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ORIGINAL RESEARCH

Association of Increased Nasal Fluids-Serum Concordance of Protein Profile with Prognosis in Nasal Polyps

Yilin Hou[1–5], Lin Sun[2–5], Changhui Chen[2–5], Mengqi Su[6], Shimin Lai[2–5], Yan Yan[2–5,7], Jieying Yan[2–5], Rongjian Zhan[2–5], Yongjin Su[1], Weiqiang Yang[1], Yi Wei[2–5], Weiping Wen[2–5,7], Hongyi Hu[1]

[1]Otorhinolaryngology, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong, People's Republic of China; [2]Department of Otorhinolaryngology, First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, People's Republic of China; [3]Department of Allergy, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, People's Republic of China; [4]Otorhinolaryngology Institute of Sun Yat-sen University, Guangzhou, Guangdong, People's Republic of China; [5]Guangzhou Key Laboratory of Otorhinolaryngology, Guangzhou, Guangdong, People's Republic of China; [6]Department of Cardiology, The University of Hong Kong-Shenzhen Hospital, Shenzhen, Guangdong, People's Republic of China; [7]Department of Otorhinolaryngology, Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, People's Republic of China

Correspondence: Hongyi Hu, Otorhinolaryngology, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, No. 1120 Lianhua Road, Futian District, Shenzhen, Guangdong, 518036, People's Republic of China, Email hyihu@sina.com

Purpose: Predicting postoperative nasal polyp recurrence (PR) remains challenging because existing approaches rely on single clinical samples and thus fail to capture the complex, multi-compartment biology of the disease. This study aims to determine whether nasal fluid (NF) and serum protein concordance, reflecting the degree of coupling between local nasal and systemic inflammation, provides a more integrated prognostic signature for PR.

Patients and Methods: Using the proximity extension assay (PEA), we quantified 92 proteins in NF and serum from 54 nasal polyps (NPs) patients (including 18 PR and 36 non-PR). In this prospective study, with PR assessed after a minimum follow-up of 6 months, NF-serum protein concordance was comprehensively assessed for associations with disease features and PR. Kaplan-Meier analysis and univariate Cox regression identified PR-predictive biomarkers, validated via bulk RNA-seq, single-cell RNA-seq (scRNAseq), immunofluorescence, and public database analysis.

Results: Proteomic analysis of NF and serum revealed distinct protein profiles between PR and non-PR groups. Elevated NF-serum concordance was observed in current smokers and asthmatics, and positively correlated with eosinophil counts (r=0.26), pre-surgery Lund-Mackay (LM) scores (r=0.34), nasal VAS (r=0.26) and SNOT-22 scores (r=0.46) (Pearson's test, all $P<0.05$). Survival analysis demonstrated that higher NF-serum concordance predicted increased PR risk (HR=7.9, 95% CI=2.4–25.6, $P<0.001$). Univariate Cox regression identified leukemia inhibitory factor receptor (LIFR) as a novel PR biomarker (HR=2.1, 95% CI=1–4.2, $P<0.05$). Bulk RNA-seq confirmed LIFR upregulation in PR ($P<0.05$), while scRNA-seq localized predominant LIFR expression to CRSwNP endothelial cells. Immunofluorescence verified LIFR-CD31 colocalization with stronger LIFR signals in PR ($P<0.05$). Transcriptional (Twist1/NR2F2) and post-transcriptional (miRNA-mediated) regulatory mechanisms were implicated in LIFR-driven pathogenesis.

Conclusion: Our study first investigated NF-serum proteomic concordance as a predictor of PR and demonstrated the potential of integrated multi-compartment analysis for predicting recurrence.

Keywords: chronic rhinosinusitis with nasal polyps, nasal fluids, proteomics, polyps recurrence, bioinformatic analysis

Introduction

Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) is a heterogeneous group of sinus disorders.[1,2] The current treatment for moderate to severe nasal polyps (NPs) involves endoscopic sinus surgery (ESS),[3] while the polyp recurrence (PR) rate remains high (14%–24%).[4] Prior attempts based on a single sample type (tissue, nasal secretions or serum)[5–7] are inherently limited because PR is a multifactorial process. A comprehensive correlation analysis is crucial

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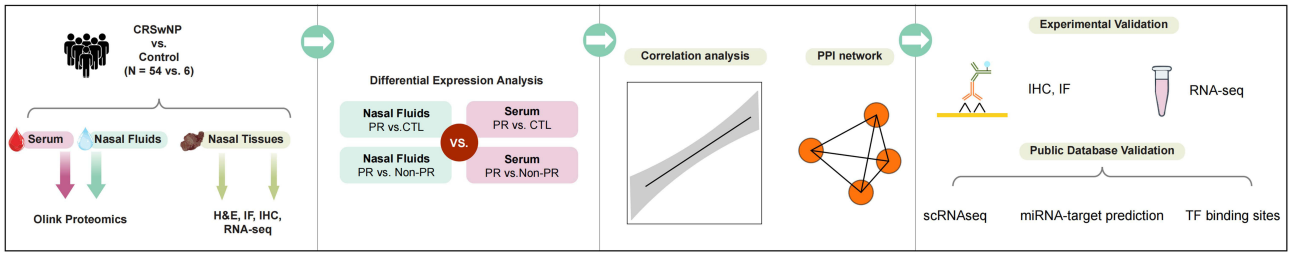
Graphical Abstract



yet currently unavailable. Therefore, emphasizing the overall interplay across the nasal mucosa, mucosal secretions, and vascular system remains a significant challenge in current research.

The nasal epithelium serves as the first barrier to the external environment. When the nasal mucosa is stimulated or infected, the submucosal vascular system participates in the immune response, transporting immune cells and cytokine mediators to the site of infection, reflecting an increase in vascular permeability.[1,8] Additionally, the protective mucus layer with antimicrobial peptides helps remove the harmful substances, bacteria, and other contents from the lumen.[9,10] Mucus layer is a complex viscous nasal fluids (NFs), which is affected by glandular secretions, goblet cells, ion/water transport, and plasma protein leakage.[11] During the pathological process of CRSwNP, chronic nasal mucosa inflammation can disrupt the tight junctions between epithelial cells, promote the activation and migration of local immune cells and cytokines, and spread from the nasal epithelium into nasal secretions.[12,13] Therefore, the changes of cytokines in NF not only reveal the inflammatory status of nasal epithelium, but may also reflect the dynamic role of the entire immune system.

This study introduces the first analysis of local-systemic compartmentalization in upper respiratory inflammatory diseases through a novel "Nasal Fluids-Serum Concordance Analysis." Using proteomic profiling of CRSwNP patients with surgical follow-up, we identified the leukemia inhibitory factor receptor (LIFR), as well as IL-5 and IL-13, as non-invasive predictive biomarkers for postoperative polyp recurrence. LIFR contributes to tissue remodeling, angiogenesis, and neuro-immune crosstalk, while IL-5 and IL-13 drive type 2 inflammation and promote eosinophil recruitment. One major difference between our research and previous proteomics studies is that we reliably analyzed the concordance in matching NF and serum through proteomics profiling analysis. Furthermore, our data emphasize that the increased consistency between NF and serum protein levels is associated with more severe disease characteristics and a higher PR probability from an entirely new perspective.

Material and Methods

Patients and Study Design

This prospective cohort study complied with the Declaration of Helsinki and was approved by the Ethics Committee on Scientific Research of the First Affiliated Hospital of Sun Yat-sen University (No. 2023–211), and written informed consent was obtained from all participants prior to surgery. Pre- and post-operative demographic, clinical, and radiological characteristics were extracted from hospital electronic medical records. Of these, CRSwNP group (n=54) diagnostic criteria was based on patient history, physical examination, nasal endoscopy, and computed tomography (CT) scans, following the European Position Paper on Sinusitis and Nasal Polyps (EPOS) guidelines.[14] The detailed exclusion criteria are available in the Supplementary Methods. The control group (n=6), consisting of patients undergoing nasal surgery for non-inflammatory conditions (eg, anatomical abnormalities or trauma). The characterization of the participants are shown in Table 1.

