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RESEARCH PAPER

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# Utilization of RNA sequencing to investigate olfactory dysfunction in chronic rhinosinusitis without nasal polyps: A pilot study

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## Abstract

**Objectives:** Prior research on olfactory dysfunction in chronic rhinosinusitis (CRS) has focused on patients with polyps and suggests that direct inflammation of the olfactory cleft mucosa plays a contributory role. The purpose of this study was to evaluate gene expression in superior turbinate mucosal specimens, comparing normosmic and dysosmic CRS patients without polyps (CRSsNP).

**Methods:** Tissue samples were obtained from the superior turbinates of patients with CRSsNP at the time of endoscopic sinus surgery. Samples subsequently underwent RNA sequencing and functional analysis to investigate biological pathways associated with differentially expressed genes between dysosmic ($n = 7$) and normosmic ($n = 4$) patients.

**Results:** Differential gene expression analysis comparing dysosmic and normosmic CRSsNP patients showed upregulation of 563 genes and downregulation of 327 genes. Using stringent criteria for multiple comparisons, one upregulated gene (Immediate Early Response 3 [*IER3*]) had an false discovery rate (FDR) correction adjusted $P$ value considered statistically significant ($P < 0.001$, fold change 2.69). Reactome functional analysis revealed eight biological pathways significantly different between dysosmic and normosmic patients ($P < 0.05$, FDR correction) including IL-4 and IL-13 signaling, IL-10 signaling, and rhodopsin-like receptors.

**Conclusions:** RNA sequencing of the superior turbinates in patients with CRSsNP can provide valuable information regarding biological pathways and genes involved in olfactory dysfunction. This study supports literature suggesting that Type 2 inflammation may play a role in olfactory dysfunction in at least some patients with CRSsNP. This study also prompts questions regarding the role of IL-10, rhodopsin-like receptors, and *IER3* in the pathogenesis of olfactory dysfunction.

KEYWORDS
chronic rhinosinusitis without nasal polyps, olfactory dysfunction, RNA sequencing

**Key points**

What are the significant findings of the study?

- Differential gene expression between normosmic and dysosmic chronic rhinosinusitis without nasal polyps (CRSsNP) patients showed upregulation of Immediate Early Response 3 (*IER3*).
- Reactome analysis showed that IL-10 signaling, IL-4/IL-13 signaling, rhodopsin-like receptors, and binding of chemokines to chemokine receptors were different between dysosmics and normosmics.

What does the study add?

- This is the first study to utilize RNA sequencing of superior turbinate mucosa to investigate olfactory dysfunction in CRSsNP patients.
- The study highlights potential areas of future investigation including IL-10 signaling, rhodopsin-like receptors, and *IER3*.

## INTRODUCTION

Olfactory dysfunction is a cardinal symptom of chronic rhinosinusitis (CRS), with up to 75% of patients reporting loss of smell.[1] Although often overshadowed by other symptoms, loss of smell can have important impacts on quality of life, including diet, feelings of safety, and even social isolation.[2,3] Olfactory loss tends to be more severe in patients with CRS with nasal polyps (CRSwNP), with research suggesting that direct inflammation of the olfactory cleft (OC) mucosa plays a contributory role.[4,5] Within CRSwNP patients, current treatments such as endoscopic sinus surgery (ESS), corticosteroids, and Type 2 biologics have been shown to improve olfaction, often returning patients to at least the hyposmic range.[6,7] However, much less is known regarding the pathophysiology of olfactory loss in patients with CRS without nasal polyps (CRSsNP). Within CRSsNP, olfactory loss is usually less severe than CRSwNP, but remains a common complaint and tends to be less responsive to current treatments.[8,9] The goal of the current study was to evaluate gene expression in superior turbinate mucosal specimens, comparing normosmic and dysosmic patients with CRSsNP. Our aim was to generate hypotheses regarding potential mechanisms of olfactory dysfunction in CRSsNP that might inform future research.

