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Research Article

Comparative analysis of inflammatory signature profiles in eosinophilic and noneosinophilic chronic rhinosinusitis with nasal polyposis

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Chronic rhinosinusitis with nasal polyposis (CRSwNP) represents a heterogeneous disorder that can be classified into either eosinophilic or noneosinophilic endotypes. However, the immunological mechanisms of each remain unclear. The purpose of the present study was to compare and analyze inflammatory signatures of eosinophilic CRSwNP (ECRSwNP) and noneosinophilic CRSwNP (NECRSwNP). Cytokine antibody array was used to identify inflammatory mediators that were differentially expressed among ECRSwNP, NECRSwNP, and control groups. Then, bioinformatics approaches were conducted to explore biological functions and signaling pathways. In addition, pairwise correlation analyses were performed among differential levels of inflammatory mediators and tissue eosinophil infiltration. The results showed that nine mediators were significantly up-regulated in ECRSwNP, including eotaxin-2, eotaxin-3, CCL18, IL-4, IL-5, IL-10, IL-12p70, IL-13, and IL-15. Bioinformatics analysis indicated that these mediators were mainly enriched in leukocyte chemotaxis and proliferation, JAK-STAT cascade, asthma, and Th1 and Th2 cell differentiation. Furthermore, seven mediators were identified to be significantly up-regulated in NECRSwNP, including CCL20, resistin, transforming growth factor (TGF)-β2, triggering receptor expressed on myeloid cells 1 (TREM-1), CD14, glucocorticoid-induced tumor necrosis factor receptor related protein (GITR), and lipocalin-2. These mediators were closely associated with LPS responses, neutrophil chemotaxis and migration, and IL-17 signaling pathway. In addition, pairwise correlation analyses indicated that differential levels of inflammatory mediators in ECRSwNP and NECRSwNP were broadly correlated with each other and with tissue eosinophil infiltration. In conclusion, we found that ECRSwNP and NECRSwNP exhibited different patterns of inflammatory signatures. These findings may provide further insights into heterogeneity of CRSwNP.

Introduction

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a complex inflammatory condition characterized by the presence of edematous masses of inflamed mucosa in the upper airways [1,2]. CRSwNP has a considerable impact on patients' quality of life and is associated with high cost of management and significant morbidity [3]. Eosinophilic inflammation has been recognized as a major pathologic hallmark of CRSwNP in Caucasians. In contrast, recent studies have indicated that over half of the patients with CRSwNP in East Asia exhibit noneosinophilic inflammation [4,5]. Due to this heterogeneity, CRSwNP can be divided into two distinct endotypes, namely eosinophilic CRSwNP (ECRSwNP) and noneosinophilic CRSwNP (NECRSwNP) [6,7]. Nevertheless, the precise mechanisms that underlie the pathogenesis of these two endotypes are still largely unknown.

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Conceptually, inflammatory mediators are small biologically active molecules that are important in the regulation of the growth, differentiation, and activation of immune cells at inflamed sites. Therefore, the mechanisms of inflammation in ECRSwNP and NECRSwNP involve a large number of inflammatory mediators and their associated pathways. Identifying and quantifying differentially expressed inflammatory mediators between ECRSwNP and NECRSwNP may expand our understanding of CRSwNP heterogeneity and aid to develop novel biological therapies [8–10]. Unfortunately, only few studies have compared inflammatory signatures between these two endotypes [11–13]. In addition, previous studies have been restricted by the limited range of inflammatory mediators tested.

Thus, to address this issue, a more comprehensive comparative analysis is needed. Antibody array is a high-throughput technique that allows detection of numerous inflammatory mediators on one membrane simultaneously. Hence, in the present study, we used a cytokine antibody array to delineate more complete inflammatory signature profiles for ECRSwNP and NECRSwNP and then employed bioinformatics tools to identify key biological functions and signaling pathways related to differential levels of inflammatory mediators.

Materials and methods

Patients and biopsy specimens

Adult subjects, including 30 patients with CRSwNP and 10 controls, were recruited from the Department of Otolaryngology Head and Neck Surgery of Xiangya Hospital of Central South University, Changsha, China. The diagnosis of CRSwNP was established by medical history, nasal endoscopy, and computed tomography (CT) scan of the sinuses according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 guidelines (EPOS 2012) [1]. Subjects with an immune deficient disease, antrochoanal polyps, fungal rhinosinusitis, cystic fibrosis, or primary ciliary dyskinesia were excluded from the study. Patients without sinus disease undergoing septoplasty or endoscopic skull base surgery were enrolled as controls. Any systemic or nasal administration of corticosteroids or antibiotics were ceased in all subjects 4 weeks preoperatively. Atopic status was assessed by using skin prick tests to a standard panel of common allergens, and patients were given a diagnosis of asthma by a pneumologist based on medical history and airway responsiveness testing. Preoperative CT scans were scored using the Lund-Mackay staging system [14]. Polyps were graded using the 0 to 3 scoring system recommended by the EPOS 2012 [1]. The study was approved by the Ethics Committee of Xiangya Hospital of Central South University, and written informed consent was obtained from all subjects before enrollment in the study.

Polyp tissues and turbinate mucosa were harvested from patients with CRSwNP and control subjects during endoscopic surgery, respectively. Tissue samples were divided into two parts: 1 part was fixed in 10% formaldehyde and subsequently embedded in paraffin wax for histological staining; the other part was snap-frozen in liquid nitrogen and stored at -80°C until use for immunoassays.

Determination of ECRSwNP and NECRSwNP

Paraffin-embedded samples were sectioned at 4 μm thickness and were stained with hematoxylin and eosin. All stained sections were examined by two independent observers who were blind to the clinical diagnosis and characteristics of the patients. The number of the infiltrating eosinophils was counted in 10 randomly selected high-power fields (HPFs). Referring to previously published criterion [4,15], CRSwNP was defined as eosinophilic when the percentage of tissue eosinophils was more than 10% of total inflammatory cells and as noneosinophilic otherwise.

Cytokine antibody array analysis

We employed a glass chip-based multiplex sandwich ELISA system (QAH-CAA-4000; RayBiotech, Norcross, GA, U.S.A.) to measure the concentrations of 200 different human inflammatory mediators (Supplementary Table S1). This array platform was a combination of five forty-cytokine arrays and each antibody was printed in quadruplicate. Measurements were performed according to the recommended protocols from the manufacturer. Briefly, tissue samples were weighed and homogenized and then the supernatants were harvested. The protein concentration of each extract was determined and normalized to 500 μg/ml. The arrays were blocked with sample buffer and then incubated with samples or serial diluted cytokine standards overnight at 4°C. After multiple washes, the arrays were incubated with a cocktail of biotinylated antibodies. The arrays were then washed and incubated with Cy3 equivalent dye-conjugated streptavidin. The images were captured using a microarray scanner (InnoScan 300; Innopsys, Carbonne, France). The fluorescence intensity data were analyzed with the array-specific Q-Analyzer Software (RayBiotech). The results were expressed as picogram per milliliter (pg/ml).

Table 1 Baseline characteristics of the subjects

	Control ($n = 10$)	NECRSwNP ($n = 18$)	ECRSwNP ($n = 12$)	P^* value
Age, years, median (IQR)	44.5 (38–50.75)	39.5 (30.25–47.75)	49 (36–58.5)	0.464, 0.539, 0.172
Female/male sex	3/7	5/13	1/11	1.000, 0.293, 0.358
Atopy	0/10	6/18	4/12	0.062, 0.096, 1.000
Asthma	0/10	0/18	0/12	1.000, 1.000, 1.000
Bilateral CT score, median (IQR)	NA	16 (8–22)	19.5 (16–20.25)	NA, NA, 0.185
Bilateral polyp score, median (IQR)	NA	4 (3–5)	3.5 (2–5.25)	NA, NA, 0.573

Abbreviations: IQR, interquartile range; CT, computer tomography; ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis; NA, not applicable.
$^*P$ value: NECRSwNP vs. Control, ECRSwNP vs. Control, and ECRSwNP vs. NECRSwNP, respectively. *P* values were obtained from the Fisher's exact test (categorical variables) or Mann–Whitney *U* test (continuous variables).

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, IL) and R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). The demographic and clinical characteristics were studied with the Fisher's exact test for categorical variables and Mann–Whitney *U* test for continuous variables. Differential mediator expression among three groups was first analyzed using the Kruskal–Wallis *H* test followed by the Benjamini–Hochberg false discovery rate (FDR) procedure for multiple testing correction [16]. If significance was found, the Mann–Whitney *U* test was then applied for between-group comparisons. The Spearman rank test was used to determine correlations, and pairwise correlation matrix was generated using the corrplot package in R 3.4.0. In addition, principal component analysis (PCA) was performed and visualized using ggbiplot package in R 3.4.0, and mediator concentrations were log2-transformed in the analysis. A *P* value of less than 0.05 was considered statistically significant.

Bioinformatics analysis

To explore the biological functions and signaling pathways of differentially levels of inflammatory mediators, we performed Gene Ontology Biological Process (GO-BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses using the clusterProfiler package in R 3.4.0 [17]. FDR-adjusted $P < 0.05$ was set as the screening criterion.

Results

Patient characteristics

The demographic and clinical characteristics of all subjects enrolled in the present study are summarized in Table 1. Among 30 CRSwNP patients, 12 (40%) were identified as ECRSwNP, and the rest ($n = 18$, 60%) were classified as NECRSwNP. The three groups were not significantly different with respect to age distribution, sex ratio, and the presence of atopy and asthma comorbidity. In addition, CT and polyp scores did not differ significantly between the ECRSwNP and NECRSwNP groups. Typical histological findings of eosinophilic and noneosinophilic polyps are shown in Figure 1. As expected, marked eosinophil infiltration was observed in ECRSwNP group ($P < 0.001$). On the other hand, most of the infiltrating cells were plasma cells and lymphocytes in the NECRSwNP group.

