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Reduced Expression of Antimicrobial PLUNC Proteins in Nasal Polyp Tissues of patients with Chronic Rhinosinusitis

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Abstract

Background—Chronic rhinosinusitis (CRS) is a disease characterized by inflammation of the nasal mucosa and paranasal sinuses. This inflammation may result in part from decreased epithelial barrier and innate immune responses, leading to frequent bacterial and fungal colonization. The objectives of this study were to investigate the expression of innate immune proteins of the Palate Lung and Nasal epithelium Clone (PLUNC) family in patients with CRS.

Methods—Nasal tissue samples were collected from control subjects and CRS patients with and without nasal polyps. Expression of the members of the PLUNC family was analyzed by real-time PCR. Expression of SPLUNC1 and LPLUNC2 proteins was analyzed by ELISA, immunoblot and immunohistochemical analysis.

Results—Levels of mRNA for most of the members of the PLUNC family were profoundly reduced in nasal polyps (NPs) compared to uncinate tissue from control subjects or CRS patients. LPLUNC2 and SPLUNC1 proteins were decreased in NPs of CRS patients compared to uncinate tissue from control subjects. Immunohistochemical data revealed that within submucosal glands of sinonasal tissues, SPLUNC1 and LPLUNC2 were differentially expressed, in serous and mucous cells, respectively. The decrease in expression of these molecules is probably explained by a decrease in the number of glands in NPs as revealed by correlations with levels of the glandular marker lactoferrin.

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Conflict of Interest
All authors declare no conflict of interest.

Author Contribution
SS and RPS designed the study. SS, DCL, MR and RGC performed the experiments; SS analyzed the data with the help of DCL and MR. AK performed the microarray analysis. JEN, LS, KEH, RKC, ATP, BKT, DBC, LCG, RCK helped in sample collection and evaluation. HWC provided SPLUNC1 standard for ELISA. MR, RKC, LCG, RCK and BKT critically revised the manuscript. All authors have read and approved the final form of the manuscript. SS, DCL and RPS wrote the manuscript.

Conclusions—Decreased SPLUNC1 and LPLUNC2 in NPs reflects a profound decrease in the number of submucosal glands. Decreased glands may lead to a localized defect in the production and release of glandular innate defense molecules.

Keywords

Chronic rhinosinusitis; innate immunity; LPLUNC2; nasal polyps; SPLUNC1

INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most highly prevalent chronic diseases. CRS afflicts up to 14 percent of Americans, resulting in an expenditure of over 4.3 billion dollars and 200,000 sinus surgeries annually (1–3). In Europe, a multicentre study involving 12 countries (GA^{2}LEN), found the overall prevalence of CRS (by EP^{3}OS criteria) to be 10.9% (4). CRS is characterized by chronic mucosal inflammation of the nose and paranasal sinuses confirmed by nasal endoscopy or sinus CT scan (5). This condition can be further subdivided into 2 entities: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).

The pathogenesis of CRS is thought to reflect a complex interplay between host factors, consisting of the innate and adaptive immune responses, and environmental factors, including fungal or bacterial colonization, biofilms, presence of superantigens, osteitis, and allergen exposure (6). Epithelium plays a role beyond providing a physical barrier as a source of constitutive and induced antimicrobial factors. We have proposed that dysregulation of defense pathways results in a defective response to environmental triggers, thus contributing to the pathogenesis of CRS (7, 8).

Short, palate, lung, and nasal epithelial clone 1(SPLUNC1) is a member of the PLUNC family of proteins, which are structural homologues of the LPS binding innate defense proteins lipopolysacccharide binding protein (LBP) and Bactericidal/permeability-increasing protein (BPI) (9, 10). SPLUNC1 is expressed in the epithelium and submucosa of the nasopharynx and is secreted into the nasal cavity, as evidenced by the high concentrations detected in nasal lavage fluid (9, 11–13). Functional studies have revealed that SPLUNC1 can bind to the lipid A portion of LPS and inhibit the growth of *Pseudomonas aeruginosa* and *Mycoplasma pneumonia* (Mp) and presumably other gram negative organisms (14–18). Moreover, SPLUNC1 has been shown to suppress inflammation and conversely, inflammatory cytokines also reduce SPLUNC1 expression and Mp clearance (16, 19). Recent evidence has elucidated a role of SPLUNC1 as an extracellular inhibitor of epithelial Na Channel (ENaC) activity, thus altering airway hydration and increasing mucous clearance (20). The hydrophobicity of SPLUNC1 allows it to act as a surfactant, capable of dispersing matrix encased-biofilms of *P. aeruginosa in vitro* (21). Thus, SPLUNC1 has immunoregulatory, antimicrobial and surfactant properties that make it an important molecule in the lining fluid of the nasal cavity.

