

Expression of Innate Immunity Genes in Epithelial Cells of Hypertrophic Adenoids with and without Pediatric Chronic Rhinosinusitis: A Preliminary Report

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Abstract

Background: Adenoid hypertrophy (AH) is associated with pediatric chronic rhinosinusitis (pCRS), but its role in the inflammatory process of pCRS is unclear. It is thought that innate immunity gene expression is disrupted in the epithelium of patients with chronic rhinosinusitis (CRS), including antimicrobial peptides and pattern recognition receptors (PRRs). The aim of this preliminary study was to detect the expression of innate immunity genes in epithelial cells of hypertrophic adenoids with and without pCRS to better understand their role in pCRS.

Methods: Nine pCRS patients and nine simple AH patients undergoing adenoidectomy were recruited for the study. Adenoidal epithelium was isolated, and real-time quantitative polymerase chain reaction (RT-qPCR) was employed to measure relative expression levels of the following messenger RNAs in hypertrophic adenoid epithelial cells of pediatric patients with and without CRS: Human β -defensin (HBD) 2 and 3, surfactant protein (SP)-A and D, toll-like receptors 1–10, nucleotide-binding oligomerization domain (NOD)-like receptors NOD 1, NOD 2, and NACHT, LRR and PYD domains-containing protein 3, retinoic acid-induced gene 1, melanoma differentiation-associated gene 5, and nuclear factor- κ B (NF- κ B). RT-qPCR data from two groups were analyzed by independent sample *t*-tests and Mann-Whitney *U*-tests.

Results: The relative expression of SP-D in adenoidal epithelium of pCRS group was significantly lower than that in AH group (pCRS 0.73 ± 0.10 vs. AH 1.21 ± 0.15 ; $P = 0.0173$, $t = 2.654$). The relative expression levels of all tested PRRs and NF- κ B, as well as HBD-2, HBD-3, and SP-A, showed no statistically significant differences in isolated adenoidal epithelium between pCRS group and AH group.

Conclusions: Down-regulated SP-D levels in adenoidal epithelium may contribute to the development of pCRS. PRRs, however, are unlikely to play a significant role in the inflammatory process of pCRS.

Key words: Adenoids; Epithelial Cell; Innate Immunity; Pattern Recognition Receptors; Pediatric Chronic Rhinosinusitis

INTRODUCTION

Pediatric chronic rhinosinusitis (pCRS) is a common condition characterized by persistent inflammation of sinonasal mucosa.^[1] Adult chronic rhinosinusitis (CRS) pathogenesis is still unclear but is known to be multifactorial. For example, host susceptibility and environmental exposure are both implicated in CRS development.^[2] pCRS, but not adults, is closely associated with adenoid hypertrophy (AH), which is another common pediatric disease.^[3] Adenoidectomy is an effective surgical procedure in children with both pCRS and AH, who fail to adequately respond to medical

treatment.^[4] Several previous studies have elucidated potential pCRS environmental risk factors such as adenoidal microbial elements and biofilms, which may lead to persistent sinonasal infections.^[5–8] However,

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little direct evidence exists that implicates adenoidal pathogens in pCRS.

Innate immunity is an integral part of our immune defense and triggers inflammation and other immune responses in individuals with CRS. Adenoidal epithelium, located at the interface of the host and environment, is a part of the innate immune function and protects against inhaled pathogens.^[9] Like other respiratory epitheliums, adenoidal epithelium may serve as the central part of the upper airway innate immunity related to mucociliary clearance,^[10] secretion of endogenous antimicrobial peptides (AMPs),^[11] interacting with the adaptive immune response, and expressing pattern recognition receptors (PRRs) to identify pathogen-associated molecular patterns (PAMPs).^[12] However, it still remains unknown whether all these characteristics of respiratory epithelium are similar with those of the adenoidal epithelium.