Differences in inflammatory mediator levels among the different groups

First, the Kruskal–Wallis *H* tests were conducted to assess whether there were any overall significant differences in the levels of inflammatory mediators among ECRSwNP, NECRSwNP, and control groups. In this analysis, 44% (88/200) of the mediators were found to be statistically significant. After Benjamini–Hochberg correction for multiple comparisons, 33% (66/200) of the mediators remained significantly different (Supplementary Table S1). Of these 66 mediators, PCA was performed to gain an overview of the differences and similarities among three groups. The results demonstrated that ECRSwNP and NECRSwNP groups separated clearly from the control group on the first principal component, while there was a partial overlap between ECRSwNP and NECRSwNP samples on the second principal component (Figure 2).

Next, the Mann–Whitney *U* tests were carried out to ascertain which pairs of groups differed significantly. The results of between-group comparisons of inflammatory mediator levels are summarized in Supplementary Table S2. A total of 56 mediators were found to be significantly different in ECRSwNP when compared with controls, 57 to

Bioscience Reports (2020) **40** BSR20193101
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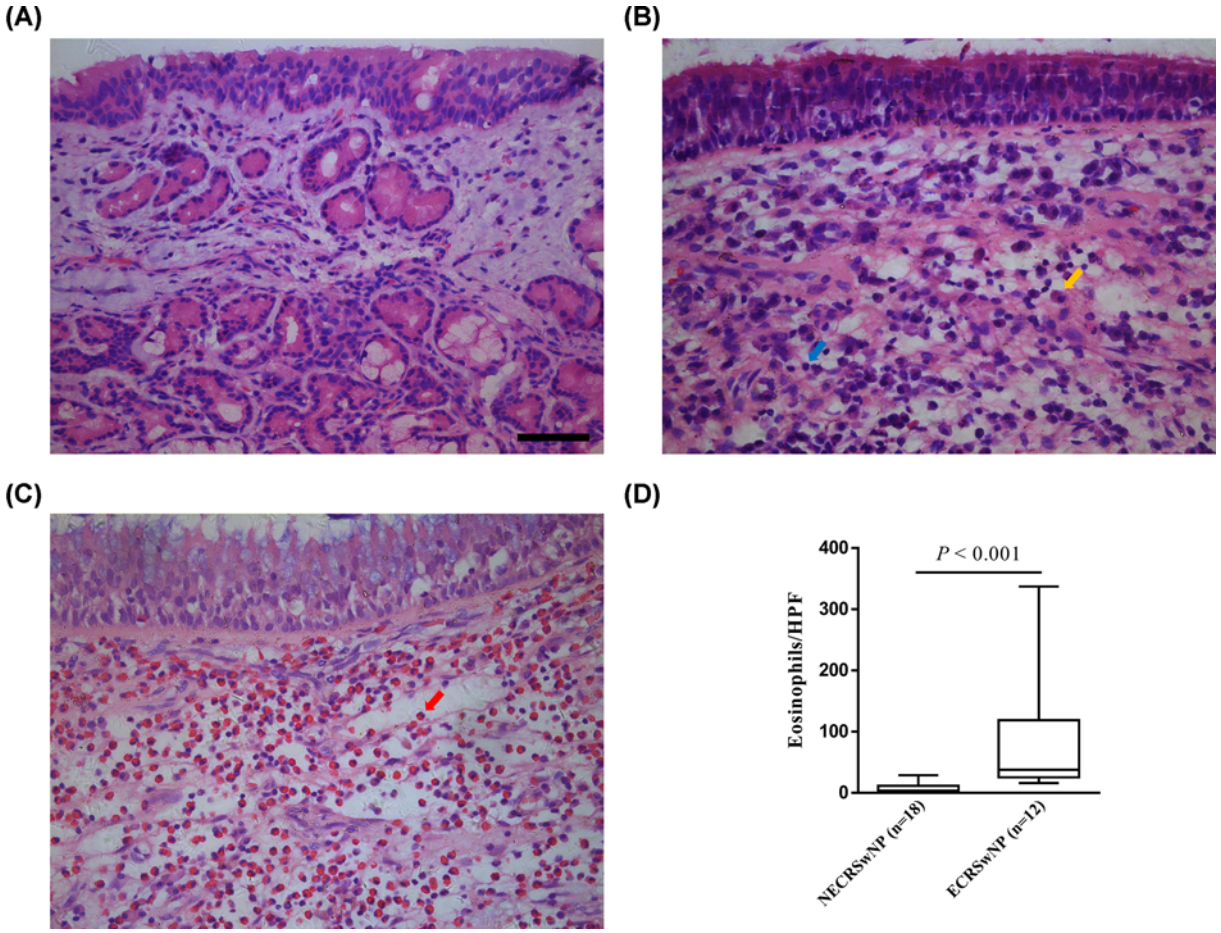

Figure 1. Representative hematoxylin and eosin staining of control tissues and nasal polyps

(**A**) Control mucosa, (**B**) noneosinophilic polyp, and (**C**) eosinophilic polyp (Original magnification: ×400; scale bar = 50 μm; plasma cell, orange arrow; lymphocyte, blue arrow; eosinophil, red arrow). (**D**) Comparison of number of tissue eosinophils per HPF between ECRSwNP and NECRSwNP. Data are presented as a box and whisker plot, and the Mann–Whitney *U* test was used for the statistical analysis. Abbreviations: ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis; HPF, high-power field.

be significantly different in NECRSwNP versus controls, and 16 to be significantly different in ECRSwNP versus NECRSwNP.

Finally, Venn analyses were used to determine inflammatory characteristics that were distinct in ECRSwNP and NECRSwNP. The results showed that nine mediators were significantly up-regulated in ECRSwNP as compared with NECRSwNP and controls (Figure 3A), including eotaxin-2, eotaxin-3, CCL18, IL-4, IL-5, IL-10, IL-12p70, IL-13, and IL-15 (Figure 4). Moreover, seven mediators were identified to be significantly up-regulated in NECRSwNP when compared with ECRSwNP and controls (Figure 5A), including CCL20, resistin, transforming growth factor (TGF)-β2, triggering receptor expressed on myeloid cells 1 (TREM-1), CD14, glucocorticoid-induced tumor necrosis factor receptor related protein (GITR), and lipocalin-2 (Figure 6).

Functional and pathway enrichment analysis

To ascertain the different immunopathologic mechanisms of ECRSwNP and NECRSwNP, distinct inflammatory signature profiles in each endotype were subjected to functional enrichment and pathway analysis, respectively. In the analysis of inflammatory signature profile in ECRSwNP, the significantly over-represented GO-BP terms included leukocyte chemotaxis and proliferation and JAK-STAT cascade (Figure 3B). The significantly enriched KEGG pathways contained cytokine–cytokine receptor interaction, JAK-STAT signaling pathway, asthma, and Th1 and Th2

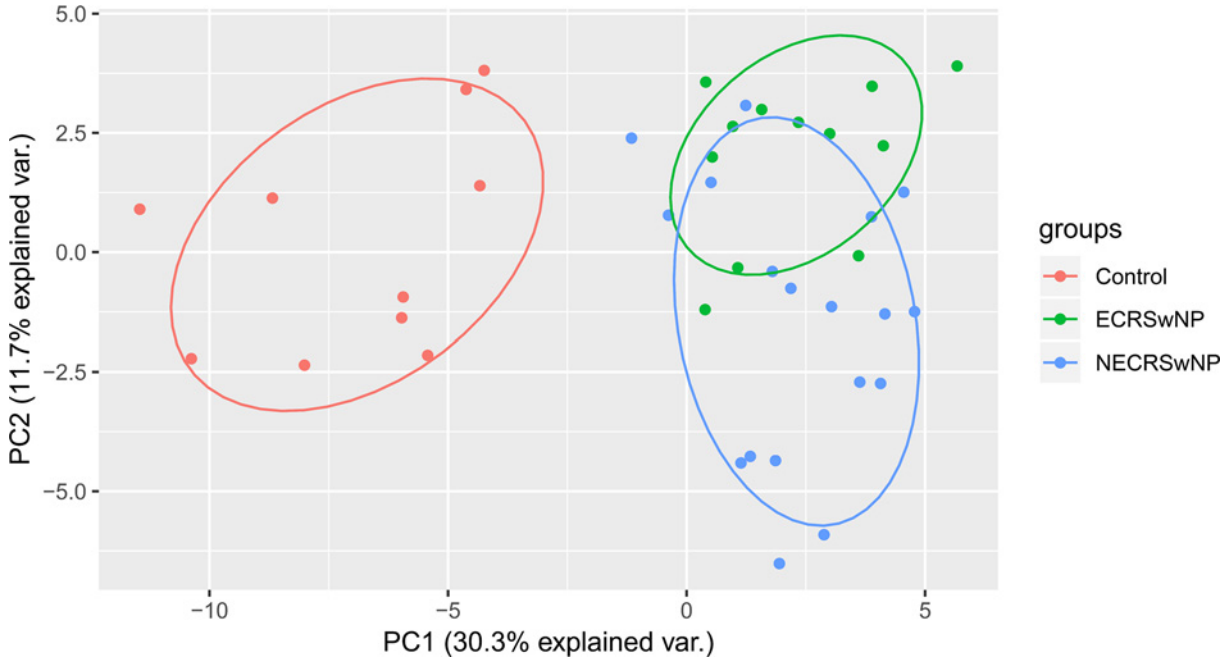

Figure 2. Principal component analysis based on the differential concentrations of inflammatory mediators among ECRSwNP, NECRSwNP, and control groups

The *X*- and *Y*-axes represent the first and second principal components, respectively. Dots stand for individual samples. The types of the samples are color indicated. Abbreviations: ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis.