Table 1 The Clinical Characteristics of CRSwNP Patients and Controls: Median [Minimum, Maximum] or Frequency (Percent)

	Control (N=6)	Non-PR (N=36)	PR (N=18)	*P Value*
Clinical Characteristic				
Age, Years	28.5 [27.0, 42.0]	44.0 [18.0, 67.0]	36.5 [21.0, 58.0]	0.092
Gender, Male (%)	5 (83.3)	25 (69.4%)	12 (66.7%)	> 0.999
Smoke, Yes (%)	0 (0)	11 (30.6%)	4 (22.2%)	0.747
Asthma, Yes (%)	0 (0)	2 (5.6%)	7 (38.9%)	**0.007**
Tissue EOS counts,/HPF	0 [0, 1.0]	7 [0, 50]	10.5 [0, 150]	**0.028**
Tissue NEU counts,/HPF	0 [0, 1.0]	5.5 [0, 120]	1.5 [0, 32]	0.119
Pre-surgery assessment				
Total LM scores	0 [0, 1.0]	14.5 [4, 24]	22 [11, 24]	**0.001**
Total MLK scores	0 [0, 1.0]	10 [4, 12]	10 [6, 12]	0.201
SNOT-22 scores	9.0 [2.0, 13.0]	19.5 [8, 82]	39.5 [12, 94]	**0.044**
VAS scores	2.0 [2.0, 4.0]	7 [2, 10]	8 [3, 10]	0.544
Post-surgery assessment				
Follow-up Time	–	373.5 [182, 761]	362 [180, 777]	0.560
Total MLK scores	–	2 [0, 8]	9.5 [4, 12]	**< 0.001**
SNOT-22 scores	–	10.5 [0, 42]	31 [1, 52]	**0.049**
VAS score	–	2 [0, 8]	5 [0, 8]	**0.006**

Notes: The Mann–Whitney *U*-test (2-tailed) was used for continuous variables, and the chi-square test was used for categorical variables. Bolded *p* values are statistically significant.

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; PR, polyp recurrence; NEU, neutrophil; EOS, eosinophil; HPF, high power field; LM, Lund-Mackay; MLK, modified Lund-Kennedy; SNOT-22, 22-item Sino-Nasal Outcome Test; VAS, Visual Analogue Scale.

Sample Collection and Protein Analysis

Nasal tissue, NF, and serum samples were collected from each patient prior to enrollment in the study. Polyp tissues were obtained from CRSwNP patients, while inferior turbinate tissues were collected from control subjects. These tissue samples were utilized for histological analysis (including H&E staining, immunohistochemistry, and immunofluorescence) and bulk RNA-seq.

NFs were collected using the Nasal Fluids Absorption Device (NFAD), which was developed in our laboratory. The procedure was conducted as previously described.[15] Briefly, the NFAD, made from polyester fiber (Xinweike Anti-STATIC Technology, Guangdong, China), was inserted into each nostril and left in place for 4 minutes. The collected fluid was subsequently centrifuged at 16,000g at 4°C for 10 minutes. Cytokine levels of different analytes in NF were analyzed using the Target 96 Inflammation panel from OLINK Proteomics® (Uppsala, Sweden) via high-sensitivity proximity expansion assay (PEA), see detailed information in https://www.olink.com/resources-support/document-download-center/.[16] Serum samples from the same participants were analyzed using the same OLINK panels. See Supplementary Table S1 for a full list of indicator markers.

Data were generated using normalized protein expression (NPX) values, expressed on a log2 scale. Higher NPX values correspond to higher protein expression levels.[17] Data containing the Serum and Nasal Fluids Proteomics data in the paper are available in Mendeley Data (DOI: 10.17632/nnpvf66x6h.2).

Clinical Assessments and Follow-Up

The clinical characteristics were assessed through CT scans, nasal endoscopy tests, and nasal symptom evaluations using the 22-item Sino-Nasal Outcome Test (SNOT-22) (range: 0–110) and the Visual Analog Scale (VAS) (range: 0–10), as well as eosinophil and neutrophil counts in nasal mucosal tissues. Polypectomy and open sinus surgery were performed based on the principles of ESS.[14] A week post-surgery, CRSwNP patients were treated with budesonide nasal spray (50 μg once daily, one spray in each nostril) for ≥3 months. Regular surgical cavity cleaning (every 4 weeks for 6 months) was also performed, which included removal of dry scabs and vesicles, and checking for recurrent polyps. At

least 6 months post-surgery, if gray, lychee-like tissue was observed in the middle nasal passage, patients received a course of double-dose intranasal corticosteroids (mometasone furoate 100 μg once daily, 2 sprays/nostril) as a rescue treatment for 2–4 weeks. The use of post-surgery antibiotics was generally conservative, prescribed only in the case of direct evidence of a pathogenic infection. Antibiotic selection, in principle, was based on bacterial culture and susceptibility testing. After 2 weeks of nasal corticosteroid therapy, if lychee-like tissues (instead of cobblestone or polypoid mucosa) were still present, fully occupying the middle meatus as seen on rhinoscopy or endoscopy, this was defined as polyp recurrence (PR, N=18). If not, it was classified as non-polyp recurrence (non-PR, N=36).

H&E, Immunohistochemistry (IHC) and Immunofluorescence (IF)

The approximately 3–5 mm^3 nasal tissues were rinsed with PBS, fixed in 4% PFA, paraffin-embedded, sectioned at 4μm and stained with H&E. Based on eosinophil infiltration quantified at 400× magnification (high-power field, HPF), samples were classified as: (1) Eosinophilic CRSwNP (E-CRSwNP): ≥10 eosinophils/HPF; (2) non-eosinophilic CRSwNP (NE-CRSwNP): <10 eosinophils/HPF. For IHC staining, the tissue sections were processed through dewaxing, antigen retrieval, and inactivation of endogenous peroxidase. After 1 h blocking with 10% BSA at room temperature, sections were incubated overnight at 4°C with primary antibodies, including neutrophil elastase (NE; Abcam #ab68672) for neutrophil quantification or LIFR (Origene #TA386958) for LIFR expression detection, followed by DAB substrate. For immunofluorescence, endothelial cells in nasal mucosa were marked using an anti-CD31 antibody (Bioss #bsm-10825M). Fluorescently labelled secondary antibodies were from Thermo Fisher Scientific. Images were analyzed quantitatively with ImageJ software.[18] All H&E and immunohistochemical slides were evaluated independently by two experienced pathologists. See details in Supplementary Methods.

Validation of Candidate Biomarkers Using Transcriptomics and Public Databases

We verified the candidate biomarkers through bulk RNA-seq analysis of our cohort (Supplementary Figure 1)[19] and by re-analyzing a public scRNA-seq dataset (GSA: HRA000772). The detailed methods are shown in Supplementary Methods. The miRNA-target regulatory relationships were predicted by Target Finder (https://jingle.shinyapps.io/TF_Target_Finder/). Transcription factor (TF) binding sites were predicted using the JASPAR Transcription Factor Binding Site database (https://jaspar.genereg.net/). Potential LIFR-targeting miRNAs were predicted using TargetScan (https://www.targetscan.org/vert_80/).

Statistical Analysis

Statistical analyses were performed using R software (version 4.1.3) and Prism 9.0 (GraphPad Software, CA, USA). All graphical graphs were generated using the "ggplot2" package. Demographic and clinical characteristics were summarized as Medians [minimum, maximum] for continuous variables and as Frequencies (percentages) for categorical variables (Table 1). The Mann–Whitney *U*-test was applied to compare continuous variables between two groups, while one-way ANOVA was used for comparisons across three groups. The Wilcoxon test was used to compare matched pairs. Fisher's exact test was used to assess categorical outcomes. Principal component analysis (PCA) was used to observe the degree of variability between samples within groups. Differentially expressed proteins (DEPs) between two groups were analyzed using the "Olink Analyze" R package. To avoid missing biologically relevant signals, proteins meeting a $P < 0.1$ in the exploratory screening were used for generating hypotheses in the differential expression analysis and were not included in the concordance analysis (see Supplementary Table S2 for results). GO_BP analysis and KEGG analysis were used to explore the roles of the DEPs using Metascape database (https://metascape.org/).[20] Protein-protein interaction (PPI) networks were predicted with STRING (http://string-db.org/).[21]

Pearson's correlation coefficient (r) was used to assess the correlation between continuous variables and was described as follows: $|r|>0.7$, strong correlation; $0.5\leq|r|\leq0.7$, medium correlation; $0.3\leq|r|<0.5$, weak correlation; and $|r|<0.3$, virtually no correlation.[22] A scatter plot comparing the PR and non-PR groups with Pearson's r revealed the potential association between concordance in NF and serum proteins. Univariate Cox regression analysis was used to identify variables predicting PR and forest plot was conducted using the Forest plot R package. Kaplan-Meier survival analysis was done by applying log rank test.