In the current study, we tested the PLUNC family of proteins for impairment in CRS. Despite the obvious importance of this family of molecules in the nasal mucosa, this represents the first comprehensive evaluation of the family in the nose and sinuses. After an initial screen of mRNA levels of PLUNC family proteins in disease and normal tissue, we focused on the two most highly expressed, SPLUNC1 and LPLUNC2, to elucidate their role in CRS.

METHODS

Patients and Specimens

CRS patients were recruited from the clinics at Northwestern University using protocols that were approved by the Institutional Review Board of Northwestern University and all subjects gave informed consent. Patients were diagnosed with CRS using task force guidelines (6, 22). Nasal tissues were obtained from defined anatomical site (uncinate and nasal polyps) by functional endoscopic sinus surgery from CRS patients who failed conservative medical therapy (saline irrigations, decongestants, prolonged treatments with antibiotic and/or steroids). Some patients had been on steroids within 2 weeks of surgery. Normal control nasal tissues were similarly obtained from patients who underwent skull based tumor excision. Control patients did not have any history of upper airway inflammatory diseases. Subjects with fungal sinusitis, established immunodeficiency, Churg-Strauss syndrome or cystic fibrosis were excluded from the study. Characteristics of the study population are shown in Table I.

Microarray and Real-time PCR

A comprehensive microarray analysis was performed as described previously and gene expression was measured with GeneChip Human U133 Plus 2.0 probe arrays (Affymetrix) (23). Detailed protocols for microarray and real-time PCR are provided in the supporting information. All microarray data has been deposited to gene expression omnibus : GSE36830

ELISA and Immunoblots

A SPLUNC1 sandwich ELISA was developed in our laboratory with anti-SPLUNC1 antibodies (R&D Systems, MN). A lactoferrin ELISA was purchased from Oxis International Inc (CA). As there was no commercially available ELISA for LPLUNC2, we used immunoblot analysis to detect LPLUNC2 (Proteintech, IL). Detailed procedures are provided in the supporting information.

Collection and extraction of proteins from sinus tissue and nasal lavage fluids (NLF)

NLF and sinonasal tissue proteins were collected and extracted as described previously (24). Detail procedures are provided in the supporting information.

Immunohistochemistry

The basic protocol for Immunohistochemistry has been described previously (24). Detailed procedures are in the supporting information.

Statistical analysis

All data are presented as mean±SEM. Comparisons were made using a Mann-Whitney U test. Correlations were assessed by Spearman Rank correlation. All statistical analyses were performed using GraphPad prism 5.0 software. A P value of less that 0.05 was considered statistically significant.

RESULTS

Screen of the PLUNC family in sinonasal tissues; decreased levels in nasal polyps

We performed a microarray analysis to compare global gene expression in uncinate tissue from control, CRSsNP, CRSwNP patients and polyp tissue from CRSwNP patients. We observed that mRNA levels of the PLUNC family were differentially expressed in various regions of sinonasal mucosa. We also found that the expression of some of these proteins

was decreased in polyps of patients with CRSwNP (Fig E1). We confirmed decreased expression of these proteins using real-time PCR (Fig 1). Expression of mRNA for SPLUNC1, LPLUNC1, LPLUNC2, LPLUNC6 and BPI was significantly reduced in the polyps of patients with CRSwNP compared to uncinates of either control subjects or patients with CRS ($P<0.05$, n=10–11). Surprisingly, we found no differences in the expression of most of these molecules when comparing only uncinates of control subjects and patients with CRS, with the exception of BPI ($P=0.05$) and LPLUNC4 ($P<0.05$), indicating a possible global decrease of these two molecules in patients with CRS. As SPLUNC1 and LPLUNC2 were most highly expressed in uncinate tissues and their expression was decreased in polyps of patients with CRSwNP, we decided to confirm the decrease in their expression at the protein level.

Decreased protein expression of SPLUNC1 and LPLUNC2 in polyps of patients with CRSwNP

To confirm the mRNA findings, we analyzed the expression of SPLUNC1 protein by ELISA in extracts of 39 sinonasal tissues from control, CRSsNP and CRSwNP patients. We detected substantial expression of SPLUNC1 but there was no difference in the expression of SPLUNC1 protein in uncinates of normal controls and patients with CRS, supporting our mRNA data. Importantly, we found a 90% reduction in the expression of SPLUNC1 protein in nasal polyps of patients with CRSwNP compared to normal control uncinate tissue (Fig 2, A, $P<0.05$, n= 9–10). We used immunoblot analysis to analyze the expression of LPLUNC2 in 25 sinonasal tissues from control, CRSsNP and CRSwNP patients. As observed for SPLUNC1, we did not find any difference in the expression of LPLUNC2 among the various uncinate tissues. However, we found polyps to have a significant decrease in the level of expression of LPLUNC2 protein compared to control uncinate tissue, supporting our mRNA data (Fig 2, B and C, $P<0.05$, $n\geq5$). Due to semi-quantitative nature of western blots, errors due to differential transfer and quantitation may affect the linearity of the assay; future studies required involve developing ELISA assays for quantifying LPLUNC2. These data collectively indicate that polyps of patients with CRSwNP have profoundly decreased expression of SPLUNC1 and LPLUNC2 compared to control uncinate tissue.