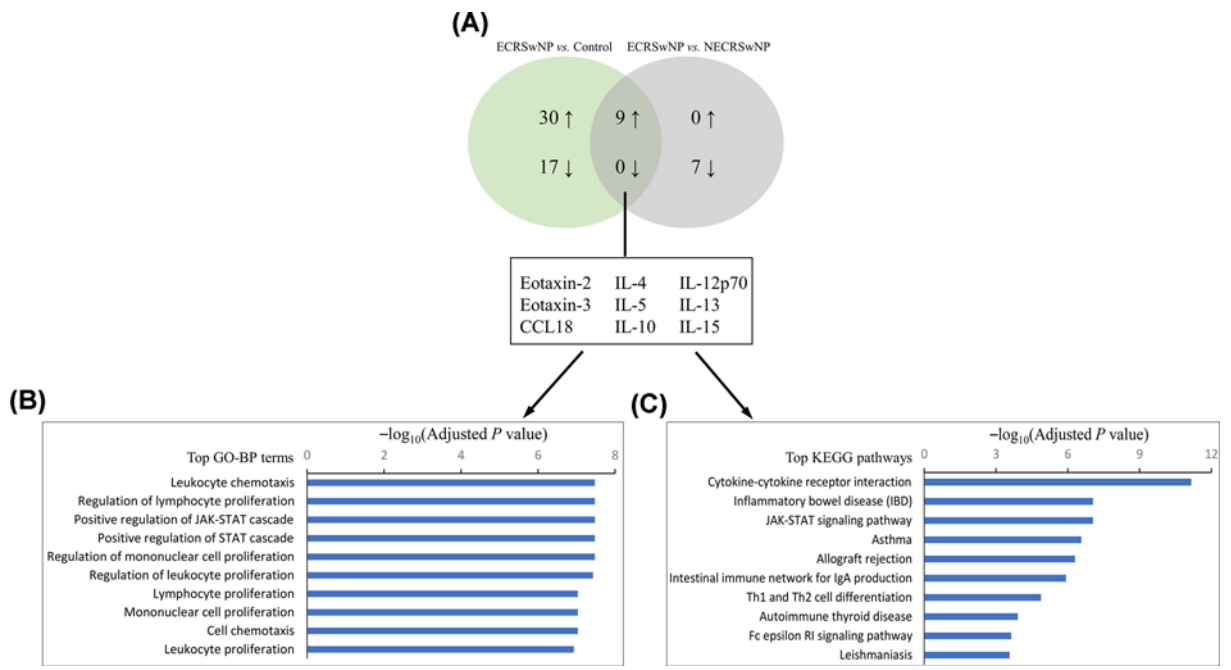

Figure 3. GO-BP and KEGG enrichment analyses of differential concentrations of inflammatory mediators in ECRSwNP in comparison with NECRSwNP and controls

(**A**) Venn diagram summarizing the number of differential concentrations of inflammatory mediators identified in ECRSwNP when compared with NECRSwNP and controls. The up- and down-arrows represent up- and down-regulated mediators, respectively. The diagram displays the names of these differential concentrations of inflammatory mediators. (**B**) Top over-represented GO-BP terms. (**C**) Top enriched KEGG pathways. Abbreviations: ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis; GO-BP, Gene Ontology Biological Process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Bioscience Reports (2020) **40** BSR20193101
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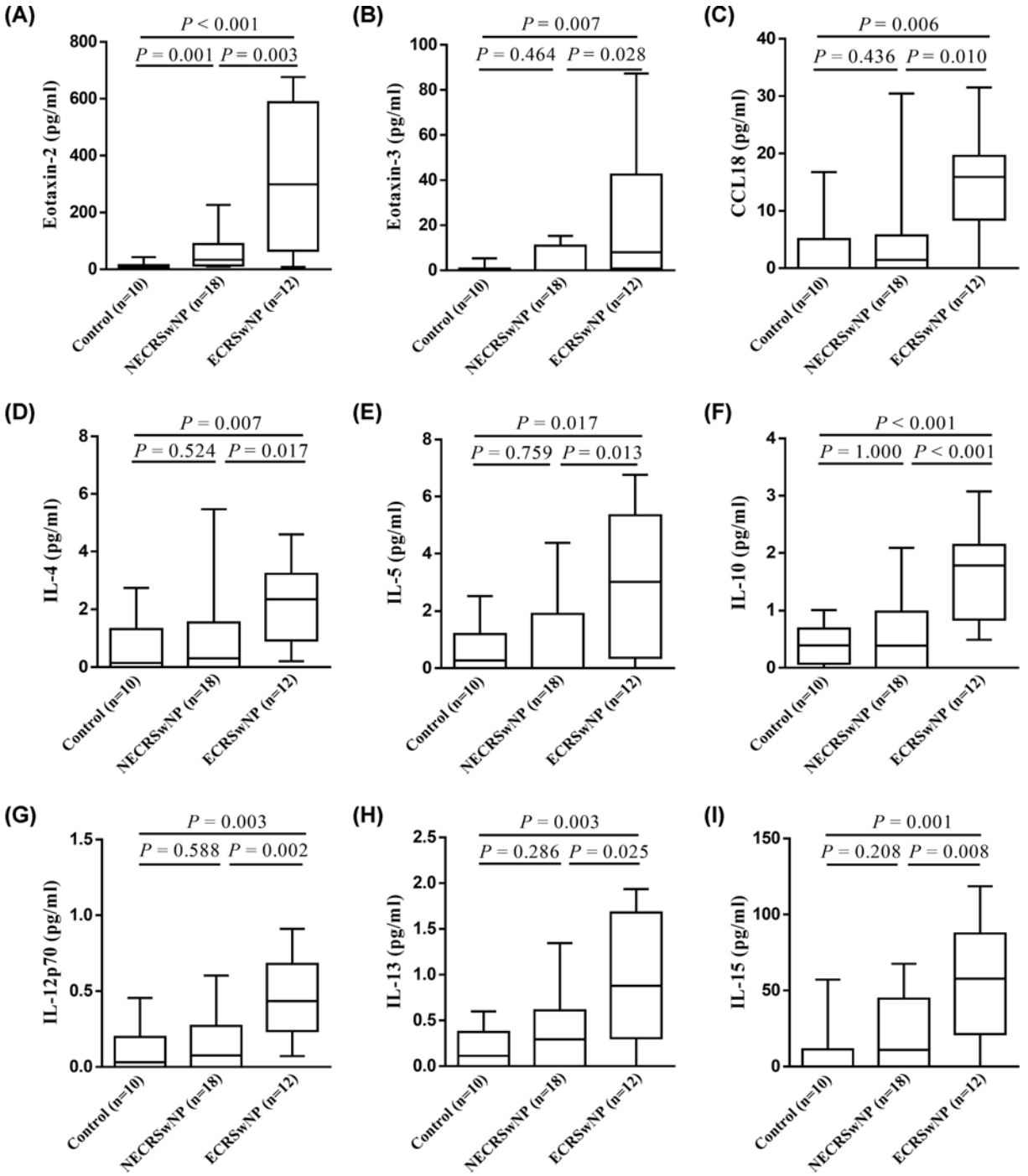

Figure 4. Protein levels of nine inflammatory mediators that were significantly elevated in ECRSwNP as compared with NECRSwNP and controls

(**A**) Eotaxin-2, (**B**) eotaxin-3, (**C**) CCL18, (**D**) IL-4, (**E**) IL-5, (**F**) IL-10, (**G**) IL-12p70, (**H**) IL-13, and (**I**) IL-15. Data are presented as box and whisker plots, and the Mann–Whitney *U* test was used for the statistical analysis. Abbreviations: ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis.

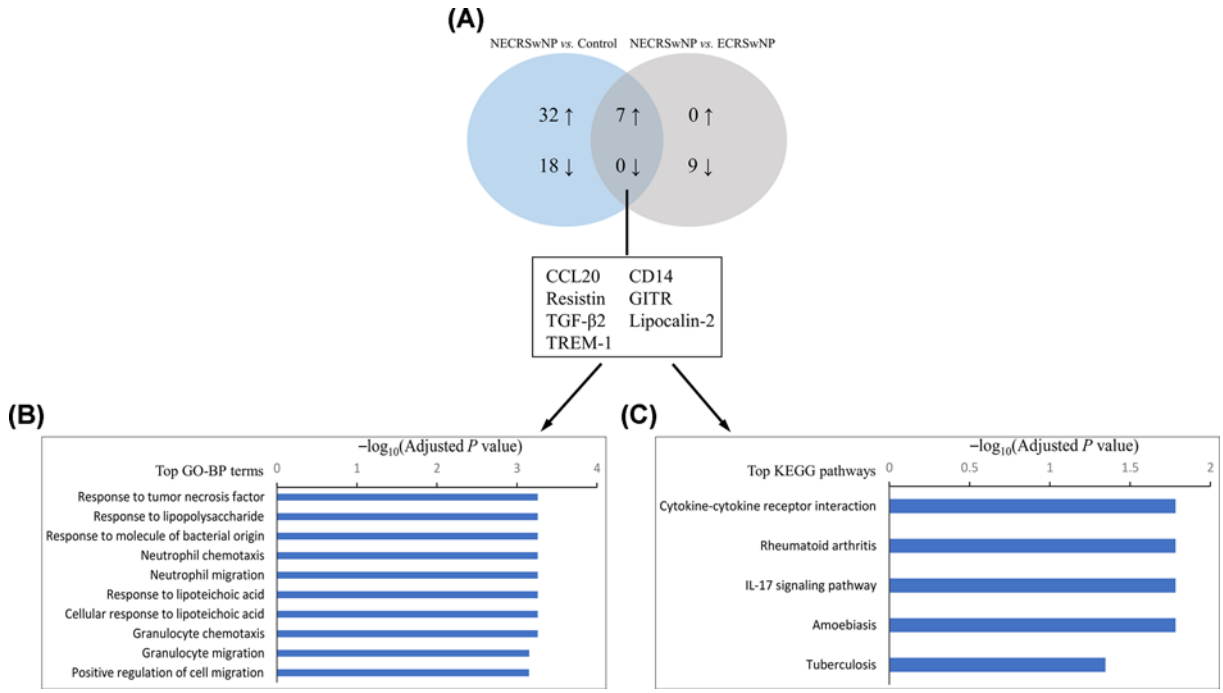

Figure 5. GO-BP and KEGG enrichment analyses of differential concentrations of inflammatory mediators in NECRSwNP in comparison with ECRSwNP and controls

