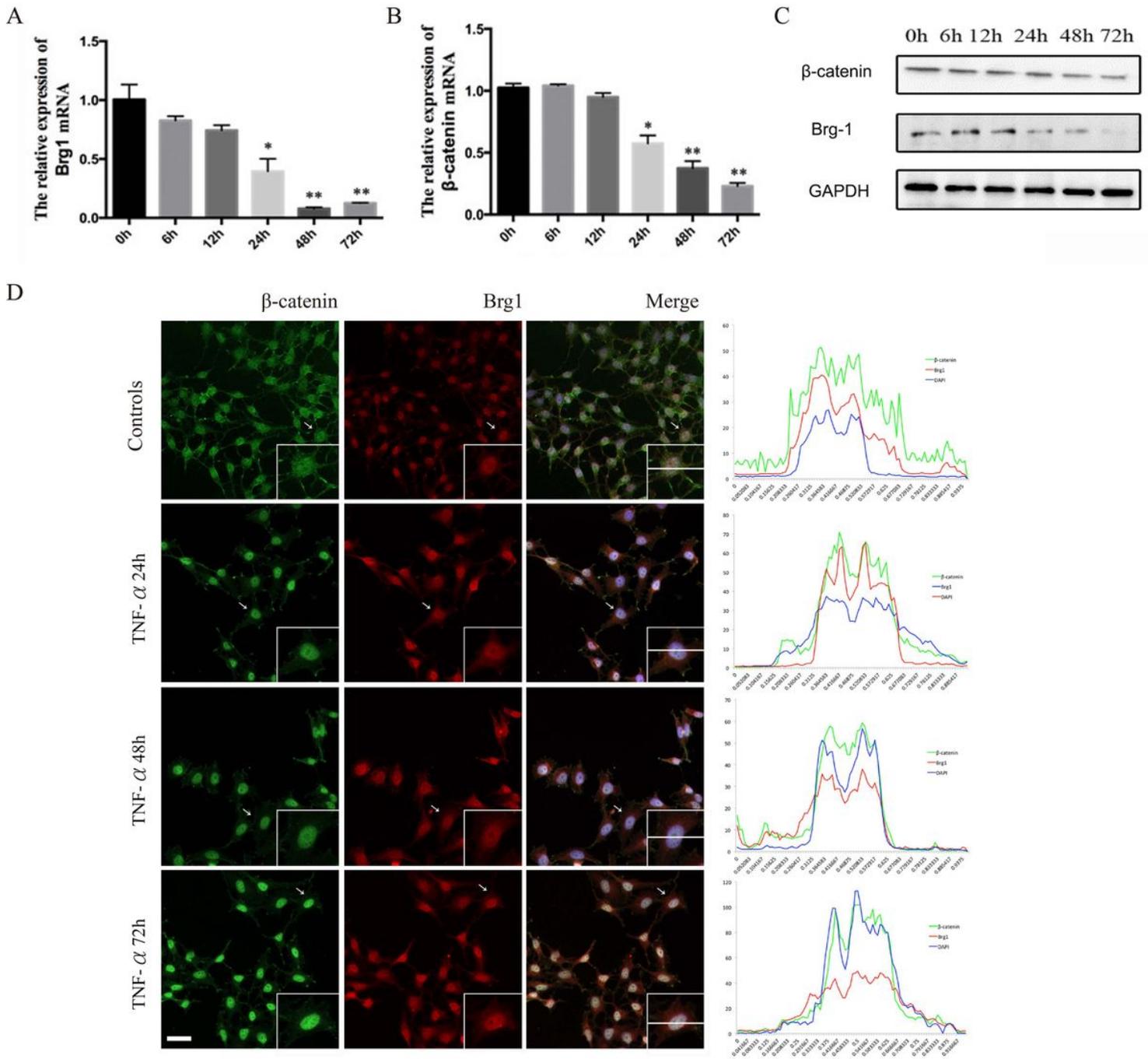
## Figure 1

The expression and localization of Brg1 and  $\beta$ -catenin in tissues detected by immunohistochemistry. A. and B. Expression pattern of Brg1 in healthy control (A) and CRSwNP (B). C. and D. Expression pattern of  $\beta$ -catenin in healthy control (C) and CRSwNP (D). ( $\times 400$  magnification; scale bar = 20 $\mu$ m). E. and F. The relative expression levels of Brg1 and  $\beta$ -catenin were assessed by AO analysis. The bar graphs and the table show quantification of the results, with each value represents the mean  $\pm$  SD of three independent experiments. Statistical significance is shown using the Student t test analysis; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Figure 2**

Brg1 and  $\beta$ -catenin are significantly reduced in CRSwNPs. A. and B. The relative mRNA levels of Brg1 and  $\beta$ -catenin in the total nasal mucosal tissue of controls and CRSwNPs were detected by real-time quantitative PCR. The bar graphs and the table show quantification of the results, with each value represents the mean  $\pm$  SD of three independent experiments. Statistical significance is shown using the Student t test analysis; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001. C. Western blotting was used to analyze the expression of Brg1 and  $\beta$ -catenin in the total nasal mucosal tissue protein of controls and CRSwNPs, where GAPDH served as a control. D. Expression patterns of Brg1 and  $\beta$ -catenin in nasal mucosal tissue of control and CRSwNP ( $\times 400$  magnification;  $\beta$ -catenin stained in green; Brg1 stained in red; nucleus stained in blue; scale bar = 20 $\mu$ m).



**Figure 3**

Stimulation of TNF- $\alpha$  causes changes in Brg1 and  $\beta$ -catenin in nasal epithelial cells. A~C. TNF- $\alpha$  was added to the medium with low concentration of serum, RNA and protein were collected at different time points. The relative expression levels of Brg1 and  $\beta$ -catenin mRNA were assessed by real-time quantitative PCR (A, B). The bar graphs and the table show quantification of the results, with each value represents the mean  $\pm$  SD of three independent experiments. Statistical significance is shown using the Student t test analysis; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001. The protein expression of Brg1 and  $\beta$ -catenin was detected by western blotting (C). D. Immunofluorescence was used to detect the expression of Brg1

and  $\beta$ -catenin in HNEpCs after induction of TNF- $\alpha$ , ( $\times 400$  magnification;  $\beta$ -catenin stained in green; Brg1 stained in red; nucleus stained in blue; scale bar = 20 $\mu$ m).