Immunohistochemical analysis indicates that SPLUNC1 and LPLUNC2 are differentially expressed in submucosal gland cell types and epithelium of sinonasal tissues

We performed immunohistochemistry to determine the localization of SPLUNC1 and LPLUNC2 in sinonasal tissues. We compared the expression of SPLUNC1 and LPLUNC2 in uncinates of controls, CRSsNP, CRSwNP patients and in polyps of CRSwNP patients. Our staining was specific, as we did not observe any staining with an IgG control (Fig 2, D and H inset). Both SPLUNC1 and LPLUNC2 were highly expressed in the submucosal glands and moderately expressed in the respiratory epithelial cells of the normal uncinate tissues. Upon scoring for staining intensity, we found no differences in glandular and epithelial intensity of SPLUNC1 staining in polyps of patients with CRSwNP compared to uncinate tissues of control subjects or CRS patients (Fig 2, D–G and Fig E2, Top). In contrast, glandular staining intensity of LPLUNC2 was slightly reduced in polyps of patients with CRSwNP compared to control uncinates ($P=0.06$, Fig 2, H–K and Fig E2, Bottom). These observations indicate that submucosal glands may be the major source of both SPLUNC1 and LPLUNC2 in sinonasal tissues.

To explore the cellular localization of SPLUNC1 and LPLUNC2 within the submucosal glands, we used both immunohistochemical analysis and AB/PAS staining in serial sections of control uncinate tissues. AB/PAS staining, which stains neutral and acidic mucins, indicated that both mucous and serous cells were found within the glands of the sinonasal tissues (Fig 3, B and E). SPLUNC1 staining was observed in regions with minimal or no

AB/PAS staining, suggesting that SPLUNC1 is mainly produced by the serous cells of the submucosal glands (Fig 3, C and F). In contrast to SPLUNC1 staining, LPLUNC2 staining was restricted to regions that stained with AB/PAS, indicating that LPLUNC2 is contained mainly within the mucous cells of the submucosal glands (Fig 3, A and D). This indicates that SPLUNC1 and LPLUNC2 are produced by different cells lining the submucosal glands. Of note, we analyzed expression of SPLUNC1 and LPLUNC2 in NLF of control subjects and patients with CRSsNP and CRSwNP by ELISA and immunoblotting, respectively. However, we did not observe any differences in the levels of SPLUNC1 or LPLUNC2 in NLF of patients with CRS compared to control (Fig E3). Thus from these observations, we conclude that the suppression of levels of SPLUNC1 and LPLUNC2 in patients with CRS is restricted to the nasal polyp itself and not global within the sinonasal cavity.

Decrease in the number of glands explains reduced SPLUNC1 and LPLUNC2 expression in polyps

We tested whether reduced expression of SPLUNC1 and LPLUNC2 may be due to a decrease in the number of glands in polyps. We stained tissues with AB/PAS and determined the number and morphology of glands. Nasal polyps from patients with CRSwNP had an 81% decrease in the number of glands compared to control uncinates (Fig 4, A–C). We also observed that polyps had glands that appeared to be stretched and elongated.

Expression of lactoferrin, a serous cell marker, is decreased in polyps of patients with CRS

To confirm the decreased number of glands in polyps, we analyzed the expression of a glandular marker lactoferrin, which is mainly produced by the serous cells of the submucosal glands (25). In support of our findings with SPLUNC1, we found reduced levels of mRNA for lactoferrin (Fig 4, D) in nasal polyps of patients with CRSwNP compared to uncinate tissues from control or CRSsNP or CRSwNP patients. We confirmed this finding at the protein level for lactoferrin (Fig 4, E). Levels of mRNA for SPLUNC1 significantly correlated with lactoferrin ($r=0.664$, $P<0.0001$, $n=42$) supporting the conclusion that lactoferrin and SPLUNC1 are contained within the serous cells of the submucosal glands (Fig E4, A). Although neutrophils are often present in sinonasal tissues and have been shown to produce lactoferrin, we observed a negative correlation between the expression of a neutrophil marker (CXCR1) and lactoferrin ($r=-0.403$, $P=0.0081$, $n=42$), suggesting that neutrophils are not a major source of lactoferrin in sinonasal tissue (Fig E4, B). These data collectively suggest that the decreased expression of SPLUNC1 in nasal polyp tissues of patients with CRSwNP is due to a decrease in the number of glands in nasal polyps.

