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ORIGINAL ARTICLE

Molecular progression of chronic rhinosinusitis: a pseudotime reconstruction of a transcriptomic dataset

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ABSTRACT

BACKGROUND
Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory disease that is classified based on the presence of nasal polyps. However, whether CRS and nasal polyps represent discrete entities or a continuous progression remains unclear. Most studies have relied on cross-sectional comparisons, which limit insight into its pathophysiology. This study aimed to test whether CRS subtypes lie on a continuous trajectory by applying pseudotime ordering to CRS transcriptomic data.

METHODS
Gene expression data were obtained from the Gene Expression Omnibus (GSE36830), which consists of control, chronic rhinosinusitis without nasal polyps (CRSsNP), uncinate tissue (CRSwNP UT), and nasal polyp tissue (CRSwNP NP) from chronic rhinosinusitis with nasal polyps. Pathway activity was quantified using Gene Set Variation Analysis (GSVA) based on gene sets for immune, inflammatory, and epithelial pathways. Highly variable pathways were selected and used as input to reconstruct a pseudotemporal disease trajectory. Statistical associations were assessed using Spearman correlation and the Jonckheere–Terpstra trend test.

RESULTS
Pseudotime reconstruction showed an ordered molecular trajectory extending from control samples through CRSsNP and CRSwNP UT, with nasal polyp samples forming a distinct end stage. Pseudotime showed a strong monotonic association with disease stage (Spearman's $\rho = 0.743$, $p < 0.001$), indicating an ordered progression of molecular changes across disease states. Progressive disease was characterized by increasing activation of immune pathways, including hypersensitivity, alongside declining activity of epithelial stress response and metabolic pathways.

CONCLUSION
These findings demonstrate that CRS represents a continuous molecular disease spectrum. Nasal polyposis emerges as a distinct molecular end state characterized by dominant immune activation. This pseudotime-based framework provides insight into CRS pathogenesis and provides evidence for specific therapeutic strategies.

Keywords: Gene set variation analysis, chronic rhinosinusitis, nasal polyps, immune pathways, inflammatory mediators, disease progression

INTRODUCTION

Chronic rhinosinusitis (CRS) is a spectrum of persistent inflammatory disorders of the nasal and paranasal sinus mucosa characterized by symptoms lasting longer than 12 weeks despite appropriate medical therapy.[1] Chronic rhinosinusitis has been recognized based on clinical signs and symptomatology, yet recent guidelines emphasize the combination of symptoms with objective evidence such as endoscopy or computed tomography.[2] This disease is then classified by phenotype into chronic rhinosinusitis with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP).[3] These phenotypes are complemented by endotypes defined by distinct inflammatory patterns like type 1, type 2, and type 3 immune responses.[4] The diversity of mechanisms within CRS reflects a multifactorial disease process,[5] emphasizing that CRS is a spectrum associated with chronic inflammatory syndrome.[1]

Chronic rhinosinusitis is among the most common chronic inflammatory diseases worldwide. The disease is estimated to affect approximately 2% to 15% of the adult population, with more females than males.[1] While most epidemiologic studies on CRS do not differentiate between CRSwNP and CRSsNP, both phenotypes occur globally. Chronic rhinosinusitis affects 13% of the population in the United States, 11% in Europe, 8% in China, 7% in South Korea, and 6% in Brazil.[6] Daily smoking, higher body mass index, and the comorbidities of asthma, diabetes mellitus, eczema, and nasal septal deviation, increase the risk of CRSwNP.[7] The disease contributes significantly to healthcare utilization, including outpatient visits, imaging, medication use, and surgical interventions.[8] Beyond the economic burden, CRS exerts a profound impact on physical functioning and quality of life.[9]

The pathophysiology of chronic rhinosinusitis is multifactorial, involving dysfunctional interactions among epithelial barriers, host immunity, and environmental exposures.[4,10] Chronic rhinosinusitis is caused by persistent infection combined with immune dysregulation, leading to chronic mucosal inflammation.[11] The main components of immunologic pathways implicated in CRS include Th1, Th2, and Th17 inflammatory responses, which vary depending on disease phenotype.[4] In CRSwNP, type 2-dominant inflammation with elevated interleukin-4, interleukin-5, and interleukin-13 has been consistently observed.[3,4] Epithelial barrier dysfunction and impaired innate immunity further perpetuate the cycle of inflammation and mucosal remodeling. Genetic susceptibility and environmental risk factors such as allergens, pollutants, and occupational exposures also modulate individual risk.[12]