AMPs are secreted by epithelial cells of sinonasal epithelium and play a major role in immediate, nonspecific defense against potential pathogens entering the body through the nasal mucosa.^[13] Human β -defensins (HBD) are one of the main AMP families and are either constitutively – (HBD-1) or inducibly-expressed (HBD-2, HBD-3) in the human nasal epithelium.^[14] Negligible levels of expression of HBD-2 and HBD-3 are found in the normal nasal mucosa,^[15] and limited studies have examined the link between HBDs and CRS. Collectins, such as surfactant protein-A (SP-A) and SP-D are also involved in innate immunity. Altered expression of SP-A or SP-D may contribute to the onset of adult CRS, which can occur with or without nasal polyps (CRSwNP or CRSsNP).^[16-18] PRRs play an especially important defensive role in epithelial cells of the respiratory tract. Further, they have been shown to allow early detection of specific environmental pathogens.^[19] The most well-characterized transmembrane PRRs are the toll-like receptors (TLRs). Intracellular PRRs include nucleotide-binding oligomerization domain-like receptors (NLRs) and retinoic acid-inducible gene-1 (RIG-1)-like receptors (RLRs). All three of these PRRs initiate intracellular signaling pathways, which eventually activate downstream transcription factors such as nuclear factor- κ B (NF- κ B) to activate inflammatory cytokines and AMPs.^[9,20] Our group and others previously demonstrated positive associations between PRRs and the development of adult CRS with and without nasal polyps.^[21-25]

As a preliminary study trying to explore the role of adenoidal innate immune function in combined pCRS, we examined messenger RNA (mRNA) levels of several innate immunity genes from adenoidal epithelial cells isolated from AH with and without pCRS. These included AMPs, namely HBD-2, HBD-3, SP-A, and SP-D, as well as multiple PRRs, namely TLRs 1 through 10, several NLRs (nucleotide-binding oligomerization domain 1 [NOD 1], NOD 2, and NACHT, LRR and PYD domains-containing protein 3 [NALP 3]), RIG-1, melanoma differentiation-associated gene 5 (MDA-5), and NF- κ B p65.

METHODS

Subjects

Nine AH with pCRS (pCRS group) and nine AH without pCRS (AH group) were recruited for the study. All patients underwent adenoidectomies between April and July 2014 at the Inpatient Service of the Department of Otorhinolaryngology at Beijing Tongren Hospital. The design of this study was reviewed and approved by the Ethics of Human Research Committee of Beijing Tongren Hospital, and informed consent was obtained from all parents or legal guardians. All patients were admitted to the ear-nose-throat department due to nasal problems such as nasal obstruction, rhinorrhea, or sleep apnea. These symptoms were secondary to pCRS inflammation or AH. All patients' relevant clinical information was recorded, including a questionnaire that addressed disease signs and symptoms, medications, and allergy history [Table 1]. All patients' quality of life (QoL) was evaluated in both groups by totaling the score of the 22-item Sino-nasal Outcome Test (SNOT-22), which has been developed and validated to assess QoL in adults with CRS.^[26]

Each patient was examined with a nasal endoscope by an experienced ENT specialist. Adenoid size was measured and recorded by subjectively evaluating the area of tissue of the nasopharynx. Adenoidal tissue volumes were categorized according to percent coverage: 0%, 1–25%, 26–50%, 51–75%, and 76–100%. These scores were then denoted as scores of 0, 1, 2, 3, and 4, respectively.^[27] All cases included scores over 3 [Table 1].

pCRS was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EP3OS) 2012,^[28] with the following criteria: Participants aged 4–14 years, children presented a history of sinonasal-related problems (nasal obstruction, rhinorrhea, headache, or cough) exceeding 12 weeks and had signs of mucopurulent drainage from the middle meatus at referral and inclusion. The control AH group had no clinical or endoscopic evidence of sinus disease, but were diagnosed with AH and underwent adenoidectomies for obstructive sleep apnea. No participants had a history of nasal allergy or asthma and had negative skin prick tests, which tested for the standard panel of allergens. Patients with nasal polyps,

Table 1: Demographic and clinical characteristics of participants in the two groups (*n* = 9 in each group)

