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Expression of Iron-Regulatory Hormone Hepcidin and Iron Transporters Ferroportin and ZIP8 in Patients With and Without Chronic Rhinosinusitis

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Abstract

Airway epithelia express intrinsic antimicrobial and nutrient-sequestering factors, which contribute to the host defense of the respiratory tract. Hepcidin is an endogenous peptide hormone that serves as a key regulator of iron metabolism, and ferroportin and ZIP8 are iron transporters. All exhibit innate antimicrobial activity. The purpose of this pilot study is to determine if molecules involved in iron regulation are expressed within sinus epithelia and to compare levels of expression between patients with and without chronic rhinosinusitis (CRS). Sinus mucosa was obtained from patients with (n = 19) and without (n = 14) CRS. Real-time polymerase chain reaction following RNA extraction was used to quantify expression of hepcidin, ferroportin, and ZIP8 mRNA. Hepcidin, ferroportin, and ZIP8 were all detected in the sinus epithelia of patients with and without CRS. However, only ZIP8 was significantly changed in CRS, with a 2.5-fold mean increase in mRNA expression relative to controls ($P = .005$). These findings suggest that ZIP8 may play a role in the innate epithelial defense of the paranasal sinuses.

Keywords

chronic rhinosinusitis; nasal polyposis; innate immunity; epithelia; iron regulation

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Author Contributions

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Competing interests: None.

Chronic rhinosinusitis (CRS) is a sinonasal inflammatory disease with significant impact on quality of life.[1] Although the etiology remains unclear, mucosal inflammation and disruption of the sinonasal microbiome have been implicated in its pathogenesis.[2] Airway epithelia express intrinsic antimicrobial/nutrient-sequestering factors, which contribute to host defense of the respiratory tract. Essential elements such as iron are indispensable for life. Host iron sequestration at the extracellular and epithelial levels has been shown to help combat infection.[3] However, pathogens have developed specialized mechanisms to siphon iron from the host through "iron piracy," facilitating biofilm formation.[3] Thus, there is a constant competition between the host and the invading microbes for important nutrients. The role of these elements, specifically iron, has not been fully elucidated in CRS.

Iron modulators, including hepcidin, ferroportin, and ZIP8, dictate iron regulation and availability during inflammation and infection. Hepcidin is an iron-regulatory hormone that blocks iron export to extracellular fluid by binding to ferroportin, a cellular iron exporter. During infection, a cytokine-driven increase in hepcidin results in downregulation of ferroportin to sequester iron into host cells.[3] ZIP8 is a transmembrane metal ion import protein that is induced during activation of monocytes.[4] It mediates uptake of nutritionally important divalent metals, including iron, and regulates iron levels during sepsis.[4] These nutrient regulatory mechanisms serve as innate immune functions to starve microbes of essential iron and attenuate infection. The purpose of this pilot study is to investigate whether molecules involved in iron regulation are expressed in sinus epithelia and to compare levels of expression between patients with and without CRS.

Methods

Specimen Collection

Maxillary mucosa was obtained during endoscopic sinus surgery from 19 adults (≥18 years) with CRS who had failed appropriate medical therapy: 11 patients had nasal polyps and 8 did not. CRS was defined per the 2015 clinical practice guidelines of the American Academy of Otolaryngology–Head and Neck Surgery. Computed tomography demonstrated partial or complete opacification of the maxillary sinus from which the specimen was procured. Sphenoid mucosa was acquired from 14 adult patients undergoing transsphenoidal pituitary surgery.[5-8] These patients were designated as controls given their lack of clinical history and endoscopic and radiographic evidence of CRS.[5-8] This study was approved by the University of California–Los Angeles Institutional Review Board, and informed consent was obtained.

RNA Isolation and Real-time Quantitative Polymerase Chain Reaction

Total RNA was isolated from sinus mucosa samples and analyzed by real-time polymerase chain reaction (PCR).[9] The mRNAs encoding the ribosomal protein L32 and the protein HPRT were used as housekeeping genes, similar to other studies.[7,10] Housekeeping genes are endogenous controls whose expression levels do not change in different tissue types or pathophysiologic conditions (eg, CRS). Primers for housekeeping and target genes (listed in Table 1) were used to reverse transcribe mRNA into cDNA, which was then amplified by real-time PCR. Levels of gene expression of hepcidin, ferroportin, and ZIP8 were

compared with each housekeeping gene per the ΔCt (threshold cycle) method, where ΔCt = Ct (housekeeping gene) – Ct (target gene).[11] Ct represents the PCR cycle at which the fluorescence level reaches a certain threshold. Higher ΔCt indicates a relatively higher target gene expression versus control. The $2^{-\Delta\Delta Ct}$ is defined as the fold change in target gene expression, in which <1.0 indicates downregulation versus housekeeping mRNA expression and >1.0 represent upregulation. Statistically significant differences ($P < .05$) in ΔCt among patients with CRS as compared with healthy controls were determined with a ranked *t* test.