## MATERIALS AND METHODS

### Recruitment

Adult patients ≥18 years old diagnosed with CRSsNP were recruited following routine rhinology clinic visits at the Medical University of South Carolina (MUSC). All patients were diagnosed with CRSsNP according to the International Consensus Statement on Allergy & Rhinology.[10] Patients included in this study had planned ESS as part of their scheduled medical care after failing medical therapy. Patients were excluded if they had cystic fibrosis, primary ciliary dyskinesia, systemic inflammatory disease (granulomatosis with polyangiitis, sarcoidosis, eosinophilic granulomatosis with polyangiitis), or had been on systemic corticosteroids within the preceding 30 days. This study was approved by the institutional review board at MUSC and all patients involved provided written informed consent before study participation.

### Clinical assessment

Demographic data and comorbidity information were collected from patients via survey and supplemented with patient medical records. A comprehensive baseline assessment was performed for included patients following routine clinic appointments. Olfactory testing was performed using Sniffin' Sticks pens (Burghart Messtechnik) which evaluate three separate domains of olfactory function including odorant threshold (T; score range 1–16), odorant discrimination (D; score range 0–16), and odorant identification (I; score range 0–16). Threshold, discrimination, and identification scores are used to create a composite TDI score (score range 1–48) with higher scores representing better olfactory function.[11] Patients were characterized as dysosmic or normosmic based on composite TDI scores with TDI <31.0 being dysosmic and TDI of 31–48 being normosmic. Additionally, patients completed the 22 item Sinonasal Outcome Test (SNOT-22) to quantify subjective disease severity.[12]

All study participants underwent bilateral sinonasal endoscopy performed using 3 mm rigid telescope (Karl Storz). Included patients were classified as CRSsNP based on the visible absence of polyps. Two treating physicians quantified the severity of discharge, edema, polyps, crusting, and scarring of the OC using a Likert scale from 0 to 2 for each attribute. Results for each side were combined to form a final Olfactory Cleft Endoscopy Scale (OCES; range 0–20) with higher

scores reflecting increased disease severity.[13] The sinonasal cavity was further graded using the Lund–Kennedy Endoscopy Scale (LKES).[14] Each subject underwent computed tomography scanning which was graded using the Lund–Mackay scoring system with reviewers blinded to olfaction data.[15]

## Tissue sampling and preparation

Tissue samples were obtained from the superior turbinate at the time of ESS. A thru-cutting Blakesley forceps was used to remove two mucosal pieces from each side, combining them into a single specimen. This was done as part of their scheduled surgery and did not subject patients to any additional intervention outside standard procedure. Samples were immediately snap-frozen and stored in RNAlater at −80°C.

## RNA sequencing and functional analysis

Superior turbinate mucosal tissue was lysed and homogenized using RLT Plus buffer, QIAshredder spin column, gDNA Eliminator spin column, and RNeasy spin column. RNA isolate was then stored at −80°C before sequencing. Paired-end sequencing was done at 150 bp read length, producing approximately 6 Gb of clean bases per sample with most having >93% at Q30 (Novogene). Alignment was done using Star[16] and differential expression analysis done with DESeq. 2.[17] Enrichment analysis was done with clusterProfiler.[18] Sequencing data was quantified against the Ensembl human gene database that includes over 19,000 coding genes as well as many other noncoding genes.[19]

## Statistical data analysis

Data regarding patient demographics and disease severity were analyzed using IBM SPSS 25.0 software package (IBM Corp). For continuous variables, results are expressed as means ± standard deviation. Categorical variables were reported as percentages. Differential gene expression is reported using both a raw *P* value and a false discovery rate (FDR) corrected adjusted *P* value. Reactome functional analysis was performed and reported using an FDR corrected adjusted *P* value. The FDR was calculated as a correction for multiple comparisons. An FDR-adjusted *P*-value ≤0.05 was considered significant differential expression, with raw *P*-values ≤0.05 considered to be of interest only.

# RESULTS

## Patient characteristics

Eleven patients with a diagnosis of CRSsNP were enrolled, of which $n = 4$ were normosmic and $n = 7$ dysosmic. Patient demographics, comorbidities, and disease severity metrics are summarized in Table 1. The average age of included patients was 47.9 ± 16.1 years. Of note, 3 patients, all in the dysosmic group, had a history of asthma. Dysosmic patients had worse average OCES, LKES, Lund–Mackay scores, and TDI scores while normosmic patients had worse SNOT-22 scores (Table 2).