Parameters	AH group	pCRS group	<i>P</i>
Gender (male/female), <i>n</i>	5/4	5/4	1.000*
Age (years), mean \pm SD	7.7 \pm 1.9	8.1 \pm 3.2	0.554†
SNOT-22 score‡, mean \pm SD	11.8 \pm 7.0	30.9 \pm 18.5	0.011†
Adenoids size (grade 3/4)	6/3	4/5	0.833*
Asthma (presence/absence), <i>n</i>	0/9	0/9	1.000*
SPT (positive/negative), <i>n</i>	0/9	0/9	1.000*

*Determined with Fisher's exact test; †Determined with *t*-test; ‡The maximum score is 110. AH: Adenoids hypertrophy; pCRS: Pediatric chronic rhinosinusitis; SNOT-22: Sino-nasal Outcome Test-22; SPT: Skin-prick test; SD: Standard deviation.

immunodeficiency, immotile ciliary syndrome, or recent common cold were excluded.

Sample collection and cell culture

Adenoidal tissue was collected during adenoidectomies and immediately placed in physiological saline solution to remove blood clots, and then in Hank's balanced salt solution containing antibiotics (200 U/ml streptomycin, 200 U/ml penicillin G, and 0.25 µg/ml amphotericin B; Gibco, Grand Island, NY, USA). Adenoidal tissue samples were later soaked in a DMEM/F12 medium (SH30023, HyClone, Logan, UT, USA) supplemented with antibiotics (100 U/ml streptomycin, 100 U/ml penicillin G, and 0.125 g/ml amphotericin B; Gibco USA) and 0.05% w/v protease type XIV (P5147, Sigma Chemical Co., St. Louis, MO, USA), and rocked overnight at 4°C for 12 h to remove the epithelium. Next, 10% foetal bovine serum (FBS; HyClone, Logan, UT, USA) was added to inactivate the protease. Superficial layers of the epithelial cells were further dispersed by pipetting, samples were centrifuged, resuspended in DMEM/F12 medium with supplements (5% FBS, Hyclone, USA; 100 U/ml streptomycin, 100 U/ml penicillin G, 0.125 g/ml amphotericin B; Gibco, USA), and seeded on 6-well tissue culture plates coated with collagen type IV (Sigma Chemical Co., St. Louis, MO, USA). Cells were incubated at 37°C with 5% CO₂. The medium was changed every 24–48 h.

Lymphocytes were initially the dominant cell type, but these nonadherent cells were gradually removed with changes of culture medium. Following epithelial cell culture, an aliquot of cells was stained with a marker for epithelial cells (cytokeratin 14) to confirm that the predominant cells were epithelial. The remainder of the cells were harvested at 120 h and then processed for RNA extraction. All cell cultures were performed in Key Laboratory of Otolaryngology-Head and Neck Surgery (Beijing Institute of Otolaryngology, China).

RNA extraction and real-time quantitative polymerase chain reaction

We measured expression levels of the molecules listed above in the isolated epithelial cells of adenoids by using real-time quantitative polymerase chain reaction (RT-qPCR). RT-qPCR and data analysis were performed using an ABI7500 (Applied Biosystems, Foster, CA, USA) according to the manufacturer's instructions. Total RNA was extracted from the isolated adenoidal epithelial cells using TRIzol Reagent (CW0580, CWbio. Co. Ltd., Beijing, China). Reverse transcription was performed using a HiFi-MMLV cDNA Kit (CW0744, CWbio. Co. Ltd., China). One microgram of total RNA was reverse transcribed in a total volume of 10 µl (about 1 µg). Expression of isolated mRNA was normalized to RNA loading for each sample using β-actin (NM_001101) mRNA as an internal standard. All primers were commercially synthesized by Invitrogen (USA), and their sequences are shown in Table 2. PCR conditions for amplifications were 45 cycles at 95°C for 15 s, 60°C for 60 s, followed by melting curve analysis. All samples were measured in triplicate. The relative levels of each gene mRNA transcripts to β-actin were determined using the 2^{-ΔΔC_t} method.