DISCUSSION

Recent studies have suggested that dysfunction of the innate immune system may play a permissive role in CRS pathogenesis, creating an environment that fosters increased microbial colonization (7, 8). The PLUNC family of proteins came into interest initially due to reports of high concentrations of SPLUNC1 in NLF and to their structural similarities to the antimicrobial protein BPI, suggesting potential antimicrobial and endotoxin neutralization functions (9). Subsequent studies demonstrated a profound antimicrobial effect of SPLUNC1 (14, 18). The present study demonstrates decreased mRNA expression for most, members of the PLUNC family in nasal polyps when compared with uncinate tissue of either control or CRS patients. This decrease in PLUNC family expression was generally restricted to the polyp itself. The PLUNC family members LPLUNC4 and BPI were exceptions, with lower expression in uncinate tissue from both CRSsNP and CRSwNP

patients. Further study will be required to assess whether there are global defects in production of these two molecules in CRS.

In the present study, we focused our efforts on the two most highly expressed PLUNC family molecules in sinonasal tissue, SPLUNC1 and LPLUNC2. Using SPLUNC1 ELISA and LPLUNC2 immunoblot analysis, we were able to confirm reduced levels in nasal polyp tissue. In a previous report using proteomics technology, Min-man et al. concluded that the intensity of SPLUNC1 staining was decreased in nasal polyp tissue (26). The current study confirms this finding and extends it to demonstrate that the decreased expression in polyp tissue was due to a decreased number of glands. It has been previously reported that some nasal polyps have a lower density of glandular structures than the surrounding ethmoid tissue (27). SPLUNC1 and LPLUNC2 were strongly expressed in submucosal glands and moderately expressed in surface epithelium. Remarkably, we observed that SPLUNC1 was localized in the serous cells and LPLUNC2 was selectively localized in the mucous cells within the submucosal glands. This suggests differential release of these host defense proteins during basal and stimulated conditions.

Based on our findings of decreased expression of glandular proteins in nasal polyp tissue, we propose that glandular hyperplasia associated with CRS, if it occurs, may be restricted to certain forms of polyps or to tissues other than the polyp itself (27). Our findings strongly suggest that the decreased SPLUNC1 in polyps is an effect of formation of the polyp without an accompanying expansion of submucosal glands. We detected substantial amounts of SPLUNC1 and LPLUNC2 in NLF and despite the decreased production of these molecules in nasal polyps, we failed to observe any differences in expression of these molecules in NLF of patients with CRS compared to control subjects. Since NLF is collected as a wash of the entire sinonasal cavity, the presumed reduction of SPLUNC1 and LPLUNC2 on the surface of the polyps may not influence the total collection. We speculate that other methods of collection of nasal lining fluid, such as using filter paper discs or sponges will detect reduced SPLUNC1 on the polyp surface (28, 29).

Some current theories of CRS pathogenesis have focused on defects in production of innate host defense proteins, barrier defects, mucociliary dysfunction, and a role for superantigens. More recently our laboratory published reduction in innate immune molecules of the S100 family in CRS (30). In the present study we show that polyps of patients with CRS have a defect in the local production of proteins of the PLUNC family and lactoferrin. Despite SPLUNC1's homology to BPI and its ability to bind LPS, it has been tested only with a few organisms (14, 16–18, 31). Recent findings, however suggest that SPLUNC1 may also be involved in suppressing *Pseudomonas* biofilm formation (21). Biofilms are a leading cause of decreased efficacy of antibiotics, which in turn may lead to increased colonization by bacteria in CRS (32, 33). Our findings thus suggest that reduced PLUNC proteins on the surface of a nasal polyp may increase susceptibility to colonization by microorganisms that form biofilms or are otherwise sensitive to PLUNCs. Based on its LPS binding ability, we speculate that SPLUNC1 may impact additional organisms other than *Mycoplasma* and *Pseudomonas*.