(**A**) Venn diagram summarizing the number of differential concentrations of inflammatory mediators identified in NECRSwNP when compared with ECRSwNP and controls. The up- and down-arrows represent up- and down-regulated mediators, respectively. The diagram displays the names of these differential concentrations of inflammatory mediators. (**B**) Top over-represented GO-BP terms. (**C**) Top enriched KEGG pathways. Abbreviations: ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis; TGF-β2, transforming growth factor beta 2; TREM-1, triggering receptor expressed on myeloid cells 1; GITR, glucocorticoid-induced tumor necrosis factor receptor related protein; GO-BP, Gene Ontology Biological Process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

cell differentiation (Figure 3C). As for inflammatory signature profile in NECRSwNP, GO-BP enrichment analysis demonstrated that response to lipopolysaccharide (LPS), response to molecule of bacterial origin, and neutrophil chemotaxis and migration were significantly over-represented (Figure 5B). The significantly enriched KEGG pathways included cytokine–cytokine receptor interaction and IL-17 signaling pathway (Figure 5C).

Correlations among inflammatory mediators and tissue eosinophil infiltration

We further assessed if distinct inflammatory signature profiles in ECRSwNP and NECRSwNP could correlate with one another and with tissue eosinophil infiltration. To do so, we performed pairwise correlation analyses among these variables (Figure 7). The results showed that the levels of inflammatory mediators were broadly associated with each other in all CRSwNP subjects. The highest positive correlation was between IL-5 and IL-13 ($r = 0.916$, $P < 0.001$), and the highest negative correlation was between IL-13 and lipocalin-2 ($r = -0.647$, $P < 0.001$). Furthermore, the levels of inflammatory mediators were generally correlated with tissue eosinophil infiltration. For instance, IL-15 concentrations were positively correlated with tissue eosinophil numbers ($r = 0.632$, $P < 0.001$), and TREM-1 levels were inversely associated with tissue eosinophil counts ($r = -0.500$, $P = 0.005$). Overall, these findings reflected complex interactions among differential levels of inflammatory mediators and tissue eosinophil infiltration.

Discussion

ECRSwNP and NECRSwNP represent two distinct endotypes of CRSwNP that exhibit different clinical and pathologic features [18–21]. For better management of ECRSwNP and NECRSwNP, there is a clear need to investigate the underlying different molecular mechanisms of these two endotypes. Differences in inflammatory signature profiles

Bioscience Reports (2020) **40** BSR20193101
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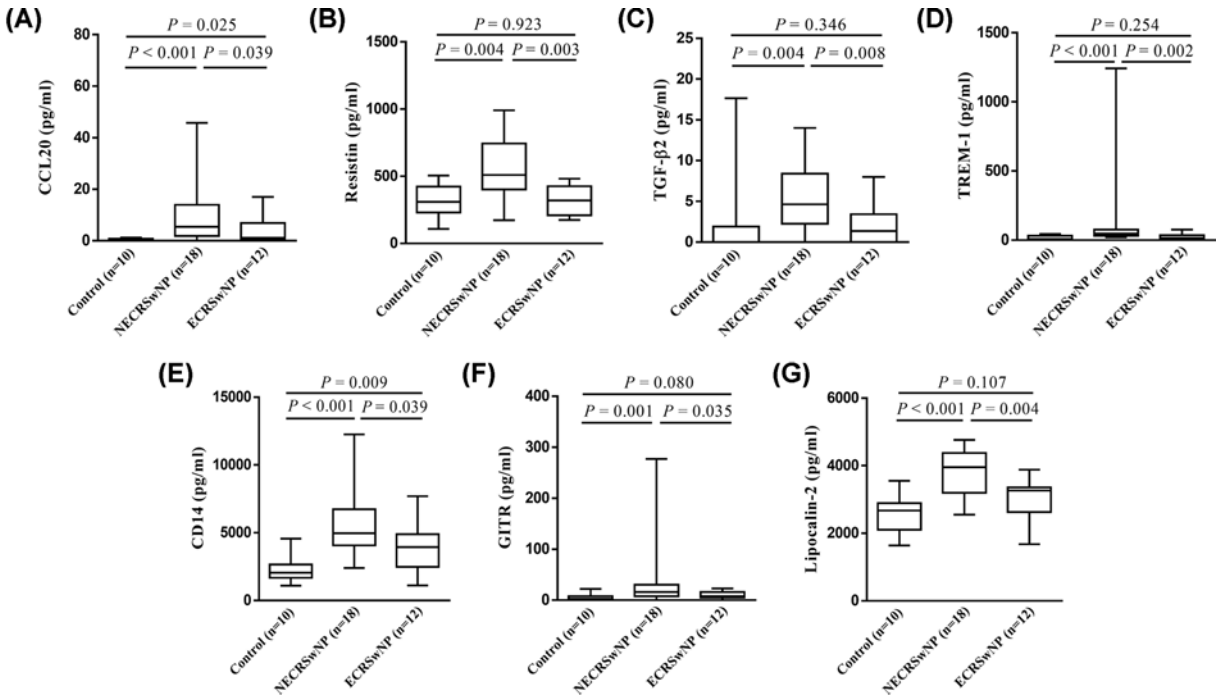

Figure 6. Protein levels of seven inflammatory mediators that were significantly elevated in NECRSwNP as compared with ECRSwNP and controls

(**A**) CCL20, (**B**) resistin, (**C**) TGF-β2, (**D**) TREM-1, (**E**) CD14, (**F**) GITR, and (**G**) lipocalin-2. Data are presented as box and whisker plots, and the Mann–Whitney *U* test was used for the statistical analysis. Abbreviations: TGF-β2, transforming growth factor beta 2; TREM-1, triggering receptor expressed on myeloid cells 1; GITR, glucocorticoid-induced tumor necrosis factor receptor related protein; ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis.

between ECRSwNP and NECRSwNP may play a critical role in CRSwNP heterogeneity. Therefore, in the present study, we performed multi-analyte profiling of various immune factors, characterized distinct inflammatory characteristics for ECRSwNP and NECRSwNP, and tested for correlations with tissue eosinophil infiltration.

In the analysis of cytokine antibody array data, a total of nine inflammatory mediators were identified to be markedly up-regulated in ECRSwNP in comparison with NECRSwNP and controls. Many of them are classical Th2-related cytokines and chemokines including IL-4, IL-5, IL-13, eotaxin-2, eotaxin-3, and CCL18, which can together promote the maturation, activation, and recruitment of eosinophils to polyp tissues [22–24]. Indeed, our pairwise correlation analyses also showed significantly positive associations of these mediator levels with each other and with mucosal eosinophilia. Thus, these results confirmed an exaggerated Th2 inflammatory signature in ECRSwNP, which are in agreement with previous literature [4,12,25].

It should be noted that the protein levels of IL-10, IL-12p70, and IL-15 were also significantly elevated in ECRSwNP. IL-10 is a potent anti-inflammatory cytokine that exerts immunosuppressive functions to limit inappropriate inflammatory responses [26]. As IL-12p70 can efficiently stimulate the differentiation of naive $CD4^+$ T cells to the Th1 subset, IL-12p70 has been described as an inhibitory factor for Th2 induction and development [27,28]. IL-15 is a pleiotropic cytokine that has immunomodulatory effects on diverse cell types [29]. Recently, several *in vivo* studies have demonstrated a protective effect of IL-15 in Th2-mediated eosinophilic airway inflammation [30]. Altogether, it is tempting to speculate that IL-10, IL-12p70, and IL-15 may play negative feedback roles in resistance to an excessive Th2 inflammatory reaction in ECRSwNP. Yet, further studies are necessary to assess this hypothesis.