Table 2: Primers used for RT-qPCR analysis

Gene	Primer sequences, 5'–3'	Amplicon size (bp)
HBD-2	For: TCTTCATATTCCTGATGCCTCTTCC Rev: AGACCACAGGTGCCAATTTGTTTAT	133
HBD-3	For: TCTTCTGTTTGCTTTGCTCTTCCCTG Rev: GCCGCCTCTGACTCTGCAATAATAT	100
SP-A	For: TCTGCCCAAAACTAAAACACTCTT Rev: TAGGCTGGGAGTCAGGAAATCTTGT	217
SP-D	For: GTGTCGGGGAGAAGATTTTCAAGA Rev: ACTCTCTGTGGGGTAGGTGAAC	216
TLR 1	For: CAGCTTTAGCAGCTTTCTGTATTG Rev: CCCCTGCTTTTATTGACCTCATCTT	107
TLR 2	For: TCTGGCATGTGCTGTGCTCTGTT Rev: GGAGCTTTCCTGGGCTTCCTTTT	131
TLR 3	For: TTAGCACGGCTCTGGAAAACAG Rev: TGTGGAGGTGAGACAGACCCTTATG	281
TLR 4	For: TGCAATGGATCAAGGACCAGAGG Rev: ACTGAGGACCGACACACCAATGAT	149
TLR 5	For: TCAAAAATCGCTTCTCCTCCTGTAGT Rev: TCCTGGTGAAGGGAATTAAGATAG	193
TLR 6	For: GTTATAGAGGAAGCCACTAAAGGAC Rev: ACAGTCACAGCCAACACCAGCA	109
TLR 7	For: GCATTTACTTGTCCCATCAGAGGC Rev: CACTCAAGGACAGAACTCCCACAG	137
TLR 8	For: GACACAAAAGATGCCTCTGTTACTG Rev: TGTCTCGGCTCTCTTCAAGGTGGTA	73
TLR 9	For: GCATCACCTCTGTGGCTGGGACCTCT Rev: CATCGTAGGGCAGGGCCTCCTCATCT	110
TLR 10	For: AAAACAACCCAAGAACAACCTCAAGA Rev: CATAAAGGCAAATCAAGATAGAACCAT	142
NOD1	For: CCAGCAGTCCTATGAGTTTTTCCAC Rev: AGAGGTTGGTGAAGTGAAGTGATC	245
NOD2	For: AGCCTCCGCAAGCACTTCCACT Rev: AAAGGCCAGGGCAGCAGCACTCA	213
NALP 3	For: TCTGGCTGTAACATTCGGAGAIT Rev: TTGCTGCTGAGGACCAAGGAGAT	90
RIG-1	For: AACCTGGCATATTGACTGGACGT Rev: CATTGCCACATACTCATAAAGGAT	192
MDA-5	For: AAGTAGGAGTCAAAGCCACCATCT Rev: GTGACGAGACCATAACGGATAACAA	196
NF-κB p65	For: GGGGACTACGACCTGAATG Rev: GGGCAGGATTGTCAAAGAT	118
β-actin	For: ACTTAGTTGCGTTACACCCTT Rev: GTCACCTCACCCTTCCA	156

HBD: Human β-defensins; SP: Surfactant protein; TLR: Toll-like receptors; NOD: Nucleotide-binding oligomerization domain; NALP 3: NACHT, LRR, and PYD domains-containing protein 3; RIG-1: Retinoic acid-induced gene 1; MDA-5: Melanoma differentiation-associated gene 5; NF-κB: Nuclear factor-κB; RT-qPCR: Real-time quantitative polymerase chain reaction; For: forward; Rev: reverse.

Statistical analysis

Data were expressed as a mean ± standard deviation (SD), or median (interquartile range). Differences of clinical data between the two groups of patients were analyzed by Fisher's exact test for dichotomous data. Independent sample *t*-tests were used for data with normal distributions. Comparison of RT-qPCR data from two groups were analyzed by independent

sample *t*-tests and Mann-Whitney *U*-tests. Spearman rank correlation coefficient (*r*) was used to assess the relationships between the parameters. Statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). A *P* < 0.05 was considered statistically significant.

RESULTS

Expression of antimicrobial peptides in adenoidal epithelium with and without pediatric chronic rhinosinusitis

RT-qPCR analyses showed no significant differences in HBD-2 or HBD-3 levels in either the AH group or the pCRS group [*P* = 0.354; *P* = 0.965, respectively; Table 3]. Decreased SP-D expression was detected in adenoidal epithelium of pCRS group compared with AH group [pCRS 0.73 ± 0.10 vs. AH 1.21 ± 0.15; *P* = 0.017, *t* = 2.654; Table 3], while no significant difference in SP-A [*P* = 0.508; Table 3] was observed between two groups.

Association between pediatric chronic rhinosinusitis severity and relative level of surfactant protein-D mRNA

The severity of pCRS symptoms was evaluated based on the total SNOT-22 score. Total SNOT-22 scores of pCRS group was significantly higher than those of AH

group [30.9 ± 18.5 vs. 11.8 ± 7.0; *P* = 0.011, *t* = 2.896; Table 1]. We also tested for a relationship between pCRS severity and the decrease in the relative level of SP-D transcripts. However, no statistically significant correlation was detected (*P* = 0.268).

Expression of toll-like receptors in adenoidal epithelium with and without pediatric chronic rhinosinusitis

Lesmeister *et al.*^[29] previously measured mRNA for TLR 1–10 in adenoid tissue, which led us to expect that isolated adenoidal epithelium may also contain mRNA for these TLRs. We measured TLR 1–10 mRNA levels in epithelial cells isolated from hypertrophic adenoids with or without pediatric CRS. We found no significant difference in TLR 1–10 mRNA levels between the AH and pCRS groups [Table 3].

Expression of nucleotide-binding oligomerization domain-like receptors in adenoidal epithelium with and without pediatric chronic rhinosinusitis

No significant differences were detected between NOD 1, NOD 2, or NALP 3 expression levels [*P* = 0.965; *P* = 0.895; and *P* = 0.627, respectively; Table 3] between the pCRS group and AH group. We also analyzed the correlation between NOD 1, NOD 2, and NALP 3 in the isolated adenoidal epithelium. We detected a strong correlation between NOD 2 and NALP 3 expression levels (*r* = 0.707, *P* < 0.001).

Expression of retinoic acid-inducible gene-1-like receptors in adenoidal epithelium with and without pediatric chronic rhinosinusitis

Next, we measured differences between RIG-1 and MDA-5 mRNA levels in the adenoidal epithelium. No statistically significant differences were found between the two groups [*P* = 0.599 and *P* = 0.508, respectively; Table 3].

Expression of nuclear factor-κB p65 in adenoidal epithelium with and without pediatric chronic rhinosinusitis

We also determined relative expression levels of NF-κB p65 in the isolated epithelial cells of adenoids by RT-qPCR. No statistically significant difference was observed between AH and pCRS group [0.85 ± 0.06 vs. 0.80 ± 0.06; *P* = 0.588, *t* = 0.553; Table 3].

DISCUSSION

We report a significant reduction in SP-D expression in the adenoidal epithelium of pCRS group compared with AH group. These findings suggest that pCRS is characterized by a down-regulation of SP-D levels in the adenoidal epithelium. Thus, the innate immune system may contribute to the onset of pCRS. Our results also reveal that the expression of all common PRRs, including TLRs, NLRs, and RLRs, and associated NF-κB show no statistically significant differences between pCRS group and AH group. These findings suggest that although PRRs are an integral part of the innate immune system, they are unlikely to contribute to the inflammatory process in pCRS.

Table 3: Relative expression levels of PRRs and AMPs in isolated adenoidal epithelium of the two groups (*n* = 9 in each group)

Gene	AH group	pCRS group	<i>P</i>
TLR 1	2.60 ± 0.59	4.40 ± 1.32	0.232
TLR 2	4.41 (3.65, 8.37)	3.70 (2.22, 9.23)	0.453*
TLR 3	3.36 (2.70, 3.63)	3.01 (1.44, 4.43)	0.691*
TLR 4	0.84 (0.40, 1.13)	1.34 (0.37, 1.81)	0.171*
TLR 5	4.57 (2.06, 5.53)	4.92 (2.24, 8.28)	0.566*
TLR 6	5.23 (2.92, 6.63)	4.00 (2.76, 6.22)	0.895*
TLR 7	3.31 (1.09, 4.83)	3.64 (0.61, 6.29)	0.825*
TLR 8	6.10 (0.59, 14.78)	6.14 (1.57, 11.55)	0.895*
TLR 9	1.69 (1.04, 2.77)	1.66 (1.22, 2.71)	0.895*
TLR 10	1.00 (0.45, 1.68)	0.69 (0.36, 1.85)	0.965*
NOD 1	0.26 (0.10, 0.63)	0.21 (0.09, 0.68)	0.965*
NOD 2	4.61 (2.57, 5.70)	4.64 (2.36, 5.66)	0.895*
NALP 3	2.58 (1.53, 9.00)	2.10 (1.34, 7.12)	0.627*
RIG-1	1.48 ± 0.31	1.28 ± 0.19	0.600
MDA-5	2.02 (1.46, 2.74)	1.48 (1.05, 3.55)	0.508*
NF-κB	0.85 ± 0.06	0.80 ± 0.06	0.588
HBD-2	38.71 (11.01, 60.15)	26.90 (0.72, 44.48)	0.354*
HBD-3	1.20 (0.12, 3.20)	1.09 (0.19, 3.13)	0.965*
SP-A	1.97 (0.84, 14.42)	1.79 (0.98, 5.30)	0.508*
SP-D	1.21 ± 0.15	0.73 ± 0.10	0.017†

*Mann-Whitney *U*-test was used for the comparison; †Statistically significant (*P* < 0.05). Normally distributed data are presented as the mean ± SD, nonnormally distributed data are presented as median (P25, P75). HBD: Human β-defensins; SP: Surfactant protein; TLR: Toll-like receptors; NOD: Nucleotide-binding oligomerization domain; NALP 3: NACHT, LRR, and PYD domains-containing protein 3; RIG-1: Retinoic acid-induced gene 1; MDA-5: Melanoma differentiation-associated gene 5; NF-κB: Nuclear factor-κB; pCRS: Pediatric chronic rhinosinusitis; PRRs: Pattern recognition receptors; AMPs: Antimicrobial peptides; SD: Standard deviation; AH: Adenoids hypertrophy.

SP-A and SP-D are hydrophilic proteins belonging to the collectin family of innate immunity proteins and are secreted from epithelial surfaces. They have long been studied in the lower airway immunity, where they bind to carbohydrate moieties present on the surface bacteria, fungi, and viruses. This leads to the clearance of pathogens by antigen-presenting cells (e.g., macrophages and dendritic cells).^[30] Previous studies investigating SP expression levels in adult CRS showed contradictory findings. One previous study found SP-D to be decreased, while the other group found increased or decreased levels of SP-A and SP-D in CRS patients when compared to controls.^[16-18,31] In another study, Salman *et al.*^[16] reported an elevation of both SP-A and SP-D levels in CRSwNP after a 3-week treatment with the oral glucocorticoid, methylprednisolone. Our findings are consistent with a few prior studies in adult CRS.^[16,17] If adenoidal epithelial cells in pCRS patients secrete less SP-D than AH patients, their innate immune defense would be compromised. As a result, pCRS patients are inflicted with persistent infections and subsequent inflammation. We propose that for this reason, AH is often comorbid with pCRS. The factors contributing to CRS development in adults or in children are not clearly understood. However, as we show here, decreased expression of important immune-associated proteins in adenoidal epithelium may play a role in the inflammatory process of pCRS.

HBD-2 and HBD-3 are key inducible AMPs in the upper airway that exhibit potent antimicrobial activity.^[14] Claeys *et al.*^[15] quantified both HBD-2 and HBD-3 in hypertrophic adenoids and nasal mucosa of adults with CRS via RT-PCR and immunohistochemistry. They found only negligible defensin expression levels in nasal biopsies and adenoids. Here we found similar results, which may suggest that inducible HBDs in adenoidal epithelium play a limited role in the onset of pCRS.

