Expression of Inflammatory Cytokines and Acquired CFTR Dysfunction in Children with Rhinosinusitis:A Cohort Study

Wenjing Liu[1], Yang Han[1], Pengpeng Wang[1], Tieshan Wang[2], Yu Qin[2], Xiaojian Yang[1], Wei Zhang[1], Xiao Xiao[1], Lixing Tang[1], and Wentong Ge[1]

[1]Beijing Children's Hospital Capital Medical University
[2]Beijing University of Chinese Medicine

August 25, 2022

Abstract

Abstract Objective: To explore the role of acquired CFTR dysfunction and inflammatory chemokines in the development of rhinosinusitis in children. Design and setting Twenty-five children who underwent endoscopic sinus surgery for acute and chronic rhinosinusitis in the Department of Otolaryngology Head and Neck Surgery at our hospital from January 2021 to December 2021 were included. Whole blood, mucosa and polyp tissues of all children were collected for study. Main outcome measures: The CFTR gene was detected by using Full-length second-generation sequencing. The expression of CFTR mRNA was measured by qRT-PCR, and the expression of inflammatory chemokines was measured by CBA. Results: There were 17 cases in the CRS group and 8 cases in the ABRS group. The expression of CFTR mRNA in the mucosa of the ABRS group was lower than that of the CRS group, and the expression of IL-6 and IL-8 in the mucosa of the ABRS group was significantly higher than that of the CRS group. IL-6, IL-8 and MCP-1 were upregulated in polyps. Conclusion: The dysfunction of acquired CFTR and the role of neutrophil chemotactic factor are more obvious in ABRS, and may play a role in the occurrence and development of CRS polyps.

Expression of Inflammatory Cytokines and Acquired CFTR Dysfunction in Children with Rhinosinusitis:A Cohort Study

Abstract

Objective:

To explore the role of acquired CFTR dysfunction and inflammatory chemokines in the development of rhinosinusitis in children.

Design and setting

Twenty-five children who underwent endoscopic sinus surgery for acute and chronic rhinosinusitis in the Department of Otolaryngology Head and Neck Surgery at our hospital from January 2021 to December 2021 were included. Whole blood, mucosa and polyp tissues of all children were collected for study.

Main outcome measures:

The *CFTR* gene was detected by using Full-length second-generation sequencing. The expression of CFTR mRNA was measured by qRT-PCR, and the expression of inflammatory chemokines was measured by CBA.

Results:

There were 17 cases in the CRS group and 8 cases in the ABRS group. The expression of CFTR mRNA in the mucosa of the ABRS group was lower than that of the CRS group, and the expression of IL-6 and

IL-8 in the mucosa of the ABRS group was significantly higher than that of the CRS group. IL-6, IL-8 and MCP-1 were upregulated in polyps.

Conclusion:

The dysfunction of acquired CFTR and the role of neutrophil chemotactic factor are more obvious in ABRS, and may play a role in the occurrence and development of CRS polyps.

Key words: CFTR, Rhinosinusitis, Inflammatory Cytokine, Children, Infection

Key points:

1 Neutrophil aggregation is associated with acquired CFTR.

2 The expression of CFTR mRNA in the mucosa of the ABRS group was lower than that of the CRS group.

3 IL-6 and IL-8 in the mucosa of the ABRS group was significantly higher than that of the CRS.

4 IL-6, IL-8 and MCP-1 were upregulated in polyps.

5 CFTR dysfunction and neutrophil chemokines IL-6 and IL-8 play more important roles in ABRS than in chronic inflammation of nasal mucosa.