Results

Proteomic Profiling of Nasal Fluids and Serum in Enrolled Patients

Proximity extension assay (PEA)-based proteomic profiling was conducted to quantify protein expression, as detailed in the Methods section. This technique enabled the measurement of 92 proteins across a wide dynamic range in NF and serum samples obtained from 54 CRSwNP patients and 6 controls (Figure 1A). Overall, the relative abundance of most proteins was higher in NF than in serum (81/92, 88.04%), with the exception of TRANCE, CCL25, FGF-19, LAP TGF-

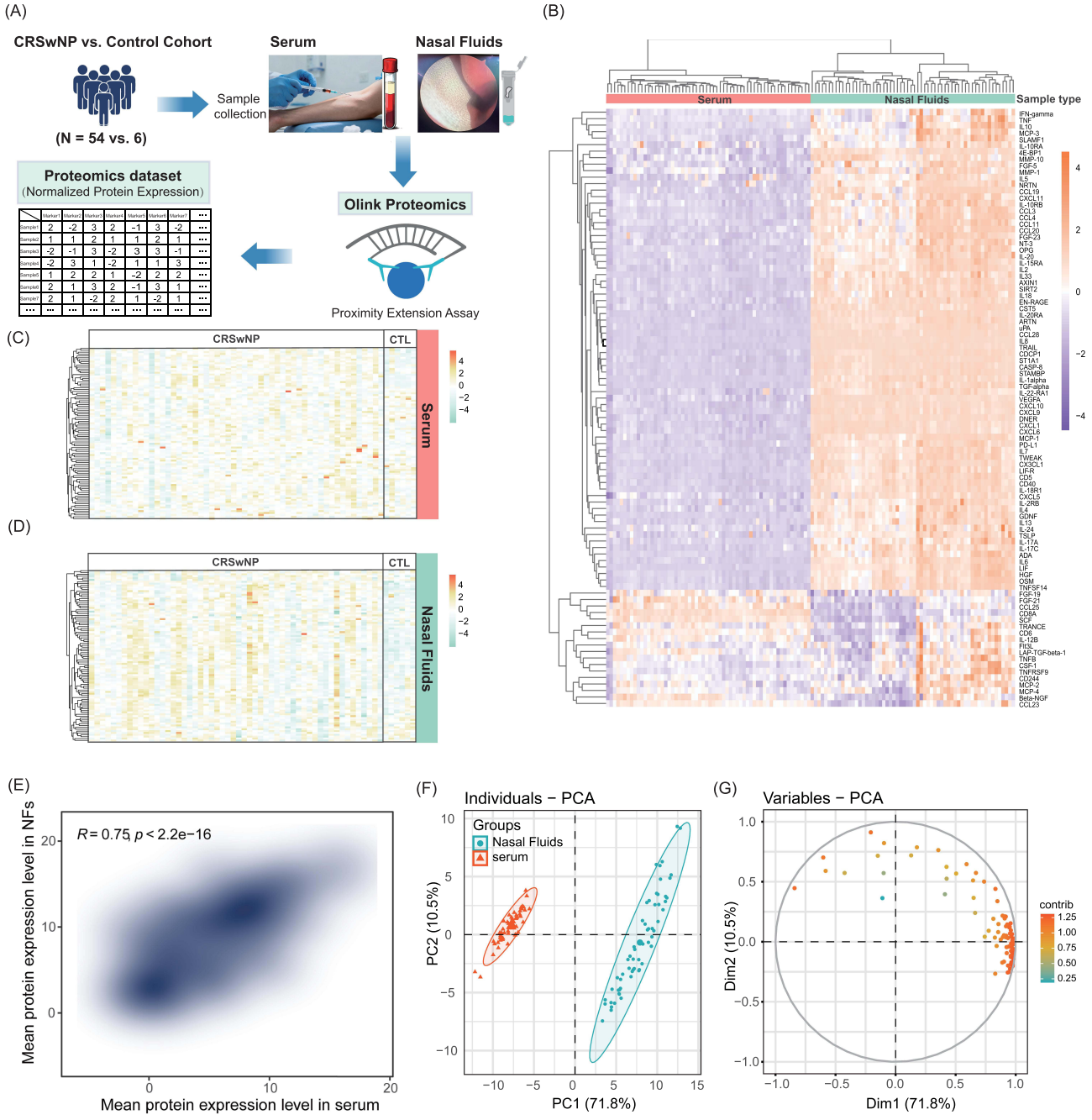


Figure 1 Quantification of Proteins in NF and Serum of Enrolled Patients. (**A**) Workflow of OLINK PEA technology used in this study. (**B**) Heatmap of the overall proteomics expression in NF and serum. Heatmap of protein expression in (**C**) serum and (**D**) NF samples from all NP patients and healthy controls. (**E**) Pearson correlation (r and *P* value) between different subjects for the detected targets. (**F**) PCA plot of serum and NF proteomics data from the enrolled patients. The first component explains 71.8% of the variation, and the second component explains 10.5%. (**G**) PCA loading plot showing the partial contribution of variables in the PCA analysis.
Abbreviations: NFs, nasal fluids; PEA, proximity extension assay; PCA, principal component analysis.

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beta-1, Beta-NGF, CD8A, SCF, IL-12B, CD6, CCL23, and FGF-21 (Figure 1B). Heatmap comparison between the CRSwNP group and the control group were shown in Figure 1C and 1D. Notably, a strong positive correlation was observed between the average protein levels in NF and serum across the entire dataset (r = 0.75, $P < 0.001$; Figure 1E), indicating consistent detection of proteins in both sample types. This correlation remained robust and was not influenced by patient subgrouping (Supplementary Figure S2). PCA was employed to differentiate the protein expression profiles of NF and serum. After normalization, the score plot (Figure 1F) and variable loading plot (Figure 1G) demonstrated clear separation between the two sample types.

Differential Protein Expression in Nasal Fluids and Serum Between PR and Non-PR CRSwNP Groups

The baseline characteristics of PR and non-PR CRSwNP patients are summarized in Table 1. Follow-up in these patients was at least 6 months. DEPs with fold changes and statistical significance are shown in the volcano plots (Figure 2A and 2B). In NFs, we identified 8 significant DEPs between PR and non-PR groups (6 up-regulated, 2 down-regulated), while serum analysis revealed 19 DEPs (17 up-regulated, 2 down-regulated; see Supplementary Table S2). Trend analysis demonstrated distinct biomarker expression patterns in NF and serum among PR, non-PR, and control groups (one-way ANOVA). Among