Treatment of CRS mainly aims to control mucosal inflammation. Other aims include facilitating sinus drainage and addressing contributing factors such as infection and allergy. First-line therapies include saline irrigations and topical intranasal corticosteroids to reduce inflammation. Systemic corticosteroids and antibiotics are considered in selected patients with a significant inflammatory burden or confirmed bacterial infection. Endoscopic sinus surgery (ESS) is recommended for patients with persistent symptoms refractory to optimal medical management. Endoscopic sinus surgery has been shown to improve symptom scores and quality of life in appropriately selected patients with CRS.[1] The advent of biologic therapies has transformed the management of severe CRSwNP, particularly for patients with type 2-dominant inflammation.[13] Biologic agents such as dupilumab, omalizumab, and mepolizumab target specific inflammatory pathways to reduce polyp size and alleviate symptoms.[14,15] Dupilumab, an IL-4Rα antagonist, has demonstrated significant improvements in nasal polyp burden, congestion, and olfactory function.[15,16] Mepolizumab can mitigate tissue edema, remodeling, and polyp growth. Omalizumab affects mast cells and basophils by binding to inbound IgE, reducing allergic signaling and IgE-mediated cellular amplification, and has also shown efficacy in selected patients with CRSwNP.[17] The use of biologic agents primarily targets upstream mediators to improve sinonasal symptoms, reduce systemic steroid and ESS use, and improve quality of life (QoL) in CRSwNP.[18]

Despite advances in understanding the pathophysiology of chronic rhinosinusitis and the

introduction of targeted biologic therapies, the biological progression of CRS remains incompletely understood. Most existing studies have characterized CRS using cross-sectional comparisons between phenotypes, implicitly assuming discrete disease entities rather than a dynamic continuum.[19] This approach limits insight into how molecular pathways evolve as the disease progresses from non-polypoid to polypoid forms. Moreover, the temporal relationships among these pathways and their role in disease progression remain unclear, even though several inflammatory endotypes have been described. As a result, the mechanisms underlying the transition from CRSsNP to CRSwNP remain incompletely elucidated.[20]

Prior studies of CRS have largely compared phenotypes cross-sectionally or applied single-cell RNA sequencing to profile the cellular composition of nasal polyps. This approach successfully identifies epithelial, fibroblast, and immune-cell alterations and reconstructs cell-differentiation trajectories within polyp tissue.[21–23] However, these studies have demonstrated overlapping molecular signatures between CRSsNP and CRSwNP but have not attempted to model the disease continuum itself.[24,25] The present study applied pseudotime reconstruction to bulk-tissue pathway profiles spanning the full clinical spectrum and ordering disease states along a single trajectory.

Therefore, this study aimed to determine whether chronic rhinosinusitis represents a continuous molecular disease spectrum by reconstructing a pseudotemporal trajectory based on pathway-level gene expression profiles. Using gene set variation analysis in combination with diffusion-based manifold learning, we sought to characterize dynamic changes in biological pathways across control, CRSsNP, and CRSwNP tissues. This study further aimed to identify key pathways associated with disease evolution and to determine whether nasal polyps represent a distinct disease entity within the CRS continuum.

METHODS

Research design

This study is an in silico bioinformatic analysis of a publicly available microarray dataset. The analysis was conducted at the Maranatha Biomedical Research Laboratory, Maranatha Christian University, Bandung, Indonesia, from November 2025 to January 2026. The gene-expression data were originally created at the Division of Allergy-Immunology and the Department of Otolaryngology, Northwestern University Feinberg School of Medicine (Chicago, Illinois, USA), and were made publicly available in March 2012.[26] In the study, uncinate tissue was collected from 6 control subjects, 6 patients with CRSsNP, and 6 patients with CRSwNP, and nasal polyp tissue from 6 patients with CRSwNP (24 samples in total). All gene expression was profiled on the Affymetrix Human Genome U133 Plus 2.0 array.

Data source and sample annotation

Gene expression data were obtained from the Gene Expression Omnibus (GEO) dataset GSE36830. [26] This dataset contains microarray profiles of tissue samples from patients with chronic rhinosinusitis and controls. Based on the sample annotation, these samples were classified into four conditions: Control uncinate tissue, chronic rhinosinusitis without nasal polyp uncinate tissue (CRSsNP UT), chronic rhinosinusitis with nasal polyp uncinate tissue (CRSwNP UT), and the nasal polyps from chronic rhinosinusitis with nasal polyps (CRSwNP NP). Since this is a re-analysis of a previously published dataset, we did not have a formal sample size calculation. However, we conducted a sensitivity analysis using G*Power 3.1 and found that, with n=24, two-sided α=0.05, and 80% power, the minimum detectable r is 0.55.[27]

The microarray platform annotation was retrieved from GEO to map probe identifiers to gene symbols. Probes lacking valid gene annotations were excluded. For genes with duplicate annotations, expression values were collapsed at the gene level using the median. This step resulted in an expression matrix used for all subsequent analyses.