There are other mechanisms by which reduced PLUNC might alter sinonasal physiology or inflammation. Published studies suggest SPLUNC1 is an anti-inflammatory protein, suppressing the ova-alum model of allergic inflammation (19). It could be speculated that the increased inflammation seen in polyps may thus relate in part to decreased SPLUNC1 expression. Also of interest is recent research showing that SPLUNC1 has a role in suppressing the activation of epithelial sodium channels (ENaC), which has implications in mucociliary clearance as well as ion and fluid influx into mucosal tissue (20, 34). A decrease of SPLUNC1 in polyp tissue may result in the local over-activation of ENaC, leading to

transepithelial water transport and contributing to polyp formation. Of note, cultured nasal polyp epithelial cells show a greater rate of transepithelial ion transport, suggesting that the movement of water into the cell and into the interstitial tissue could explain the edema commonly seen in polyps (35). The dysregulation of ENaC and surface liquid levels also impairs mucociliary clearance, another mechanism by which pathogens are able to thrive and cause chronic inflammation. Recent studies have implicated SPLUNC1 in mucociliary clearance *in vivo* (31). Based on the multifaceted functional abilities of SPLUNC1, we hypothesize that reduction in SPLUNC1 may contribute to CRS pathogenesis via loss of its physicochemical effects as much as via loss of its antimicrobial or LPS neutralizing effects.

The functions of other proteins of the PLUNC family are not yet elucidated. Based on protein sequence homology with BPI, and their abundance at mucosal surfaces and NLF, it is thought that this family may be involved in mucosal innate host defense (7, 8). Further functional analysis needs to be undertaken to elucidate the specific roles of each of these molecules in host defense and in CRS. Our results and their implications for localization of host defense molecules are summarized in Figure 5. To the extent that they are important in immunity, the decrease in the expression of PLUNCs, and other glandular proteins such as lactoferrin and lysozyme, in nasal polyps may play a contributory role in the increased bacterial colonization of the nasal mucosa in CRSwNP patients. In conclusion, this study demonstrates markedly suppressed expression of PLUNC family members in nasal mucosa of CRS patients, particularly in nasal polyp tissue. Decreased SPLUNC1, theoretically associated with decreased ability to clear pathogens and dysregulation of ionic balance, may contribute to the chronic inflammatory response and subsequent pathogenesis of nasal polyps in CRSwNP patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CRS	Chronic rhinosinusitis
CRSwNP	CRS with nasal polyps
CRSsNP	CRS without nasal polyps
UT	Uncinate tissue
PLUNC	Palate LUng Nasal epithelial Clone
SPLUNC1	Short PLUNC1
LPLUNC2	Long PLUNC2
BPI	Bactericidal/Permeability-Increasing Protein
NLF	Nasal lavage fluid

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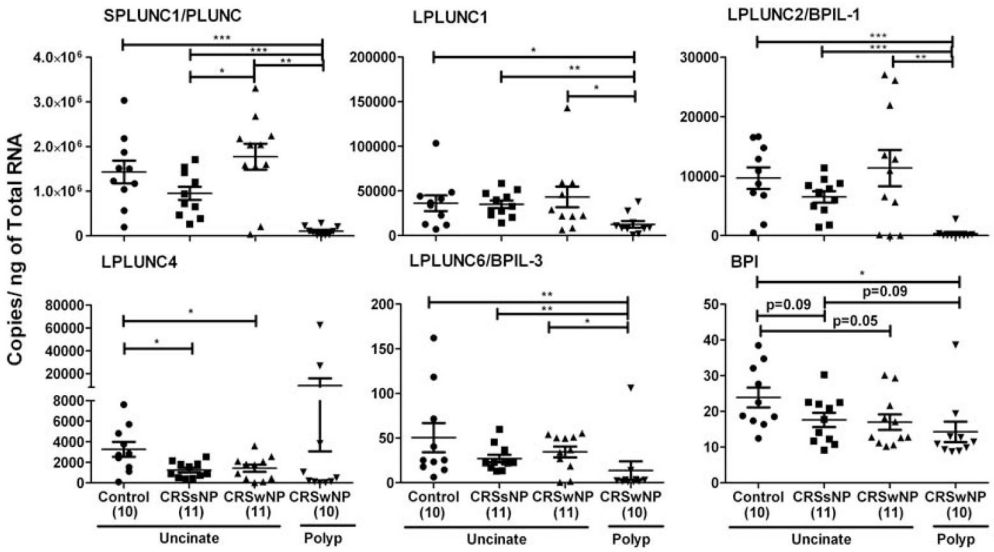

Figure 1. Evaluation of mRNA expression of the BPI-PLUNC family of proteins in sinonasal tissues

Total RNA was extracted from uncinates of control subjects and patients with CRSsNP or CRSwNP, and polyps of patients with CRSwNP. Expression of mRNA was analyzed by real-time PCR. Expression was normalized to the median value of β-glucuronidase and expressed as copies/ng of total RNA. *P<0.05, **P<0.01, ***P<0.001