Although NECRSwNP is a more prevalent endotype of CRSwNP in East Asian population [4,5], few publications have focused on the immunological characteristics of noneosinophilic nasal polyps. In the present study, we found a total of seven inflammatory mediators to be significantly up-regulated in NECRSwNP compared with ECRSwNP and controls, including CCL20, resistin, TGF-β2, TREM-1, CD14, GITR, and lipocalin-2. The roles of most of these mediators have not been characterized before in NECRSwNP. Using GO-BP and KEGG enrichment analyses, we found

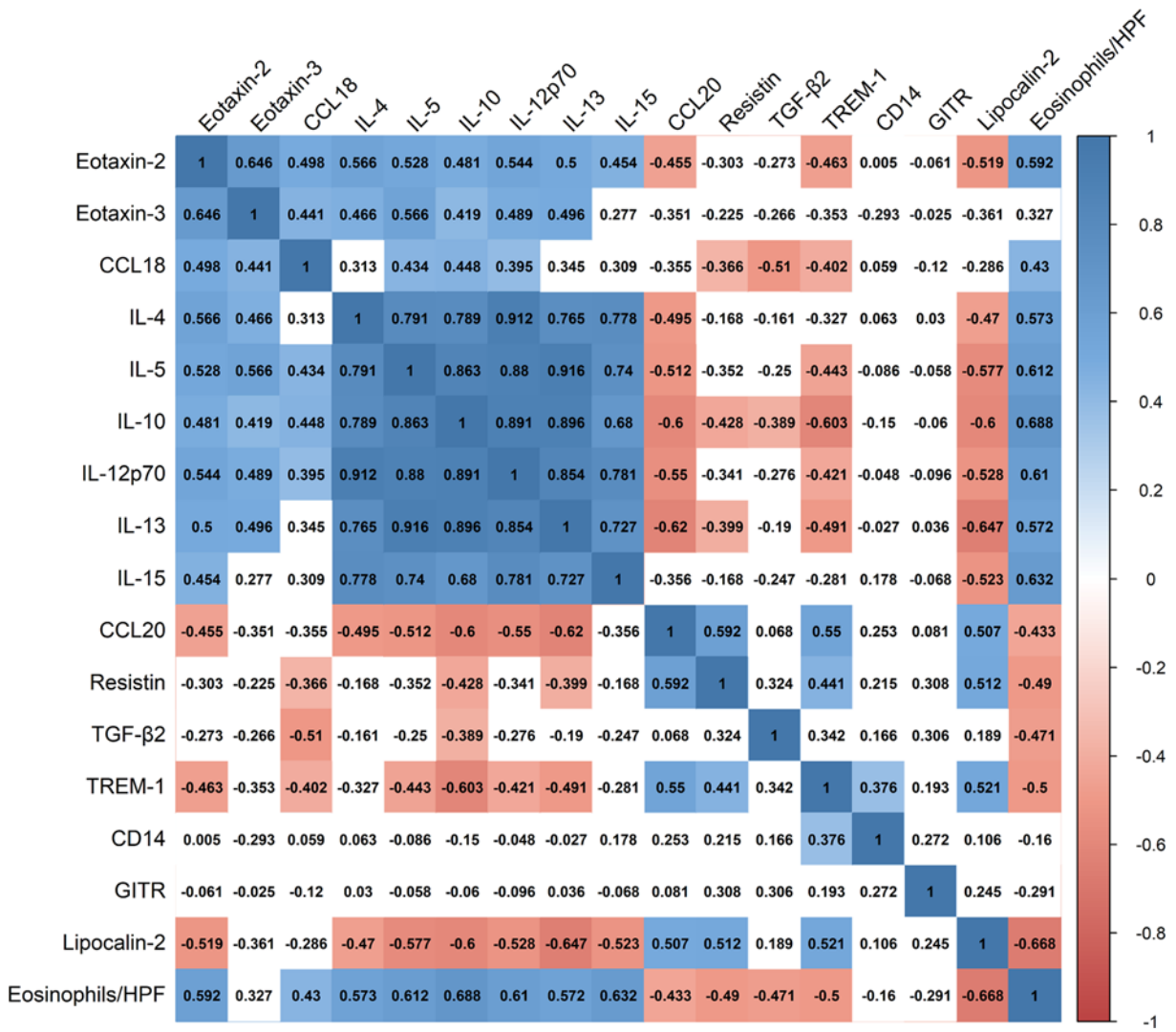

Figure 7. Correlation matrix showing pairwise associations of inflammatory mediators and tissue eosinophil infiltration

Color bar represents *r* values. Positive and negative correlations are indicated by blue and red colors, respectively. Non-significant correlations are uncolored. Abbreviations: TGF-β2, transforming growth factor beta 2; TREM-1, triggering receptor expressed on myeloid cells 1; GITR, glucocorticoid-induced tumor necrosis factor receptor; HPF, high-power field.

that these inflammatory mediators were mainly concentrated in LPS responses, neutrophil chemotaxis and migration, and IL-17 signaling pathway. In addition, our pairwise correlation analyses suggested that the concentrations of these mediators were generally negatively associated with polyp eosinophil numbers.

LPS is a ubiquitous cell wall constituent of gram-negative bacteria. CD14, an important receptor in the innate immune system, plays a pivotal role in sensing and binding of LPS [31,32]. Elevated protein level of CD14 in noneosinophilic nasal polyps might be indicative of high LPS exposure in NECRSwNP [33]. Importantly, it has been documented that the expression of resistin, TREM-1, lipocalin-2, and CCL20 are strongly up-regulated upon stimulation with LPS [34–38]. Resistin has the potential to induce neutrophil proinflammatory responses and promote neutrophil extracellular trap (NET) formation [39]. TREM-1, expressed on neutrophils and monocytes/macrophages, has been shown to amplify innate immune responses via triggering neutrophil degranulation and enhancing phagocytic activity [36,40,41]. Furthermore, lipocalin-2, also known as neutrophil gelatinase-associated lipocalin (NGAL), has a bacteriostatic effect in the innate immune responses against invading pathogens [37,42]. CCL20 is expressed on epithelial cells and can function as a potent chemotactic factor for the recruitment of CCR6-expressing Th17 cells to sites of inflammation [38,43,44]. Collectively, our findings indicated that NECRSwNP is highly related to neutrophilic Th17 inflammatory responses to LPS exposure.

Some limitations in the present study should be acknowledged. First, it was designed as a cross-sectional study, in which dynamic changes of immune mediator levels in the occurrence and development of ECRSwNP and NECRSwNP could not be determined and compared. Second, the sample size of the present study was relatively small. This may limit statistical power to reveal differences in inflammatory mediator levels among ECRSwNP, NECRSwNP, and control groups. Thus, further investigation in another independent larger cohort is required to confirm the findings of the present study.

Conclusions

In summary, using the antibody array technique, we found that ECRSwNP and NECRSwNP presented distinct types of inflammatory signature profiles. ECRSwNP is characterized by a predominant Th2 milieu, whereas NECRSwNP is closely associated with LPS responses and neutrophilic Th17-driven inflammation. The results of the present study may contribute to a better understanding of CRSwNP heterogeneity and provide new insight into the development of personalized therapeutic strategies for patients with CRSwNP.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

Y.Y.: experiment work, data analysis, data interpretation, and manuscript preparation. C.Y., X.Y. and S.X.: experiment work, literature research, and data collection. H.S.: study design, project management, data interpretation, and manuscript review.

Abbreviations

CRSwNP, chronic rhinosinusitis with nasal polyposis; ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; FDR, false discovery rate; GITR, glucocorticoid-induced tumor necrosis factor receptor related protein; GO-BP, Gene Ontology Biological Process; HPF, high-power field; KEGG, Kyoto Encyclopedia of Genes and Genomes; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis; NET, neutrophil extracellular trap; PCA, principal component analysis; TGF-β2, transforming growth factor beta 2; TREM-1, triggering receptor expressed on myeloid cells 1.

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Bioscience Reports (2020) **40** BSR20193101
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Supplementary Table S1 Comparisons of 200 inflammatory mediator levels among ECRSwNP, NECRSwNP, and control groups using cytokine antibody array

Mediators	Description	Overall *P* value	Adjusted *P* value
CCL21	C-C motif chemokine 21	0.276	0.383
Axl	AXL oncogene	**0.025**	0.066
Betacellulin	Betacellulin	0.445	0.520
CCL28 (MEC)	C-C motif chemokine 28 (Mucosae-associated epithelial chemokine)	0.275	0.383
CCL27 (CTACK)	C-C motif chemokine 27 (Cutaneous T-cell-attracting chemokine)	0.586	0.634
CXCL16	C-X-C motif chemokine 16	**0.001**	**0.007**
CXCL5 (ENA-78)	C-X-C motif chemokine 5 (Epithelial-derived neutrophil-activating protein 78)	0.365	0.468
CCL26 (Eotaxin-3)	C-C motif chemokine 26	**0.007**	**0.027**
CXCL6 (GCP-2)	C-X-C motif chemokine 6 (Granulocyte chemotactic protein 2)	0.180	0.286
CXCL1 (GRO)	C-X-C motif chemokine 1 (Growth-regulated alpha protein)	**0.001**	**0.007**
CCL14 (HCC-1)	C-C motif chemokine 14	**<0.001**	**<0.001**
CCL16 (HCC-4)	C-C motif chemokine 16	**0.027**	0.070
IL-9	Interleukin 9	0.341	0.452
IL-17F	Interleukin 17F	0.097	0.188
IL-18BPα	Interleukin-18-binding protein alpha	0.900	0.909
IL-28A (IFNL2)	Interleukin 28A (Interferon lambda 2)	0.390	0.481
IL-29 (IFNL1)	Interleukin 29 (Interferon lambda 1)	0.303	0.415
IL-31	Interleukin 31	0.208	0.313
CXCL10 (IP-10)	C-X-C motif chemokine 10 (10 kDa interferon gamma-induced protein)	0.127	0.217
CXCL11 (I-TAC)	C-X-C motif chemokine 11 (Interferon-inducible T-cell alpha chemoattractant)	0.423	0.507
LIF	Leukemia inhibitory factor	0.151	0.248
TNFSF14 (LIGHT)	Tumor necrosis factor ligand superfamily member 14	0.545	0.601
XCL1 (Lymphotactin)	C motif chemokine 1	**0.017**	0.051
CCL8 (MCP-2)	C-C motif chemokine 8 (Monocyte chemotactic protein 2)	**0.003**	**0.014**