Pathway activity scores using Gene Set Variation Analysis

We then conducted Gene Set Variation Analysis to characterize biological programs. Gene sets were downloaded from the Molecular Signatures Database (MSigDB) [28,29] for *Homo sapiens*, specifically the Hallmark, the Reactome canonical pathways (C2:CP), and the Gene Ontology biological processes (C5:BP). Pathways were filtered using keywords based on regular-expression patterns designed to specifically focus on immune and inflammatory pathways and epithelial function. Pathway names and

descriptions were filtered, and to ensure the validity of the results, pathways were scored only if they contained at least ≥10 expressed genes. Pathway activity was quantified using Gene Set Variation Analysis (GSVA).[30] This method estimated sample-wise enrichment scores for each pathway. Gene Set Variation Analysis was applied to the gene-level expression matrix using a Gaussian kernel suitable for microarray data. This approach yielded a pathway-sample matrix of GSVA scores. The pathway activity scores indicate the relative activation of each biological pathway across individual samples.

Feature selection and redundancy control

Pathways were ranked by variance across samples, and the top 20% most variable pathways were retained. This step was conducted to reduce noise and to focus on pathways that carry biologically informative variation. We then assessed pathway redundancy by computing pairwise Spearman correlations. A correlation-based distance metric was used to hierarchically cluster pathways, and highly correlated pathways (correlation ≥ 0.70) were grouped. Representative pathways from each correlation cluster were used for subsequent downstream analysis.

Pseudotime construction using non-linear manifold learning

We then aimed to model disease progression as a continuous process, from control samples to CRSsNP and ending with CRSwNP by applying the so-called diffusion maps algorithm. Diffusion maps are a nonlinear manifold-learning technique that captures nonlinear relationships by constructing a graph that represents local similarities between samples and modeling the transition (diffusion) of states across it. This approach allows us to model nonlinear transitions in pathways and observe how key pathways, such as the immune system and tissue remodeling, change as the disease progresses.

Pseudotime was then computed from the diffusion embedding to assign each sample an arbitrary scalar value representing its position along the inferred biological trajectory. This value does not correspond to chronological time but rather to inferred progression along a disease continuum. This approach allowed us to summarize the relative position of each sample along the trajectory inferred from the data. The control group was identified as the starting point for the trajectory construction.

Statistical analysis

To formally test our primary hypothesis that these conditions evolve along a continuum, disease groups were encoded as an ordinal variable (Control=0, CRSsNP UT=1, CRSwNP UT=2, CRSwNP NP=3). We then tested the assumption that, as pseudotime increases, samples are progressively more likely to be classified as CRSwNP NP. This association was evaluated using Spearman's rank correlation, and monotonic trends across the ordered disease groups were further assessed using the Jonckheere–Terpstra test.

To identify pathways associated with disease progression, pathway scores were correlated with pseudotime using Spearman's correlation. False discovery rates were controlled using the Benjamini–Hochberg adjustment. Pathway scores along pseudotime were visualized by plotting their scores against pseudotime. This enables direct interpretation of how the pathways evolve across the inferred continuum of chronic rhinosinusitis.

Ethical Clearance

Ethical approval was not sought for this research, as it involves only the reanalysis of a de-identified publicly available transcriptomic dataset.

RESULTS

This research aims to investigate whether chronic rhinosinusitis represents a disease spectrum and, if so, which biological pathways are involved and how they progress along the continuum. We performed a pathway-based analysis of nasal tissue gene expression profiles from a publicly available microarray dataset (GSE36830). Sample-level pathway activity was quantified using gene set variation analysis (GSVA), and unsupervised, non-linear manifold learning was applied to reconstruct a disease progression trajectory. The trajectory was anchored to control samples to determine whether the evolution of pathway activity followed an ordered manner from Control through CRSsNP toward CRSwNP. Additionally, we also wanted to identify pathway changes along this continuum.

The demographic and clinical characteristics of subjects included in the microarray analysis are summarized in Table 1. The study samples consisted of four groups: Control uncinate tissue (UT), CRSsNP UT, CRSwNP UT, and CRSwNP polyp tissue, with 6 subjects per group.