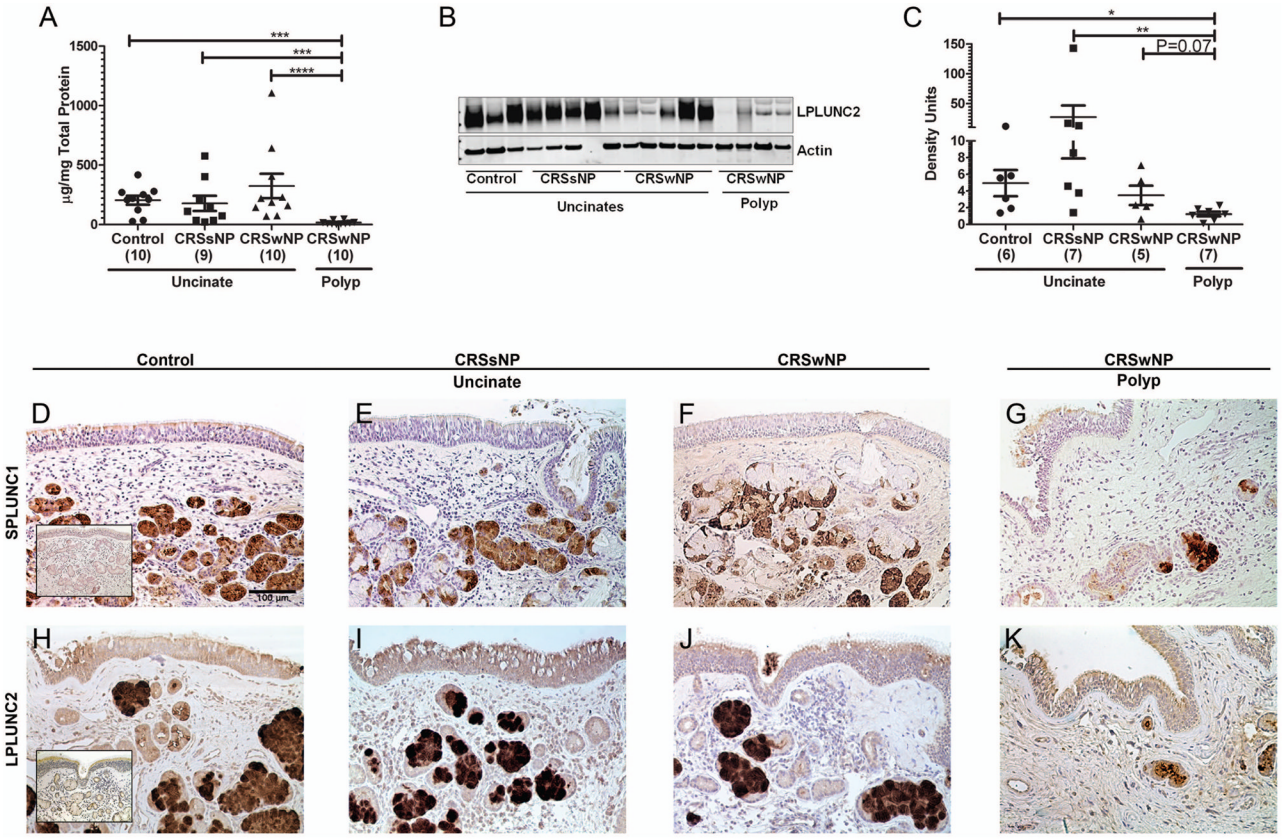

Figure 2. Evaluation of protein expression and localization of the two highly expressed PLUNC family members by ELISA (SPLUNC1), immunoblot (LPLUNC2) and immunohistochemistry

Tissue extracts of uncinates from control subjects and patients with CRSsNP or CRSwNP, and polyps from patients with CRSwNP were used to analyze the concentration of SPLUNC1 protein by ELISA (A) and LPLUNC2 protein by immunoblot analysis (B and C). A representative immunoblot for LPLUNC2 (49 kDa) and Actin (42 kDa) (B). Densitometry analysis of LPLUNC2 immunoblots (C). Representative SPLUNC1 (D–G) and LPLUNC2 (H–K) immunostaining in uncinate of a control subject (D and H), a CRSsNP patient (E and I), a CRSwNP patient (F and J) and a polyp from a CRSwNP patient (G and K). Inset in D and H shows control IgG staining for SPLUNC1 and LPLUNC2 respectively. Representative of 7–10 donors per group shown as 200X. *P<0.05, **P<0.01, ***P<0.001

