The differences between sinonasal respiratory epithelial adenomatoid hamartoma and nasal polyps: insights into immunopathology

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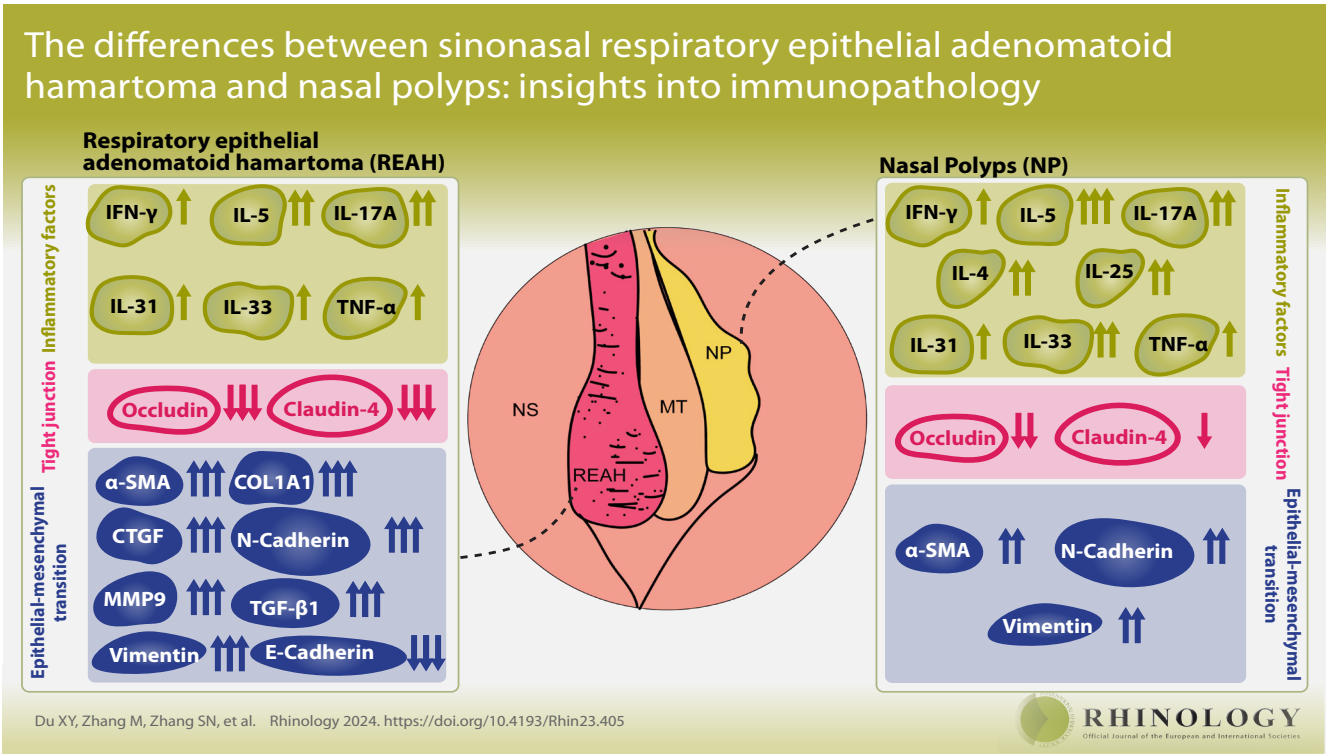

Abstract

Background: Respiratory epithelial adenomatoid hamartoma (REAH) is a benign lesion commonly occurring in the nasal cavity and sinuses. It is often accompanied by nasal polyps (NP). While the histological features of these two conditions have been studied, there is limited knowledge about their differences in the underlying immunopathology. **Methods**: Nasal tissue specimens were collected from 8 patients with concurrent REAH and NP and 10 controls. The expression levels of inflammatory cytokines, tight junctions (TJ), and epithelial-mesenchymal transition (EMT)-related factors in the tissues were analyzed. The mRNA expression of the aforementioned factors was measured using qRT-PCR, while the expression of TJ and EMT-related proteins was analyzed through Western blotting and immunohistochemistry. **Results**: Compared to the control group, levels of inflammatory cytokines (IFN-γ, IL-5, IL-17A, IL-31, IL-33, and TNF-α) and EMT-related factors (α-SMA, COL1A1, MMP9, TGF-β1, and Vimentin) were significantly increased in both REAH and NP tissues. Conversely, E-Cadherin and TJ-related factors (Claudin-4 and Occludin) significantly decreased. When comparing REAH with NP, it was observed that the expression of IL-4, IL-5, and IL-33 was lower in REAH, while TNF-α was higher. Regarding TJ-related factors, the expression of Occludin was lower in REAH. Furthermore, in terms of EMT-related factors, except for E-Cadherin, the expressions of α-SMA, COL1A1, CTGF, MMP9, TGF-β1, and Vimentin were higher in REAH. **Conclusion**: REAH and NP exhibit different immunopathological mechanisms. NP demonstrates a more severe inflammatory response, whereas REAH is characterized by a more pronounced TJ and EMT breakdown than NP.

Key words: epithelial-to-mesenchymal transition, inflammation, nasal polyps, respiratory epithelial adenomatoid hamartoma, tight junctions

Introduction

Respiratory epithelial adenomatoid hamartoma (REAH) is a benign lesion that develops in the nasal cavity and sinuses. Wenig and Heffner first reported it in 1995 [1]. REAH typically originates from olfactory clefts and can exist alone in the sinonasal tract and nasopharynx [2]. Initially considered a rare disorder with limited cases reported in the literature [3], REAH is characterized by glandular proliferation of the respiratory surface epithelium and growth into the deeper submucosa [4-6]. As a result, more cases are being diagnosed and treated due to otolaryngologists' and pathologists' continuous in-depth understanding of REAH [2]. Clinically, REAH is frequently associated with nasal polyps (NP) [3, 7]. Notably, these two disorders share similar clinical manifestations, such as nasal obstruction, rhinorrhea, loss of smell, and facial pain/headache [4]. In the past, REAH has often been misdiagnosed as NP due to their similar nasal symptoms and lack of awareness [3-5]. While significant progress has been made in understanding the histopathology and imaging of REAH, research on its pathogenesis remains limited. From what is understood, the host immune response, mucociliary clearance, epithelial barrier dysfunction, and epithelial-mesenchymal transition (EMT) play crucial roles in the pathophysiological mechanism of chronic rhinosinusitis with nasal polyps (CRSwNP)[8-11]. However, whether the immunopathological mechanisms are the same in both diseases is unclear. Therefore, this study aimed to investigate the differences in inflammatory response, tight junctions (TJ), and EMT between REAH and NP.