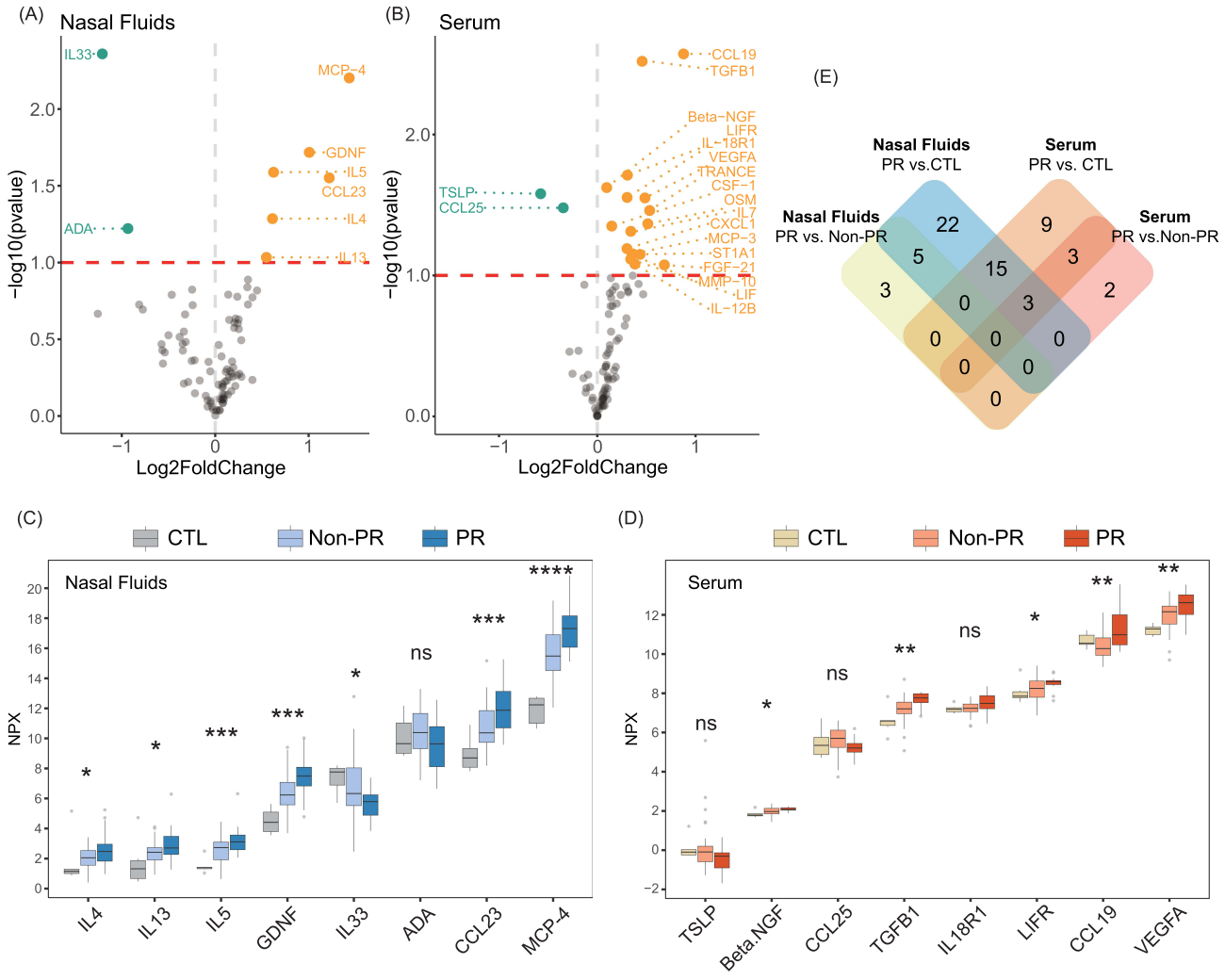

Figure 2 Differential Expression Analysis in NF and Serum of PR and non-PR CRSwNP Patients. Significant differences in biomarkers were observed between the PR and non-PR groups in NF (**A**) and serum (**B**), respectively. The expression of the top DEPs in the control, non-PR, and PR groups for both NF (**C**) and serum (**D**). Values are presented as median ± IQR. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns, not significant, analyzed by one-way ANOVA. (**E**) Venn diagram of DEPs.
Abbreviations: NFs, nasal fluids; PR, polyps recurrence; DEPs, differentially expressed proteins; IQR, interquartile range.

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the top 8 DEPs in NF, IL4, IL5, IL13, GDNF, IL33, CCL23, and MCP-4 exhibited increasing expression (all Anova $P<0.05$), whereas ADA showed no significant difference between PR and controls (Figure 2C). Similarly, serum analysis revealed sequential increases in Beta-NGF, LAP TGF-β1, LIFR, CCL19, and VEGFA (all Anova $P<0.05$), while TSLP, CCL25 and IL18R1 expression remained unchanged between PR and controls (Figure 2D).

Notably, Venn diagram analysis identified no overlapping DEPs between NF and serum (Figure 2E). This suggests that these proteins may function together in distinct pathways or biological processes. To explore potential clinical implications, we further assessed whether protein expression correlation between NF and serum correlated with disease characteristics or recurrence outcomes.

Enhanced Correlation Between Nasal Fluid and Serum Protein Abundance as a Disease-Associated Feature

We investigated the correlation between NF-serum protein concordance values and clinical features in CRSwNP patients. The Ranking Correlation Coefficient (RCC) is defined as Pearson correlation between NF and serum protein expression ranking vectors.[23] An elevated RCC indicates a higher average Pearson's r between the NF and serum proteomes. Consistent with this, our analysis revealed significantly higher global concordance in PR patients (r=0.74) versus non-PR (r=0.72) and controls (r=0.67, $P=0.002$; Figure 3A and Supplementary Table S3). The stability of these results was confirmed through bootstrap resampling of proteome subsets (Supplementary Figure S3), supporting the robustness of the methodology.

As shown in Figure 3B, RCC levels were higher in smoking CRSwNP patients compared to non-smoking CRSwNP patients. Likewise, CRSwNP patients with comorbid asthma exhibited higher global RCC levels than those without asthma (Figure 3C). We further identified functional correlations between increased NF-serum concordance and both histological and clinical parameters. Tissue eosinophil counts positively correlated with RCC (r=0.261, $P<0.05$; Figure 3D), while neutrophil counts showed no association (r=0.042, $P=0.752$; Figure 3E). Pre-ESS clinical scores, including nasal VAS, SNOT-22, and LK scores also demonstrated significant positive correlations with RCC (r=0.257, 0.456, and 0.343 respectively; all $P<0.05$; Figure 3F–3H), except for pre-ESS MLK score (r=0.153, $P=0.244$; Figure 3I).

Given the observed association between NF-serum concordance and PR risk, we stratified CRSwNP patients into high ($>2/3$) and low ($<2/3$) concordance groups to assess differences in PR probability. Survival analysis indicated that elevated global concordance was significantly associated with a higher risk of PR (HR=7.98, 95% CI=2.48–25.67, $P<0.001$; Figure 3J). This tendency was also observed when the concordance scores were categorized into high (>2/3), middle (1/3-2/3), and low (<1/3), see Supplementary Figure S4. Further, an RCC effect plot confirmed strong model fit and predictive capability ($P=0.006$, AIC=62.44; Figure 3K).

Biomarkers for Polyp Recurrence Prediction Based on Nasal Fluid-Serum Protein Concordance

Both PR and non-PR groups exhibited distinct NF–serum protein concordance patterns. The PR group demonstrated significantly higher concordance scores for 48 inflammatory markers (designated as Part I), which were characterized by their larger size and predominance on the left side of the scatterplot, reflecting greater fold changes (Figure 4A and 4B). Conversely, significantly lower concordance was observed for 44 proteins (Part II; Figure 4A and 4C). KEGG pathway enrichment analysis revealed that the highly concordant proteins in PR patients were primarily involved in immune-activating pathways, including cytokine–cytokine receptor interaction, JAK-STAT signaling, PI3K-Akt signaling, and NF-κB signaling (Figure 4D). Meanwhile, the most significantly enriched GO_BP terms included positive regulation of response to external stimulus, positive regulation of type 2 immune response, and eosinophil chemotaxis (Figure 4E; Supplementary Tables S4 and S5). Protein–protein interaction (PPI) network analysis further indicated strong modular connectivity among these proteins (Figure 4F). Given the association between globally elevated NF–serum concordance and disease prognosis, we next sought to identify hub biomarkers capable of predicting polyp recurrence outcomes. Univariate Cox regression