## Differential gene expression analysis

Differential gene expression analysis comparing dysosmic and normosmic CRSsNP patients showed upregulation of 563 genes and downregulation of 327 genes (Supporting Information: Table 1). Differential gene expression between groups is shown via volcano plot and heat map

**TABLE 1** Patient demographic data and baseline characteristics ($n = 11$).

| Characteristics | | Count | Column (%) |
|---|---|---|---|
| Demographics | | | |
| Olfactory category | Dysosmic | 7 | 63.6 |
| | Normosmic | 4 | 36.4 |
| Age (year, mean ± SD) | | 47.90 ± 16.10 | |
| Gender | Female | 4 | 36.4 |
| | Male | 7 | 63.6 |
| Race | African American/Black | 1 | 9.1 |
| | American Indian/Alaska Native | 1 | 9.1 |
| | White/Caucasian | 9 | 81.8 |
| Years of education | Graduate school/ professional degree | 2 | 18.2 |
| | High school | 4 | 36.4 |
| | Postsecondary | 5 | 45.5 |
| | College/university | | |
| Previous surgery | 1 | 1 | 9.1 |
| | 4 | 1 | 9.1 |
| | None | 9 | 81.8 |
| Smoking history | Former smoker | 2 | 18.2 |
| | None | 9 | 81.8 |
| Septal deviation | Yes | 7 | 63.6 |
| Asthma | Yes | 3 | 27.3 |
| GERD | Yes | 3 | 27.3 |
| Diabetes | Non-insulin dependent | 1 | 9.1 |
| | None | 10 | 90.9 |
| Depression | Yes | 3 | 27.3 |
| Allergic rhinitis | Yes | 4 | 36.4 |
| Allergy testing history | Yes | 5 | 45.5 |

**TABLE 2** Patient olfaction and disease severity measures ($n$ = 11, mean [SD]).

| Olfaction | Normosmic | Dysosmic |
|---|---|---|
| Total TDI | 33.0 (2.3) | 22.0 (5.1) |
| Threshold | 7.0 (0.4) | 3.0 (1.5) |
| Discrimination | 13.3 (1.0) | 9.6 (2.8) |
| Identification | 12.8 (1.5) | 9.4 (4.0) |
| QOD-NS total | 7.3 (2.5) | 7.9 (7.4) |
| Disease severity measures | | |
| SNOT-22 | 48.8 (14.3) | 37.7 (18.6) |
| Olfactory cleft endoscopy score | 0.3 (0.5) | 1.7 (1.4) |
| Lund–Kennedy endoscopy score | 3.0 (1.8) | 3.9 (2.0) |
| Lund–Mackay score | 10.3 (3.0) | 12.1 (4.7) |
| Sinus control test | 8.8 (3.4) | 6.6 (1.9) |
| PHQ-9 | 4.0 (0.8) | 4.7 (5.0) |

Abbreviation: SNOT-22, 22 item sinonasal outcome test.