CCL7 (MCP-3)	C-C motif chemokine 7 (Monocyte chemotactic protein 3)	0.431	0.513
CCL13 (MCP-4)	C-C motif chemokine 13 (Monocyte chemotactic protein 4)	**0.030**	0.075
CCL22 (MDC)	C-C motif chemokine 22 (Macrophage-derived chemokine)	0.095	0.186
MIF	Macrophage migration inhibitory factor	0.523	0.582
CCL20 (MIP-3α)	C-C motif chemokine 20 (Macrophage inflammatory protein 3 alpha)	**<0.001**	**<0.001**
CCL19 (MIP-3β)	C-C motif chemokine 19 (Macrophage inflammatory protein 3 beta)	0.108	0.202
CCL23 (MPIF-1)	C-C motif chemokine 23 (Myeloid progenitor inhibitory factor 1)	0.275	0.383
MSP	Macrophage stimulating protein	**0.001**	**0.007**
CXCL7 (NAP-2)	C-X-C motif chemokine 7 (Neutrophil-activating peptide 2)	0.143	0.238
Osteopontin	Osteopontin	0.498	0.567
CCL18 (PARC)	C-C motif chemokine 18 (Pulmonary and activation-regulated chemokine)	**0.006**	**0.025**
Platelet factor 4	Platelet factor 4	**0.001**	**0.007**
SDF-1α	Stromal cell-derived factor 1 alpha	0.102	0.194
CCL17 (TARC)	C-C motif chemokine 17 (Thymus and activation-regulated chemokine)	0.127	0.217
CCL25 (TECK)	C-C motif chemokine 25 (Thymus-expressed chemokine)	0.421	0.507
TSLP	Thymic stromal lymphopoietin	0.576	0.626
Activin A	Activin A	**0.003**	**0.014**
AgRP	Agouti-related protein	**0.044**	0.101
Angiogenin	Angiogenin	0.221	0.323
Angiopoietin-1	Angiopoietin 1	**<0.001**	**<0.001**
Angiostatin	Angiostatin	0.142	0.238
Cathepsin S	Cathepsin S	0.676	0.708
CD40 (TNFRSF5)	CD40 (Tumor necrosis factor receptor superfamily member 5)	0.054	0.120
Cripto-1 (TDGF1)	Teratocarcinoma-derived growth factor 1	**0.042**	0.098
DAN (NBL1)	Neuroblastoma suppressor of tumorigenicity 1	0.196	0.299
DKK-1	Dickkopf-related protein 1	**0.019**	0.055

E-Cadherin	Epithelial cadherin	0.110	0.202
TROP1 (EpCAM)	Epithelial cell adhesion molecule	0.082	0.164
Fas Ligand (TNFSF6)	Fas ligand (Tumor necrosis factor ligand superfamily member 6)	0.183	0.286
FcγRIIB/C	Low affinity immunoglobulin gamma Fc region receptor II b/c	**0.007**	**0.027**
Follistatin	Follistatin	0.182	0.286
Galectin-7	Galectin-7	0.281	0.388
ICAM-2 (CD102)	Intercellular adhesion molecule 2	0.059	0.130
IL-13Rα1	Interleukin-13 receptor subunit alpha 1	0.752	0.779
IL-13Rα2	Interleukin-13 receptor subunit alpha 2	0.072	0.153
IL-17B	Interleukin 17B	**0.022**	0.060
IL-2Rα	Interleukin 2 receptor subunit alpha	**<0.001**	**<0.001**
IL-2Rβ	Interleukin 2 receptor subunit beta	**0.011**	**0.036**
IL-23	Interleukin 23	**0.004**	**0.018**
LAP (TGF-β1)	Latency-associated peptide (Transforming growth factor beta 1)	0.495	0.567
NrCAM	Neuronal cell adhesion molecule	0.311	0.420
PAI-1	Plasminogen activator inhibitor 1	**0.022**	0.060
PDGF-AB	Platelet-derived growth factor AB	**0.003**	**0.014**
Resistin	Resistin	**0.002**	**0.011**
SDF-1β	Stromal cell-derived factor 1 beta	**<0.001**	**<0.001**
gp130	Membrane glycoprotein 130	**0.037**	0.089
Shh-N	Sonic hedgehog protein N-product	**0.018**	0.053
Siglec-5 (CD170)	Sialic acid-binding Ig-like lectin 5	**0.035**	0.085
ST2 (IL-1 R4)	Interleukin 1 receptor 4	**0.030**	0.075
TGF-β2	Transforming growth factor beta 2	**0.003**	**0.014**
Tie-2	Tyrosine-protein kinase receptor TIE-2	**0.021**	0.060
Thrombopoietin	Thrombopoietin	**<0.001**	**<0.001**

TRAILR4	TNF-related apoptosis-inducing ligand receptor 4	**0.011**	**0.036**
TREM-1	Triggering receptor expressed on myeloid cells 1	**0.001**	**0.007**
VEGF-C	Vascular endothelial growth factor C	0.200	0.303
VEGFR1	Vascular endothelial growth factor receptor 1	**0.048**	0.109
Amphiregulin	Amphiregulin	0.080	0.163
BDNF	Brain-derived neurotrophic factor	0.503	0.568
bFGF	Basic fibroblast growth factor	**<0.001**	**<0.001**
BMP-4	Bone morphogenetic protein 4	0.101	0.194
BMP-5	Bone morphogenetic protein 5	0.266	0.377
BMP-7	Bone morphogenetic protein 7	0.387	0.481
β-NGF	Beta nerve growth factor	**0.039**	0.093
EGF	Epidermal growth factor	**0.001**	**0.007**
EGFR	Epidermal growth factor receptor	**0.002**	**0.011**
EG-VEGF (PK1)	Endocrine-gland-derived vascular endothelial growth factor (Prokineticin 1)	0.653	0.696
FGF-4	Fibroblast growth factor 4	0.166	0.268
FGF-7 (KGF)	Fibroblast growth factor 7 (Keratinocyte growth factor)	0.374	0.473
GDF-15	Growth differentiation factor 15	**0.001**	**0.007**
GDNF	Glial cell line-derived neurotrophic factor	0.602	0.647
Growth hormone	Growth hormone	0.251	0.361
HB-EGF	Heparin-binding EGF-like growth factor	0.547	0.601
HGF	Hepatocyte growth factor	**<0.001**	**<0.001**
IGFBP-1	Insulin-like growth factor-binding protein 1	0.122	0.216
IGFBP-2	Insulin-like growth factor-binding protein 2	**0.008**	**0.030**
IGFBP-3	Insulin-like growth factor-binding protein 3	**0.002**	**0.011**
IGFBP-4	Insulin-like growth factor-binding protein 4	0.968	0.968
IGFBP-6	Insulin-like growth factor-binding protein 6	0.370	0.471

IGF-1	Insulin-like growth factor 1	0.699	0.728
Insulin	Insulin	**0.010**	**0.035**
M-CSFR	Macrophage colony stimulating factor 1 receptor	**0.001**	**0.007**
NGFR (TNFRSF16)	Nerve growth factor receptor (Tumor necrosis factor receptor superfamily member 16)	0.388	0.481
NT-3	Neurotrophin 3	0.165	0.268
NT-4	Neurotrophin 4	0.794	0.819
Osteoprotegerin	Osteoprotegerin	0.890	0.904
PDGF-AA	Platelet-derived growth factor AA	**<0.001**	**<0.001**
PLGF	Placenta growth factor	0.663	0.702
SCF	Stem cell factor	0.568	0.621
CD117 (SCFR)	CD117 (Stem cell factor receptor)	0.385	0.481
TGF-α	Transforming growth factor alpha	0.838	0.855
TGF-β1	Transforming growth factor beta 1	0.125	0.217
TGF-β3	Transforming growth factor beta 3	0.524	0.582
VEGF-A	Vascular endothelial growth factor A	**0.006**	**0.025**
VEGFR2	Vascular endothelial growth factor receptor 2	0.404	0.493
VEGFR3	Vascular endothelial growth factor receptor 3	0.360	0.465
VEGF-D	Vascular endothelial growth factor D	0.654	0.696
CXCL13 (BLC)	C-X-C motif chemokine 13 (B lymphocyte chemoattractant)	**0.005**	**0.022**
CCL11 (Eotaxin-1)	C-C motif chemokine 11	**0.003**	**0.014**
CCL24 (Eotaxin-2)	C-C motif chemokine 24	**<0.001**	**<0.001**
G-CSF	Granulocyte colony-stimulating factor	**<0.001**	**<0.001**
GM-CSF	Granulocyte-macrophage colony-stimulating factor	**0.035**	0.085
I-309 (TCA-3/CCL1)	C-C motif chemokine 1	0.408	0.495
CD54 (ICAM-1)	CD54 (Intercellular adhesion molecule 1)	0.253	0.361
IFN-γ	Interferon gamma	0.111	0.202

IL-1α	Interleukin 1 alpha	0.218	0.323
IL-1β	Interleukin 1 beta	0.079	0.163
IL-1ra	Interleukin 1 receptor antagonist	**0.013**	**0.041**
IL-2	Interleukin 2	**0.022**	0.060
IL-4	Interleukin 4	**0.014**	**0.043**
IL-5	Interleukin 5	**0.015**	**0.045**
IL-6	Interleukin 6	0.060	0.130
IL-6R	Interleukin 6 receptor subunit alpha	0.356	0.464
IL-7	Interleukin 7	**0.023**	0.061
IL-8 (CXCL8)	Interleukin 8 (C-X-C motif chemokine 8)	0.170	0.272
IL-10	Interleukin 10	**0.001**	**0.007**
IL-11	Interleukin 11	0.052	0.117
IL-12p40	Interleukin 12 p40	0.443	0.520
IL-12p70	Interleukin 12 p70	**0.003**	**0.014**
IL-13	Interleukin 13	**0.010**	**0.035**
IL-15	Interleukin 15	**0.002**	**0.011**
IL-16	Interleukin 16	0.669	0.704
IL-17A	Interleukin 17A	**0.002**	**0.011**
CCL2 (MCP-1)	C-C motif chemokine 2 (Monocyte chemotactic protein 1)	**<0.001**	**<0.001**
M-CSF	Macrophage colony-stimulating factor 1	0.082	0.164
CXCL9 (MIG)	C-X-C motif chemokine 9 (Monokine induced by interferon gamma)	0.357	0.464
CCL3 (MIP-1α)	C-C motif chemokine 3 (Macrophage inflammatory protein 1 alpha)	0.190	0.294
CCL4 (MIP-1β)	C-C motif chemokine 4 (Macrophage inflammatory protein 1 beta)	0.126	0.217
CCL15 (MIP-1δ)	C-C motif chemokine 15 (Macrophage inflammatory protein 1 delta)	**0.010**	**0.035**
PDGF-BB	Platelet-derived growth factor BB	**0.011**	**0.036**
CCL5 (RANTES)	C-C motif chemokine 5 (Regulated upon activation, normal T cell expressed and secreted factor)	0.930	0.935