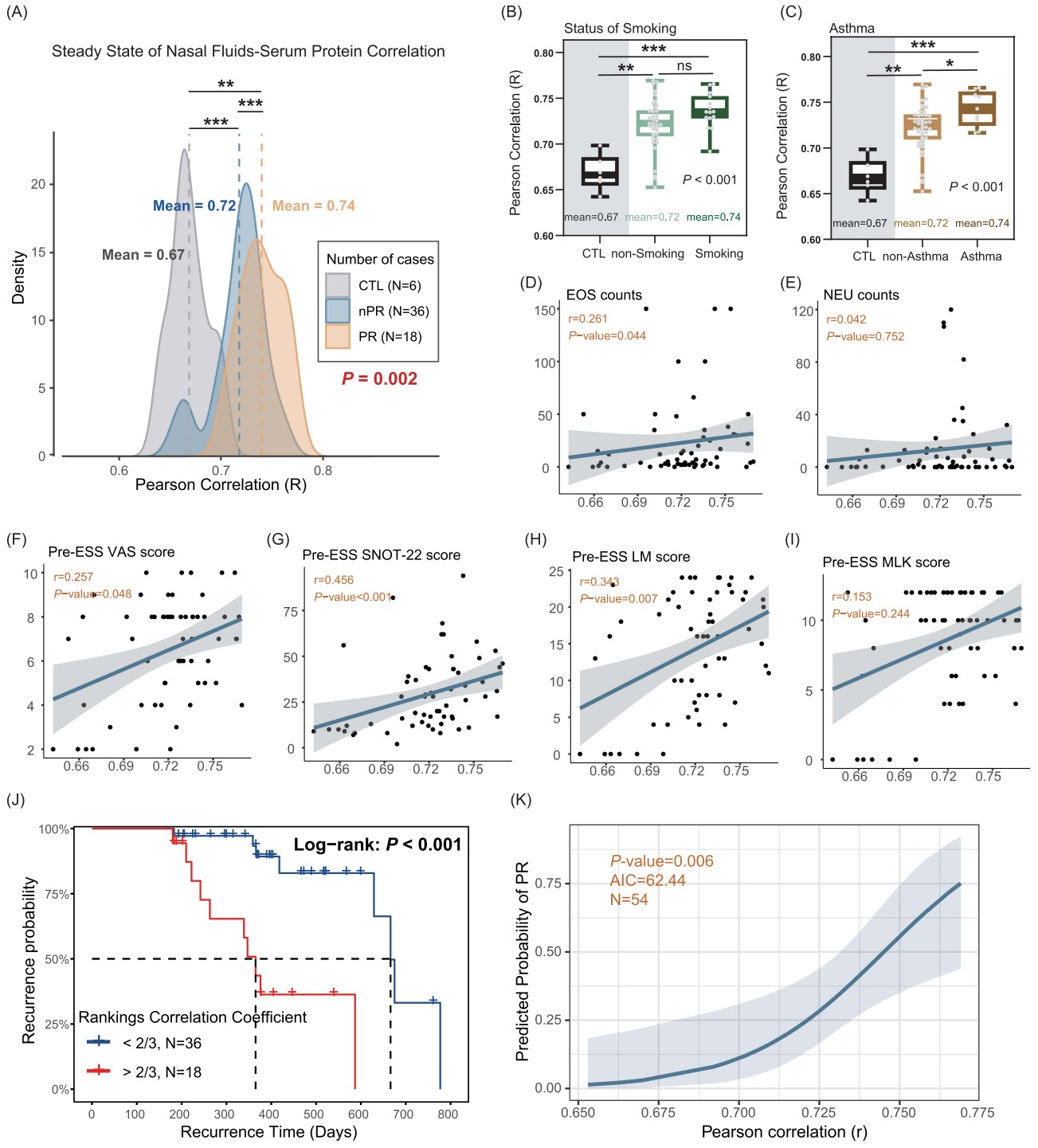

Figure 3 Correlation between NF-serum protein concordance and its association with disease characteristics. (**A**) Density plot showing the global Pearson correlation (r) for NF-serum protein pairs within the PR group (n=18), non-PR group (n=36), and control group (n=6). The NF-serum protein concordance values in with or without (**B**) smoking status, and (**C**) comorbid asthma. Scatter plot between global NF-serum protein concordance values and (**D**) tissue EOS counts, (**E**) tissue NEU counts, (**F**) pre-ESS VAS score, (**G**) SNOT-22 score, (**H**) LM score, and (**I**) MLK score. (**J**) Kaplan-Meier analysis stratified by NF-serum protein concordance revealed significantly higher recurrence in high-concordance CRSwNP patients (P<0.001). (**K**) Effect plot demonstrates positive correlation between global NF-serum protein concordance and PR probability (P=0.006). *P < 0.05, **P < 0.01, ***P < 0.001, ns, no significant difference (p > 0.05).
Abbreviations: EOS, eosinophils; NEU, neutrophils; ESS, Endoscopic Sinus Surgery; VAS, Visual Analog Scale; LM, Lund-Mackay; VAS, visual analog scale.

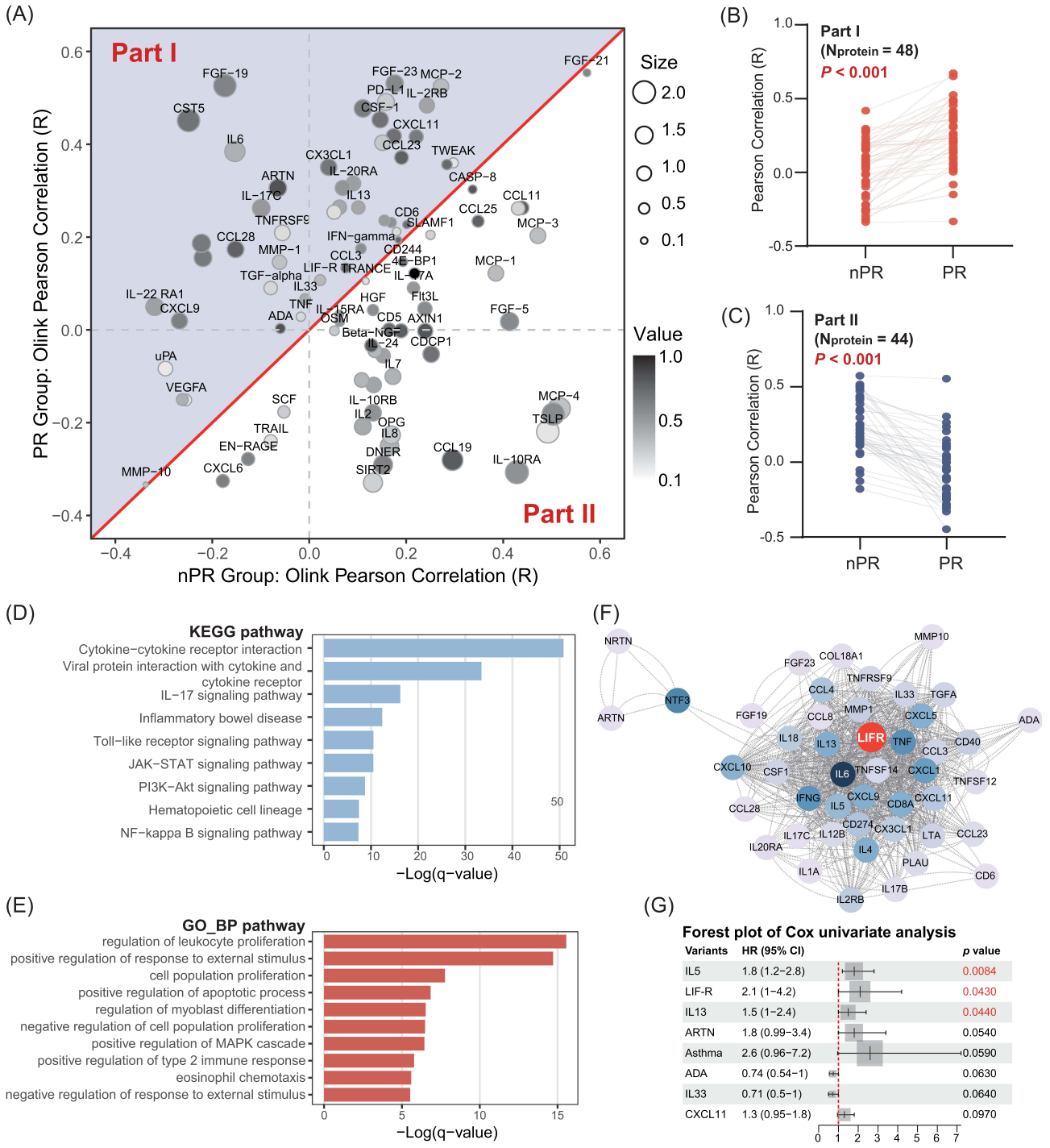

Figure 4 Prognostic value of increased concordance of NF-serum protein biomarkers. (**A**) Pearson correlation scatterplots showing NF-serum protein concordance scores for the PR group (y-axis) versus the non-PR group (x-axis). Points above the diagonal indicate higher concordance in the PR group (Part I), while points below indicate lower concordance (Part II). Circle color represents the absolute difference in concordance scores, and circle size reflects the vertical distance from the diagonal. Comparison of Pearson correlation in NF-serum protein concordance scores between the PR and non-PR groups for the protein set in (**B**) Part I, $N_{protein}$=48 and (**C**) Part II, $N_{protein}$=40. The Wilcoxon test was used to compare matched pairs. (**D**) KEGG, (**E**) GO-BP enrichment analysis, and (**F**) protein-protein interaction (PPI) network of Part I biomarkers. (**G**) Forest plot of univariate Cox analysis.

Abbreviations: AUC, Area under the curve; ROC, Receiver operating characteristic.

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analysis identified three prognostic biomarkers for PR, see Figure 4G: IL-5 (HR = 1.8, 95% CI = 1.2–2.8), LIFR (HR = 2.1, 95% CI = 1.0–4.2), and IL-13 (HR = 1.5, 95% CI = 1.0–2.4); see Supplementary Table S6 for details.