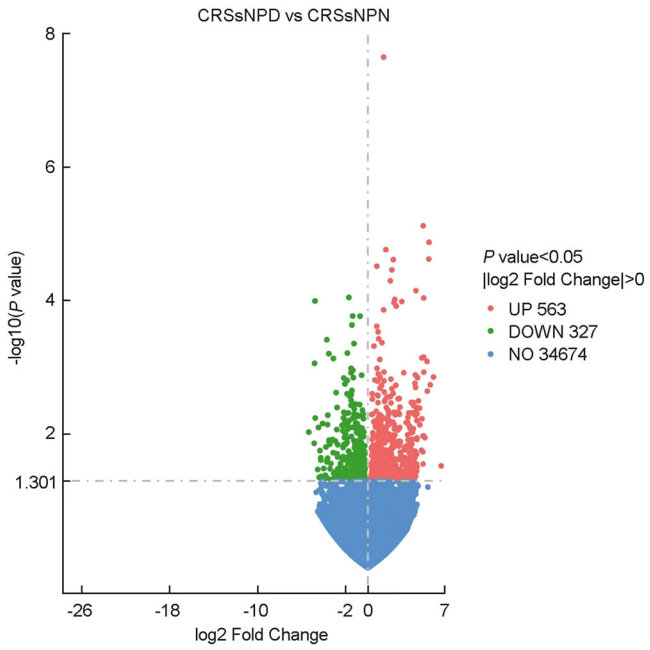


**FIGURE 1** Volcano plot of differential gene expression in CRSsNPD versus CRSsNPN. Red and green dots represent genes which are upregulated and downregulated, respectively, when comparing CRSsNPD and CRSsNPN. CRSsNP, chronic rhinosinusitis without nasal polyps.

(Figures 1 and 2). Stratified by *P*-value, the top 10 upregulated and downregulated genes in this analysis are presented in Table 3. Using stringent criteria for multiple comparisons, one upregulated gene (Immediate Early Response 3, *IER3*) had an FDR correction adjusted *P* value < 0.05 ($P$ = 7.9E−4, fold change 2.69). None of the downregulated genes met this criteria (Table 3a and b).

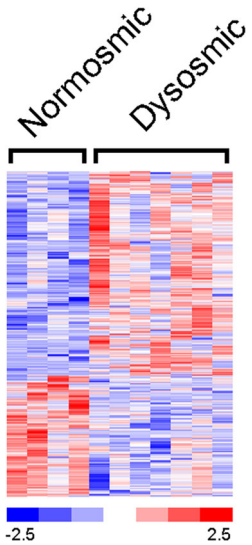


**FIGURE 2** Heat map of genes differentially expressed between CRSsNPD and CRSsNPN patients. Red and blue color represent relative upregulation and downregulation, respectively. Scale bar, colorimetric scaling to $z$ standardized values. CRSsNP, chronic rhinosinusitis without nasal polyps.

## Reactome functional analysis

Reactome functional analysis revealed eight biological pathways significantly different between dysosmic and normosmic patients with CRSsNP ($P$ < 0.05, FDR correction) (Figure 3). Of note, IL-10 signaling, IL-4/IL-13 signaling, rhodopsin-like receptors, and the binding of chemokine receptors to chemokines were some of the pathways most significantly enriched.

# DISCUSSION

Olfactory dysfunction has significant consequences as it relates to quality of life, diet, and nutrition.[20] While research investigating the mechanisms underlying olfactory dysfunction in patients with CRSwNP has increased in recent years, CRSsNP has received substantially less attention. Our study used RNA sequencing of superior turbinate tissue samples to explore how gene expression varies among dysosmic and normosmic patients with CRSsNP. Human studies on olfaction utilizing RNA sequencing are currently limited and focus on age-related olfactory loss[21] and Alzheimer's Disease.[22] To the best of our knowledge, this is the first study to utilize RNA sequencing from the superior turbinate in CRSsNP patients to generate new hypotheses for our understanding of the mechanisms of olfactory dysfunction.

## IL-4 and IL-13 signaling

Reactome functional analysis showed that IL-4 and IL-13 signaling was significantly affected in the dysosmic patients. This finding suggests that

**TABLE 3a** Top 10 upregulated genes in CRSsNP dysosmics.

| Gene | *P* value | FDR adjusted *P* value | Log fold change | Log *P* value |
|---|---|---|---|---|
| *IER3* | 2.23E−08 | 0.000789886 | 1.431521126 | 7.651991 |
| *TRBV27* | 7.53E−06 | 0.133431512 | 5.030940079 | 5.123267 |
| *AL157700.1* | 1.32872E−05 | 0.142745016 | 5.561352243 | 4.876566 |
| *TRIM52-AS1* | 1.712E−05 | 0.142745016 | 1.63531719 | 4.766497 |
| *TRBJ1-6* | 2.36845E−05 | 0.142745016 | 5.542495102 | 4.625536 |
| *CXCL2* | 2.41634E−05 | 0.142745016 | 2.311600651 | 4.616843 |
| *RDH10* | 3.03654E−05 | 0.152498073 | 0.817437293 | 4.517622 |
| *GDF15* | 3.44191E−05 | 0.152498073 | 2.187486893 | 4.463201 |
| *IL20RB* | 5.04134E−05 | 0.198544624 | 2.042592111 | 4.297454 |
| *SMR3A* | 7.04672E−05 | 0.236526746 | 4.370085157 | 4.152013 |

Abbreviations: CRSsNP, chronic rhinosinusitis without nasal polyps; FDR, false discovery rate; IER3, immediate early response 3.