TIMP-1	Tissue inhibitor of metalloproteinases 1	0.333	0.444
TIMP-2	Tissue inhibitor of metalloproteinases 2	0.220	0.323
TNF-α	Tumor necrosis factor alpha	0.244	0.354
TNF-β	Tumor necrosis factor beta	0.499	0.567
TNFRI	Tumor necrosis factor receptor 1	**0.003**	**0.014**
TNFRII	Tumor necrosis factor receptor 2	**0.011**	**0.036**
CD137	CD137	0.087	0.172
CD166 (ALCAM)	CD166 (Activated leukocyte cell adhesion molecule)	0.074	0.156
CD80	CD80	0.803	0.824
BCMA (TNFRSF17)	B-cell maturation protein (Tumor necrosis factor receptor superfamily member 17)	0.350	0.461
CD14	CD14	**<0.001**	**<0.001**
CD30 (TNFRSF8)	CD30 (Tumor necrosis factor receptor superfamily member 8)	0.308	0.419
CD40 Ligand	CD40 ligand	**0.029**	0.074
CEACAM-1	Carcinoembryonic antigen-related cell adhesion molecule 1	**0.002**	**0.011**
DR6 (TNFRSF21)	Death receptor 6 (Tumor necrosis factor receptor superfamily member 21)	**0.023**	0.061
Dtk (TYRO3)	Tyrosine-protein kinase receptor TYRO3	0.215	0.321
CD105 (Endoglin)	CD105 (Endoglin)	**0.007**	**0.027**
ErbB3	Receptor tyrosine-protein kinase erbB-3	**<0.001**	**<0.001**
E-Selectin	E-Selectin	0.447	0.520
Fas	Apoptosis-mediating surface antigen FAS	0.110	0.202
Flt-3 Ligand	Fms-related tyrosine kinase 3 ligand	**0.008**	**0.030**
GITR (TNFRSF18)	Glucocorticoid-induced tumor necrosis factor receptor related protein (Tumor necrosis factor receptor superfamily member 18)	**0.003**	**0.014**
HVEM (TNFRSF14)	Herpes virus entry mediator A (Tumor necrosis factor receptor superfamily member 14)	0.440	0.520
CD50 (ICAM-3)	CD50 (Intercellular adhesion molecule 3)	0.148	0.245
Contactin-2	Contactin-2	0.468	0.541
IL-1R1	Interleukin 1 receptor type 1	0.109	0.202

IL-2Rγ	Interleukin 2 receptor gamma	0.191	0.294
IL-10Rβ	Interleukin 10 receptor beta	0.128	0.217
IL-17RA	Interleukin 17 receptor A	**0.014**	**0.043**
IL-21R	Interleukin 21 receptor	0.080	0.163
LIMPII	Lysosomal integral membrane protein 2	**0.002**	**0.011**
Lipocalin-2 (NGAL)	Lipocalin 2 (Neutrophil gelatinase-associated lipocalin)	**<0.001**	**<0.001**
L-Selectin	L-Selectin	**0.002**	**0.011**
LYVE-1	Lymphatic vessel endothelial hyaluronic acid receptor 1	**0.013**	**0.041**
MICA	MHC class I polypeptide-related sequence A	**0.008**	**0.030**
MICB	MHC class I polypeptide-related sequence B	0.112	0.202
NRG1-β1	Neuregulin 1 beta 1	0.117	0.209
PDGFRβ	Platelet-derived growth factor receptor beta	0.065	0.140
CD31 (PECAM-1)	CD31 (Platelet endothelial cell adhesion molecule 1)	0.515	0.579
RAGE	Receptor for advanced glycation end products	**<0.001**	**<0.001**
TIM-1 (KIM-1)	T cell immunoglobulin mucin receptor 1 (Kidney injury molecule 1)	**0.004**	**0.018**
TRAILR3	TNF-related apoptosis-inducing ligand receptor 3	**<0.001**	**<0.001**
Trappin-2	Trappin-2	0.397	0.487
uPAR	Urokinase plasminogen activator surface receptor	**<0.001**	**<0.001**
VCAM-1	Vascular cell adhesion molecule 1	**0.041**	0.096
XEDAR	X-linked ectodysplasin-A2 receptor	0.325	0.436

Overall *P* values were determined among ECRSwNP, NECRSwNP, and control groups using the Kruskal-Wallis *H* tests. Adjusted *P* values were calculated by Benjamini-Hochberg false discovery rate correction for multiple comparisons.

The significant values are indicated in bold.

Supplementary Table S2 Protein levels of 66 inflammatory mediators among ECRSwNP, NECRSwNP, and control groups

Mediators (pg/ml)	Control	NECRSwNP	ECRSwNP	*P** value
CXCL16	143.47 (122.89-158.78)	252.31 (197.12-329.94)	190.85 (171.52-261.17)	<**0.001**, **0.003**, 0.134
CCL26 (Eotaxin-3)	0 (0-0)	0 (0-8.81)	8.08 (2.67-34.2)	0.464, **0.007**, **0.028**
CXCL1 (GRO)	26.26 (15.8-63.47)	100.04 (84.91-127.67)	124.6 (51.19-188.85)	<**0.001**, **0.004**, 0.787
CCL14 (HCC-1)	237.82 (222.88-245.3)	141.68 (126.79-168.75)	158.01 (133.81-184.09)	<**0.001**, <**0.001**, 0.602
CCL8 (MCP-2)	12.16 (9.11-20.61)	0 (0-4.74)	5.26 (0-9.43)	**0.002**, **0.009**, 0.285
CCL20 (MIP-3α)	0.31 (0.04-0.74)	5.45 (2-12.17)	1.2 (0.8-3.64)	<**0.001**, **0.025**, **0.039**
MSP	0 (0-0)	13.55 (0.53-60.85)	14.45 (10.75-46.83)	**0.003**, <**0.001**, 0.662
CCL18 (PARC)	0 (0-1.86)	1.46 (0-5.52)	15.9 (11.99-19.53)	0.436, **0.006**, **0.010**
Platelet factor 4	9846.86 (7440.35-11411.69)	1172.11 (298.36-3618.96)	2953.91 (1059.65-7153.99)	<**0.001**, **0.002**, 0.215
Activin A	6.39 (0-24.18)	211.03 (141.65-318.62)	97.81 (0-278.76)	<**0.001**, 0.123, 0.172
Angiopoietin-1	366.09 (326.93-455.6)	145.55 (75.77-191.61)	160.78 (109.36-193.12)	<**0.001**, <**0.001**, 0.662
FcγRIIB/C	502.46 (407.69-812.31)	1136.36 (911.07-1400.57)	970.12 (597.32-1281.46)	**0.001**, 0.050, 0.232
IL-2Rα	9.52 (7.84-11.92)	35.72 (26.64-65.06)	55.8 (31.75-78.81)	<**0.001**, <**0.001**, 0.267
IL-2Rβ	6.89 (0.34-20.93)	54.63 (36.57-87.13)	46.01 (22.62-79.98)	**0.003**, **0.030**, 0.391
IL-23	10.86 (0.27-13.48)	28.51 (21.44-34.2)	26.07 (21.85-43.12)	**0.001**, **0.004**, 0.983
PDGF-AB	36.11 (15.18-71.58)	2.44 (0-25.07)	0.22 (0-1.01)	**0.040**, <**0.001**, 0.104
Resistin	309.96 (243.06-421.5)	509.28 (409.08-729.51)	319.09 (222.24-414.29)	**0.004**, 0.923, **0.003**
SDF-1β	2.49 (0.59-4.1)	13.83 (10.57-19.48)	8.87 (7.27-13.86)	<**0.001**, <**0.001**, 0.124
TGF-β2	0 (0-1.38)	4.63 (2.48-8.13)	1.37 (0-2.84)	**0.004**, 0.346, **0.008**
Thrombopoietin	480.67 (334.25-608.13)	1013.39 (785.01-1253.54)	813.27 (696.13-922.02)	<**0.001**, <**0.001**, 0.285
TRAILR4	0 (0-2.45)	5.48 (3.45-15.64)	4.84 (2.06-8.25)	**0.004**, **0.036**, 0.249
TREM-1	7.61 (0-30.73)	47.33 (35.04-69.51)	14.3 (9.16-32.96)	<**0.001**, 0.254, **0.002**
bFGF	3250.94 (2080-4428.12)	687.85 (278.86-919.84)	317.59 (164.41-575.21)	<**0.001**, <**0.001**, 0.172
EGF	5.34 (3.67-7.21)	1 (0.33-2.57)	0.91 (0.54-1.25)	**0.003**, <**0.001**, 0.723