Results

The 2 housekeeping genes were expressed and their mRNA concentrations correlated closely (R^2 [correlation coefficient] = 0.95). As compared with HPRT mRNA, hepcidin mRNA was expressed in healthy epithelia at an 8-fold lower level (ΔCt = −3.0 ± 2.5, mean ± SD), ferroportin at a 3-fold higher level (ΔCt = 1.7 ± 0.6), and ZIP8 at about the same level (ΔCt = 0.1 ± 0.9; Figure 1). Only ZIP8 was significantly changed in CRS, with an approximately 2.5-fold mean increase in mRNA (ΔCt = 1.3 ± 1.1 in CRS vs ΔCt = 0.1 ± 0.9 in control, $P = .005$) relative to controls. Similar findings were found when compared with L32 mRNA (Figure 2). There were no statistically significant differences in hepcidin, ferroportin, or ZIP8 mRNA expression between patients with CRS with and without nasal polyps.

Discussion

This pilot study is the first to explore the involvement of hepcidin, ferroportin, and ZIP8 in iron regulation in sinus epithelia and to compare levels of mRNA expression in patients with and without CRS. Iron regulation occurs at the extracellular level and epithelial surface. In response to infection/inflammatory stimuli, a cytokine-driven upregulation in serum hepcidin occurs, leading to downregulation of ferroportin.[3] This, in turn, reduces iron transport into extracellular fluid and decreases levels of plasma iron available for microorganisms. In our study, hepcidin and ferroportin were detected in sinus epithelia of patients with CRS and healthy controls in moderate amounts. However, there was no statistically significant difference in hepcidin or ferroportin mRNA expression between the groups. This supports the finding that hepcidin and ferroportin are directly interconnected in iron regulation, as they were not significantly different. However, given that they primarily function in plasma and not in epithelial cells, they may not play an important role in iron regulation in CRS at the epithelial level.

In contrast, ZIP8 was shown to have a 2.5-fold mean increase ($P = .005$) in the sinus epithelia of patients with CRS versus controls. ZIP8 is a transmembrane protein that mediates cellular uptake of divalent metal ions, including iron.[4] It is highly expressed in lung tissue and is upregulated in primary airway epithelial cells, monocytes, and alveolar macrophages in response to lipopolysaccharide and inflammatory cytokines in vitro.[4,12] Thus, the increased expression of ZIP8 observed in the sinus epithelia of patients with CRS is consistent with prior studies examining lower airway tissue in response to inflammation.[12] ZIP8 activity is further coupled with and potentiated by element metabolism and sequestration. Once activated, ZIP8 acts in a feedback loop as a potent

negative regulator of NF-κB, a transcription factor essential for innate immune function. The complex interplay between ZIP8 upregulation and NF-κB inhibition may contribute to pathogenesis of CRS. There were no statistically significant differences in mRNA levels of hepcidin, ZIP8, and ferroportin between patients with CRS with and without nasal polyps. However, the negative findings may be due to the small sample size. Additional studies with a larger number of specimens for each CRS subtype are necessary to delineate their potential role in CRS with and without nasal polyps. Another study limitation was the procurement of mucosa from the sphenoid versus maxillary sinus in controls, as the latter was not opened during pituitary surgery. Although the sphenoid has been used as a control in other studies, further research is needed to determine if there are differences in iron-regulatory protein gene expression among the various sinuses. In our study, we also observed upregulation of ferroportin and ZIP8 as compared with HPRT but downregulation of these same target genes when compared with L32. This finding is due to differences in HPRT and L32 baseline levels of expression. Comparative analysis to both housekeeping genes still corroborated that ZIP8 was upregulated in CRS versus controls, with no differences in hepcidin and ferroportin.

Prior studies have suggested that iron regulation is involved in CRS pathophysiology. Psaltis et al found that patients with CRS have reduced lactoferrin (an iron-binding protein involved in host sequestration of iron from invading pathogens) versus controls, hinting that lower lactoferrin levels may predispose to CRS.[7,8] Kim et al found ferritin (an iron-storing and acute-phase protein) was upregulated in CRS with nasal polyps, suggesting that ferritin may function as a systemic marker of chronic inflammation in CRS with nasal polyps.[13] While these studies underscore the importance of iron regulation in CRS pathogenesis, further research is needed to determine which additional iron-regulatory proteins are involved and how these proteins interact in CRS. An understanding of these mechanistic pathways may aid in developing therapeutic targets that will upregulate host iron sequestration from invading microbes in treatment of CRS.

Conclusion

This pilot study is the first to demonstrate that the iron-regulatory molecules hepcidin, ferroportin, and ZIP8 are present in sinus epithelia of patients with CRS and healthy controls. However, only ZIP8 mRNA expression was significantly increased in the sinus epithelia of patients with CRS relative to controls. These findings suggest that ZIP8 may play a role in the innate epithelial defense of the paranasal sinuses in CRS, thus serving as a springboard for future studies.