**TABLE 3b** Top 10 downregulated genes in CRSsNP dysosmics.

| Gene | *P* value | FDR adjusted *P* value | Log fold change | Log *P* value |
|---|---|---|---|---|
| *ANGPTL4* | 8.90914E−05 | 0.236526746 | −1.72397842 | 4.050164 |
| *VWFP1* | 0.000100847 | 0.236526746 | −4.80535181 | 3.996339 |
| *AC048382.5* | 0.000169347 | 0.301334612 | −1.38238376 | 3.771224 |
| *PARM1* | 0.000170029 | 0.301334612 | −0.70763906 | 3.769476 |
| *C21orf62-AS1* | 0.000230828 | 0.388680998 | −1.452005 | 3.636711 |
| *HSPB9* | 0.000383296 | 0.543436516 | −3.73070049 | 3.416466 |
| *ANGPT1* | 0.000438234 | 0.575303255 | −1.25494197 | 3.358294 |
| *JPH2* | 0.000608371 | 0.732998021 | −1.86288518 | 3.215832 |
| *NGF-AS1* | 0.000620396 | 0.732998021 | −3.54817707 | 3.207331 |
| *GDF3* | 0.00073342 | 0.787759447 | −3.13419516 | 3.134647 |

Abbreviations: CRSsNP, chronic rhinosinusitis without nasal polyps; FDR, false discovery rate.

Type 2 inflammation may play a role in olfactory dysfunction in some patients with CRSsNP. Multiple studies in patients with CRSwNP have shown an association between Type 2 inflammation in the sinonasal mucus and olfactory dysfunction.[23–25] Additionally, biological medications that target Type 2 inflammation improve olfaction in CRSwNP patients, particularly dupilumab which targets IL4/IL13.[26,27] While research in CRSsNP has received less attention, one recent study investigating OC mucosal protein concentrations showed that 5.6% and 14.8% of patients with CRSsNP had abnormal protein expression of IL-4 and IL-13, respectively.[28] Another study showed that Type 2 inflammation played a major role in 34% of patients with CRSsNP.[5] Our study adds to existing literature in supporting the possible role of Type 2 inflammation in olfactory dysfunction among a subset of CRSsNP patients. While previous studies have shown only a minority of patients with CRSsNP exhibit Type 2 inflammation, it is possible that this pattern of inflammation was more common among our cohort, which was comprised of patients with asthma and a higher disease burden requiring surgical intervention. If this relationship can be further established, biological medications targeting Type 2 inflammation could eventually play a role in medical management of olfactory dysfunction in some patients with CRSsNP, particularly in those with comorbid asthma.

## IL-10 signaling

The Reactome functional analysis also showed that IL-10 signaling was affected in CRSsNP patients with dysosmia. The IL-10 cytokine is classically considered an anti-inflammatory cytokine.[29] However, multiple recent studies have demonstrated an association between higher sinonasal mucus levels of IL-10 and reduced olfactory function.[25,28,30] The exact role of IL-10 in olfactory dysfunction is unclear at this time. It is possible that this differential regulation of the IL-10 signaling pathway is simply a harbinger of chronic Type 2 inflammation in general, given that IL-10 is a known product of Th2

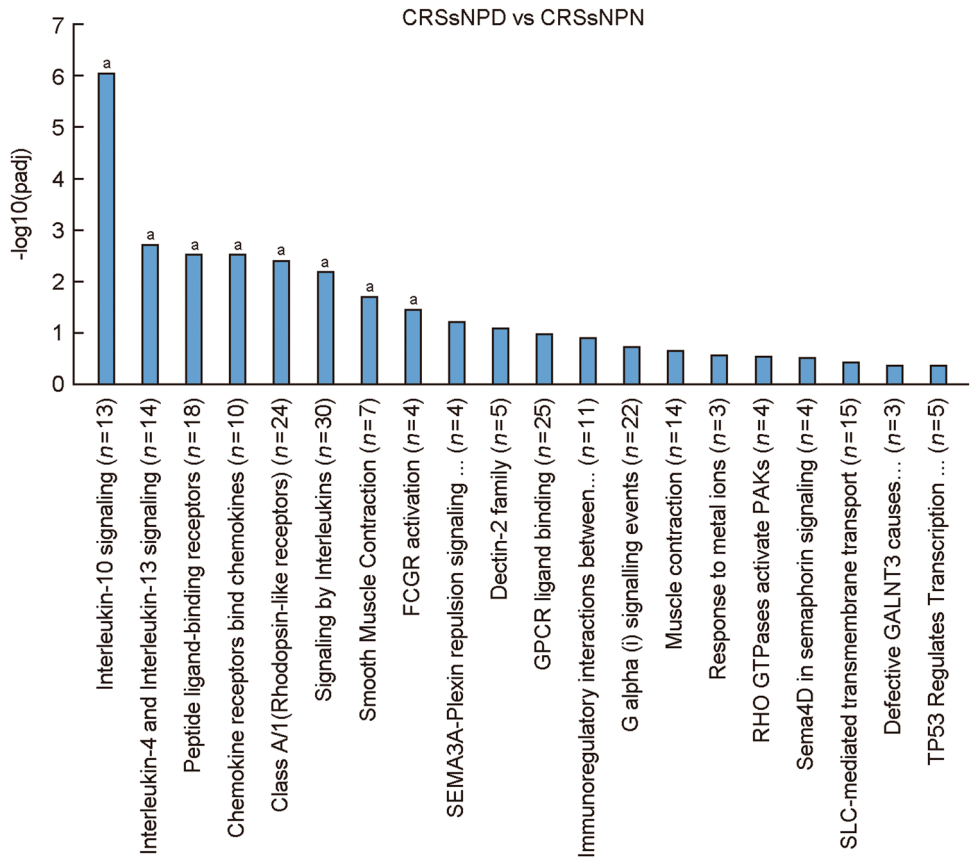


**FIGURE 3** Reactome functional analysis ([a] had significantly different between dysosmic and normosmic patients with CRSsNP). CRSsNP, chronic rhinosinusitis without nasal polyps.

cells necessary for inhibition of concurrent Th1 signaling.[29,31] That being said, the role of IL-10 is complex. The repeated association between elevated sinonasal IL-10 levels and reduced objective olfactory metrics does bring forth the question of whether or not IL-10 dysregulation is playing a meaningful role in the pathogenesis of olfactory dysfunction. This is a possible topic of interest for future mechanistic investigations.

## Rhodopsin-like receptors

Our Reactome functional analysis also highlighted rhodopsin-like receptors as being significantly affected in CRSsNP patients with dysosmia. These receptors make up the largest family of G-protein coupled receptors and have been shown to include olfactory receptors.[32] The role that this differential gene expression plays, if any, is unclear at this time. There are examples of chronic inflammatory states leading to genetic downregulation, including specific processes taking place in the sinonasal mucosa. A 2013 study by Hupin et al.[33] showed downregulation of polymeric Ig receptor in ethmoid tissue in Th2 mediated eosinophilic inflammatory states including CRSwNP patients. While it is biologically plausible that the chronic inflammatory state seen in CRS patients could result in genetic downregulation and decreased olfactory receptor expression, this is speculative at present. Future cell surface receptor quantification studies or RNA sequencing analysis on a much larger scale population would be needed to support such a claim.

## Binding of chemokines to chemokine receptors

Additionally, Reactome functional analysis showed that binding of chemokines to chemokine receptors was affected in our dysosmic patients. Previous research has illustrated the active role which olfactory epithelial stem cells play in the production of chemokines to maintain immune defense and has explored the possible role this plays in olfactory dysfunction in CRS.[34] This potential mechanism of dysosmia is supported by multiple studies highlighting the relationship between abnormal levels of OC chemokines and worse olfactory function in CRSwNP[24] and CRSsNP.[35] The current study adds to the existing literature and supports the possible role of chemokine activity in olfactory dysfunction among CRSsNP patients.