EGFR	1828.94 (1637.76-2741.6)	1152.51 (982.37-1564.14)	1248.74 (916.54-1462.7)	<**0.001**, **0.003**, 0.787
GDF-15	30.92 (25.98-43.16)	187.71 (107.44-426.93)	169.62 (111.88-302.34)	<**0.001**, <**0.001**, 0.755
HGF	405.89 (300.73-461.66)	177.31 (156.05-250.76)	153.8 (77.54-187.21)	<**0.001**, <**0.001**, 0.113
IGFBP-2	429.06 (298.35-657.38)	808.92 (403.5-1512.67)	1012.34 (718.34-1205.77)	**0.010**, **0.002**, 0.787
IGFBP-3	648.86 (349.57-1103.96)	2591.49 (1060.39-3952.26)	3295.19 (2087.64-4625.84)	**0.004**, <**0.001**, 0.368
Insulin	135.68 (102.86-159.6)	53.23 (0-104.07)	96.79 (52.91-150.74)	**0.003**, 0.180, 0.087
M-CSFR	118.56 (69.78-169.87)	319.59 (250.22-576.62)	239.84 (195.94-293.56)	<**0.001**, <**0.001**, 0.095
PDGF-AA	12.39 (9.1-17.36)	1.89 (0.66-3.46)	1.7 (1.41-2.29)	<**0.001**, <**0.001**, 0.851
VEGF-A	7.41 (6.42-10.89)	4.09 (3.19-5.69)	4.9 (4.21-5.89)	**0.002**, **0.009**, 0.573
CXCL13 (BLC)	0.08 (0-0.61)	2.72 (1.02-16.11)	2.03 (0.54-20.07)	<**0.001**, **0.017**, 0.632
CCL11 (Eotaxin-1)	0 (0-0.29)	3.66 (0.53-7.22)	9.79 (3.26-22.46)	**0.012**, **0.003**, 0.087
CCL24 (Eotaxin-2)	6.93 (5.2-14.26)	33.87 (14.05-84.89)	299.19 (91.9-582.69)	**0.001**, <**0.001**, **0.003**
G-CSF	0 (0-0)	78.72 (30.94-279.51)	36.08 (16.15-56.16)	<**0.001**, <**0.001**, 0.158
IL-1ra	0.14 (0-13.21)	29.03 (10.93-37.65)	34.72 (25.28-49.38)	**0.024**, **0.004**, 0.285
IL-4	0.14 (0-0.81)	0.3 (0.03-1.32)	2.34 (1.25-2.91)	0.524, **0.007**, **0.017**
IL-5	0.27 (0-1.1)	0 (0-1.56)	3.01 (0.69-5.17)	0.759, **0.017**, **0.013**
IL-10	0.39 (0.14-0.63)	0.39 (0-0.96)	1.78 (1.04-2.12)	1.000, <**0.001**, <**0.001**
IL-12p70	0.03 (0-0.14)	0.08 (0-0.23)	0.43 (0.28-0.66)	0.588, **0.003**, **0.002**
IL-13	0.11 (0-0.34)	0.29 (0-0.5)	0.88 (0.42-1.66)	0.286, **0.003**, **0.025**
IL-15	0 (0-5.01)	10.88 (0-42.62)	57.83 (29.13-82.73)	0.208, **0.001**, **0.008**
IL-17A	0.08 (0-0.39)	0.66 (0.32-1.53)	1.08 (0.68-1.23)	**0.006**, <**0.001**, 0.439
CCL2 (MCP-1)	12.48 (9.68-14.37)	75.8 (32.36-97.24)	42.26 (31.29-61.95)	<**0.001**, <**0.001**, 0.368
CCL15 (MIP-1δ)	2.17 (0.14-3.61)	11.59 (0.73-15.77)	10.28 (6.56-16.65)	0.057, <**0.001**, 0.632
PDGF-BB	23.97 (10.75-41.06)	8.04 (2.46-11.47)	6.37 (4.38-7.99)	**0.024**, **0.002**, 0.518
TNFRI	111.61 (50.02-131.63)	206.96 (160.96-308.87)	159.73 (85.65-198.15)	<**0.001**, 0.093, 0.113
TNFRII	287.01 (224.28-398.46)	818.35 (477.13-1449.62)	412.38 (203.91-686.44)	**0.003**, 0.418, 0.065

CD14	2062.31 (1748.39-2383.65)	4971.86 (4071.34-6423.26)	3938.16 (2716.8-4757.09)	<**0.001**, **0.009**, **0.039**
CEACAM-1	183.01 (159.99-201.84)	238.61 (190.6-342.18)	298.18 (247.29-592.78)	**0.031**, <**0.001**, 0.072
CD105 (Endoglin)	3048.71 (2840.73-3611.05)	2154.96 (1438.25-2856.68)	1514.3 (1264.28-2165.2)	**0.016**, **0.002**, 0.267
ErbB3	5891.85 (4846.56-7997.64)	1433.21 (541.37-1924.77)	1011.79 (121.97-2105.73)	<**0.001**, <**0.001**, 0.602
Flt-3 Ligand	13.24 (8.41-24.84)	3.91 (2.24-6.47)	2.48 (1.81-9.39)	**0.006**, **0.004**, 0.632
GITR (TNFRSF18)	2.99 (0-7.01)	16.06 (9-29.15)	7.72 (4.92-15.6)	**0.001**, 0.080, **0.035**
IL-17RA	9 (6.57-12.73)	14.98 (11.39-21.31)	20.7 (13.24-26)	**0.024**, **0.004**, 0.346
LIMPII	30.02 (16.56-35.36)	7.89 (2.82-12.82)	7.85 (1.93-11.34)	**0.001**, <**0.001**, 0.632
Lipocalin-2 (NGAL)	2673 (2263.17-2761.73)	3957.21 (3290.64-4334.54)	3261.41 (2693.88-3306.59)	<**0.001**, 0.107, **0.004**
L-Selectin	1267.05 (897.5-1701.16)	3263.37 (2183.15-3872.34)	2457.56 (1799.68-4108.86)	<**0.001**, **0.006**, 0.692
LYVE-1	149.16 (81.94-238.25)	450.46 (251.05-647.07)	313.42 (259.86-846.24)	**0.005**, **0.014**, 0.787
MICA	8.43 (5.58-12.35)	17.54 (13.41-24.65)	13.43 (12.23-17.41)	**0.004**, **0.025**, 0.172
RAGE	41.63 (24.44-74.78)	2.82 (1.35-10.37)	10 (5.33-16.28)	<**0.001**, <**0.001**, 0.095
TIM-1 (KIM-1)	1.03 (0-4.67)	11.91 (7.05-15.01)	6.15 (4.04-11.56)	**0.001**, **0.011**, 0.249
TRAILR3	0.79 (0.05-1.84)	24.59 (12.94-39.36)	45.71 (29.23-96.41)	<**0.001**, <**0.001**, 0.124
uPAR	15.97 (0-27.53)	320.44 (139.9-507.79)	210.83 (163.84-393.89)	<**0.001**, <**0.001**, 0.573

Data are expressed as medians (interquartile ranges).

* *P* value: NECRSwNP *vs.* Control, ECRSwNP *vs.* Control, and ECRSwNP *vs.* NECRSwNP, respectively. *P* values were obtained from the Mann-Whitney *U* test.

The significant values are indicated in bold.

Abbreviations: CXCL, C-X-C motif chemokine; CCL, C-C motif chemokine; GRO, growth-regulated alpha protein; MCP-2, monocyte chemotactic protein 2; MIP-3α, macrophage inflammatory protein 3 alpha; MSP, macrophage stimulating protein; PARC, pulmonary and activation-regulated chemokine; FcγRIIB/C, low affinity immunoglobulin gamma Fc region receptor II b/c; IL-2Rα, interleukin 2 receptor subunit alpha; IL-2Rβ, interleukin 2 receptor subunit beta; PDGF-AB, platelet-derived growth factor AB; SDF-1β, stromal cell-derived factor 1 beta; TGF-β2, transforming growth factor beta 2; TRAILR4, TNF-related apoptosis-inducing ligand receptor 4; TREM-1, triggering receptor expressed on myeloid cells 1; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GDF-15, growth differentiation factor 15; HGF, hepatocyte growth factor; IGFBP-2, insulin-like growth factor-binding protein 2; IGFBP-3, insulin-like growth factor-binding protein 3; M-CSFR, macrophage colony stimulating factor 1 receptor; PDGF-AA, platelet-derived growth factor AA; VEGF-A, vascular endothelial growth

factor A; BLC, B lymphocyte chemoattractant; G-CSF, granulocyte colony-stimulating factor; IL-1ra, interleukin 1 receptor antagonist; MCP-1, monocyte chemotactic protein 1; MIP-1δ, macrophage inflammatory protein 1 delta; PDGF-BB, platelet-derived growth factor BB; TNFRI, tumor necrosis factor receptor 1; TNFRII, tumor necrosis factor receptor 2; CEACAM-1, carcinoembryonic antigen-related cell adhesion molecule 1; ErbB3, receptor tyrosine-protein kinase erbB-3; Flt-3 Ligand, fms-related tyrosine kinase 3 ligand; GITR, glucocorticoid-induced tumor necrosis factor receptor related protein; TNFRSF18, tumor necrosis factor receptor superfamily member 18; IL-17RA, interleukin 17 receptor A; LIMPII, lysosomal integral membrane protein 2; NGAL, neutrophil gelatinase-associated lipocalin; LYVE-1, lymphatic vessel endothelial hyaluronic acid receptor 1; MICA, MHC class I polypeptide-related sequence A; RAGE, receptor for advanced glycation end products; TIM-1, T cell immunoglobulin mucin receptor 1 ; KIM-1, kidney injury molecule 1; TRAILR3, TNF-related apoptosis-inducing ligand receptor 3; uPAR, urokinase plasminogen activator surface receptor; ECRSwNP, eosinophilic chronic rhinosinusitis with nasal polyposis; NECRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyposis.