Published in final edited form as:

Am J Otolaryngol. 2014 ; 35(3): 377–383. doi:10.1016/j.amjoto.2013.11.011.

Mucosal expression of aquaporin 5 and epithelial barrier proteins in chronic rhinosinusitis with and without nasal polyps

Alan H. Shikani, MD, FACS^{a,b,*}, Venkataramana K. Sidhaye, MD^c, Randall J. Basaraba, DVM, PhD^d, Henry J. Shikani, ScM^e, Mohammed A. Alqudah, MD^b, Natalie Kirk^d, Emily Cope^f, and Jeff G. Leid, PhD^f

^aDirector, Maryland Nose & Sinus Center Rhinology Fellowship Program and of the Department of Otolaryngology-Head and Neck Surgery, Union Memorial Hospital and Sinai Hospital, Baltimore, MD, USA

^bDepartment of Otolaryngology-Head and Neck Surgery, Union Memorial Hospital, Baltimore, MD, USA

^cDivision of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore

^dDepartment of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA

^eDepartment of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore

^fCenter for Microbial Genetics and Genomics, Northern Arizona University; Flagstaff

Abstract

Objectives—The purpose of this study is to characterize the association between altered epithelial barrier function, represented by changes in histology and differential expression of the mucosal water membrane permeability protein aquaporin 5 (AQP5), and the pathophysiology of chronic refractory sinusitis (CRS) in patients with and without nasal polyposis.

Study design—Prospective clinical study.

Setting—Tertiary rhinology referral center.

Participants—Sinonasal samples were obtained from seven CRS subjects with nasal polyps (CRSwNP), seven CRS without nasal polyposis (CRSsNP), and five control healthy patients.

Methods—Mucosal membrane changes were evaluated through hematoxylin and eosin staining of the membrane barrier and immunohistochemical staining of AQP5 expression, a membrane channel protein that affects trans-epithelial water permeability and tissue edema. AQP5 expression

Corresponding author at: Maryland Nose & Sinus Center Rhinology Fellowship Program and the Department of Otolaryngology-Head and Neck Surgery, The Union Memorial Hospital, 201 East University Parkway, Baltimore, MD 21218. Tel.: +410 554 4455; fax: 410 554 2171. ashikani@aol.com (A.H. Shikani).

Authors' disclosure: This study was partially supported through an unrestricted educational grant provided by ASL Pharmacy (Camarillo, California).

was confirmed by real-time PCR (rt-PCR) and western blot. Levels of other membrane proteins, including E-cadherin and Septin-2, were also assessed.

Results—CRSwNP patients showed substantial histologic evidence of membrane remodeling with increased edema and glandular hyperplasia. The epithelial expression of AQP5 was significantly lower in CRSwNP as compared to CRSsNP or control. There was no significant difference in the expression of E-cadherin and Septin-2.

Conclusions—Collectively, these data suggest that the mucosal epithelial barrier is compromised in the context of CRS (predominantly in CRSwNP) when compared to control and that AQP5 acts as a key tight junction protein in the maintenance of mucosal water homeostasis. We hypothesize that AQP5 plays a possible role in the pathophysiology of mucosal edema and polyp formation.

1. Introduction

The human immune response is a layered defense system against infection. It starts with a physical barrier which acts as a shield against potential environmental pathogens and is followed, first, by a non-specific innate response and, next, by a more specific adaptive response with both a cellular and humoral arm. The mucosal surface of the sinonasal tract interfaces with a considerable number of viruses and bacteria, which are usually eliminated by a mucociliary clearance mechanism. The ‘epithelial barrier hypothesis’ of chronic rhinosinusitis (CRS) posits that an intact barrier with tight epithelial junctions is necessary for a healthy nasal mucosa. Defects in this protective barrier may result in passage of pathogenic microbes across the epithelium and a subsequent dysregulated inflammatory cascade [1]. It has been reported that the integrity of the healthy mucosal epithelium is maintained by neighboring cells, with 4 types of cell–cell contacts: tight junctions, adherens junctions, gap junctions and desmosomal junctions [2]. The epithelial tissue in the respiratory tract also contains many important proteins, including the protease inhibitor SPINK5, the S100 family of proteins [3,4] and claudins and occludins, all of which regulate the tight junction function and permeability of the barrier.

Aquaporins (AQPs) are another group of proteins found in the epithelial barrier of certain body tissues. They control tissue water transport and homeostasis and have been detected in lung tissue [5,6], but their association with the pathophysiology of CRS has not been previously studied. To date, thirteen main aquaporins have been characterized (AQP 1–13), and their expression and function correlate with tissue and cell type [5]. Aquaporin 5 (AQP5) is found in type I pulmonary alveolar epithelial cells and submucosal gland acinar cells [6]. Both AQP1 and AQP5 are important in water transport in the lungs [7,8] and the deletion or decreased expression of these tight junction proteins is often associated with disease [8–10]. AQPs 1–5 are all expressed in the human nasal mucosa, however only AQP5 is found in the apical sites of epithelial cells and acinar cells [11]. A relationship between AQPs and polyps, the chronically inflamed sinus mucosa of CRS, has not been defined to date.

CRS is a common upper respiratory tract disease caused by a dysregulated inflammatory response to microbes that invade the mucosa of the nose and paranasal sinuses, although the

full complement of microorganisms that cause CRS remains to be characterized, and the exact pathophysiology behind the inflammatory process is still a work in progress [12]. CRS has been divided into CRS with nasal polyps (CRSwNP), which has a tendency towards T-helper type 2 (TH2) cytokine polarization, and CRS without nasal polyps (CRSSNP), which is associated with more of a TH1 type response [13]. While the inflammatory profile is known to differ dramatically between CRSwNP and CRSSNP, little is understood about the epithelial differences between these two groups [1,3,14,15]. In this work, we demonstrate that epithelial barrier function proteins are impaired in the context of an inflamed sinus mucosa when compared to control, and that the expression of AQP5 differs between CRSwNP and CRSSNP. These data provide additional insight into the pathophysiology of CRS with and without nasal polyps.

2. Materials and Methods

This is a prospective study that evaluates sinonasal tissue samples from 7 patients with CRSwNP, 7 CRSSNP patients undergoing endoscopic sinus surgery and 5 healthy controls undergoing transphenoidal or septal surgery. None of the CRS patients had previous surgery or had been taking systemic corticosteroids at the time of sample harvesting. The CRS patients had completed at least three courses of oral antibiotics and nasal corticosteroids sprays prior to surgery. Control patients were not on antibiotics or nasal corticosteroids sprays. Sinus tissue samples were taken from the ethmoids through a small Tru-cut Blakesley forceps and were about 5 mm³ in size. Control specimens were collected, with patients' permission, either from healthy sphenoids during trans-sphenoidal surgery for pituitary tumor or from the anterior ethmoids area during a routine septoplasty. Samples were evaluated by hematoxylin and eosin (H&E), immunohistochemistry, western blot and real-time PCR. The study was approved by the Liberty-institutional review board (IRB).

2.1. RNA isolation from human sinonasal tissue

Total RNA was isolated using the Qiagen TissueLyser LT protocol which was modified slightly for explanted sinonasal tissue. To begin RNA extraction, a 2 mL microcentrifuge tube containing 1 stainless steel bead (5 mm in diameter) was placed on dry ice for 15 min. Human sinus samples were placed into the precooled 2 mL microcentrifuge tube and cooled further for 15 min on dry ice. The tubes containing the tissue were placed into the TissueLyser adapter and incubated at RT for 2 min. After incubation, 500 µl of TriPure RNA buffer was added in addition to 500 µl sterile glass beads (0.1 mm in diameter). The TissueLyser was run at 30 Hz for 5 min. The samples were then centrifuged briefly in a cooled microcentrifuge. Two volumes of chloroform were added to the supernatant fluid, and the samples were again incubated at RT, this time for 10 min. The samples were centrifuged at 12,000 × g for 1 min at 4 °C. The top aqueous layer was removed and placed into a fresh RNase free Eppendorf tube. To this supernatant, 5 vol of 100% EtOH was added, and this mixture was pipetted into an RNA spin column (Ribopure-Bacteria, Ambion). The RNA was washed 3× with Ribopure Wash Buffer and subsequently eluted from the column. The isolated RNA was then treated with DNase I for 30 min at 37 °C, following the manufacturer protocol (Ribopure-Bacteria Kit, Ambion). Lastly, samples were placed into sterile, RNase-free Eppendorf tubes for storage at –80 °C until needed.

2.2. Real-time PCR for selected human sinonasal genes

Real-time PCR was conducted by SYBRgreen assay (Applied Biosystems) on a BioRad MyiQ5 Real-Time PCR detection system. PCR conditions may need to be adjusted per assay; however, the initial PCR master mix was 1× SYBRMastermix (Applied Biosystems), 1 ng/ml DNA, and 1 μM of each primer of the appropriate primer pair. In all assays, a positive control of 100 ng purified target DNA, and a negative control consisting of water was used. If primer specificity had not been previously determined, we validated the PCR assay based on several criteria: precision of primers, accuracy, specificity, range of detection, linearity, ruggedness, and robustness of the assay. All primers were blasted against the GeneBank database in silico to ensure that their sequences are specific to each organism.

2.3. Hematoxylin and Eosin Tissue staining for histological analysis

At the time of collection, all tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with H&E. The severity of histopathologic changes was scored with the evaluator (RJB) blinded to the treatment groups and patient identifications. Sections were scored on a scale of 0–5 with 5 being the most severe. The following parameters for each section were evaluated: degree and type of inflammation, edema, epithelial attenuation, epithelial hyperplasia, epithelial squamous metaplasia, fibrosis, and glandular or goblet cell hyperplasia. The summed total scores (35 total possible) were compiled and compared between treatment groups.

2.4. Aquaporin 5 Immunohistochemistry

Paraffin-embedded sections of human sinonasal tissue were collected, deparaffinized, rehydrated and subjected to antigen retrieval by incubation in Target Retrieval solution, pH 6.0 (DAKO, Carpinteria, CA) for 25 min at 90 ° C, followed by a 20 min cooling period at room temperature. The sections were then treated with 0.3% hydrogen peroxide in water for 15 min to quench endogenous peroxidase activity. Following a rinse in Tris buffered saline with 1% Tween-20 (TTBS), the slides were subjected to two blocking steps: (i) 15 min incubation with 0.15 mM glycine in PBS, and (ii) 30 min incubation with 1% normal horse serum with a rinse in TTBS in between. The slides were then incubated with rabbit polyclonal antibody to human Nrf2, Guanylate Cyclase GCS (Santa Cruz Biotechnology, Santa Cruz, CA), MDA, NQO1 (Abcam, Cambridge, MA), monoclonal antibody against AQP5, mouse monoclonal antibody against glutathione (Virogen, Watertown, MA) or respective isotype IgG controls at a 1:100 dilution in blocking buffer followed by several rinses in TTBS. This was followed by 30 min incubation with biotinylated goat-anti rabbit-IgG (Vector Laboratories) and visualization of bound antibody by the Avidin-Biotin system (Vectastain; Vector Laboratories) and diaminobenzidine substrate (Dako; Carpinteria, CA). The sections were counterstained with Meyer's hematoxylin (Scytek Laboratories; Logan, Utah), mounted with coverslips, and examined on an Olympus BX41 light microscope. Photomicrographs were acquired with an Olympus DP70 camera and the associated computer software. Microscopic lesions stained with H&E and sections stained by immunohistochemistry (IHC) were scored by the reviewer blinded to the treatment groups.

2.5. Immunohistochemical scoring

For immunohistochemical scoring purposes, each section was graded on a scale of 0–5 with 5 representing the most severe. Within the tissues sections, the overall immunohistochemical scoring was determined based on extent of staining and staining intensity. IHC for AQP5 was divided into epithelial and non-epithelial (mesenchymal) expression, but only epithelial scores were considered in the evaluation of immunoreactivity.

2.6. Western Blot

The overall (global) expression of AQP5 in all tissue samples was determined by western blot. Specimens were homogenized with Ultra-Turrax T-25 homogenizer (Janke & Kunkel, Germany), treated with RIPA buffer (50 mM Tris HCl pH 8.0, 150 mM NaCl, 1% Triton X-100, protease and phosphatase inhibitor), and spun at $800 \times g$ for 10 min. The supernatant was spun at 55,000 rpm with Beckman Coulter T-72Ti Centrifuge Rotor for one hour at 4 °C. After decanting the residual supernatant, the pellets were resuspended with 5× Laemmli buffer. The protein samples were assayed using the BCA reagents (Thermo Scientific Pierce) and subjected to SDS-PAGE on 10% acrylamide gels. The gels were then transferred onto Immuno-Blot PVDF membranes (Bio-Rad) and probed for Septin-2, E-cadherin and AQP5.

3. Results

A total of nineteen patients were examined in this study. The cohorts were control (patients undergoing pituitary surgery or septoplasty) ($n = 5$), CRS with nasal polyps ($n = 7$) and CRS without nasal polyps ($n = 7$) undergoing endoscopic sinus surgery. As expected, CRS patients in general had more severe inflammation than healthy control. CRSwNP samples scored higher when evaluated for edema, inflammation, eosinophilic infiltration and epithelial attenuation, which is representative of remodeling. Conversely, epithelial cell hyperplasia, often referred to as “thickening”, was greater in those with CRSsNP. The scoring results for these samples are listed in graphical form in Fig. 1. An example of H&E histology is shown in Fig. 2.

When these same samples were analyzed for expression of AQP5 by immunohistochemistry, epithelial levels of AQP5 were significantly lower in CRSwNP as compared to control or CRSsNP (Fig. 3). There was also a relative decrease in AQP5 epithelial expression in CRSsNP as compared to healthy control (although not as striking as CRSwNP). These data are shown in Fig. 4. AQP5 was expressed at relatively higher levels in the sub-epithelial (primarily glandular) tissue of CRSwNP patients when compared to cases of CRSsNP. Both real-time PCR and western blot analysis confirmed our IHC findings on the decrease in the global expression of AQP5 in the context of disease (Figs. 5 and 6).

These results suggest that expression and regulation of AQP5 are complex within sinonasal tissue, with different levels of the protein on epithelial and non-epithelial sides of the barrier. Whether this variability is contributing to disease, or being caused by it, is uncertain.

The samples were also evaluated for expression of Septin-2 and E-cadherin, both of which have been reported to participate in epithelial barrier function [1,2]. No significant

differences in the levels of these proteins were observed between the three patient groups, suggesting that they may not be involved in CRS.

4. Discussion

Chronic rhinosinusitis is divided into CRSwNP and CRSsNP. The etiology of nasal polyps' formation is not well understood and many theories have been proposed as to their origination. One theory hypothesizes that epithelial damage occurs, with subsequent prolapse of the lamina propria and epithelialization of the prolapse [1]. The precise pathophysiology of changes to the epithelium and lamina propria, as well as the basis for mucosal edema with polyp formation, is to date unknown.

This study reports on the expression of AQP5 in CRS and its potential association with tissue edema and polyp formation. AQPs are a family of small, hydrophobic membrane proteins that, among other things, transport water across and in between cell membranes. In theory, it would be logical to expect that water permeability and alteration in AQP expression may correlate with diseased edematous mucosa. Our western blot data indicate an overall trend towards *decreased* global protein expression of AQP5 in CRSwNP as compared to normal tissue and CRSsNP, but the difference does not meet statistical significance. IHC localization studies on the other hand, do show significantly decreased epithelial mucosal expression of AQP5 in nasal polyps, with increased sub-epithelial expression. Analysis of mRNA expression also demonstrates a significant reduction in the global expression of AQP5. We hypothesize that *epithelial* AQP5 plays an important role in the regulation of tight junction barrier function. Decreased levels of the protein in the context of CRSwNP may contribute to the pathophysiology of the edematous and inflammatory process of nasal polyp formation, possibly by affecting the integrity of the epithelial barrier of the nasal mucosa. Whether this is caused by loss or internalization of epithelial AQP5 is not clear.

Failure of epithelial barrier function and decreased expression of tight junction proteins have been reported in other infectious and immune-related diseases, such as those affecting the intestinal mucosa [1,4,10,11,16]. Marchiando et al. linked tumor necrosis factor (TNF)-induced tight junction regulation, barrier loss and increased permeability in the intestinal mucosa to the endocytosis of occludin [16]. Other studies have shown that occludin over-expression preserves tight junction barrier function and limits barrier loss in the intestine [4,10,11,16]. One would not be surprised by similarities between AQP5 and occludin expression and function, as both proteins are expressed in the membrane and cytoplasm, both are regulated by kinases and both exhibit a pattern of endocytosis that affects tight junction regulation [3,4,6,9–13].

One interesting observation with these data was the difference seen between the global expression of AQP5 and the epithelial vs sub-epithelial expression of the protein. Our global data suggested that there was no significant difference in AQP5 expression between control and CRSsNP. The reduction in global AQP5 in CRSwNP as observed by immunohistochemistry was on the other hand quite significant. We can only speculate whether the loss of epithelial AQP5 with disease is due to endocytosis or down-regulation.

This may suggest that it is the actual regulation of AQP5 expression that matters, not just the total levels of the protein in the tissue. Intriguing as this is, an argument could be made that the decreased levels of epithelial AQP5 and the increased level of sub-epithelial AQP5 in polyp tissue are functional differences that contribute to tissue edema and are factors that lead to the formation of polyps in these CRS patients. Additional studies are warranted, comparing larger numbers of CRSwNP and CRSsNP, to determine the significance of these observations.

There were important differences also collectively observed by histology between CRSsNP and CRSwNP in our study. As expected, scoring of tissue edema was greater in polyp patients versus non-polyps. Epithelial hyperplasia (EA), which is a sign of tissue repair, was markedly different between these samples. In CRSsNP, the average score for EH was 1.6, whereas, in CRSwNP it was 0.7 ($p < 0.1$). Conversely, for epithelial attenuation, which is a measure of cell and epithelial layer distortion, CRSwNP had an average score of 3 versus 1.5 for CRSsNP. These histological parameters confirm that tissue edema and epithelial attenuation are increased in nasal polyps and suggest that the repair mechanisms are more active in patients without polypoid disease.

Overall, it is clear there are marked differences between epithelial barrier parameters in CRS patients with and without polyps. Our studies suggest that AQP5 is a membrane integrity protein which is significantly decreased in patients with polypoid disease, as compared to healthy sinus mucosa. It may be theoretically possible to utilize the mucosal expression of AQP5 as a measure of restoration of sinus tissue health after surgery for nasal polyps.

5. Conclusions

These data support the concept that the mucosal epithelial barrier is compromised in the context of CRS disease, especially CRSwNP, and that loss of the junctional protein AQP5 may act as a tight junction protein that plays a role in the development of mucosal edema and the pathophysiology of nasal polyp formation. These findings suggest that the treatment of patients with polyps may necessitate strategies that target the epithelium, and possibly modulate AQP5.

References

1. Kern RC, Conley DB, Walsh W, et al. Perspectives on the etiology of chronic rhinosinusitis: an immune barrier hypothesis. *Am J Rhinol.* 2008; 22(6):549–59. Epub 2008/09/13. [PubMed: 18786300]
2. Zuckerman JD, Lee WY, DelGaudio JM, et al. Pathophysiology of nasal polyposis: the role of desmosomal junctions. *Am J Rhinol.* 2008; 22(6):589–97. Epub 2009/01/31. [PubMed: 19178795]
3. Schleimer RP, Kato A, Peters A, et al. Epithelium, inflammation, and immunity in the upper airways of humans: studies in chronic rhinosinusitis. *Proc Am Thorac Soc.* 2009; 6(3):288–94. Epub 2009/04/24. [PubMed: 19387032]
4. Tieu DD, Kern RC, Schleimer RP. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. *J Allergy Clin Immunol.* 2009; 124(1):37–42. Epub 2009/06/30. [PubMed: 19560577]
5. Nielsen S, Frokiaer J, Marples D, et al. Aquaporins in the kidney: from molecules to medicine. *Physiol Rev.* 2002; 82(1):205–44. Epub 2002/01/05. [PubMed: 11773613]

6. Verkman AS, Mitra AK. Structure and function of aquaporin water channels. *Am J Physiol Ren Physiol*. 2000; 278(1):F13–28. Epub 2000/02/08.
7. Verkman AS. Aquaporin water channels and endothelial cell function. *J Anat*. 2002; 200(6):617–27. Epub 2002/08/07. [PubMed: 12162729]
8. Verkman AS, Matthay MA, Song Y. Aquaporin water channels and lung physiology. *Am J Physiol Lung Cell Mol Physiol*. 2000; 278(5):L867–79. Epub 2000/04/27. [PubMed: 10781416]
9. Wang K, Feng YL, Wen FQ, et al. Decreased expression of human aquaporin-5 correlated with mucus overproduction in airways of chronic obstructive pulmonary disease. *Acta Pharmacol Sin*. 2007; 28(8):1166–74. Epub 2007/07/21. [PubMed: 17640479]
10. Wang D, Iwata F, Muraguchi M, et al. Correlation between salivary secretion and salivary AQP5 levels in health and disease. *J Med Invest*. 2009; 56(Suppl):350–3. Epub 2009/01/01. [PubMed: 20224222]
11. Seno S, Ogawa T, Shibayama M, et al. Expression and localization of aquaporin 1, 2, 3, 4, and 5 in human nasal mucosa. *Am J Rhinol Allergy*. May-Jun;2012 26(3):167–71. [PubMed: 22643939]
12. Benninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngology-head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery*. 2003; 129(3 Suppl):S1–S32. [PubMed: 12958561]
13. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *Otolaryngology-head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery*. 2004; 131(6 Suppl):S1–S62. [PubMed: 15577816]
14. Kato A, Peters A, Suh L, et al. Evidence of a role for B cell-activating factor of the TNF family in the pathogenesis of chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2008; 121(6):1385–92. 92 e1–2. Epub 2008/04/16. [PubMed: 18410958]
15. Richer SL, Truong-Tran AQ, Conley DB, et al. Epithelial genes in chronic rhinosinusitis with and without nasal polyps. *Am J Rhinol*. 2008; 22(3):228–34. Epub 2008/07/01. [PubMed: 18588753]
16. Ooi EH, Wormald PJ, Tan LW. Innate immunity in the paranasal sinuses: a review of nasal host defenses. *Am J Rhinol*. 2008; 22(1):13–9. Epub 2008/02/21. [PubMed: 18284853]

Fig. 1. Graphical representation of scored histological parameters between control and CRS tissue. The Y-axis represents the mean score (on a scale of 0–5 with 5 being the most severe). CRSwNP patients showed higher edema and glandular/goblet cell hyperplasia.

Fig. 2.

Representative section of Control (A), CRSsNP (B) and CRSwNP (C). CRS with and without polyposis has an attenuated surface epithelium with disruption of barrier function. (A) Normal sinus mucosa has a pseudostratified, columnar epithelium (arrow) with mild submucosal mononuclear cell infiltrates. (B) CRSsNP sinus mucosa has segmental loss and attenuation of the surface epithelium (arrow) with mild accumulations of mononuclear cell infiltrates and marked submucosal edema (E). (C) CRSwNP sinus mucosa also has segmental attenuation of the surface epithelium (black arrow) with marked submucosal edema (E) and substantial eosinophil infiltrates (white arrow). *Hematoxylin and eosin stain, Mag 400×*.

Fig. 3.

Representative section of Control (A), CRSsNP (B) and CRSwNP (C). CRS with and without polyposis has an attenuated surface epithelium with variable expression of epithelial and submucosal aquaporin. (A) Normal sinus mucosa has a pseudostratified, columnar epithelium (arrow) with strong nuclear and cytoplasmic staining (dark brown) for AQP5. Submucosal mononuclear cell infiltrates are mild and submucosal glands also stain positive for AQP5. (B) CRSsNP sinus mucosa demonstrates a mild surface epithelial hyperplasia with decreased AQP5 staining (arrow). Dense infiltrates of mononuclear cells in the submucosa show evidence of macrophages and decreased staining of AQP5 in lymphocytes. (C) CRSwNP sinus mucosa demonstrating light staining for AQP5 in the surface epithelium (arrow). *Immunohistochemical stain, Mag 400×*.

Fig. 4. Immunohistochemical analysis demonstrating AQP5 expression in healthy, CRSwNP and CRSsNP tissue. Note the decrease in epithelial AQP5 expression in CRSwNP.

Fig. 5. Western blot analysis shows a trend towards decreased global expression of AQP5 in CRSwNP (although not statistically significant). The global expression of AQP5 in CRSsNP was slightly increased, but not significant, when compared to control.

Sample	SEPT2	AQP5	Cadherin
CRSwPolyps	29.58	32.7	32.37
CRSsPolyps	29.43	30.9	32.95
Control	28.92	28.8	32.14

Fig. 6.

RT-PCR analysis of selected genes from human sinonasal control and CRS samples. The CRSwNP group shows a significantly higher CT value than control and CRSsNP, which indicates a lower AQP5 expression. (Note: the *higher* the average CT value, the *lower* the expression of that particular gene). Our data show that CRSwNP patients had about 2-fold less AQP5 gene expression than CRSsNP cases, and about 10-fold less than control patients.