## Differential gene expression analysis

Our differential gene expression analysis yielded 563 upregulated and 327 downregulated genes. Of these, only *IER3* also had an FDR

adjusted $P \leq 0.05$. Given our small sample size, the lack of genes surviving FDR correction was not surprising; however, the continued significance of *IER3* upregulation even with FDR correction makes it unlikely that this is a false positive. *IER3* is a stress-inducible gene shown to play a role in extracellular signal-regulated kinase signaling pathways.[36] It appears to have differing functions based on the tissue where it is expressed, likely acting as a tumor suppressor[37] in certain areas and an oncogene in others.[36,38] *IER3* has not been previously investigated in the CRS literature. Its role at this time is unclear and it may be of interest in future studies.

## Strengths and limitations

Strengths of this study include the utilization of RNA sequencing to analyze superior turbinate mucosa in patients with CRS, as well as objective assessment of olfactory function. To the best of our knowledge, this is the first study using RNA sequencing to generate hypotheses for the mechanisms underlying olfactory dysfunction in CRSsNP. The main limitation of this study was its small sample size, which limits the statistical power for individual gene comparisons, particularly when adjusting for multiple comparisons on a genome-wide scale. For this reason, it is highly likely that there are other important differentially expressed genes involved in olfactory dysfunction in CRSsNP patients that were not identified statistically. The sample size also made it impossible to analyze the statistical significance of differences in comorbidities between the dysosmic and normosmic groups. Another limitation is the lack of histopathological analysis to differentiate whether the superior turbinate mucosa analyzed was olfactory or respiratory epithelium. Bulk superior turbinate biopsy likely has a mix of olfactory and respiratory epithelial cells.[39] Further insight may be provided using single cell RNA sequencing to focus on olfactory cells specifically. Additionally, superior turbinate tissue was only available for collection after sinus surgery, meaning that our population was comprised entirely of CRS patients who failed initial medical management. This patient population was likely skewed toward those with a more severe disease presentation and may not have been representative of CRSsNP as a whole.

## CONCLUSIONS

RNA sequencing of the superior turbinates in patients with CRSsNP can provide valuable information regarding biological pathways and genes involved in olfactory dysfunction among this patient population. The findings in this study support recent literature suggesting that Type 2 inflammation may play a role in olfactory dysfunction in at least some patients with CRSsNP. This study also prompts a number of questions regarding the role of IL-10, rhodopsin-like receptors, chemokine activity, and *IER3* in the pathogenesis of olfactory dysfunction. Further study with a more robust sample size is needed to better characterize how these different factors contribute to dysosmia.

### AUTHOR CONTRIBUTIONS

This manuscript has not been published elsewhere, nor is it currently under consideration for publication elsewhere. Each of the authors listed has contributed significantly to the creation of this manuscript in keeping with the latest guidelines of the International Committee of Medical Journal Editors. Jorge A. Gutierrez III was involved in the design, data collection, analysis, drafting, and approval of the final manuscript. Jeremy L. Barth was involved in the design, analysis, drafting, and approval of the final manuscript. Rodney J. Schlosser was involved in the design, analysis, drafting, and approval of the final manuscript. Thomas S. Edwards was involved in the design, analysis, drafting, and approval of the final manuscript. Timothy L. Smith was involved in the design, analysis, drafting, and approval of the final manuscript. Zachary M. Soler was involved in the design, conception, data collection, analysis, drafting, and approval of the final manuscript. All authors are in agreement with the content of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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### CONFLICTS OF INTEREST STATEMENT

R. J. S.: Consultant for Stryker, Medtronic, Optinose, Cyrano. Medical Director, Healthy Humming. Z. M. S.: Consultant for Lyra, Optinose, and Regeneron. Medical Director, Healthy Humming. The remaining authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data is available from the authors upon request.

### ETHICS STATEMENT

This study was conducted in accordance with the principles expressed in the Declaration of Helsinki. The study was performed with the approval of the Medical University of South Carolina Institutional Review Board.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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