Expression Validation of Key LIFR Biomarker as Polyp Recurrence

Notably, univariate COX regression identified leukemia inhibitory factor receptor (LIFR) as a novel biomarker in NP pathogenesis. Transcriptomic profiling of polyp tissues showed significantly elevated expression of LIFR, its ligand LIF, as well as IL5 and IL13 (established prognostic markers in nasal polyposis) in the PR group ($P < 0.05$; Figure 5A). This upregulation was confirmed at the protein level, as ELISA quantification revealed significantly higher LIFR concentrations in both NFs and serum of PR patients compared to non-PR and control groups ($P < 0.05$; Figure 5B and 5C). IHC analysis confirmed markedly increased membrane-localized LIFR expression in the PR group compared to non-PR controls, within both the nasal epithelium and submucosa ($P < 0.05$; Figures 5D and 5E). In PR tissues, there is more severe epithelial disruption and dense inflammatory infiltration. Intense, granular LIFR staining is predominantly localized to the epithelial cells and is focally present on inflammatory cells within the edematous stroma. Re-analysis of public scRNA-seq data (GSA: HRA000772; Wang et al[24] indicated predominant LIFR expression in CRSwNP endothelial cells (Figure 5F and 5G). Immunofluorescence assays further validated LIFR colocalization with CD31 (Pecam1) endothelial cells, with markedly stronger LIFR signal exhibiting continuous distribution along the CD31-positive vascular walls in the PR group ($P < 0.05$; Figure 5H).

Bioinformatics Reveals Twist1/NR2F2 and miRNA-Mediated Regulation of LIFR

To elucidate the regulatory mechanisms of LIFR expression in NP pathogenesis, we conducted comprehensive bioinformatics analyses. TF prediction using TF-Target Finder,[25] integrating FIMO-JASPAR, KnockTF, ChIP-Atlas, GTRD, and PWMEnrich-JASPAR databases, identified Twist1 and NR2F2 as consensus transcriptional regulators of LIFR (Figure 6A). In silico analysis of the LIFR promoter using JASPAR predicted putative binding sites for both TFs, supporting their potential role in transcription regulation (Figure 6B). Additionally, TargetScan revealed three miRNAs (miR-143-3p, miR-4770, and miR-6088) targeting the LIFR 3'UTR, suggesting miRNA-mediated post-transcriptional regulation (Figure 6C). Together, these findings suggest a multi-layered regulatory network involving both transcriptional (Twist1/NR2F2) and post-transcriptional (miRNA-mediated) mechanisms that may regulate LIFR-driven pathology in nasal polyposis.

Discussion

Our study provides the first comprehensive proteomic characterization of paired NF and serum samples in CRSwNP, identifying distinct inflammatory cytokine patterns between NF and serum, consistent with prior reports.[26,27] Importantly, we found that increased NF-serum protein consistency is a novel pathophysiological feature of CRSwNP. Furthermore, we identified LIFR as the first NF-derived prognostic biomarker for postoperative polyp recurrence and analyzed its transcriptional (Twist1/NR2F2) and post-transcriptional (miR-143-3p/miR-4770/miR-6088) regulation through bioinformatics analysis.

To the best of our knowledge, the relationship between NF and serum proteomic abundance as a prognostic marker in CRS has not been previously reported. However, similar patterns of protein concordance between distinct anatomical regions associated with disease aggressiveness have been reported in other diseases. For example, in intermittent explosive disorder (IED), plasma and cerebrospinal fluid (CSF) levels of CRP, IL-8, and TNF-α correlated significantly with each other, with higher expression being found to be relatively more susceptible to aggressive behavior and/or aggressive tendencies.[28] By dynamically observing changes in cytokines in fracture blister fluid (FBF) and plasma, Li et al found that different cytokine activity states from the inflammatory stage to the proliferative and repair stages in fracture blisters.[23] Additionally, in rheumatoid arthritis patients, certain inflammatory proteins may play an important role in the inflammatory response, such as IL-35, with higher concentrations in synovial fluid than in serum, and is positively correlated with serum C-reactive protein (CRP) levels and the Disease Activity Score for 28 Joints (DAS28).[29]

The lower average concordance in CRSwNP patients may be attributed to endotype heterogeneity, or it could be due to differences in individual immune status, as our data show that concordance between NF-serum protein pairs is related

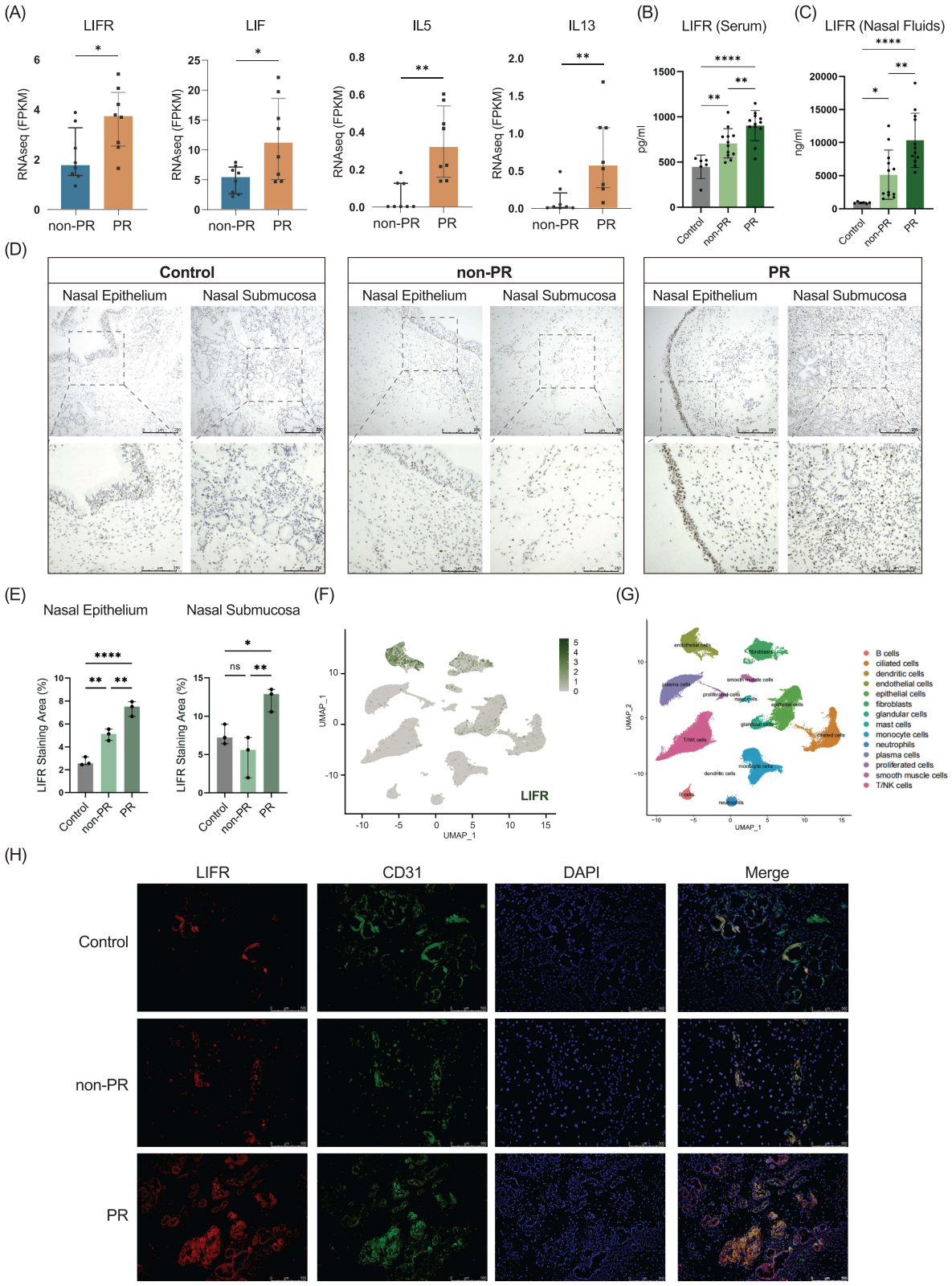

Figure 5 Expression validation of LIFR in CRSwNP. (**A**) Transcriptional levels of biomarkers (LIFR, LIF, IL-5, IL-13) in PR versus non-PR groups. ELISA quantification of LIFR concentrations in both (**B**) serum and (**C**) NF of PR patients compared to non-PR and control groups. (**D**) Representative images of H&E and LIFR immunohistochemical staining (Magnification ×400). (**E**) Quantification of LIFR protein expression intensity (n=6/group). (**F** and **G**) scRNA-seq analysis of CRSwNP tissues showing LIFR expression patterns across cell populations (left: cell type clusters; right: LIFR expression). (**H**) Immunofluorescence of LIFR (red) co-localization with vascular endothelial cells (CD31+, green) and nuclear staining (DAPI, blue) at ×400 magnifications (n=6/group). *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$.
Abbreviation: ns, not significant.

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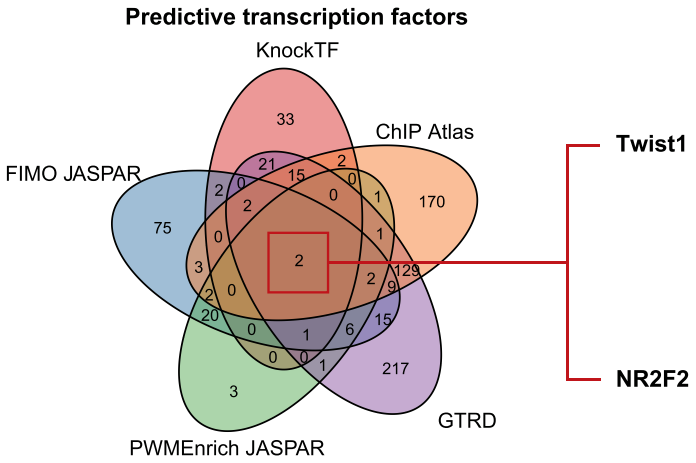
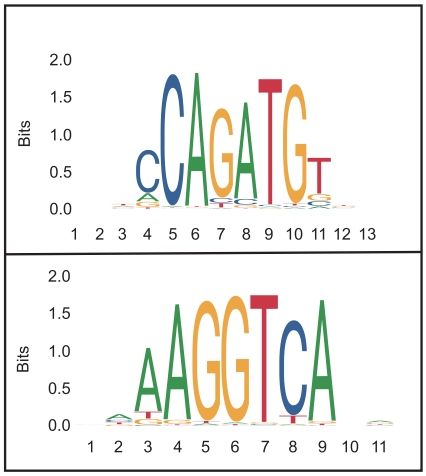
(A)



(B)



(C)

Predicted position	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}	Predicted relative K_D
Position 28-34 of LIFR 3' UTR hsa-miR-143-3p	5' ...GUCACUUCAGUCAGCCAUCUCAA... 3' CUCGAUGUCACGAAGUAGAGU	7mer-A1	-0.14	84	-0.14	6.093	0.26	-2.993
Position 28-34 of LIFR 3' UTR hsa-miR-4770	5' ...GUCACUUCAGUCAGCCAUCUCAA... 3' UCGAUGUCACAGUAGAGU	7mer-A1	-0.12	80	-0.12	6.093	0.26	-3.059
Position 28-34 of LIFR 3' UTR hsa-miR-6088	5' ...GUCACUUCAGUCAGCCAUCUCAA... 3' GCGGGGGGGCGAAGUAGAGA	7mer-A1	-0.1	79	-0.1	6.093	0.26	-2.434

Figure 6 Bioinformatics Analysis of LIFR Transcriptional and Post-transcriptional Regulation. (**A**) The miRNA-target regulatory relationships were predicted by Target Finder. (**B**) Transcription factor binding site analysis using JASPAR and NCBI databases identified Twist1 and NR2F2 as potential regulators of the LIFR promoter region. (**C**) miRNA target prediction via TargetScan revealed three miRNAs (miR-143-3p, miR-4770, and miR-6088) with putative binding sites in the LIFR 3'UTR.

to comorbidities.CRS endotypes can be classified by the dominant inflammatory cell type (eg, eosinophilic, neutrophilic, lymphocytic, mixed cellular, or paucicellular types) or immune response profile (eg, Th1, Th2, or Th17).[30] The local and systemic characteristics of the polyp recurrence group found, as we did, that the enrichment pathways of chemotaxis and immune activation tend to show high NF-serum protein concordance scores, indicating enhanced coupling between NF and serum in the inflammatory response. These findings suggest that recurrent NPs may implicate the development of an ongoing immune response pattern, where systemic immune responses could persist and influence the local microenvironment, even when local inflammation is under control. Previous studies have shown that the interaction between the local immune microenvironment and the peripheral immune system is a complex and multi-level process, involving multiple cell types, signaling molecules and anatomical structures. In barrier tissues such as the gut, bidirectional interactions between the nervous and immune systems are identified to play a crucial role in maintaining tissue homeostasis and responding to environmental challenges.[31] The specific location of mast cells enables them to rapidly release pre-formed and newly synthesized mediators, such as histamine, serotonin, TNF-α, and tryptase, which modulate inflammation and neuronal activation.[32] Additionally, specific sensory neurons modulate the IL-23/IL-17 pathway by interacting with dendritic cells, thereby controlling the immune response in psoriasiform skin inflammation.[33] This neuro-immune interaction also exhibits similar regulatory effects in other barrier tissues.[34] In summary, the interaction between the local immune microenvironment and the peripheral immune system occurs through various mechanisms, including intercellular signaling, anatomical specificity, and tissue-specific immune regulation. Future studies should focus on how the local immune microenvironment interacts with the peripheral immune system, and explore whether protein concordance reflects a feedback loop between different compartments, contributing to chronic inflammation in CRS.

Biomarker analysis is commonly employed to detect and predict diseases, as well as to monitor recurrence closely. In patients with CRSwNP, NFs provide a more accessible sampling method that can be non-invasively collected from the nasal mucosal surface. Our study revealed LIFR, IL-5, and IL-13 as significant markers for polyp recurrence in CRSwNP patients. The leukemia inhibitory factor receptor (LIFR), a key receptor for the neurotrophic factor LIF, exists in two isoforms (membrane-bound and soluble) and is highly expressed in various central nervous system disorders.[35] Interestingly, our previous work also revealed elevated levels of glial cell line-derived neurotrophic factor (GDNF), a member of the GDNF family of ligands, in type 2 CRSwNP, suggesting potential neuro-immune interactions in disease pathogenesis.[36] Studies have shown that LIFR forms molecular complexes with STAT4, driving the sustained production of inflammatory mediators like IL-6 in fibroblasts. This autocrine circuit is particularly prominent in inflammatory conditions such as rheumatoid arthritis.[37] Additionally, the LIFR signaling pathway significantly promotes tumor development in obesity-driven endometrial cancer and triple-negative breast cancer. Inhibiting LIFR can effectively prevent tumor progression.[38,39] Therefore, the LIFR signaling pathway plays multiple roles in disease progression. Through in-depth research on LIFR and its related signaling pathways, new therapeutic targets and strategies can be identified for NP recurrence. Limitations include the limited sample size, the use of a focused 92-protein Olink panel that may miss broader pathways, and a recurrence endpoint (≥6 months) that may not reflect the true biological recurrence dynamics. Future research should be conducted to address these limitations.

Conclusion

In summary, our study establishes NFs as a non-invasive biomarker and reveals that NF-serum protein concordance reflects local-systemic interactions, offering new insights for monitoring PR and personalized therapy.

Data Sharing Statement

The data that support the findings of this study are openly available in Mendeley Data atdoi:10.17632/nnpvf66x6h.2 (URL: https://data.mendeley.com/datasets/nnpvf66x6h/2).

Author Contributions

Yilin Hou: Conceptualization, Data curation, Project administration, Visualization, Writing – original draft. Lin Sun and Changhui Chen: Formal analysis, Investigation, Writing – review & editing. Mengqi Su: Data curation, Funding acquisition, Writing – review & editing. Shimin Lai and Yan Yan: Methodology, Resources, Validation, Writing – review & editing. Jieying Yan and Rongjian Zhan: Validation, Writing – review & editing. Yongjin Su and Weiqiang Yang: Methodology, Resources, Investigation, Writing – review & editing. Yi Wei: Funding acquisition, Data curation, Resources, Writing – review & editing. Weiping Wen - Funding acquisition, Conceptualization, Data curation, Writing – review & editing. Hongyi Hu: Funding acquisition, Conceptualization, Writing – review & editing. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Bachert C, Marple B, Schlosser RJ. et al. Adult chronic rhinosinusitis. *Nat Rev Dis Primers*. 2020;6(1):86. doi:10.1038/s41572-020-00218-1
2. Bachert C, Zhang L, Gevaert P. Current and future treatment options for adult chronic rhinosinusitis: focus on nasal polyposis. *J Allergy Clin Immunol*. 2015;136(6):1431–1440. doi:10.1016/j.jaci.2015.10.010
3. Alsharif S, Jonstam K, van Zele T, et al. Endoscopic Sinus Surgery for Type-2 CRS wNP: an Endotype-Based Retrospective Study. *Laryngoscope*. 2019;129(6):1286–1292. doi:10.1002/lary.27815
4. Loftus CA, Soler ZM, Koochakzadeh S, et al. Revision surgery rates in chronic rhinosinusitis with nasal polyps: meta-analysis of risk factors. *Int Forum Allergy Rhinol*. 2020;10(2):199–207. doi:10.1002/alr.22487
5. Li Z, Lu T, Sun L, et al. Factors for predicting the outcome of surgery for non-eosinophilic chronic rhinosinusitis with nasal polyps. *Ann Allergy Asthma Immunol*. 2024;133(5):559–567.e553. doi:10.1016/j.anai.2024.05.023
6. Qi S, Yan B, Liu C, et al. Predictive significance of Charcot-Leyden Crystal mRNA levels in nasal brushing for nasal polyp recurrence. *Rhinology*. 2020;58(2):166–174. doi:10.4193/Rhin19.296
7. Chen CL, Zhao JF, Guo CL, et al. Nasal secretion tissue plasminogen activator: a novel effective predictor of nasal polyp recurrence. *J Allergy Clin Immunol Pract*. 2022;10(8):2191–2194.e2193. doi:10.1016/j.jaip.2022.04.031
8. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. *J Allergy Clin Immunol*. 2020;145(6):1499–1509. doi:10.1016/j.jaci.2020.04.010
9. Radek K, Gallo R. Antimicrobial peptides: natural effectors of the innate immune system. *Semin Immunopathol*. 2007;29(1):27–43. doi:10.1007/s00281-007-0064-5
10. Zhao X, Yu F, Li C, et al. The use of nasal epithelial stem/progenitor cells to produce functioning ciliated cells in vitro. *Am J Rhinol Allergy*. 2012;26(5):345–350. doi:10.2500/ajra.2012.26.3794
11. Kaliner MA. Human nasal respiratory secretions and host defense. *Am Rev Respir Dis*. 1991;144(3):S52–56. doi:10.1164/ajrccm/144.3_pt_2.S52
12. Zhang N, Van Crombruggen K, Gevaert E, et al. Barrier function of the nasal mucosa in health and type-2 biased airway diseases. *Allergy*. 2016;71(3):295–307. doi:10.1111/all.12809
13. Hill DB, Button B, Rubinstein M, et al. Physiology and pathophysiology of human airway mucus. *Physiol Rev*. 2022;102(4):1757–1836. doi:10.1152/physrev.00004.2021
14. Fokkens WJ, Lund VJ, Hopkins C, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. *Rhinology*. 2020;58(Suppl S29):1–464. doi:10.4193/Rhin20.600
15. Hou Y, Chen C, Li Z, et al. Comparing Protein and Gene Expression Signature between Nasal Polyps and Nasal Fluids in Chronic Rhinosinusitis. *Int Arch Allergy Immunol*. 2024;185(3):274–285. doi:10.1159/000534226
16. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9(4):e95192. doi:10.1371/journal.pone.0095192
17. Wik L, Nordberg N, Broberg J, et al. Proximity Extension Assay in Combination with Next-Generation Sequencing for High-throughput Proteome-wide Analysis. *Mol Cell Proteomics*. 2021:20100168. doi:10.1016/j.mcpro.2021.100168.
18. Li P, Li T, Luo J, et al. IL-13 May Could Enhance the Proliferation and Affect the Differentiation of Nasal Epithelium Basal Cells Through the mTOR/p70S6K1 Pathway in Chronic Rhinosinusitis With Nasal Polyps. *Mediators Inflamm*. 2025;2025:8108993. doi:10.1155/mi/8108993
19. Chung M, Bruno VM, Rasko DA, et al. Best practices on the differential expression analysis of multi-species RNA-seq. *Genome Biol*. 2021;22(1):121. doi:10.1186/s13059-021-02337-8
20. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1):1523. doi:10.1038/s41467-019-09234-6
21. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023;51(D1):D638–D646. doi:10.1093/nar/gkac1000
22. Yuan G, Yang S, Yang S. RGS12 represses oral squamous cell carcinoma by driving M1 polarization of tumor-associated macrophages via controlling ciliary MYCBP2/KIF2A signaling. *Int J Oral Sci*. 2023;15(1):11. doi:10.1038/s41368-023-00216-5
23. Li Y, Long Y, Chen X, et al. Cytokine patterns in the blister fluid and plasma of patients with fracture blisters. *Int Immunopharmacol*. 2023:123110738. doi:10.1016/j.intimp.2023.110738.
24. Wang W, Xu Y, Wang L, et al. Single-cell profiling identifies mechanisms of inflammatory heterogeneity in chronic rhinosinusitis. *Nat Immunol*. 2022;23(10):1484–1494. doi:10.1038/s41590-022-01312-0
25. Wang J. TFTF: an R-Based Integrative Tool for Decoding Human Transcription Factor-Target Interactions. *Biomolecules*. 2024;14(7). doi:10.3390/biom14070749
26. Chen Y, Gao R, Liu H, et al. Serum Proteomic Analysis Revealed Biomarkers for Eosinophilic Chronic Rhinosinusitis with Nasal Polyps Pathophysiology. *J Inflamm Res*. 2024:17805–17821. doi:10.2147/jir.S444280.
27. Kao SS, Bassiouni A, Ramezanpour M, et al. Proteomic analysis of nasal mucus samples of healthy patients and patients with chronic rhinosinusitis. *J Allergy Clin Immunol*. 2021;147(1):168–178. doi:10.1016/j.jaci.2020.06.037
28. Coccaro EF, Lee R, Breen EC, et al. Plasma and cerebrospinal fluid inflammatory markers and human aggression. *Neuropsychopharmacology*. 2023;48(7):1060–1066. doi:10.1038/s41386-023-01541-3
29. Šenolt L, Šumová B, Jandová R, et al. Interleukin 35 Synovial Fluid Levels Are Associated with Disease Activity of Rheumatoid Arthritis. *PLoS One*. 2015;10(7):e0132674. doi:10.1371/journal.pone.0132674
30. Klingler AI, Stevens WW, Tan BK, et al. Mechanisms and biomarkers of inflammatory endotypes in chronic rhinosinusitis without nasal polyps. *J Allergy Clin Immunol*. 2021;147(4):1306–1317. doi:10.1016/j.jaci.2020.11.037
31. Veiga-Fernandes H, Mucida D. Neuro-Immune Interactions at Barrier Surfaces. *Cell*. 2016;165(4):801–811. doi:10.1016/j.cell.2016.04.041
32. van Diest SA, Stanisor OI, Boeckxstaens GE, et al. Relevance of mast cell-nerve interactions in intestinal nociception. *Biochim Biophys Acta*. 2012;1822(1):74–84. doi:10.1016/j.bbadis.2011.03.019
33. Riol-Blanco L, Ordovas-Montanes J, Perro M, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature*. 2014;510(7503):157–161. doi:10.1038/nature13199
34. Jakob MO, Murugan S, Klose CSN. Neuro-Immune Circuits Regulate Immune Responses in Tissues and Organ Homeostasis. *Front Immunol*. 2020;11308. doi:10.3389/fimmu.2020.00308

35. Viswanadhapalli S, Dileep KV, Zhang KYJ, et al. Targeting LIF/LIFR signaling in cancer. *Genes Dis*. 2022;9(4):973–980. doi:10.1016/j.gendis.2021.04.003
36. Hou Y, Chen C, Li Z, et al. Proteomic profiling of nasal fluids and serum for type 2 nasal polyps diagnosis. *Ann Allergy Asthma Immunol*. 2025;135(2):186–196.e130. doi:10.1016/j.anai.2025.04.007
37. Nguyen HN, Noss EH, Mizoguchi F, et al. Autocrine Loop Involving IL-6 Family Member LIF, LIF Receptor, and STAT4 Drives Sustained Fibroblast Production of Inflammatory Mediators. *Immunity*. 2017;46(2):220–232. doi:10.1016/j.immuni.2017.01.004
38. Blankenship L, Pratap UP, Yang X, et al. Inhibition of LIFR Blocks Adiposity-Driven Endometrioid Endometrial Cancer Growth. *Cancers*. 2022;14(21). doi:10.3390/cancers14215400
39. Randolph L, Joshi J, Rodriguez Sanchez AL, et al. Significance of LIF/LIFR Signaling in the Progression of Obesity-Driven Triple-Negative Breast Cancer. *Cancers*. 2024;16(21). doi:10.3390/cancers16213630