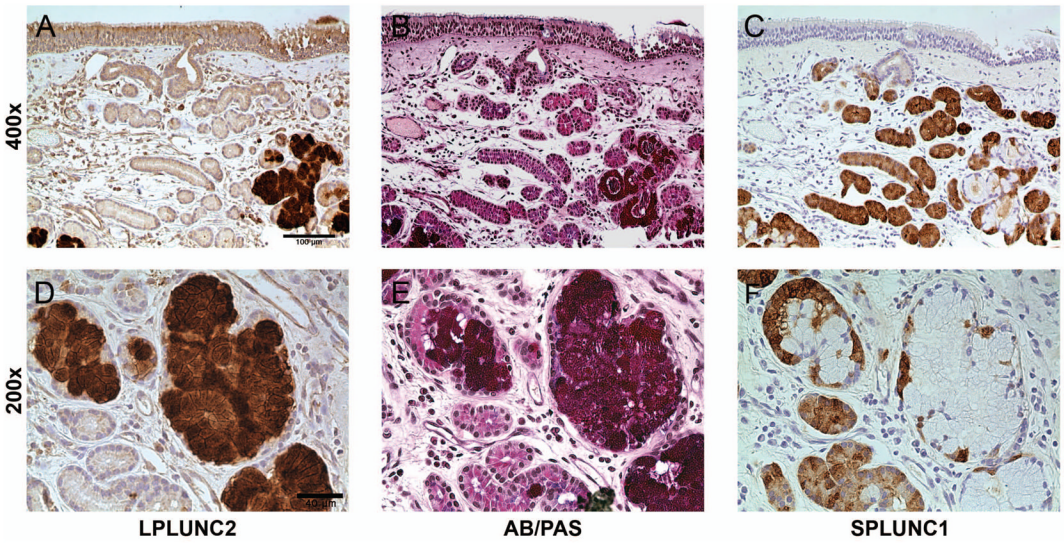

Figure 3. Evaluation of sequential staining for AB/PAS, SPLUNC1 and LPLUNC2 in sinonasal tissue

Serial sections (3 μm) of uncinate tissues from normal patients were used to stain with LPLUNC2 antibody (A and D), AB/PAS (B and E) or SPLUNC1 antibody (C and F). Shown is a representative subject out of 4 control subjects, represented as 200X (A–C) and 400X (D–F).

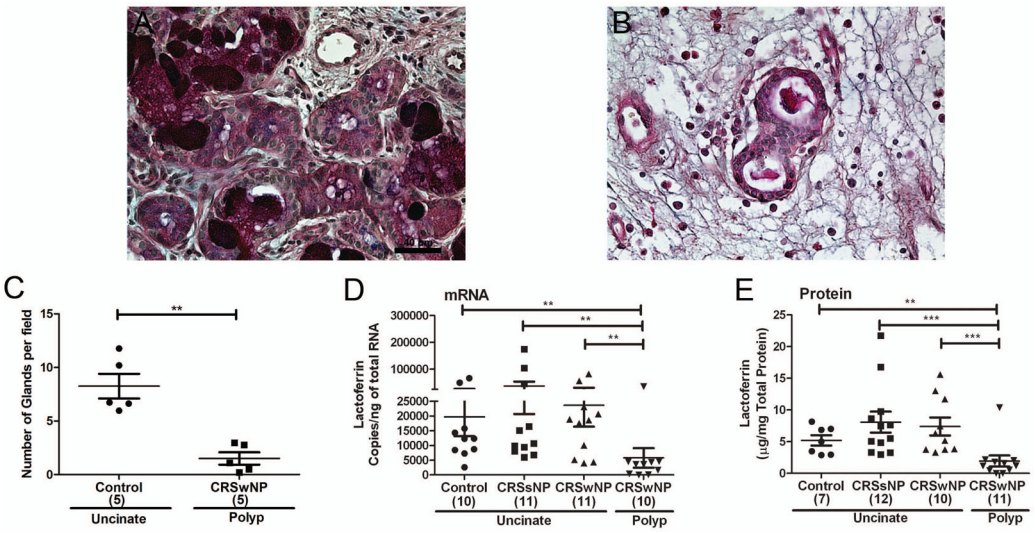

Figure 4. Evaluation of the number of glands and expression of lactoferrin in sinonasal tissue

Representative AB/PAS staining in uncinate from a control subject (A) and a polyp from a patient with CRSwNP (B). Quantitation of the number of glands in tissues stained with AB/PAS in uncinate from control subjects and polyps from patients with CRSwNP (C) (n=5, 10 fields per slide). Lactoferrin expression in sinonasal tissue was assessed by real-time PCR (D) and ELISA (E). **P<0.01, ***P<0.001

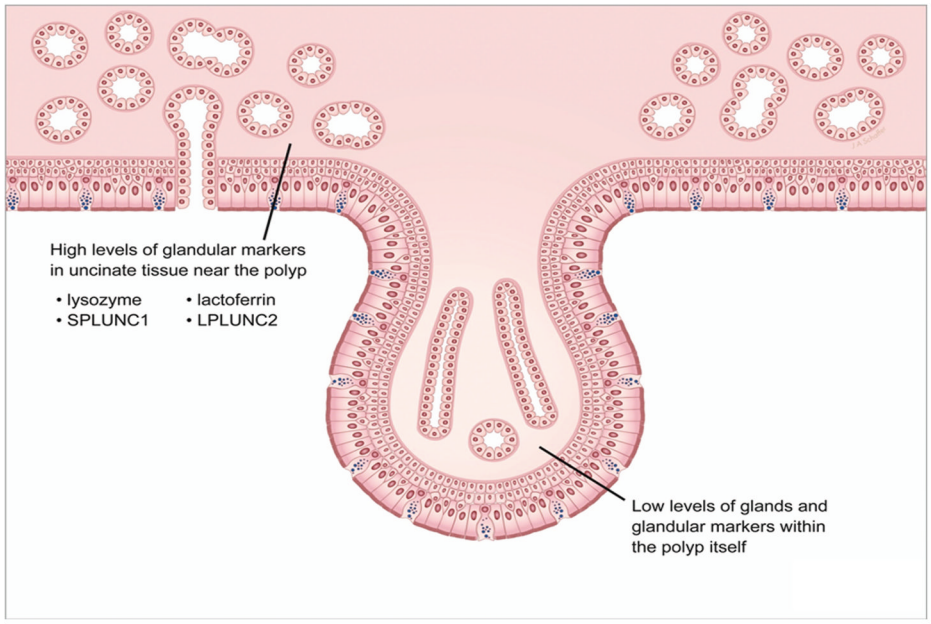

Figure 5.

Decrease in the number of glands in nasal polyps of patients with CRSwNP may lead to a localized defect in the production of antimicrobial proteins such as lactoferrin, lysozyme and proteins of the PLUNC family.

Table I

Subjects' characteristics

	Control			CRSsNP			CRSwNP		
Total no. of subjects	**48 (25M)**			**59(27M)**			**81 (59M)**		
Age (y), median (range)	**43 (16–77)**			**38(18–64)**			**41(22–74)**		
	Y	**N**	**U**	**Y**	**N**	**U**	**Y**	**N**	**U**
Atopy	3	44	1	30	23	6	45	26	10
Asthma	0	47	1	11	44	4	38	43	0
PCR	Uncinate			Uncinate			Uncinate		Polyp
No. of subjects	10 (3M)			11 (6M)			11 (9M)		10 (7M)
Age (y), median (range)	42 (16–62)			34 (23–64)			39 (27–61)		48.5 (28–74)
SPLUNC1 (PLUNC)									
ELISA-Lavage									
No. of subjects	18 (9M)			18 (6M)			15 (11M)		
Age (y), median (range)	33(17–63)			38 (28–60)			44 (30–70)		
ELISA-Extracts									
No. of subjects	10 (4M)			9 (1M)			10 (7M)		10 (7M)
Age (y), median (range)	52.5 (16–72)			36 (28–62)			40 (22–74)		41 (26–74)
IHC									
No. of subjects	10 (3M)			9 (5M)			9 (8M)		10 (7M)
Age (y), median (range)	54 (25–77)			27 (21–50)			35 (29–53)		35.5 (22–63)
LPLUNC2 (BPIL-1)									
IB-Lavage									
No. of subjects	10 (6M)			10 (4M)			6 (5M)		
Age (y), median (range)	33 (21–44)			38.5 (28–60)			52.5 (33–68)		
IB-Extracts									
No. of subjects	6 (1M)			7 (3M)			5 (3M)		7 (7M)
Age (y), median (range)	48.5 (16–63)			49 (18–63)			41 (34–52)		42 (32–74)
IHC									
No. of subjects	9 (6M)			10 (6M)			8 (6M)		7 (6M)

	Control			CRSsNP			CRSwNP		
Total no. of subjects	**48 (25M)**			**59(27M)**			**81 (59M)**		
Age (y), median (range)	**43 (16–77)**			**38(18–64)**			**41(22–74)**		
	Y	**N**	**U**	**Y**	**N**	**U**	**Y**	**N**	**U**
Age (y), median (range)	58 (19–64)			34 (21–54)			54 (34–72)		50 (27–72)
Lactoferrin									
ELISA-Extracts									
No. of subjects	7 (5M)			12 (5M)			10 (6M)		11 (8M)
Age (y), median (range)	45 (19–62)			36 (21–62)			40 (30–68)		40 (26–64)

M:male, Y:yes, U: unknown, IHC: immunohistochemistry, IB: immunoblot