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ORIGINAL RESEARCH

Chemokine CCL19 and Its Receptors CCR7 and CCRL1 in Chronic Rhinosinusitis

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Background: CCL19 has been shown to predict disease severity in COVID-19 and treatment response in rheumatoid arthritis. CCL19 can exert both pro- and anti-inflammatory effects and is elevated in chronic rhinosinusitis (CRS). However, its role in CRS remains unknown. This study sought to determine the transcriptional changes in CCL19, its receptors, and associated cytokines and their association with disease severity in CRS.

Methods: A clinical database of control subjects and patients with CRS was examined. Lund-Kennedy, Lund-Mackay, Sinonasal Outcomes Test 22 (SNOT-22), and rhinosinusitis disability index (RSDI) scores were collected at enrollment. mRNA was extracted from sinonasal tissues and subjected to multiplex gene expression analysis. Gene transcript differences between patients with CRS and controls were compared and correlated with disease severity metrics. Immunohistochemical analyses of CCL19, CCR7, and CCRL1 were conducted to compare differences in protein expression between cohorts. A subgroup analysis was performed to compare transcriptional and protein expression difference between patients with (CRSwNP) and without (CRSsNP) nasal polyps and controls.

Results: Thirty-eight subjects (control group, n=7; CRS group, n=31) were included in this study. *CCRL1* (p=0.0093) and *CCR7* (p=0.017) levels were significantly elevated in CRS compared to those in controls. *CCL19* (p=0.038) and *CCR7* (p=0.0097) levels were elevated in CRSwNP and *CCRL1* was elevated in CRSsNP (p=0.0004). CCR7 expression was significantly elevated in sinonasal epithelial cells in CRSwNP (p=0.04). *CCL19* expression was positively correlated with *TNFA* expression (p<0.0002). *CCL19 and CCR7* expression was positively correlated with SNOT-22 and RSDI scores (p<0.05).

Conclusion: CCL19 and CCR7 may modulate TNF-α-driven pro-inflammatory signaling and contribute to increased disease severity in CRS. Mechanistic studies are required to further elucidate the role of CCRL1 in CRS.

Keywords: chronic rhinosinusitis with nasal polyps, chemokines, cytokines, gene expression, protein expression

Introduction

Chemokines have been widely studied to play a pivotal role in the pathogenesis of chronic rhinosinusitis (CRS), a heterogeneous inflammatory disease of the sinonasal cavity. Encompassing a varied group of proteins, chemokines expediate the recruitment of inflammatory cells to tissue sites through the binding of G protein-coupled receptors present on their target cells.[1,2] Increased expression of chemokines can lead to tissue damage and inflammatory diseases by facilitating an overabundance of leukocyte mobilization.[2] The levels of macrophage inflammatory protein-1 alpha (MIP-1α/CCL3), eotaxin-1 (CCL11), and pulmonary and activation-regulated chemokine (PARC/CCL18), for example, have been demonstrated to be elevated in sinonasal tissues in patients with CRS with nasal polyps (CRSwNP) compared to those with CRS without nasal polyps (CRSsNP).[2–4] Additionally, monocyte chemoattractant protein-3 (MCP-3/CCL7) has been shown to increase expression in CRS overall, with no significant differences observed between CRSwNP and CRSsNP subtypes.[5] Chemokine dysregulation in CRS has been demonstrated to possess a genetic correlation with

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several gene family polymorphisms identified in CRS.[6] These previous works underscore the significant role of chemokines in CRS, making chemokine research an attractive area in understanding the pathogenesis of CRS, as well as in identifying potential therapeutic targets.

CCL19 is a hemostatic chemokine that regulates the cellular trafficking of circulating and bone marrow-derived leukocytes to and from lymphoid tissues through interactions with its typical receptor, CCR7.[7–11] Depending on the biological context, CCL19 has demonstrated dual functional roles as either a pro- or anti-inflammatory mediator in response to homeostasis, infection, or tissue insult[11] by modulating interleukin production and antigen presentation.[12,13] During the COVID-19 pandemic, CCL19 gained notoriety, as studies suggested that circulating CCL19 levels were positively correlated with worsening disease severity.[14,15] Additionally, elevated plasma levels of CCL19 have been shown to predict treatment response in patients with rheumatoid arthritis.[16] Additional studies have also implicated CCL19 in rheumatoid arthritis pathology, and other work has suggested that CCL19 may play a role in mediating plaque destabilization in atherosclerosis disease mechanisms.[16–18]

The roles of CCL19 and its typical (CCR7) and atypical (CCRL1) receptors in the upper airway remain poorly understood. CCL19 is reported to exert pro-inflammatory effects through CCR7 binding and the initiation of interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) production.[12,19] The atypical receptor, CCRL1, is thought to bind and rapidly internalize CCL19 for degradation, leading to protective responses in host immune defense.[20] mRNA expression of *CCL19* and *CCR7* has been reported to be elevated in sinonasal tissues of patients with CRSwNP, whereas protein expression was shown to be significantly elevated in both CRSwNP and CRSsNP.[21,22] Additionally, patients with CRSwNP and asthma exhibit distinctive signs of airway remodeling, primarily driven by the heightened activity of matrix metalloproteinases (MMPs). Upregulation of MMPs have been observed in the migration of bone marrow mesenchymal cells via the interaction of CCL19 and CCR7, and hence, may be responsible for the effect seen in CRSwNP.[6,23] How these elevated levels of CCL19 affect inflammatory pathways, disease severity, and whether CCL19 acts through CCR7 and/or CCRL1 in CRS remain unknown.

This study sought to define the gene transcriptional and protein expression changes of CCL19 and its receptors and downstream mediators in patients with CRS, as well as to determine whether these CCL19-related changes were different between CRSwNP and CRSsNP and correlated with CRS-specific disease severity measures. Given the purported pathological significance of CCL19 in other inflammatory disorders, such as COVID-19 and rheumatoid arthritis, the findings herein have the potential to enhance our understanding of the pathophysiology, clinical significance, and therapeutic potential of CCL19 and its receptors in CRS disease subtypes. This knowledge may ultimately contribute to the development of more effective treatment approaches for this challenging inflammatory disorder.

Materials and Methods

Study Design and Human Subjects

Study participants were enrolled into the study after receiving informed consent using procedures authorized by the Institutional Review Board (IRB) at the University of Utah (IRB_00074325). All study activities were conducted according to the principles outlined in the Declaration of Helsinki. This investigation involved adult study subjects, 18 years of age or older, with CRS (study subjects) and non-CRS (controls). All participants had undergone formal clinical evaluation, including complete medical history, head and neck physical examination, computed tomography (CT) scan review, and nasal endoscopy. The inclusion criteria for study subjects were adults with a diagnosis of CRS, as defined by the American Academy of Otolaryngology-Head and Neck Surgery guidelines, who had elected to undergo endoscopic sinus surgery (ESS) between 2013 and 2020.[24] Those adults who had elected ESS for non-CRS and non-inflammatory disease intervention were included as control participants. At the time of enrollment, patient demographics (*i.e.*, age, sex, race, comorbidities, and polyp status), objective CRS disease-specific severity metrics–Lund-Mackay (LM) CT[25] and Lund-Kennedy (LK) endoscopy scores[26]–and subjective CRS disease-specific quality of life metrics–rhinosinusitis disability index (RSDI)[27] and sinonasal outcome test-22 (SNOT-22)[28] scores–were collected. To avoid potential confounding results due to other inflammatory disease and medication use, exclusion criteria for all subjects included recurring acute rhinosinusitis, allergic fungal rhinosinusitis, autoimmune disease, rheumatologic disorders, and cystic

fibrosis, as well as the use of oral steroids within two weeks of specimen collection. In addition, control subjects with comorbid asthma or allergic diseases were excluded to avoid potential inflammatory confounders. Participants meeting inclusion were subjected to sinonasal tissue biopsy, followed by mRNA gene and protein expression analysis as outlined in Figure 1.

Statistical Analysis

STATA statistical software (StataCorp, College Station, Texas, Version 18-BE) was used for statistical analysis. Continuous variables, such as age, Lund-Mackay CT, Lund-Kennedy endoscopy, SNOT-22 and RSDI scores, were reported as the mean ± standard deviation for each cohort. Categorical variables, including demographics, polyp status, and medical history, are presented as the number and percentage of patients in each patient group. Normally distributed data were compared using Welch's *t*-test, and non-parametrically distributed data were analyzed using the Wilcoxon rank-sum test. Linear regression analysis was used to compare the presence of more than two groups were present. Spearman correlation tests were performed to determine associations between gene expression and disease severity measures, as well as associations between gene expression variables, as the data were non-normally distributed. All tests were two-tailed, and the significance level was set at $p<0.05$.

Anterior Ethmoid Tissue Processing

Sinonasal tissue biopsies, approximately 4.0 mm^2, were obtained from the anterior ethmoid sinus during ESS per the standard of care. Tissue specimens were either immediately frozen and stored at −80°C until being subjected to mRNA extraction and gene expression analysis, or fixed in 4% neutral formalin for 48 hours until being subjected to tissue processing by the Molecular Diagnostics Core at the Huntsman Cancer Institute (Salt Lake City, UT) and protocols for immunohistochemical analysis, as previously detailed.[29,30]

Gene Expression Analysis in Anterior Ethmoid Tissues

The repository included a database containing the expression of 594 adaptive and innate immunity genes from the analysis of anterior ethmoid tissue in controls and patients diagnosed with CRS. The Human Immunology v2 Gene Panel and nCounter® MAX Analysis System (NanoString Technologies, Inc., Seattle, WA, USA) were used to generate the

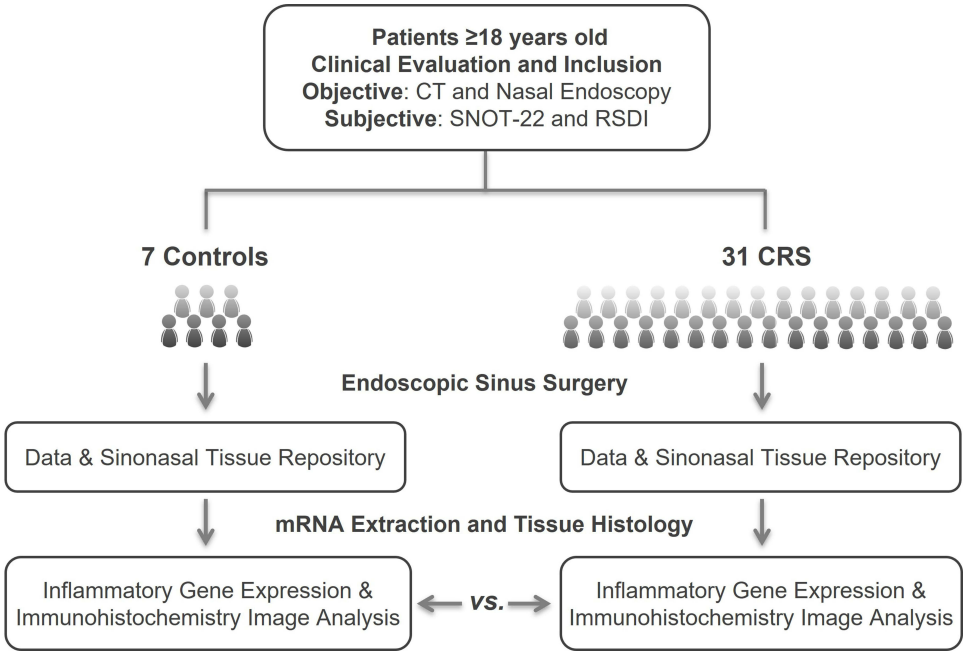

Figure 1 A flowchart demonstrating the study design and procedures employed in this investigation.

database. mRNA extraction and multiplex gene expression analysis were performed as previously reported.[29,30] Briefly, after being thawed at 4°C, ethmoid tissues were placed in 0.6 mL of lysis buffer containing 5-mm stainless-steel beads and homogenized using a Qiagen Tissuelyser LT (Qiagen; Hilden, Germany). Nucleic acid was extracted using a Qiagen AllPrep RNA microRNA Universal Kit and a QiaCube, following the manufacturer's instructions. A NanoDrop 8000 (Thermofisher Scientific; Pittsburg, PA) was employed to quantify the amount of mRNA.

An nCounter Digital Analyzer and nSolver version 3.0 software (NanoString Technologies, Inc., Seattle, WA) were employed to determine the raw mRNA transcript copy counts of the target genes. Background subtraction and normalization of the raw mRNA transcript copy counts were conducted using the geometric mean of fifteen internal reference genes: *GAPDH, ABCF1, EEF1G, G6PD, ALAS1, POLR2A, PPIA, POLRIB, TUBB, SDHA, TBP, RPL19, GUSB, OZA1*, and *HPRT1*. After normalization, the ratio of the difference in the means of the log-transformed normalized data to the square root of the sum of the variances of the samples in the two groups was deduced. Normalized log-transformed data were then subjected to a 2-tailed *t*-test, under the assumption of unequal variance. In each treatment group, the results were presented as the mean mRNA transcript copy count ± standard deviation of each gene with respect to cohort. To address the objective of this study, the mRNA transcript copy numbers for *CCL19*, its receptors *CCR7* and *CCRL1* and known associated interleukins, pro-inflammatory *IL1A, IL1B, IL12A, IL12B, TNFA*, and anti-inflammatory *IL10*, were compared between the CRS and control cohorts.[31] Only the genes with differences in mRNA transcript copy numbers between the overall cohort and controls, or CRS subgroup analysis, were used for further analyses [*ie* significant differences ($p<0.05$), differences that might be approaching significance ($p<0.1$)].

Immunohistochemical Staining for Co-Expression of CCL19 and CCR7

Slide-mounted anterior ethmoid tissues were sequentially deparaffinized in xylenes and rehydrated in ethanol (Thermo Fisher Scientific; Pittsburg, PA) and ddH_2O, as previously described.[30] Tissues were then subjected to heated-antigen retrieval in citrate-based antigen unmasking solution (pH 6.0), followed by antigen blocking with BLOXALL (20 min) and 2.5% goat serum (20 min), all of which were obtained from Vector Laboratories (Burlingame, CA, USA). The tissues were then incubated with polyclonal rabbit anti-human CCR7 (1:200, 4°C, overnight) (Bioss Antibodies, bs-1305R; Woburn, MA, USA), followed by incubation with ImmPRESS^{TM}HRP anti-rabbit IgG (20 min) and detection with ImmPACT DAB Peroxidase kits from Vector Laboratories (Burlingame, CA, USA). This staining protocol was repeated for monoclonal mouse anti-human CCL19 (1:150, 4°C, overnight) (Invitrogen, OTI2A12; Carlsbad, CA, USA) detection, employing heated antigen retrieval with Tris-based antigen unmasking solution, ImmPRESS^{TM}HRP anti-mouse IgG (clone #238446), and ImmPACT SG Peroxidase kits from Vector Laboratories.

Immunohistochemical Staining for Co-Expression of CCL19 and CCRL1

After rehydration, heated antigen retrieval in citrate-based antigen unmasking solution (pH 6.0), and antigen blocking with BLOXALL (20 min) and 2.5% goat serum (20 min), the tissues were incubated with polyclonal rabbit anti-human CCRL1 (1:100, 2 h, RT) (LS Bio, LS-A1441-50; Seattle, WA), followed by ImmPRESS^{TM}HRP anti-rabbit IgG incubation (20 min) and detection with ImmPACT DAB Peroxidase kits from Vector Laboratories (Burlingame, CA, USA). This staining protocol was repeated for monoclonal mouse anti-human CCL19 (1:150, 4°C, overnight) (Invitrogen, OTI2A12; Carlsbad, CA, USA) detection, employing heated antigen retrieval with Tris-based antigen unmasking solution, ImmPRESS^{TM}HRP anti-mouse IgG (clone #238446), and ImmPACT SG Peroxidase kits from Vector Labs. In both studies, tissues were incubated with and without primary and secondary antibodies for all antigens to serve as staining controls. After detection of CCL19 with ImmPACT SG Peroxidase, tissues were counterstained with hematoxylin (Vector Labs; Burlingame, MA), dehydrated, and slide mounted with Permount (ThermoFisher Scientific; Pittsburg, PA), as previously described.[30] An Olympus BX43 upright microscope (Olympus Inc.; Pittsburg, PA) equipped with an EOS Rebel T2i digital SLR camera (Canon Inc.; Melville, NY) was used to image all samples. Images were processed using the Digital Photo Professional version 4.0 software (Canon Inc., Melville, NY).[30]

Image Acquisition & Analysis

Images were captured at x10 magnification using an Olympus BX43 light microscope fitted with a Canon EOS Rebel T7i camera attached to a computer. Images were acquired using Digital Photo Professional software (version 4.17.30.0). The color density and white balance were standardized for all images before image capture. Fields with optimal light and image contrast demonstrating DAB and SG colocalization, as well as hematoxylin counterstaining, were selected as the regions of interest for each representative tissue sample. All images were saved in JPEG format for further processing.

A head and neck anatomical pathologist reviewed all slides and determined that CCL19, CCR7, and CCRL1 staining was primarily observed in the sinonasal epithelium in all tissue specimens. Image J 1.53 k [Java 3.0.6 (64 bit), National Institutes of Health; Bethesda, MD] was then used to quantify the respective co-expression of CCL19 (SG) with CCR7 or CCRL1 (DAB) via image analysis methods described by Crowe and Yue with modifications.[32] The images were imported into Image J and converted into 8-bit images. The threshold was adjusted with the minimum set to zero and the maximum set to 100 for all images to prevent bias. The threshold setting was established in advance by selecting slides with anticipated maximum and minimum staining intensities based on observed staining patterns. After applying these settings, the stained tissue areas were measured in all tissues. Statistical comparison of the data was executed using the two-tailed Kruskal–Wallis test, with significance set at $p<0.05$. The resulting data were then analyzed and represented as individual values accompanied by the mean ± standard deviation of the co-expression area as a percentage of the total area for each cohort using GraphPad Prism 9 (Version 9.5.1). As the target proteins were primarily expressed in epithelial cells, images were captured and analyzed using sections of tissues with continuous intact epithelium for each sample, which was limited in some tissues.

Results

Patient Demographics

A total of 38 patients (control, n=7; CRS, n=31) were included in this study. There were no statistically significant differences in age, sex, or race between the two groups (Table 1). As expected, there were significant differences in asthma ($p=0.012$) and reported allergies ($p<0.008$) between CRS and controls, as patients with comorbid asthma and/or

Table 1 Demographic Data for the Overall Study Cohort (n=38)

	CRS (n=31)	Controls (n=7)	*p*-value
Age, Mean (SD)	47.58 (17.73)	43.57 (16.63)	0.55
Sex Female, n (%) Male, n (%)	 15 (48) 16 (52)	 2 (29) 5 (71)	0.6
Race, n (%) White Other	 31 (100) 0 (0)	 6 (86) 1 (14)	0.37
Asthma, n (%)	18 (58.07)	0 (0)	0.012*
N-ERD, n (%)	1 (3.23)	0 (0)	>0.99
Nasal polyps, n (%)	14 (45)	0 (0)	0.055
#Allergy, n (%)	19 (61)	0 (0)	0.008**
Lund-Mackay CT Score, Mean (SD)	12.5 (5.3)	1.3 (1.9)	<0.0001***
Lund-Kennedy Endoscopy Score, Mean (SD)	5.2 (4.3)	2.0 (1.6)	0.011*
SNOT-22 Total Score, Mean (SD)	58.6 (18.3)	27.2 (18.9)	0.0022**
RSDI Total Score, Mean (SD)	52.9 (30.0)	16.6 (13.2)	0.0036**

Notes: *$p<0.05$; **$p<0.01$; ***$p<0.001$; #Patient-reported allergy.
Abbreviations: CRS, chronic rhinosinusitis; SD, standard deviation; N-ERD, nonsteroidal anti-inflammatory exacerbated respiratory disease; CT, computed tomography; SNOT-22, Sinonasal outcome test-22; RSDI, rhinosinusitis disability index.

allergies were excluded from the control cohort. Significant differences in the Lund-Mackay CT ($p<0.0001$), Lund-Kennedy endoscopy ($p=0.011$), SNOT-22 ($p=0.0022$), and RSDI ($p=0.0036$) scores were observed between the groups.

CRS patients were further subdivided into CRSwNP (n=14) and CRSsNP (n=17) groups to determine whether there was any variation in gene expression with the phenotypic subtypes of CRS. No significant differences in demographic variables were observed between the CRS subgroups, whereas the Lund-Mackay CT ($p=0.005$), Lund-Kennedy endoscopy ($p=0.0016$), and RSDI ($p=0.033$) scores were significantly higher in the CRSwNP cohort compared to in the CRSsNP (Table 2).

Gene Expression Analysis

The gene expression of the atypical receptor *CCRL1* ($p=0.0093$) and typical receptor *CCR7* ($p=0.017$) was significantly elevated in sinonasal tissues obtained from patients with CRS compared to that in controls (Table 3). Increased *CCL19* expression in the CRS cohort approached statistical significance ($p=0.077$), whereas the difference in *TNFA* expression between CRS and controls was not significant. When examining the differential expression of these genes between CRS subtypes, *CCL19* ($p=0.038$) and *CCR7* ($p=0.0097$) levels were significantly upregulated in patients with CRSwNP, whereas *CCRL1* levels were significantly upregulated in CRSsNP ($p=0.0004$) compared to controls (Table 4). Linear

Table 2 Demographic Data for the CRS Cohort with Respect to Clinical Phenotype: CRSwNP and CRSsNP

	CRSwNP (n=14)	CRSsNP (n=17)	*p*-value
Age, mean (SD)	45 (18.87)	49.70 (17.01)	0.46
Sex Female, n (%) Male, n (%)	 7 (50) 7 (50)	 8 (53) 9 (47)	>0.99
Race White, n (%) Other, n (%)	 14 (100) 0 (0)	 17 (100) 0 (0)	>0.99
Asthma, n (%)	9 (64)	9 (53)	0.79
N-ERD, n (%)	1 (7)	0 (0)	0.90
#**Allergy**, n (%)	8 (57)	11 (65)	0.95
Lund-Mackay CT Score, Mean (SD)	15.57 (5.24)	10.06 (3.88)	0.005**
Lund-Kennedy Endoscopy Score, Mean (SD)	7.57 (4.88)	3.06 (2.17)	0.0016**
SNOT-22 Total Score, Mean (SD)	65.50 (22.02)	53.31 (13.34)	0.2
RSDI Total Score, Mean (SD)	70.63 (33.01)	41.92 (22.97)	0.033*

Notes: *$p<0.05$; **$p<0.01$; #Patient-reported allergy.
Abbreviations: CRS, chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; SD, standard deviation; N-ERD, nonsteroidal anti-inflammatory exacerbated respiratory disease; CT, computed tomography; SNOT-22, Sinonasal outcome test-22; RSDI, rhinosinusitis disability index.

Table 3 Gene Expression Analysis in the Overall Study Cohort

	CRS (n=31)	Controls (n=7)	*p*-value
CCL19, mean (SD)	605.14 (771.16)	133.36 (100.37)	0.077
CCRL1, mean (SD)	171.47 (138.55)	62.01 (39.13)	0.0093**
CCR7, mean (SD)	96.02 (141.91)	20.78 (10.14)	0.017*
TNFA, mean (SD)	41.69 (39.15)	23.97 (11.58)	0.48

Notes: *$p<0.05$; **$p<0.01$.
Abbreviation: CRS, chronic rhinosinusitis.

regression analysis demonstrated a significant difference in *CCL19* expression among the CRSwNP, CRSsNP, and control groups (Table 4). The difference in *TNFA* expression between the groups was not statistically significant (p=0.091). No significant differences were observed in the gene expression levels of the known pro-inflammatory cytokines, *IL1A, IL1B, IL12A, IL12B*, or the anti-inflammatory interleukin, *IL10* between the CRS and controls (Supplemental Table 1), or between CRSsNP and CRSwNP (Supplemental Table 2).

Three genes with significant differences in gene expression between cohorts, *CCL19*, *CCR7*, and *CCRL1*, were used in the subsequent correlation analysis. *TNFA* was also included given its involvement in the corresponding inflammatory pathways as a gene of particular interest. In the CRS cohort, a significant positive correlation between *CCL19* mRNA transcription levels and *CCR7* (p<0.0001) and *TNFA* (p=0.0002) levels (Table 5). *CCR7* expression was also strongly and significantly correlated with *TNFA* levels (p<0.0001) (Table 5).

CCL19 gene expression levels were significantly correlated with the SNOT-22 (p=0.0073) and RSDI (p=0.023) scores (Table 6). *CCR7* gene expression levels also correlated with SNOT-22 (p=0.025) and RSDI (p=0.023) scores. *CCRL1* expression did not show any significant correlation with clinical variables in the overall cohort. The correlations between *TNFA* and SNOT-22 (p=0.053) and RSDI (p=0.0628) scores were not statistically significant (Table 6).

Table 4 Gene Expression Analysis with Respect to CRS Clinical Phenotype Compared to Controls

	CRSwNP (n=14)	*p*-value vs controls	CRSsNP (n=17)	*p*-value vs controls	Linear Regression *p*-value
CCL19, mean (SD)	783.51 (856.32)	0.038*	458.26 (684.45)	0.23	0.043*
CCRL1, mean (SD)	87.50 (43.40)	0.25	240.61 (152.36)	0.0004***	0.66
CCR7, mean (SD)	107.42 (141.62)	0.0097**	86.64 (145.80)	0.075	0.18
TNFA, mean (SD)	50.65 (41.87)	0.22	34.31 (36.36)	0.90	0.091

Notes: *p<0.05; **p<0.01; ***p<0.001.
Abbreviations: CRS, chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps.

Table 5 Spearman Correlations Between Genes in the Overall Study Cohort

	CCL19	CCRL1	CCR7	TNFA
CCL19, R (*p*-value)	-	−0.1224 (0.4622)	0.828 (<0.0001)*	0.5722 (0.0002)*
CCRL1, R (*p*-value)	-	-	−0.0113 (0.9462)	−0.1452 (0.3827)
CCR7, R (*p*-value)	-	-	-	0.6873 (<0.0001)*

Note: *p<0.001.

Table 6 Spearman Correlations Between Genes and CRS-Specific Disease Severity Metrics in the Overall Study Cohort

	Lund-Mackay CT Score	Lund-Kennedy Endoscopy Score	SNOT-22 Score	RSDI Score
CCL19, R (*p*-value)	0.2752 (0.11)	0.053 (0.76)	0.4921 (0.0073)**	0.4388 (0.023)*
CCRL1, R (*p*-value)	−0.2738 (0.11)	−0.1016 (0.57)	0.0106 (0.96)	−0.0922 (0.65)
CCR7, R (*p*-value)	0.2608 (0.13)	0.0605 (0.73)	0.4171 (0.025)*	0.4397 (0.023)*
TNFA, R (*p*-value)	0.2544 (0.14)	−0.025 (0.89)	0.3639 (0.053)	0.3634 (0.063)

Note: *p<0.05; **p<0.01.
Abbreviations: CT, computed tomography; SNOT-22, Sinonasal outcome test-22; RSDI, rhinosinusitis disability index.

CCL19/CCR7 and CCL19/CCRL1 Co-Expression Protein Analysis

Immunohistochemical staining demonstrated increased co-expression of CCL19 and its typical (CCR7) and atypical (CCRL1) receptors in the sinonasal epithelium of patients with CRSwNP (n=7) compared with CRSsNP (n=6) and controls (n=6) (Figure 2A). Uniform co-expression of CCL19 and its receptors was observed in CRSwNP, whereas co-expression in controls and CRSsNP was primarily observed in apical epithelial cells with less consistent expression patterns within the sinonasal epithelium. Quantitative image analysis showed that the degree of CCL19/CCR7 co-expression was significantly higher in patients with CRSwNP than in those with CRSsNP (p=0.04). Although not statistically significant, CCL19/CCR7 co-expression was elevated in CRSwNP compared to controls (p=0.06) (Figure 2B). While there appeared to be elevated co-expression of CCL19/CCRL1 in CRSwNP compared to CRSsNP and controls, these differences were not significant (Figure 2C).

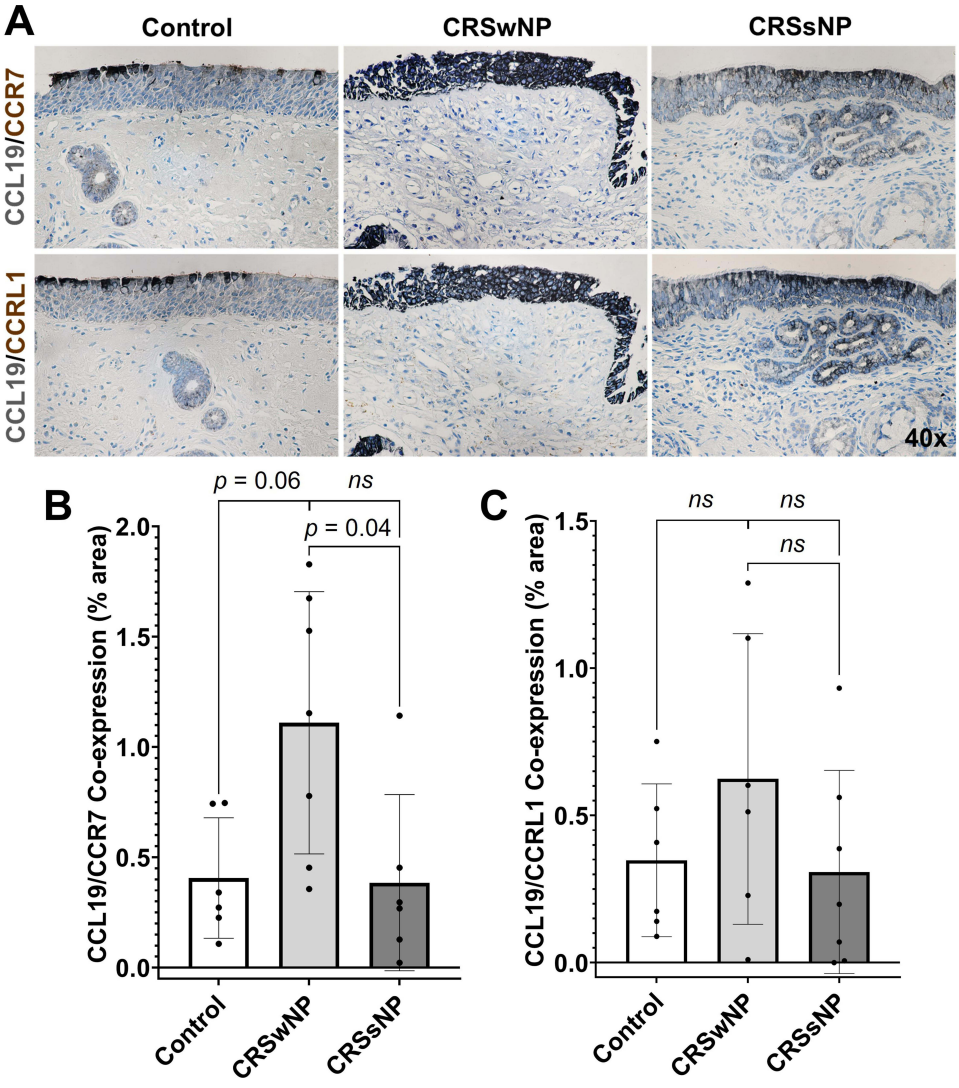

Figure 2 Sinonasal epithelial cell expression of CCL19 and its receptors CCR7 and CCRL1 is elevated in CRSwNP (n=7) compared to in CRSsNP (n=6) and controls (n=6). (**A**) Representative immunohistochemical images demonstrate the increased co-expression of CCL19 and typical receptor CCR7 (top), as well as of CCL19 and its atypical receptor CCRL1, in the sinonasal epithelium in patients with CRSwNP compared to in CRSsNP and controls. CCL19 appears gray, whereas its receptors appear dark brown. (**B**) CCL19 and CCR7 co-expression in the sinonasal epithelium, represented as the % total area via Image J analysis, is significantly elevated in CRSwNP compared to CRSsNP (p=0.04) and controls (p=0.06). (**C**) Although not significant, CCL19 and CCRL1 co-expression is elevated in CRSwNP compared to in CRSsNP and controls, represented as the % total area via Image J analysis. Data is represented by individual values with the mean ± SD with respect to cohort.

Table 7 Summary of Key Results

Study Component	Key Findings

Gene Expression	• *CCRL1* and *CCR7* expression is elevated in CRS vs controls
	• Gene expression varies by CRS subtype
	  ○ *CCL19* and *CCR7* expression is elevated in CRSwNP
	  ○ *CCRL1* expression is elevated in CRSsNP

Gene Correlation Analysis	• *CCL19* expression is correlated with increased *CCR7* and*TNFA* expression in CRS • *CCL19* and *CCR7* expression is correlated with worsening disease severity (SNOT-22 and RSDI)
Protein Analysis	• Co-expression of CCL19 and its receptors CCR7 and CCRL1 is increased in CRSwNP

Discussion

This study, which included 31 patients with CRS (CRSwNP=14, CRSsNP=17) and 7 controls, identified statistically significant increases in sinonasal tissue expression of *CCRL1* and *CCR7* in patients with CRS. *CCL19* and *CCR7* expression was significantly increased in patients with CRSwNP, whereas *CCRL1* expression was significantly elevated in patients with CRSsNP. *CCL19* expression levels were strongly correlated with *CCR7* levels, and both were significantly correlated with *TNFA* levels. A significant increase in sinonasal epithelial cell co-expression of CCL19 and CCR7 was observed in patients with CRSwNP compared to CRSsNP and controls. *CCL19* and *CCR7* levels were significantly correlated with SNOT-22 and RSDI scores. The results of this study suggest that CCL19 may exert pro-inflammatory effects through its typical receptor CCR7 and associated TNFA-mediated signaling in CRSwNP, whereas in CRSsNP, *CCL19* may interact with its atypical receptor *CCRL1*, which is thought to sequester and degrade CCL19. A summary of the key findings in this investigation is listed in Table 7.

Few studies have examined the roles of CCL19 and CCR7 in CRS. Ocampo et al demonstrated that *CCL19* and *CCR7* mRNA expression was elevated in CRSwNP.[22] Zou et al found that the protein expression of CCL19 in sinonasal tissues was significantly increased in CRSsNP and CRSwNP compared to controls.[21] These findings are consistent with the results of this study, and are particularly in keeping with our findings in CRSwNP. The data from this study demonstrated a significant increase in sinonasal tissue gene expression levels of *CCL19* and *CCR7*, as well as in the co-expression of CCL19 and CCR7 in the sinonasal epithelium of patients with CRSwNP. These data suggest that CCL19 and its typical receptor CCR7 are biologically active in the sinonasal epithelium of CRSwNP patients. The subsequent significant and positive correlations between *CCL19, CCR7*, and *TNFA*, a cytokine associated with CCL19-mediated pro-inflammatory cascades, and the correlation between SNOT-22 and RSDI suggest that the CCL19/CCR7 complex may play a potential role in pathological inflammation in patients with CRS.

Although *TNFA* levels were not found to be significantly elevated in CRS patients in this study, its mRNA expression was strongly correlated with *CCL19* and *CCR7* levels in the overall cohort. Despite its potent pro-inflammatory and immunomodulatory effect, the role of TNFA in CRS is incomplete, and conflicting studies have been published.[33–35] This uncertainty is further compounded by ongoing debate regarding whether the TNFA inhibitor class of medications predispose patients to sinusitis.[36] To further define the role of CCL19/CCR7-mediated inflammatory consequences in CRSwNP and potential association with TNA-α, additional mechanistic studies are warranted.

While *CCL19* was found to be significantly elevated in CRSwNP, it was not significantly elevated in CRSsNP compared to that in the controls. However, *CCRL1*, an atypical receptor for CCL19, was elevated in patients with CRSsNP. CCRL1 is one of the four known atypical chemokine receptors (ACRs).[37,38] ACRs are thought to sequester and degrade their associated chemokines and, as a result, are also known to be decoy or scavenger receptors.[37,38] The identification of elevated levels of *CCRL1* in CRSsNP is novel; however, little is known about the role of *CCRL1* in inflammatory airway disease, and additional mechanistic studies are needed to understand the potential interactions between CCL19 and CCRL1 in CRS. The novel finding of elevated CCRL1 levels in CRSsNP suggests a distinct regulatory mechanism involving CCRL1-mediated sequestration and degradation of CCL19. Interpreting expression patterns in the clinical context of CRSwNP versus CRSsNP provides valuable insights into the molecular mechanisms

underlying each disease endotype and informs the development of targeted therapeutic strategies tailored to the specific pathophysiological features of each endotype.

CCL19 has been associated with disease severity and may potentially serve as a prognostic marker in RA.[14–16] This study demonstrates a significant correlation between *CCL19* and *CCR7* and CRS disease-specific quality-of-life metrics SNOT-22 and RSDI. While *CCL19* and *CCR7* were associated with these quality-of-life metrics, there was no association between these gene levels and objective CRS-specific disease severity measures, Lund-Mackay CT, or Lund-Kennedy endoscopy scores. The lack of a relationship between objective disease metrics and systemic markers of inflammation is common in CRS and continues to be a challenge in the search for sensitive and specific biomarkers for objective disease severity in CRS.[29,39]

The study design/methodology demonstrates adherence to ethical guidelines, including obtaining informed consent and approval from the IRB, ensuring participant welfare and research integrity. Participants undergo thorough clinical evaluation, including medical history, physical examination, and imaging studies, enhancing the accuracy of CRS diagnosis and classification. Clear inclusion criteria based on established guidelines for CRS diagnosis and selection of patients undergoing ESS improve the homogeneity of the study population, enhancing the relevance and applicability of findings to clinical practice. The primary limitations of this study are the small sample size and potential selection bias in only including patients who elected to proceed with endoscopic sinus surgery due to the requirement for a sinonasal tissue sample. This may limit the generalizability of findings to broader CRS populations and overlook less severe cases managed conservatively. Stringent exclusion criteria, such as the use of oral steroids and specific comorbidities, may restrict the representativeness of the study cohort and limit the extrapolation of findings to diverse CRS populations. While participants were excluded if they had used oral corticosteroids within 2 weeks of enrollment, literature of the duration of action of these medications is limited and should be considered when interpreting these results. Despite promising correlations with disease-specific quality-of-life metrics, challenges persist in establishing sensitive and specific biomarkers for objective disease severity in CRS, underscoring the need for further research and larger, more inclusive cohorts.

As innovative techniques have been developed and validated for biospecimen collection, future work should aim to include both surgical and nonsurgical cohorts to avoid the associated selection bias, as these data represent a subset of patients with diseases unresponsive to medical therapy. A more inclusive cohort would aid in the generalization of the results. Recognizing that CRS is a heterogeneous inflammatory disorder, a larger sample size will allow for an endotypically-driven analysis, which may provide a stronger foundation for subgroup analyses. Quantitative protein and mechanistic analyses of CCL19 pathway-associated mediators in a larger sample size are needed to elucidate the pathophysiology and clinical implications of these molecules in CRS. To validate the findings and advance understanding of CRS pathogenesis, priorities include conducting mechanistic studies to elucidate the role of dysregulated chemokine signaling, particularly involving CCL19, CCR7, and TNF-α. Additionally, targeted therapeutic interventions should be evaluated through animal models and clinical trials, with a focus on longitudinal assessments and subgroup analyses to assess biomarker potential and treatment responsiveness across different CRS phenotypes. Validating animal models of CRS will be crucial for translating preclinical findings into clinical applications, ultimately facilitating the development of personalized therapeutic approaches for CRS management.

Conclusion

In conclusion, CCL19 and CCR7 may modulate TNF-α-driven pro-inflammatory signaling in CRS, particularly in patients with CRSwNP. While *CCL19* expression in the overall CRS cohort compared to controls approached but did not reach significance, this study demonstrated a statistically significant correlation between *CCL19, CCR7*, SNOT-22, and RSDI in CRS, suggesting that the CCL19/CCR7 signaling axis may contribute to worse disease severity in CRS. While the study contributes valuable insights into CRS pathogenesis by identifying associations between chemokine expression and disease severity metrics, several unanswered questions remain regarding the underlying mechanisms and clinical implications of these findings. Addressing these gaps in knowledge through further research will be crucial for advancing our understanding of CRS pathophysiology and improving patient care.

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Disclosure

Abigail Pulsipher has a financial interest in GlycoMira Therapeutics. Jeremiah Alt and financial interests in GlycoMira Therapeutics, Inc. and is a consultant for GSK, Medtronic ENT, and OptiNose. None of these potential conflicts of interest are affiliated with this research. The authors report no other conflicts of interest in this work.

References

1. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006;354(6):610–621. doi:10.1056/nejmra052723
2. Peterson S, JA P, DR N, et al. Increased expression of CC chemokine ligand 18 in patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2012;129(1):119–127.e9.
3. König K, Klemens C, Haack M, et al. Cytokine patterns in nasal secretion of non-atopic patients distinguish between chronic rhinosinusitis with or without nasal polyps. *Allergy Asthma Clin Immunol*. 2016;12(1). doi:10.1186/s13223-016-0123-3
4. Olze H, Forster U, Zuberbier T, Morawietz L, Luger E. Eosinophilic nasal polyps are a rich source of eotaxin, eotaxin-2 and eotaxin-3. *Rhinology*. 2006;44(2):145.
5. Wright ED, Frenkiel S, Ghaffar O, Al-Ghamdi K. Monocyte chemotactic protein expression in allergy and non-allergy-associated chronic sinusitis. *J Otolaryngol*. 1998;27(5):281.
6. Antonino M, Nicolò M, Jerome Renee L, et al. Single-nucleotide polymorphism in chronic rhinosinusitis: a systematic review. *Clin Otolaryngol*. 2022;47(1):14–23. doi:10.1111/coa.13870
7. Forster R, Schubel A, Breitfeld D, et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell*. 1999;99(1):23–33. doi:10.1016/s0092-8674(00)80059-8
8. Bromley SK, Thomas SY, Luster AD. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol*. 2005;6(9):895–901. doi:10.1038/ni1240
9. Reif K, Ekland EH, Ohl L, et al. Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. *Nature*. 2002;416 (6876):94–99. doi:10.1038/416094a
10. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol*. 2000;18:593–620. doi:10.1146/annurev.immunol.18.1.593
11. Yan Y, Chen R, Wang X, et al. CCL19 and CCR7 expression, signaling pathways, and adjuvant functions in viral infection and prevention. *Front Cell Dev Biol*. 2019;7:212. doi:10.3389/fcell.2019.00212
12. Marsland BJ, Battig P, Bauer M, et al. CCL19 and CCL21 induce a potent proinflammatory differentiation program in licensed dendritic cells. *Immunity*. 2005;22(4):493–505. doi:10.1016/j.immuni.2005.02.010
13. Takamura K, Fukuyama S, Nagatake T, et al. Regulatory role of lymphoid chemokine CCL19 and CCL21 in the control of allergic rhinitis. *J Immunol*. 2007;179(9):5897–5906. doi:10.4049/jimmunol.179.9.5897
14. Nayak N, Pati A, Pavani Y, Sahu S, Ranjan S, Panda AK. CCL19 (rs3136658) and CCL21 (rs2812377) variants are associated with susceptibility and related mortality of SARS-CoV-2 Infection. *J Infect Dis*. 2023;228(1):101–102. doi:10.1093/infdis/jiad032
15. Tveita A, Murphy SL, Holter JC, et al. High circulating levels of the homeostatic chemokines CCL19 and CCL21 predict mortality and disease severity in COVID-19. *J Infect Dis*. 2022;226(12):2150–2160. doi:10.1093/infdis/jiac313
16. Ellingsen T, Hansen I, Thorsen J, et al. Upregulated baseline plasma CCL19 and CCR7 cell-surface expression on monocytes in early rheumatoid arthritis normalized during treatment and CCL19 correlated with radiographic progression. *Scand J Rheumatol*. 2014;43(2):91–100. doi:10.3109/03009742.2013.803149
17. Damas JK, Smith C, Oie E, et al. Enhanced expression of the homeostatic chemokines CCL19 and CCL21 in clinical and experimental atherosclerosis: possible pathogenic role in plaque destabilization. *Arterioscler Thromb Vasc Biol*. 2007;27(3):614–620. doi:10.1161/01.ATV.0000255581.38523.7c
18. Pickens SR, Chamberlain ND, Volin MV, Pope RM, Mandelin AM 2nd, Shahrara S. Characterization of CCL19 and CCL21 in rheumatoid arthritis. *Arthritis Rheum*. 2011;63(4):914–922. doi:10.1002/art.30232
19. Bose F, Petti L, Diani M, et al. Inhibition of CCR7/CCL19 axis in lesional skin is a critical event for clinical remission induced by TNF blockade in patients with psoriasis. *Am J Pathol*. 2013;183(2):413–421. doi:10.1016/j.ajpath.2013.04.021

20. Comerford I, Milasta S, Morrow V, Milligan G, Nibbs R. The chemokine receptor CCX-CKR mediates effective scavenging of CCL19 in vitro. *Eur J Immunol*. 2006;36(7):1904–1916. doi:10.1002/eji.200535716
21. Zou Y, Wang Y, Wang SB, et al. Characteristic expression and significance of CCL19 in different tissue types in chronic rhinosinusitis. *Exp Ther Med*. 2016;11(1):140–146. doi:10.3892/etm.2015.2897
22. Ocampo CJ, Norton J. Elevated Expression of mRNA for CCL2, CCL19, CCR7 and CXCR3 in Chronic Rhinosinusitis with Nasal Polyposis. *J Allergy Clin Immunol*. 2012;2012:1.
23. TG ZW, Lv C, Long J, Cong L, Han Y. Matrix metalloproteinase-9 is up-regulated by CCL19/CCR7 interaction via PI3K/Akt pathway and is involved in CCL19-driven BMSCs migration - PubMed. *Biochem Biophys Res Commun*. 2014;451(2). doi:10.1016/j.bbrc.2014.07.112
24. Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, et al. Clinical practice guideline (update): adult sinusitis. *Otolaryngol Head Neck Surg*. 2015;152(2 Suppl):S1–S39. doi:10.1177/0194599815572097
25. Lund VJ, Mackay IS. Staging in rhinosinusitis. *Rhinology*. 1993;31(4):183–184.
26. Lund VJ, Kennedy DW. Staging for rhinosinusitis. *Otolaryngol Head Neck Surg*. 1997;117(3):S35–40. doi:10.1016/S0194-59989770005-6
27. Benninger MS, Senior BA. The development of the Rhinosinusitis Disability Index. *Arch Otolaryngol Head Neck Surg*. 1997;123(11):1175–1179. doi:10.1001/archotol.1997.01900110025004
28. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP. Psychometric validity of the 22-item Sinonasal Outcome Test. *Clin Otolaryngol*. 2009;34 (5):447–454. doi:10.1111/j.1749-4486.2009.01995.x
29. Orb Q, Pulsipher A, Smith KA, Ashby S, Alt JA. Correlation between systemic inflammatory response and quality of life in patients with chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2019;9(5):458–465. doi:10.1002/alr.22289
30. Blight BJ, Gill AS, Sumsion JS, et al. Cell adhesion molecules are upregulated and may drive inflammation in chronic rhinosinusitis with nasal polyposis. *J Asthma Allergy*. 2021;14:585–593. doi:10.2147/JAA.S307197
31. Gowhari Shabgah A, Al-Obaidi ZMJ, Sulaiman Rahman H, et al. Does CCL19 act as a double-edged sword in cancer development? *Clin Exp Immunol*. 2022;207(2):164–175. doi:10.1093/cei/uxab039
32. Crowe A, Yue W. Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated protocol. *BIO-PROTOCOL*. 2019;9(24). doi:10.21769/bioprotoc.3465
33. Mfuna Endam L, Cormier C, Bosse Y, Filali-Mouhim A, Desrosiers M. Association of IL1A, IL1B, and TNF gene polymorphisms with chronic rhinosinusitis with and without nasal polyposis: a replication study. *Arch Otolaryngol Head Neck Surg*. 2010;136(2):187–192. doi:10.1001/archoto.2009.219
34. Fajardo-Dolci G, Solorio-Abreu J, Romero-Alvarez JC, et al. DQA1 and DQB1 association and nasal polyposis. *Otolaryngol Head Neck Surg*. 2006;135(2):243–247. doi:10.1016/j.otohns.2006.03.034
35. Erbek SS, Yurtcu E, Erbek S, Atac FB, Sahin FI, Cakmak O. Proinflammatory cytokine single nucleotide polymorphisms in nasal polyposis. *Arch Otolaryngol Head Neck Surg*. 2007;133(7):705–709. doi:10.1001/archotol.133.7.705
36. Piski Z, Gerlinger I, Nepp N, Farkas K, Weber R. TNF-alpha inhibitors and rhinosinusitis-a systematic review and meta-analysis. *Am J Rhinol Allergy*. 2020;34(3):436–442. doi:10.1177/1945892419898988
37. Ulvmar MH, Hub E, Rot A. Atypical chemokine receptors. *Exp Cell Res*. 2011;317(5):556–568. doi:10.1016/j.yexcr.2011.01.012
38. Bonecchi R, Graham GJ. Atypical chemokine receptors and their roles in the resolution of the inflammatory response. *Front Immunol*. 2016;7:224. doi:10.3389/fimmu.2016.00224
39. Smith KA, Gill AS, Pollard CE, et al. An eosinophil peroxidase activity assay accurately predicts eosinophilic chronic rhinosinusitis. *J Allergy Clin Immunol*. 2023;152(2):400–407. doi:10.1016/j.jaci.2023.04.012