PRRs such as TLRs, NLRs, and RLRs have been shown to play an essential role in the early stage of innate immunity. Activation of PRRs at epithelial surfaces allows the rapid initiation of defensive immune responses. Each protein recognizes a different PAMP. TLR 1, TLR 2, TLR 4, TLR 5, TLR 6, TLR 10, and NLRs can recognize certain components of bacteria, while TLR 3, TLR 7, TLR 8, TLR 9, and RLRs recognize viral DNA or RNA. The signaling pathway of TLRs, NLRs, and RLRs eventually activate NF- κ B-dependent transcription and induce production of inflammatory cytokines and AMPs.^[20]

Among all of the PRRs, TLRs are the best characterized transmembrane PRRs. Our group and others have found that sinonasal epithelium TLRs are associated with the pathogenesis of CRS in adults.^[21-24] However, few studies have examined the role of TLRs in pCRS. This is due in part by less pCRS characterization as well as potential ethical risks (a large tissue sample would be required from healthy patients). Since an intimate association between AH and pCRS was known,^[3] we attempted to uncover the role of TLRs on hypertrophic adenoids in the development of pCRS.

Lesmeister *et al.*^[29] previously detected TLRs in human adenoids tissue. However, the adenoid tissue contained both epithelium and germinal centers of lymphocytes.^[32] Mature and immature lymphocytes express TLRs on their cell membranes. Therefore, the relationship between constitutive TLRs of the tissue and associated diseases are rather complicated. For this reason, we focused on the epithelium. We were surprised to find no significant difference in TLR 1–10 mRNA levels between pCRS group and AH group. These results are inconsistent with CRS in adults.^[21-24]

Both NLRs and RLRs are newly discovered intracellular PRRs. They detect various PAMPs, which triggers an immune response. Limited studies have examined expression levels of these receptors in the nasal mucosa. Månsson *et al.*^[25] measured the expression of NOD 1, NOD 2, and NALP 3 via RT-qPCR and immunohistochemistry in the human upper airway, including hypertrophic adenoids and CRSwNP. They found moderate levels of all three NLRs in adenoids, and found correlations between NOD 1 and NOD 2 expression levels, as well as between NOD 1 and NALP 3 in adenoids. Here we found no statistically significant difference in the relative expression levels of NOD 1, NOD 2, or NALP 3 in the isolated adenoidal epithelium. However, we did find a strong correlation between expression levels of NOD 2 and NALP 3. This difference may due to the difference in tissue examined (adenoids tissue vs. adenoidal epithelium). Tengroth *et al.*^[33] recently found mRNA and corresponding protein expression of RIG-1 and MDA-5 in nasal epithelium using RT-qPCR, immunohistochemistry and flow cytometry. We found no statistically significant difference of RIG-1 or MDA-5 expression levels in adenoidal epithelium between two groups.

Following the detection of PAMPs by PRRs, an immune response is usually triggered simultaneously with the activation of NF- κ B-dependent transcription. We measured NF- κ B expression levels between groups in order to confirm the correlation with PRRs. In our study, the relative expression levels of PRRs in both bacteria and viruses showed no significant difference between groups. The relative expression levels of all PRRs and NF- κ B showed no statistically significant difference in isolated adenoidal epithelium between pCRS group and AH group. These results suggest that PRRs are unlikely to contribute to the inflammatory process of pCRS.

We also acknowledge a few limitations in our study. First, we used simple AH patients as controls. AH itself is a common disease in children, which does not represent the normal condition of the epithelium in the nasopharynx. Ideally, controls should have been nasopharynx epithelium in subjects without any nasal inflammation. However, such controls are challenging to recruit, and such a large sample may be considered unethical to collect from healthy individuals. Second, RT-qPCR alone was used to determine the relative levels of AMP and PRR transcripts. This is due to limited sample amount. Our future studies should utilize immunohistochemistry and/or ELISA to detect featured AMPs

and PRRs. Third, our preliminary study had a relatively small sample size after removing confounding factors. Therefore, further studies in larger populations are necessary to further delineate the pathogenic role of adenoids in pCRS.

In conclusion, our study shows that down-regulated SP-D levels in adenoidal epithelium may contribute to the development of pediatric CRS. This preliminary study and future studies may lead to new strategies to treat pediatric CRS by using SP-D analogues. Although PRRs seem to play a limited role in the inflammatory process of pediatric CRS, these findings may provide us with a better understanding of the development of pediatric CRS.

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Conflicts of interest

There are no conflicts of interest.

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