Materials and methods

Patient tissue samples

The study included 8 patients with concurrent REAH and CRSwNP and 10 who underwent concha bullosa surgery at the Affiliated Hospital of Qingdao University between January 2022 and December 2022. The study protocol was approved by the medical ethics committee of the Affiliated Hospital of Qingdao University (approval number: QYFY WZLL 28132).

All patients underwent transnasal endoscopic surgery at our hospital after nasal corticosteroids therapy failed. REAH was diagnosed and confirmed by two pathologists following the WHO criteria [12]. REAH and NP tissue samples from the same patient were obtained from the ipsilateral side olfactory cleft and middle nasal meatus, respectively (Figure 1). Furthermore, control mucosal samples were taken from the middle turbinate during the concha bullosa surgery. Patient demographic information is shown in Supplementary Table 1.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissue samples using the RNA Extraction Kit (TaKaRa Biotechnology, Dalian, Liaoning, China) following the manufacturer's instructions. The quality of the total RNA was assessed using Nanodrop-2000 (Thermo Fisher Scientific). Single-stranded cDNA was synthesized using the TaqMan RNA Reverse Transcription Kit (TaKaRa Biotechnology). qRT-PCR was also conducted using the SYBR Premix Ex Taq kit (TaKaRa Biotechnology) under the following conditions: 95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds and 60°C for 1 minute. The primer sequences used in this study are provided in Supplementary Table 2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to normalize gene expression. The relative quantification of all gene expressions was performed using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Proteins were extracted from the collected tissues using RIPA buffer (Solarbio, China). Protein quantification was performed using the BCA method. The proteins (30μg) were separated by electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel (Epizyme, China) and transferred to a 0.45μm polyvinyl difluoride membrane (Millipore, France). The membranes were then incubated with a 5% fat-free skim milk solution in Tris-buffered saline with Tween-20 (TBST; Solarbio, China) for 1 hour at room temperature, followed by overnight incubation with primary antibodies listed in Supplementary Table 3. Following incubation, the membranes were washed three times with TBST and incubated for 1 hour with a secondary goat anti-rabbit antibody (E-AB-1003, Elabscience, USA). The immunoblots were visualized using an enhanced chemiluminescence kit (MA0186-1, Meilunbio, China). GAPDH (E-AB-20059, Elabscience, USA) and tubulin (AC007, ABclonal, China) were used as internal controls. Furthermore, the protein bands were visualized using the Chemiluminescence Gel Imaging Analysis System OdysseyR Fc Image System and quantified using Image J software (National Institute of Health, Bethesda, MD, USA).

Immunohistochemistry (IHC)

After dewaxing and antigen retrieval, paraffin sections (4 μm) were used for IHC staining. The sections were incubated in 3% hydrogen peroxide to block endogenous peroxidase activity, followed by blocking with 3% BSA (Servicebio, China) for 30 minutes at room temperature. Subsequently, the blocking solution was removed, and primary antibodies as listed in Supplementary Table 4 were added, with overnight incubation at 4°C. After washing, the diluted secondary antibody was added and incubated at room temperature for 50 minutes. Following three washes with PBS, the diaminobenzidine reagent (Servicebio, China) was added, and the color development time was controlled under the microscope. Positive results appeared brown-yellow, and the sections were rinsed with deionized H_2O to stop the color development. Nuclei were restained with hematoxylin for 3 minutes. Then, the sections were dehydrated with gradient ethyl alcohol, made transparent in xylene, and mounted with mounting media. Furthermore, images were subjected to semi-quantitative

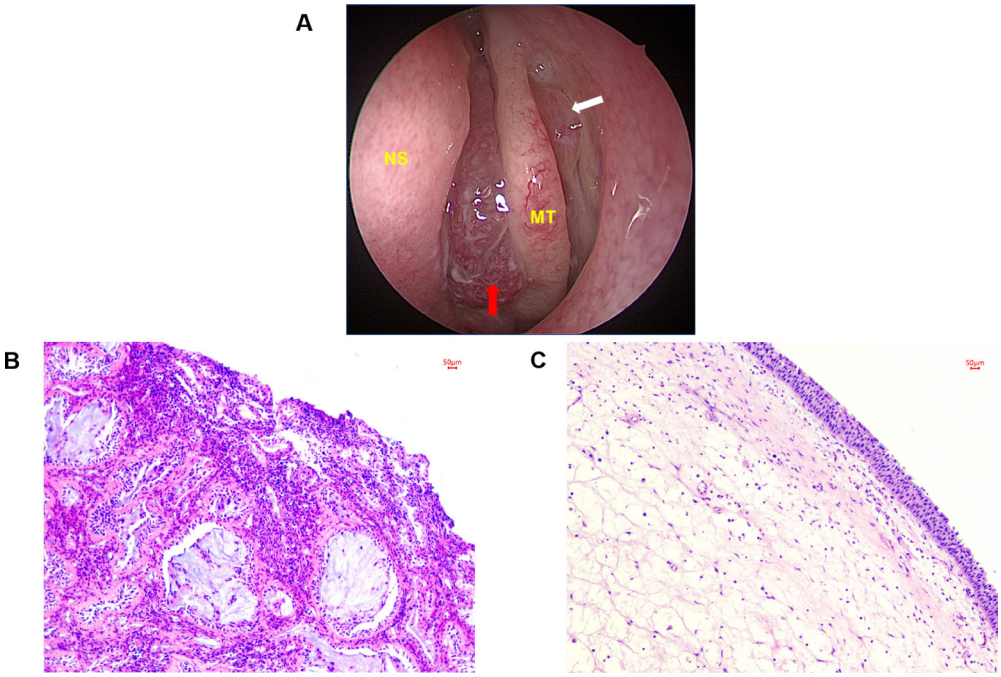

Figure 1. Endoscopic and histological differences between REAH and NP. (A) Representative image of REAH and NP in the ipsilateral nasal cavity of the same patient. The red arrow indicates REAH and the white arrow NP. (B) Histopathology of REAH (magnification, ×100). (C) Histopathology of NP (magnification, ×100). REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; NS, nasal septum; MT, middle turbinate.

analysis using Image J software (National Institute of Health, Bethesda, MD, USA).