Introduction

Rhinosinusitis is an inflammation of the nasal mucosa, which can be divided into Acute rhinosinusitis (ARS) and Chronic rhinosinusitis (CRS) according to the length of medical history [1]. At present, it is considered that viral infection is one of the most important causes of acute rhinosinusitis. The nasal mucosa epithelium is the first line of defense of the respiratory tract and is also the main entrance and initial reaction site of respiratory virus invasion[2]. The viruses that cause acute rhinosinusitis in children are mainly respiratory viruses, such as *Rhinovirus* ,*Respiratory Syncytial Virus* and *Influenza Virus* [3]. Viruses enter cells through receptor-mediated endocytosis, and then express and replicate the viral genome within hours of infection, causing damage to the nasal mucosa epithelium[4, 5]. Children's immune systems are not yet mature and are therefore more susceptible to pathogens that can induce ARS. Acute Bacterial sinusitis (ABRS) is a complication of viral upper respiratory tract infection. Mucosal injury and mucociliary dysfunction caused by viral infection may be the main causes of secondary bacterial infection in the nasal mucosa. In sinusitis, the most common bacteria are *Streptococcus pneumoniae* , *Haemophilus influenzae* , and*Moraxella catarrhalis* [6]. Recurrent ARS or prolonged ABRS can lead to CRS. In China, the prevalence of CRS is approximately 2.1% in children[1]. CRS significantly affects the child's quality of life.

CFTR is a glycoprotein expressed at the tip of mucosal epithelial cells. It is an important Cl channel protein in the mucosal epithelium which composed of 1480 amino acids. The airway epithelial surface is covered with a thin layer of airway surface liquid (ASL)[7]. The volume and viscosity of ASL are mainly regulated by CFTR. The dysfunction of CFTR will lead to the decrease of $Cl^-$and $HCO3^-$ to the extracellular transport, and the excessive absorption of Na^+ and H_2O into the cell. Thus, ASL is dehydrated, resulting in the decrease of Mucociliary Clearance (MCC)[8]. Increased mucus viscosity on the airway surface makes the mucosal surface more susceptible to bacterial colonization and induces or exacerbates chronic infection[9]. However, CFTR dysfunction can be either primary or acquired. Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by *CFTR* mutation, which is the cause of primary CFTR dysfunction. Therefore, the role of CFTR protein in airway mucosa has been widely discussed. In this study, we investigate the role of acquired CFTR dysfunction in acute and chronic rhinosinusitis in children.

Method

2.1 Patient and sample grouping

Twenty-five children diagnosed with rhinosinusitis and requiring endoscopic sinus surgery were included in this study from January 2021 to December 2021 in the Department of Otolaryngology Head and Neck Surgery at our hospital. According to EPOS 2020, 8 patients were diagnosed with ABRS with orbital complications,

and 17 were diagnosed with Chronic Rhinosinusitis with Nasal Polyps (CRSwNP). None of the patients had CF, congenital disease, or paranasal sinus masses. The children with ABRS were treated with anti-infection and comprehensive therapy before operation, but their symptoms did not improve significantly. The CRS patients received regular drug treatment in the outpatient department for 3 months, but their symptoms did not improve significantly. All the children were admitted to hospital for endoscopic sinus surgery and nasal secretions were collected for bacterial culture during surgery, and whole blood, nasal mucosa and polyp samples were collected for follow-up experiments. Whole blood samples were collected from parents of some children. All patients' guardians denied that they had CF-related diseases or had relatives diagnosed as CF in their families.

2.2 Whole exome sequencing (WES), parental generation verification and rearrangement deletion detection

200ul of whole blood samples were taken from all subjects for second-generation sequencing of all exons and flanking sequences of*CFTR* , and DNA was extracted from all samples using QIAGEN DNA Blood Midi/Mini kit (Qiagen GmbH, Hilden, Germany).

200ul was taken from the whole blood samples of the parents of some subjects for the first-generation verification of *CFTR* . A blood / tissue / cell genome extraction kit (Tiangen biochemical Technology Co., Ltd., DP304-03) was used to extract genomic DNA.

2.3 Quantitative real-time PCR

The expression of CFTR mRNA was measured by qRT-PCR in all polyps and mucosa. Fluorescent quantitative PCR was performed using a non-specific fluorescent staining method (SYBR Green I) using Lamboride Taq SYBR Green Qpcr Remix (R0202). The primer sequence was CFTR:"F:AGCTGTCAAGCCGTGTTCTAGATA; R: ATGAGGAGTGCCACTTGCAAA",β-actin:"F:TTCTACAATGAGCTGCGTGTG;R: AGAGGCGTACAGGGATAGCA".

2.4 Cytometric Bead Array

CBA was used to detect chemokine content in all polyps and mucosal specimens. The selected markers included neutrophil chemokines: IL-6, IL-8, IFN-γ, MCP-1; chemotactic factor for eosinophil: IL-4, IL-5, IL-13, RANTES, GMCSF and IgE. Testing was performed using the BD kit.

2.5 Statistics

SPSS 26.0 software was used to analyze all data. The significance of differences between the count data groups was analyzed using $\chi 2$ test or Fisher's exact test. Continuous variables were tested for normal distribution using the Shapiro-Wilk test, and the results were expressed as mean ± SD. An independent sample T test (two-tailed) or nonparametric Mann-Whitney U test was used for comparison between the two groups, and all results with $P<0.05$ were considered significant differences. GraphPad Prism version 9.0.0 was used for drawing.

Results

3.1 Clinical characteristics of patients

In this study, there were 17 patients in the CRS group and 8 patients in the ABRS group. The clinical characteristics of the patients are shown in Table 1. The Lund-Kennedy and Lund-Mackay score was significantly higher in the CRS group. The most common species in nasal secretions was*Staphylococcus* in both the CRS and ABRS group.

3.2 *CFTR* mutation carrying status

CFTR mutation was not detected in 7 subjects, and 14 *CFTR*mutations were detected in the remaining 18 subjects. The relationship between the detected mutations and disease was evaluated according to the general criteria of United States College of Medical Genetics and Genomics (ACMG). We found that one

sites was Likely Pathogenic, five were a Variant of Uncertain Significance (VUS), and the rest were graded as Benign. These sites were searched in ClinVar database, and no CF pathogenic sites were found. The China population database provided by Beijing Mygenostics co., Ltd. (included 46648 Han people in the Chinese mainland area) was selected to query the carrying rate of these mutations. The MAF of 6 sites was>0.05, which was the SNPs in the population (Table S1). The whole blood of the parents of the 7 patients with non-SNPs was collected for first-generation verification, and it was found that these mutations came from one parent (Table S2). The patients with no *CFTR* mutation or only one mutation were tested for Multiplex Ligation-dependent Probe Amplification (MLPA), and the results were negative. In combination with past medical history, family history, sweat chloride level test (SCLs, all lower than 60 mmol/L) and preoperative chest X-ray, it can be determined that all the enrolled children in this study were excluded from the diagnosis of CF.

3.3 Expression of CFTR mRNA

There was no statistical difference in the expression of CFTR mRNA in mucosa between the CRS group and the ABRS group, but it could be seen that the expression of CFTR mRNA was lower in ABRS compared with CRS (Table 2, Figure 1A).

The expression of CFTR mRNA was significantly lower in the polyps than in the mucosa ($P<0.05$) (Table 2, Fig. 1B).

3.4 Expression of inflammatory chemokines

Comparing the expression of inflammatory chemokines in the mucosa of the CRS group and the ABRS group, the neutrophil related chemokines IL-6 and IL-8 were significantly increased in the ABRS group ($P<0.05$), but there was no statistical difference in eosinophil related chemokines between the two groups (Table 3, Fig. 2). Comparison of chemokine expression in mucosa and polyps on the affected side of CRS showed that the neutrophil-associated chemokines IL-6, IL-8 and MCP-1 were significantly increased in polyps (Table 3, Fig. 3).

In all the samples, the contents of IL-5 and GM-CSF were lower than the detection limit (10pg/ml). Considering the low content of them in tissues, no valid data could be displayed.

Discussion

ABRS is a common condition and it is difficult to estimate its exact incidence. About 0.5-2% of children with ARS develop secondary bacterial infections that lead to ABRS, but most of them can be cured by anti-infective treatment [1, 10]. Studies have found that when people are stimulated by rhinovirus, local inflammatory reaction will be caused, IL-6 and IL-8 in nasal lavage fluid will increase[11]. RV-1B infection can promote the endocytosis of *Staphylococcus aureus* by lung cells cultured with semi-permeable membranes, and induce the release of IL-6 and IL-8 and the overexpression of ICAM-1[12]. In our study, the results of bacterial culture of nasal sinus secretion showed that the most common bacteria were *Staphylococcus* (12 cases), followed by *Streptococcus* (5 cases), and the rest were multiple infections.

In order to rule out the presence of primary CFTR dysfunction caused by potential CF patients in the CRS group, SCLs and WES were performed, and parental generation verification was performed in patients with non-SNPs. CF diagnosis was ruled out after analyzing the results. In other words, children with these mutations do not normally affect the amount or function of CFTR protein in the body. However, in the case of exposure to allergens or invasion of microorganisms such as viruses, the anti-strike ability and recovery ability of CFTR may be weakened[13].

Acquired CFTR dysfunction is mostly associated with epithelial mucosal injury caused by acute and chronic infection, inflammation, hypoxia and environmental pollution[8]. Virus invasion causes nasal mucosal epithelial injury, and stimulates the immune response and repair mechanism to prevent the virus from causing persistent airway injury[14]. However, when the immunity of the body is not enough to clear the virus or the viral load

is too high and the virulence is too strong, the CFTR function of mucosal epithelium cannot be restored in time, and the mucus fluidity under the airway mucosa is reduced, which leads to acute and chronic infection.

The immune response of the nasal mucosal epithelium is not limited to release of antimicrobial surfactants and mucus to delay the spread of pathogens in the airway, but it also expresses and secretes various cytokines and chemokines to drive the immune response against invading pathogens in the airway[14]. In an in vitro study, infection of human primary nasal epithelial cells with H3N7 resulted in an exponential increase in IFN α, IL 28A, and IL 29. The chemokines and the inflammatory markers such as CXCL11, RANTES, IL-1, IL-6, IL-8, are rapidly produced and released [14]. In this study, the expression of CFTR mRNA in the ABRS group was lower than that in the CRS group, and the IL-6 and IL-8 associated with neutrophils were significantly increased in the ABRS group. When activated in an inflammatory response, neutrophils can kill pathogens directly by phagocytosis or degranulation and indirectly by releasing TNF-α, IL-1, IL-8, IL-6, IFN-γ [11, 15]. These factors stimulate the production of downstream factors and recruit more neutrophils or other leukocytes to the site of infection or inflammation, exerting anti-inflammatory effects [16, 17]. In combination with the results of this study, we speculate that the extremely low CFTR mRNA caused by acute inflammation of nasal mucosa makes CFTR function seriously impaired, which not only easily aggravates the proliferation of pathogens such as bacteria, but also plays a certain role in the chemotaxis of neutrophil.

The etiology of nasal polyps is complex, which is a chronic inflammatory lesion of nasal mucosa. Eosinophils, macrophages and dendritic cells can participate in the activation, release, induction and regulation of a variety of cytokines to mediate inflammatory processes. Tissue remodeling caused by chronic inflammation and immune response eventually leads to nasal polyps [18]. In the CRS group, we compared the expression of CFTR mRNA and inflammatory chemokines in the ipsilateral mucosa and polyps of the same patient. It was found that the expression of CFTR mRNA in polyps was significantly lower than in mucosa. Thi et al. showed the expression of CFTR in nasal polyps was significantly lower than that in nasal mucosa, which was also supported by our results[19]. The formation of nasal polyps, epithelial barrier damage and rupture is an important feature, and colonized bacteria and other pathogens, such as Staphylococcus aureus, etc., aggravate the epithelial barrier damage[20]. Moreover, the hypoxic environment of the sinuses in CRS patients may also cause locally acquired CFTR dysfunction when polyps block the nasal or sinus drainage pathways, and may initiate a cascade of persistent fluid and electrolyte abnormalities in the sinuses[8]. Some findings suggest that local persistent hypoxia may lead to acquired CFTR dysfunction in nasal mucosa, resulting in mucociliary dysfunction in CRS[21, 22].

In recent years, more and more data show that the endotype of CRS in different races is not the same. CRSwNP is mainly eosinophilic inflammation in western countries. However, CRS is characterized by non-eosinophilic inflammation in Asia[23, 24]. In this study, we also observed that the neutrophil-mediated chemokines IL-6, IL-8 and MCP-1 were significantly increased in polyps, while the expression of eosinophil-associated chemokines was not significantly different between mucosa and polyps. Some studies found that IL-6 was elevated in CRS polyps and GRO, IL-8 were positively correlated with IL-6, suggesting that IL-6 may enhance neutrophil recruitment to the site of infection[25]. Therefore, we hypothesized that chronic inflammation of the nasal mucosa and pathogen infection not only cause CFTR dysfunction, but also cause neutrophil chemotaxis. And because of local mucosal hyperplasia or swelling, resulting in local hypoxia microenvironment, which further lead to CFTR dysfunction: The intracellular H2O could not be discharged out of the cell along with Cl^-, which participated in the formation of nasal polyps.

This study is a single-center study, so the sample size is limited. Due to the limitation of sample collection, local mucosa and polyps may not represent the inflammation status of all mucosa. Besides, baseline data on CFTR expression in the nasal mucosa of children are lacking due to the unavailability of nasal mucosa tissue from healthy children. However, we still observed differences in CFTR expression in different inflammatory states of nasal mucosa. In this study, the detection results of eosinophil chemokines IL-5 and CSF were below the lower limit of detection. Considering their low amount of content in the sample, the data were unreliable, so they could not be included in the analysis and discussion. Future studies with larger sample

sizes and more sensitive reagents and assays will be considered to explore the role of neutrophil chemotactic factor and eosinophil chemotactic factor in rhinosinusitis.

Conclusion

Acquired CFTR dysfunction and neutrophil chemokine play a role in the development of acute and chronic rhinosinusitis in children. CFTR dysfunction and neutrophil chemokines IL-6 and IL-8 play more important roles in ABRS than in chronic inflammation of nasal mucosa and may play a role in the occurrence and development of nasal polyps.

Reference

1. J. FW, J. LV, Hopkins C. ea. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. 2020.

2. Davies G, Lam M, Harris SE, et al. Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. *Nat Commun.* May 29 2018;9(1):2098.

3. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl.* Mar 2012;23:3 p preceding table of contents, 1-298.

4. J M Greve, G Davis, A M Meyer, et al. The major human rhinovirus receptor is ICAM-1. *Cell* 1989;56:839-947.

5. Sugrue RJ. Interactions between respiratory syncytial virus and the host cell: opportunities for antivirus strategies? *Expert Rev Mol Med.* Sep 5 2006;8(21):1-17.

6. van Cauwenberge P, Ingels K. Effects of viral and bacterial infection on nasal and sinus mucosa. *Acta Otolaryngol.* Mar 1996;116(2):316-321.

7. A Wanner, M Salathé, O'Riordan TG. Mucociliary clearance in the airways. *American journal of respiratory and critical care medicine* 1996;154:1868-1902.

8. Banks C, Freeman L, Cho DY, Woodworth BA. Acquired cystic fibrosis transmembrane conductance regulator dysfunction. *World J Otorhinolaryngol Head Neck Surg.* Sep 2018;4(3):193-199.

9. Cabrini G, Rimessi A, Borgatti M, et al. Role of Cystic Fibrosis Bronchial Epithelium in Neutrophil Chemotaxis. *Front Immunol.* 2020;11:1438.

10. J. FW, J. LV, Mullol J. ea. European position paper on rhinosinusitis and nasal polyps 2007. 2007.

11. Basharat U, Aiche MM, Kim MM, Sohal M, Chang EH. Are rhinoviruses implicated in the pathogenesis of sinusitis and chronic rhinosinusitis exacerbations? A comprehensive review. *Int Forum Allergy Rhinol.* Oct 2019;9(10):1159-1188.

12. Passariello C, Schippa S, Conti C, et al. Rhinoviruses promote internalisation of Staphylococcus aureus into non-fully permissive cultured pneumocytes. *Microbes Infect.* Mar 2006;8(3):758-766.

13. Groman JD, Meyer ME, Wilmott RW, Zeitlin PL, Cutting GR. Variant cystic fibrosis phenotypes in the absence of CFTR mutations.*N Engl J Med.* Aug 8 2002;347(6):401-407.

14. Yan Y, Tan KS, Li C, et al. Human nasal epithelial cells derived from multiple subjects exhibit differential responses to H3N2 influenza virus infection in vitro. *J Allergy Clin Immunol.* Jul 2016;138(1):276-281 e215.

15. Kasama T, Miwa Y, Isozaki T, Odai T, Adachi M, Kunkel SL. Neutrophil-derived cytokines: potential therapeutic targets in inflammation. *Curr Drug Targets Inflamm Allergy.* Jun 2005;4(3):273-279.

16. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol.* Mar 2013;13(3):159-175.

17. Mott LS, Park J, Murray CP, et al. Progression of early structural lung disease in young children with cystic fibrosis assessed using CT. *Thorax.* Jun 2012;67(6):509-516.

18. Guo T, Ma H, Zhou Y. Bioinformatics analysis of microarray data to identify the candidate biomarkers of lung adenocarcinoma.*PeerJ.* 2019;7:e7313.

19. Nguyen TN, Do BH, Kitamura T, et al. Expression of Cl(-) channels/transporters in nasal polyps. *Eur Arch Otorhinolaryngol.*Aug 2020;277(8):2263-2270.

20. Stevens WW, Schleimer RP, Kern RC. Chronic Rhinosinusitis with Nasal Polyps. *J Allergy Clin Immunol Pract.* Jul-Aug 2016;4(4):565-572.

21. Woodworth BA. Resveratrol ameliorates abnormalities of fluid and electrolyte secretion in a hypoxia-Induced model of acquired CFTR deficiency. *Laryngoscope.* Oct 2015;125 Suppl 7:S1-S13.

22. Guimbellot JS, Fortenberry JA, Siegal GP, et al. Role of oxygen availability in CFTR expression and function. *Am J Respir Cell Mol Biol.* Nov 2008;39(5):514-521.

23. Huabin L, Yuting L, J W. Endotypes and precision medicine in chronic sinusitis reatment. *J Otolaryngol Ophthalmol Shandong Univ.* 2019;33:9-13.

24. Wang X, Zhang N, Bo M, et al. Diversity of TH cytokine profiles in patients with chronic rhinosinusitis: A multicenter study in Europe, Asia, and Oceania. *J Allergy Clin Immunol.* Nov 2016;138(5):1344-1353.

25. Zheng H, Tang L, Song B, et al. Inflammatory patterns of antrochoanal polyps in the pediatric age group. *Allergy Asthma Clin Immunol.* 2019;15:39.

Hosted file

`Figure.docx` available at https://authorea.com/users/437166/articles/583343-expression-of-inflammatory-cytokines-and-acquired-cftr-dysfunction-in-children-with-rhinosinusitis-a-cohort-study

Hosted file

`Table.docx` available at https://authorea.com/users/437166/articles/583343-expression-of-inflammatory-cytokines-and-acquired-cftr-dysfunction-in-children-with-rhinosinusitis-a-cohort-study