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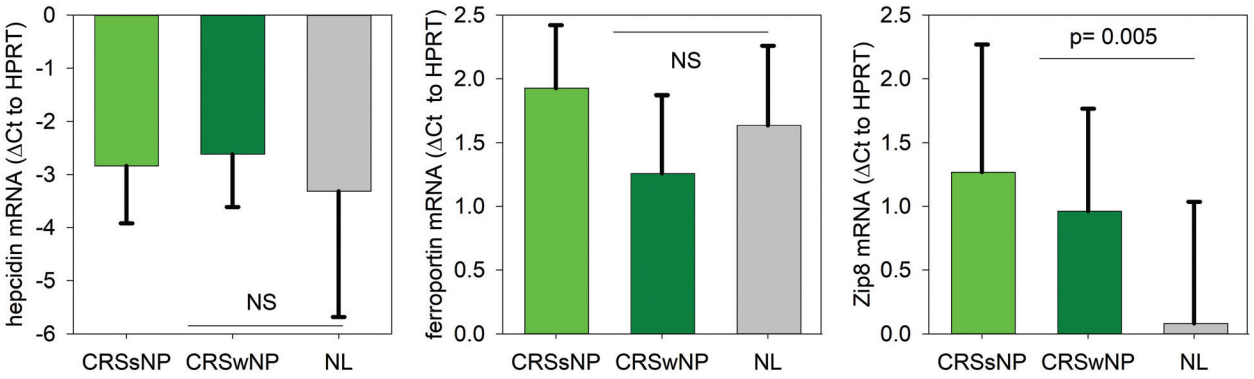

Figure 1.

With HPRT as comparator gene, hepcidin mRNA, ferroportin mRNA, and ZIP8 mRNA expression. ΔCt = Ct (HPRT) – Ct (target gene): relative level of mRNA expression vs HPRT. Mean ± SD values are shown. *P* value by ranked t test, comparing CRSsNP and CRSwNP against NL. CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; NL, normal; NS, not significant.

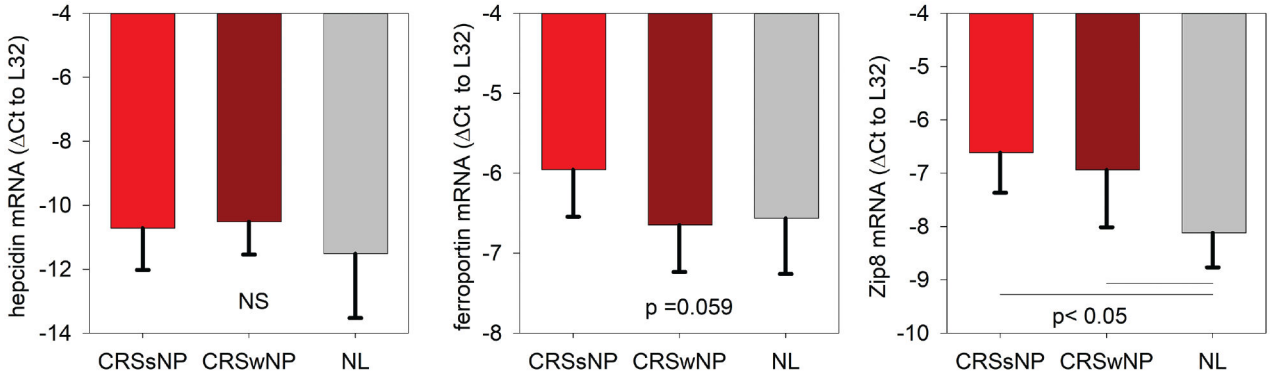

Figure 2.

With L32 as comparator gene, hepcidin mRNA, ferroportin mRNA, and ZIP8 mRNA expression. ΔCt = Ct (L32) – Ct target gene: relative level of mRNA expression vs L32. Mean ± SD values are shown. *P* value by ranked *t* test, comparing CRSsNP and CRSwNP against NL. CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; NL, normal; NS, not significant.

Table 1.

Target and Housekeeping Gene Primers Used for Real-time Polymerase Chain Reaction.

Gene	Forward primer	Reverse primer
Hepcidin	CCAGCTGGATGCCCATGTT	GCCGCAGCAGAAAATGCA
Ferroportin	CATTGCTGCTAGAATCGGTCTT	GCAACTGTGTCACCGTCAAAT
Zip8	CAGTGTGGTATCTCTACAGGATGGA	CAGTTTGGGCCCCTTCAAA
L32	AGTTCCTGGTCCACAACGTC	AGCGATCTCGGCACAGTAAG
HPRT	TGCTTTCCTTGGTCAGGCAG	AAGCTTGCGACCTTGACCAT