Statistical analyses

Statistical analyses were conducted using GraphPad Prism (Prism 8; GraphPad Software Inc., La Jolla, CA, USA). According to the normality of the data distribution, we employed either a one-way analysis of variance (ANOVA) or the Kruskal-Wallis rank sum test to compare the differences among the three groups. We used the t-test or Wilcoxon rank sum test to compare the differences between the two groups. The REAH and NP groups were compared using a paired test. A statistically significant result was defined as $P < 0.05$.

Results

Differences in expression of inflammatory cytokines among REAH, NP, and control

First, we compared the expression of inflammatory factors in the nasal samples of three groups. At the transcription level, the expression of multiple inflammatory cytokines in both the REAH and NP groups was significantly higher than that in the control group, including interferon (IFN)-γ, interleukin (IL) -5, IL-17A, IL-31, IL-33, and tumor necrosis factor (TNF)-α (Figure 2). When comparing the REAH and NP groups, the expression of IL-4, IL-5, and IL-33 was higher in the NP group. In contrast, the expression of TNF-α was lower in the NP group (Figure S1). This indicates that both REAH and NP groups show apparent inflammatory responses in the nasal mucosa compared to healthy controls. Furthermore, the inflammation level in the NP group is higher than that in the REAH group, predominantly characterized by type 2 inflammation.

Differences in the expression of TJ-associated factors among REAH, NP, and control

Subsequently, we compared the expression differences of TJ-associated factors in the nasal tissues of the three groups. At the mRNA level, the expression of TJ-associated factors decreased in the NP and REAH groups compared to the control group. Statistical differences were observed in Claudin-4 and Occludin (Figure 3A-F). Additionally, the expression level of Occludin in the REAH group was lower than that in the NP group (P=0.039) (Figure S2). To compare the protein expression levels of Claudin-4 and Occludin, we further analyzed the data from the three groups of nasal tissues. Our results revealed that the protein expression levels of both factors were as follows: the control group had significantly higher levels than the NP and REAH groups. In comparison, the NP group had higher protein expression levels than the REAH group (Figure 3G-J). IHC also showed that the expression of Claudin-4 and Occludin in the REAH group was significantly lower than in the NP group (Figure 3K-N). These results indicate that both NP and REAH exhibited epithelial barrier disruption compared with healthy controls, with REAH showing more severe TJ damage than NP.

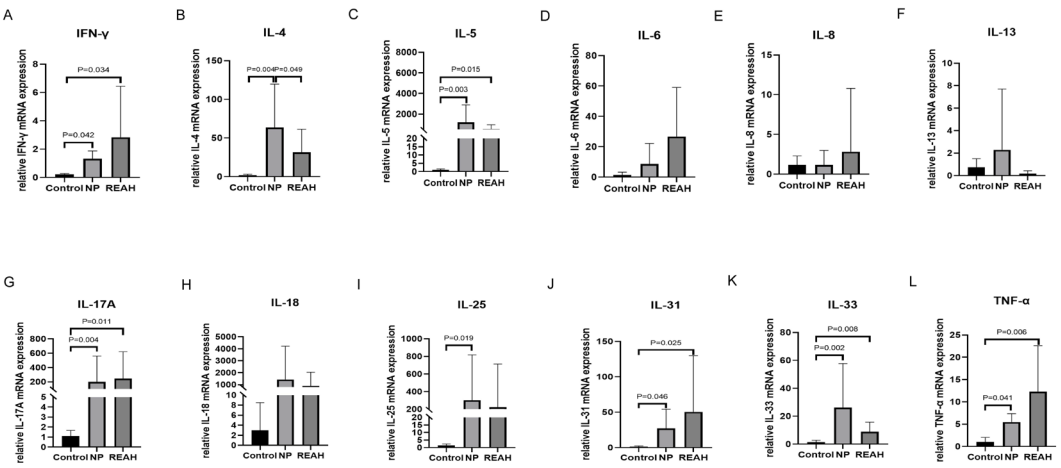

Figure 2. Relative expression of inflammatory factors in nasal tissues in REAH, NP, and control groups. Bars show the mean and vertical lines represent the standard deviation. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α.

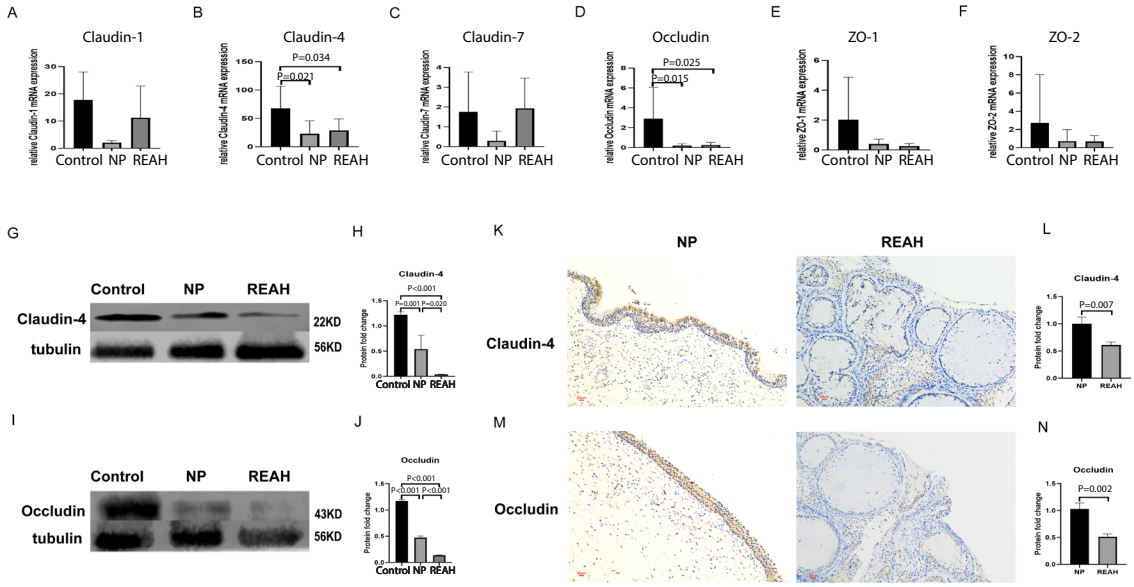

Figure 3. Expression of tight junction-associated factors in nasal tissues in REAH, NP, and control groups. A-F, mRNA levels. G-J, protein levels. K-N, representative immunohistochemistry stainings (magnification, ×100) and quantitative analysis in NP and REAH group. Bars show the mean and vertical lines represent the standard deviation. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; ZO, zona occludens.

Differences in expression of EMT-related factors among REAH, NP, and control

In our study, we investigated the differences in the expression of EMT-related factors in the nasal tissues of three groups. The results indicate that the expression levels of multiple factors in the NP and REAH groups significantly differed from those in the control group. At the mRNA level, the expression of α- smooth muscle actin (SMA), Collagen 1A1 (COL1A1), connective tissue growth factor (CTGF), matrix metalloproteinases (MMP)9, N-Cadherin, transforming growth factor (TGF)-β1, and vimentin was significantly higher in the REAH group compared to the control group. In comparison, the expression of E-Cadherin was lower. Similarly, the expression of α-SMA, TGF-β1, and vimentin was significantly higher in the NP group than in the control group, and E-Cadherin expression was lower (Figure 4). When REAH and NP were compared in pairs, except for E-Cadherin, all other factors mentioned above were expressed at higher levels in the REAH group (Figure S3). We further examined the protein levels of these factors and found that E-Cadherin expression was lowest in the REAH group, followed by the NP group. Additionally, E-Cadherin expression was highest in the healthy control group. The expressions of α-SMA, COL1A1, CTGF, MMP9,

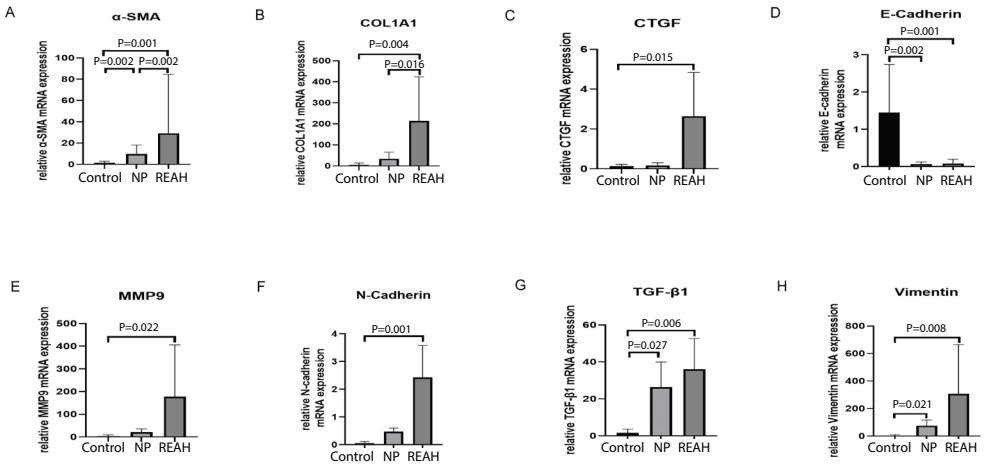

Figure 4. Relative expression of epithelial-mesenchymal transition-related factors mRNA levels in nasal tissues in REAH, NP, and control groups. Bars show the mean and vertical lines represent the standard deviation. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1.

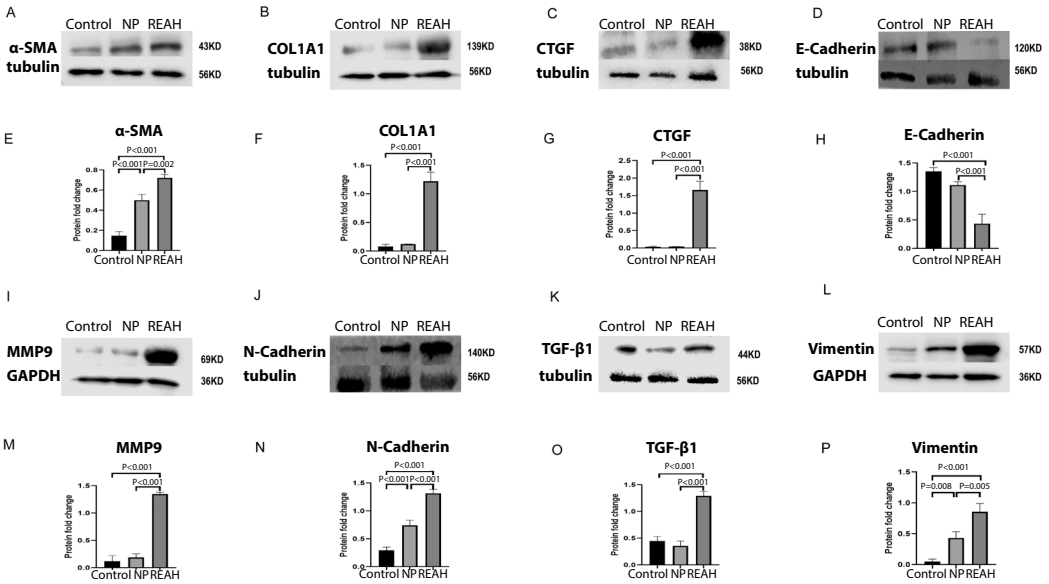

Figure 5. Expression of epithelial-mesenchymal transition-related factors protein levels in nasal tissues in REAH, NP, and control groups. Bars show the mean and vertical lines represent the standard deviation. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

N-Cadherin, TGF-β1, and Vimentin in the REAH group were significantly higher than those in the control and NP groups (Figure 5). Notably, these protein level changes were consistent with the mRNA level findings. Similarly, the above results were further confirmed by IHC (Figure 6). Our results suggest that REAH and NP exhibit significant EMT compared to healthy controls, with REAH showing more severe EMT and tissue remodeling than NP.

Discussion

REAH is a benign tumor-like lesion that occurs in the sinonasal tract. Although it is common in clinical practice, it often goes unnoticed [13]. REAH is more commonly observed in middle-aged and older men, with a male-to-female ratio of 3:2 [4, 13]. Additionally, REAH can occur independently or in association with other inflammatory processes, with NP being the most common [13-15]. Previous reports have shown that the origin of REAH is most frequently found in the olfactory clefts, seen in approximately 30 to 55 percent of patients who undergo primary or revision surgery for NP [2, 4, 5, 16, 17]. It is also important to note that REAH and NP can be easily mistaken for each other due to similar clinical presentation, imaging features, and biological behavior [14, 16, 18]. The exact cause of REAH is still unclear and is believed to

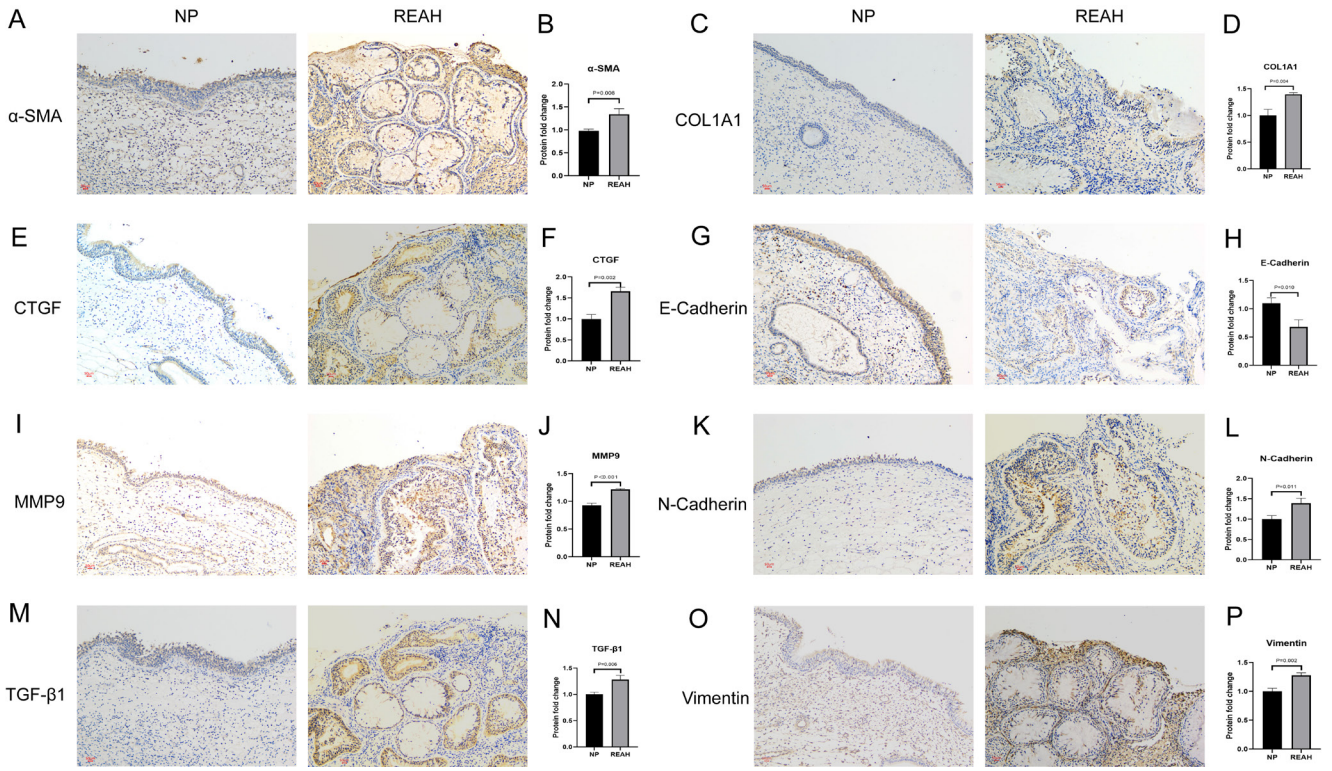

Figure 6. Immunohistochemistry of epithelial-mesenchymal transition-related factors in NP and REAH groups. A,C,E,G,I,K,M,O, representative images (magnification, ×100). B,D,F,H,J,L,N,P, quantitative analysis. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1.

be influenced by multiple factors.

Most scholars believe that REAH occurs in the setting of inflammatory polyps and is likely due to a long-standing mucosal inflammatory process [5, 18, 19]. Likewise, Gu et al. found that Th9 cells and IL-9 were involved in the pathogenesis of REAH, confirming the role of inflammatory response in its development and progression [20]. Additionally, our research reveals that both REAH and NP exhibit significant mixed inflammatory responses in the sinus mucosa, including type 1 inflammation represented by IFN-γ and TNF-α, type 2 inflammation represented by IL-4, IL-5, and IL-33, and type 3 inflammation represented by IL-17A. However, the key distinction between REAH and NP is that NP's type 2 inflammatory response is more severe. It is important to note that Dupilumab, a fully human anti-IL-4 receptor alpha monoclonal antibody, effectively blocks the signaling of IL-4 and IL-13, key drivers of type 2 inflammation. This medication has been approved for the treatment of CRSwNP [21]. A study conducted by Takeda et al. [22] discovered that REAH affects the effectiveness of dupilumab in treating eosinophilic CRSwNP. Our study comprehensively explains this clinical phenomenon by elucidating the underlying inflammatory mechanism.

The epithelial barrier in the nasal mucosa serves as the initial defense against external threats. TJ plays a crucial role in strengthening the mechanical integrity of the nasal mucosal epithelium and reducing the paracellular ion and molecular transport [23, 24]. In the case of CRSwNP, an abnormal expression of TJ proteins, such as occludin, claudin3, and zonula occludens 1, has been identified as a contributing factor to its pathogenesis [23, 25, 26]. However, there has been no previous research on the abnormal expression of TJ-associated factors in REAH. Thus, our study is the first to demonstrate that occludin and claudin4 are expressed at low levels in REAH, with occludin expression being even lower than NP. This indicates the presence of epithelial barrier damage and dysfunction in REAH.

The disassembly of TJ is an early event in EMT [27] that subsequently participates in tissue remodeling of the nasal mucosa [8, 9, 28]. Previous studies have shown that EMT is a distinctive feature of CRSwNP and is closely associated with the formation of NP [10, 29]. In patients with CRSwNP, E-Cadherin expression in nasal mucosal epithelial cells is downregulated, while the expression of TGF-β1, α-SMA, fibronectin, and Vimentin is upregulated [30]. Consistent with these findings, our results also demonstrated increased expression of multiple EMT-related factors in NP tissues. In REAH, previous histological studies have observed structural changes such as thickening of the basement membrane and hyperplasia of goblet cells [31, 32]. Gauchotte et al. also found constant expression of MMP9 in epithelial cells of REAH through immunohistochemistry [32]. Our study found evidence of EMT in

REAH at the mRNA and protein levels, including increased expression of TGF-β1, Vimentin, COL1A1, α-SMA, N-Cadherin, CTGF, and MMP9. Notably, their abnormal expression was more severe compared to NPs. These findings suggest that type 2 EMT, associated with tissue regeneration and fibrosis caused by inflammatory damage, plays a crucial role in the immunopathological mechanism of REAH and is closely linked to tissue remodeling. To the best of our knowledge, this study is the first to investigate the molecular-level pathophysiological mechanisms of REAH, shedding light on the distinct immunopathological mechanisms of REAH and NP and providing further insights into the true relationship between these two diseases. However, it is important to note that this study has recognized limitations, such as a small sample size and inadequate mechanism research. In future studies, it is crucial to expand the sample size and endeavor to establish robust in vivo and in vitro disease models to facilitate more comprehensive mechanism research.

Conclusions

We investigated the variation in inflammatory response, TJ, and EMT between individuals with REAH, NP, and healthy controls at both the mRNA and protein levels. Our findings highlight the immunopathological mechanisms and that REAH and NP are distinct diseases. Specifically, we observed that REAH exhibits a less severe type 2 inflammation than NP but demonstrates more pronounced TJ destruction and EMT.

Acknowledgements

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Authorship contribution

XD, MZ, SZ, XY, LW, and ZZ performed the experiments and analyzed the data. NL and YJ provided nasal tissue specimens. YJ and LY were responsible for the overall design, data analysis, and manuscript preparation.

Conflict of interest

The authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Demographic characteristics of subjects.

	REAH&NP	Control	P value
Subject number	8	10	
Gender, male	6 (75.0)	6 (60.0)	0.183#
Age (years)	48.5±13.2	46.7±12.7	0.876*
Prior sinus procedure	1 (12.5)	0 (0)	0.444#

Values are presented as number (%) or mean ± standard deviation. # Data were analyzed by using the Chi-square test. * Data were analyzed by using an independent-sample t-test. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps.

Table S2. Primers used for quantitative RT-PCR analysis.

Gene	Sequence
IFN-γ	(F)5′-AGGAAGCCGAGGTTTTAACTG-3′
	(R)5′-AGGACGCTCATAAGTGTCACC-3′
IL-4	(F)5′-AGTGTCCCCCATGCACTGA-3′
	(R)5′-CAGGGGCACAAGTTCCACTG-3′
IL-5	(F)5′-AGCTGCCTACGTGTATGCCA-3′
	(R)5′-GTGCCAAGGTCTCTTTCACCA-3′
IL-6	(F)5′-ACTCACCTCTTCAGAACGAATTG-3′
	(R)5′-CCATCTTTGGAAGGTTCAGGTTG-3′
IL-8	(F)5′-GAGAGTGATTGAGAGTGGACCAC-3′
	(R)5′-CACAACCCTCTGCACCCAGTTT-3′
IL-13	(F)5′-GTGACGGTGTTGATGGTAAGAT-3′
	(R)5′-AGCTCCACAGAGTGTTCCTTG-3′
IL-17A	(F)5′-CAAGACTGAACACCGACTAAG-3′
	(R)5′-TCTCCAAAGGAAGCCTGA-3′
IL-18	(F)5′-ATCGCTTCCTCTCGCAACAA-3′
	(R)5′-GAGGCCGATTTCCTTGGTCA-3′
IL-25	(F)5′-CCAGGTGGTTGCATTCTTGG-3′
	(R)5′-TGGCTGTAGGTGTGGGTTCC-3′
IL-31	(F)5′-GTGCTCGTGTCCCAGAATTAC-3′
	(R)5′-TGTCTTGAGATATGCCCGGAT-3′
IL-33	(F)5′-GTGACGGTGTTGATGGTAAGAT-3′
	(R)5′-GCTCCACAGAGTGTTCCTTG-3′
TNF-α	(F)5′-CTCTTCTGCCTGCTGCACTTTG-3′
	(R)5′-ATGGGCTACAGGCTTGTCACTC-3′
Claudin-1	(F)5′-CAGTCAATGCCAGGTACGAATTT-3′
	(R)5′-AAGTAGGGCACCTCCCAGAAG-3′
Claudin-4	(F)5′-TGTACCAACTGCCTGGAGGAT-3′
	(R)5′-GACACCGGCACTATCACCATAA-3′
Claudin-7	(F)5′-GGGCATGAAGTGCACGCGCT-3′
	(R)5′-CGGCAAGACCTGCCACGATG-3′
Occludin	(F)5′-CTGGATACCGCAGCTAGGAA-3′
	(R)5′-TGAACCCCAGTACAATGGCA-3′
ZO-1	(F)5′-CCACACTGTGCGTCCATGA-3′
	(R)5′-GGATCTCCGGGAAGACACTT-3′
ZO-2	(F)5′-CGGTTAAATACCGTGAGGCAAA-3′
	(R)5′-GGGAACCACTGGGTGTAATTCA-3′
α-SMA	(F)5′-TGCCAACAACGTCATGTCG -3′
	(R)5′-CAGCGCGGTGATCTCTTTCT -3′
COL1A1	(F)5′-GAGGGCCAAGACGAAGACATC-3′
	(R)5′-CAGATCACGTCATCGCACAAC-3′
CTGF	(F)5′-ATGGCTCTATTTGCAGTCTTTCA-3′
	(R)5′-CACCCAGATGACATTGGATGTT-3′
E-Cadherin	(F)5′- GTCTCCTCTTGGCTCTGCC -3′
	(R)5′-TCGACCGGTGCAATATTCAA -3′
MMP9	(F)5′-TCTGGAGGTTCGACGTGAAGG -3′
	(R)5′-GAACTCACGCGCCAGTAGAA -3′
N-Cadherin	(F)5′-CTCCTGCGTGAGACCAGAAA-3′
	(R)5′-TCCGTGATAAAACGGCAGCA-3′
TGF-β1	(F)5′-GGGACTTCTACAACCCCGTG -3′
	(R)5′-CCGTGTACTTCTTCTCGCGT -3′
Vimentin	(F)5′-GACGCCATCAACACCGAGTT-3′
	(R)5′-CTTTGTCGTTGGTTAGCTGGT-3′
18s rRNA	(F)5′-CTGGATACCGCAGCTAGGAA-3′
	(R)5′-GAATTTCACCTCTAGCGGCG-3′
GAPDH	(F)5′-TCGACAGTCAGCCGCATCTT-3′
	(R)5′-GAGTTAAAAGCAGCCCTGGTG-3′

IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α; ZO, zona occludens; α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table S3. Primary antibodies used in Western blotting.

Antibody	Species	Concentration	Reference	Source
Claudin-4	Rabbit	1:2000	D160402-0025	Sangon Biotech
Occludin	Rabbit	1:3000	DF7504	Affinity
α-SMA	Rabbit	1:8000	14395-1-AP	Proteintech
COL1A1	Mouse	1:10000	67288-1-Ig	Proteintech
CTGF	Rabbit	1:2000	PB0570	Boster
E-Cadherin	Mouse	1:1000	bs-10 R,009	Bioss
MMP9	Rabbit	1:2000	E-AB-70247	Elabscience
N-Cadherin	Rabbit	1:1000	T55015	Abmart
TGF-β1	Rabbit	1:1000	346599	Zenbio
Vimentin	Mouse	1:2000	bs-0756 R	Bioss

α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1.

Table S4. Primary antibodies used in immunohistochemistry.

Antibody	Species	Concentration	Reference	Source
Claudin-4	Rabbit	1:500	SC-376643	Santa cruz
Occludin	Rabbit	1:500	SC-133256	Santa cruz
α-SMA	Rabbit	1:1000	14395-1-AP	proteintech
COL1A1	Rabbit	1:600	GB 11022-3	Servicebio
CTGF	Rabbit	1:100	PB0570	BOSTER
E-Cadherin	Rabbit	1:600	GB 11082	Servicebio
MMP9	Rabbit	1:1000	GB 11132	Servicebio
N-Cadherin	Rabbit	1:600	GB 111009	Servicebio
TGF-β1	Rabbit	1:400	GB 11179	Servicebio
Vimentin	Rabbit	1:600	GB 11192	Servicebio

α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1.

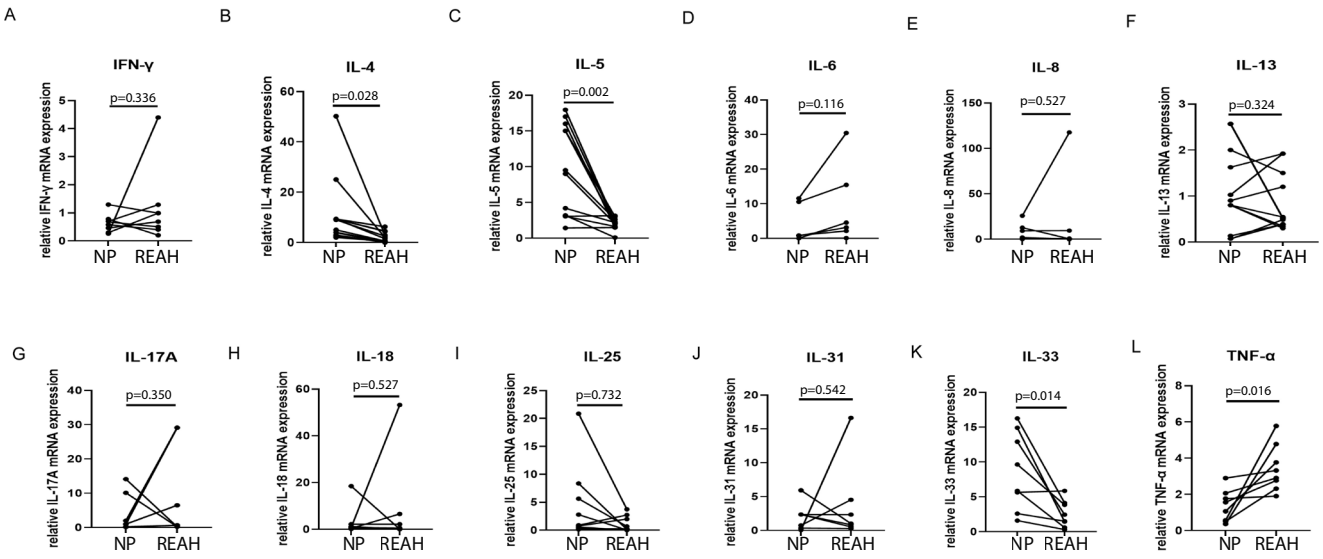

Figure S1. Relative expression of inflammatory factors in nasal tissues in REAH and NP. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α.

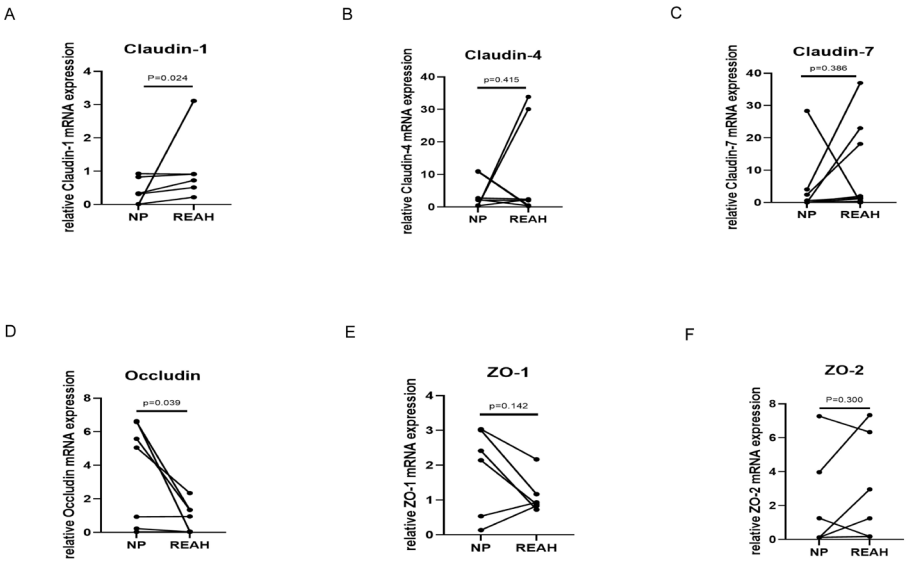

Figure S2. Relative expression of tight junction-associated factors in nasal tissues in REAH and NP. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; ZO, zona occludens.

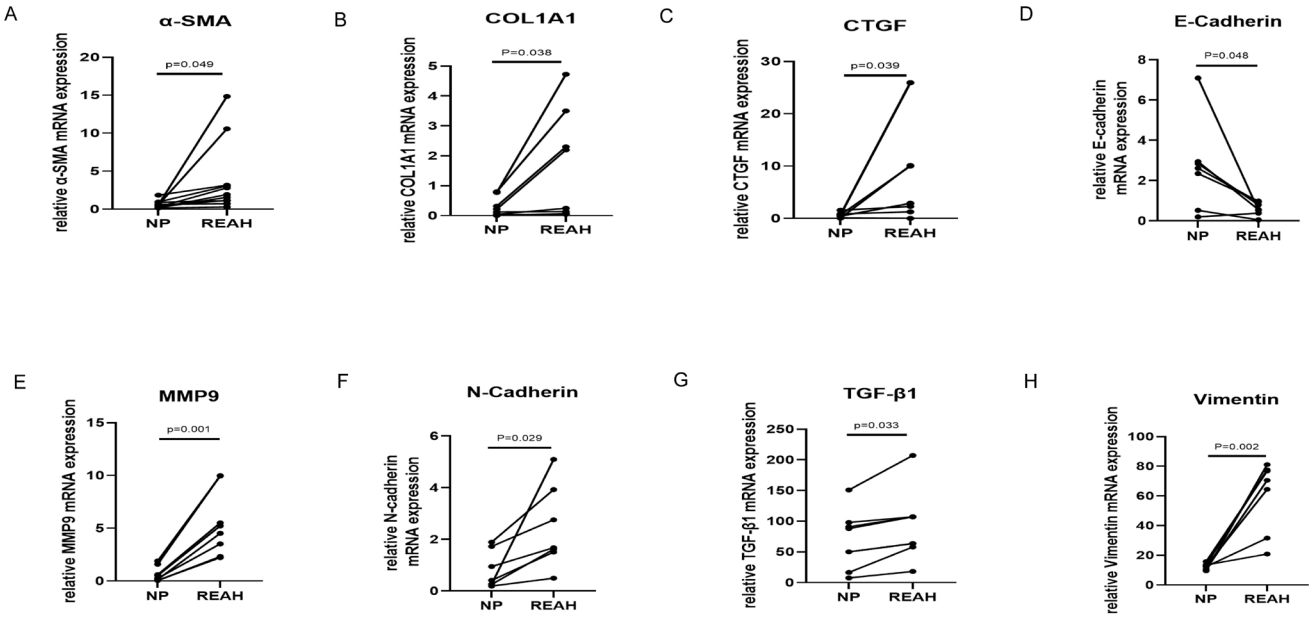

Figure S3. Relative expression of epithelial-mesenchymal transition-related factors in nasal tissues in REAH and NP. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1.

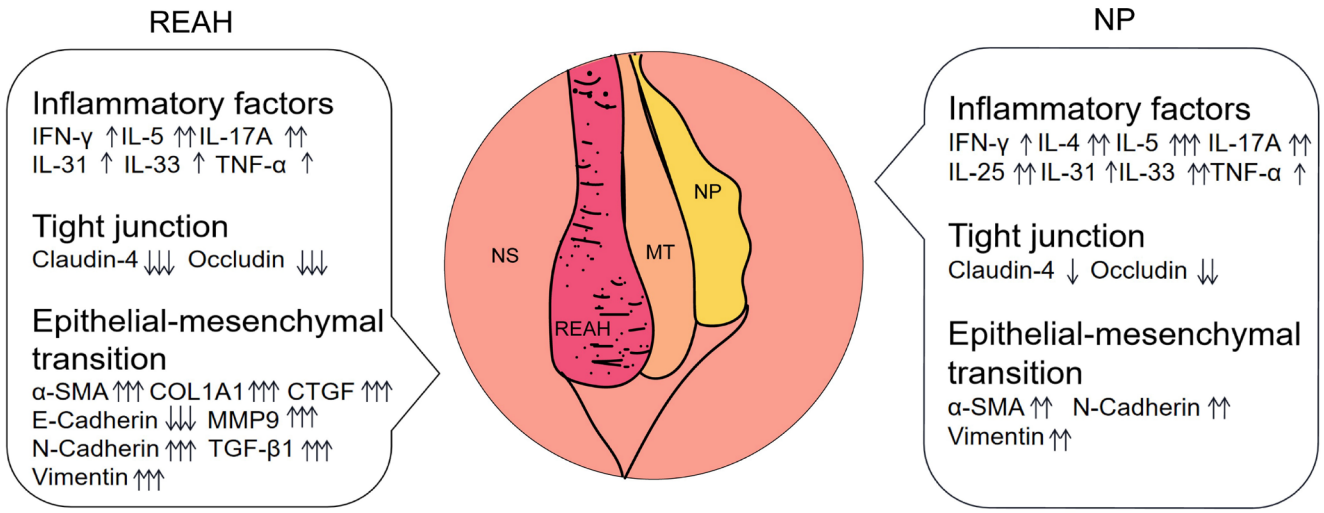

Figure S4. Graphical abstract. REAH, respiratory epithelial adenomatoid hamartoma; NP, nasal polyps; NS, nasal septum; MT, middle turbinate; IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α; α-SMA, α-smooth muscle actin; COL1A1, collagen 1A1; CTGF, connective tissue growth factor; MMP9, matrix metalloproteinases 9; TGF-β1, transforming growth factor-β1. ZO, zona occludens.