Table 1. Distribution of subjects characteristics (n=24)

Characteristic	Control UT (n = 6)	CRSsNP UT (n = 6)	CRSwNP UT (n = 6)	CRSwNP Polyp (n = 6)	p value
Gender Male Female	2 (33.3)	2 (33.3)	4 (66.7)	4 (66.7)	0.542
Age (years)	36.0 ± 14.7	47.0 ± 14.7	38.0 ± 12.2	38.0 ± 12.2	0.511
History of Atopy	0 (0.0)	1 (16.6)	1 (16.6)	1 (16.6)	1.000
History of Asthma	0 (0.0)	0 (0.0)	1 (16.6)	1 (16.6)	1.000
Oral steroid	0 (0,0)	2 (33.3)	1 (16.6)	1 (16.6)	0.879
Intranasal steroid	0 (0.0)	0 (0.0)	1 (16.6)	1 (16.6)	1.000
Leukotriene antagonist	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—
Preoperative antibiotic use	0 (0.0)	1 (16.6)	2 (33.3)	2 (33.3)	0.695

Note: data presented as n (%), except age as mean ± SD, p-value is calculated using Fisher's exact test and one-way ANOVA for the age variable; UT = uncinate tissue; CRSsNP = chronic rhinosinusitis sans nasal polyps = CRS without NP

Across groups, the distributions of sex and age were comparable, although CRSwNP samples tended to include a higher proportion of male subjects. Atopic disease and asthma were absent in Control subjects, but were slightly higher in CRSsNP and CRSwNP groups. Use of intranasal corticosteroids was comparable among subjects, whereas use of leukotriene antagonists was uncommon across all groups.

Microarray profiling of nasal tissue yielded expression for 22,880 unique genes after mapping and median collapse. We then summarized the transcription data at the level of pathway activity to reduce the high dimensionality of gene expression data. Pathway activity scores were inferred using predefined gene sets. Gene sets were sourced from multiple collections in the Molecular Signatures Database. In total, 7,652 gene sets were downloaded. To focus on pathways relevant to chronic rhinosinusitis, gene sets were filtered using regular expression patterns that capture immune/inflammatory processes and epithelial barrier or tissue remodeling programs. This filtering reduced the set to 1,395 pathways. Pathways were retained only if they had at least 10 overlapping genes, yielding 975 pathways. We next selected the top 20% most variable pathways, resulting in 195 pathways. Finally, to address redundancy arising from highly correlated pathway activity, pathways were clustered based on autocorrelation, and representative pathways were selected from each cluster, producing a final set of 44 pathways used for downstream analysis.

The 44 representative pathways were used as input to the diffusion map–based manifold learning, which reconstructed a low-dimensional representation of sample-to-sample similarity. This algorithm analyzed the sample without disease-label information. The only information introduced was the control samples, which defined the trajectory's starting point. Despite the absence of disease labels, the inferred pseudotime revealed an ordered trajectory (Figure 1A). Control samples consistently occupied the earliest pseudotime positions, followed by CRSsNP uncinate tissue samples at intermediate positions. CRSwNP samples extended further along the trajectory, with uncinate tissue samples partially overlapping with CRSsNP. These results suggest a gradual transition between these disease states instead of clear-cut differences. However, a pattern emerged for CRSwNP polyp samples that localized to a distinct region at later pseudotime. This indicates a comparatively abrupt divergence in pathway activity patterns associated with polyp tissue. Our results showed that pathway-level information alone is sufficient for unsupervised manifold learning to recover an ordered progression structure, capturing both continuous transitions across disease states and a distinct molecular state associated with nasal polyps.

We then statistically tested whether pseudotime followed an ordered progression across disease states. We initially assessed trends across the ordered disease categories (Control → CRSsNP → CRSwNP (UT) → CRSwNP (NP)) using the Jonckheere–Terpstra test, which evaluates whether increasing pseudotime is associated with worsening disease status. This analysis demonstrated a significant increase in

pseudotime across disease states (JT=183, p<0.001). To further support these findings, disease status was encoded as an ordinal variable and correlated with pseudotime using Spearman's rank correlation, revealing a strong positive association (ρ=0.743, p<0.001). Together, these results provide evidence that pseudotime increases progressively from Control to CRSsNP and further to CRSwNP (UT) and CRSwNP (NP).

We then characterized the biological pathways underlying this trajectory by examining GSVA scores for each pathway along the pseudotime. The top highly correlated pathways with statistical significance (8 positive and 4 negative) are shown in Figure 1B. Several pathways showed strong associations with pseudotime, with activity scores consistently increasing or decreasing as the disease progressed.

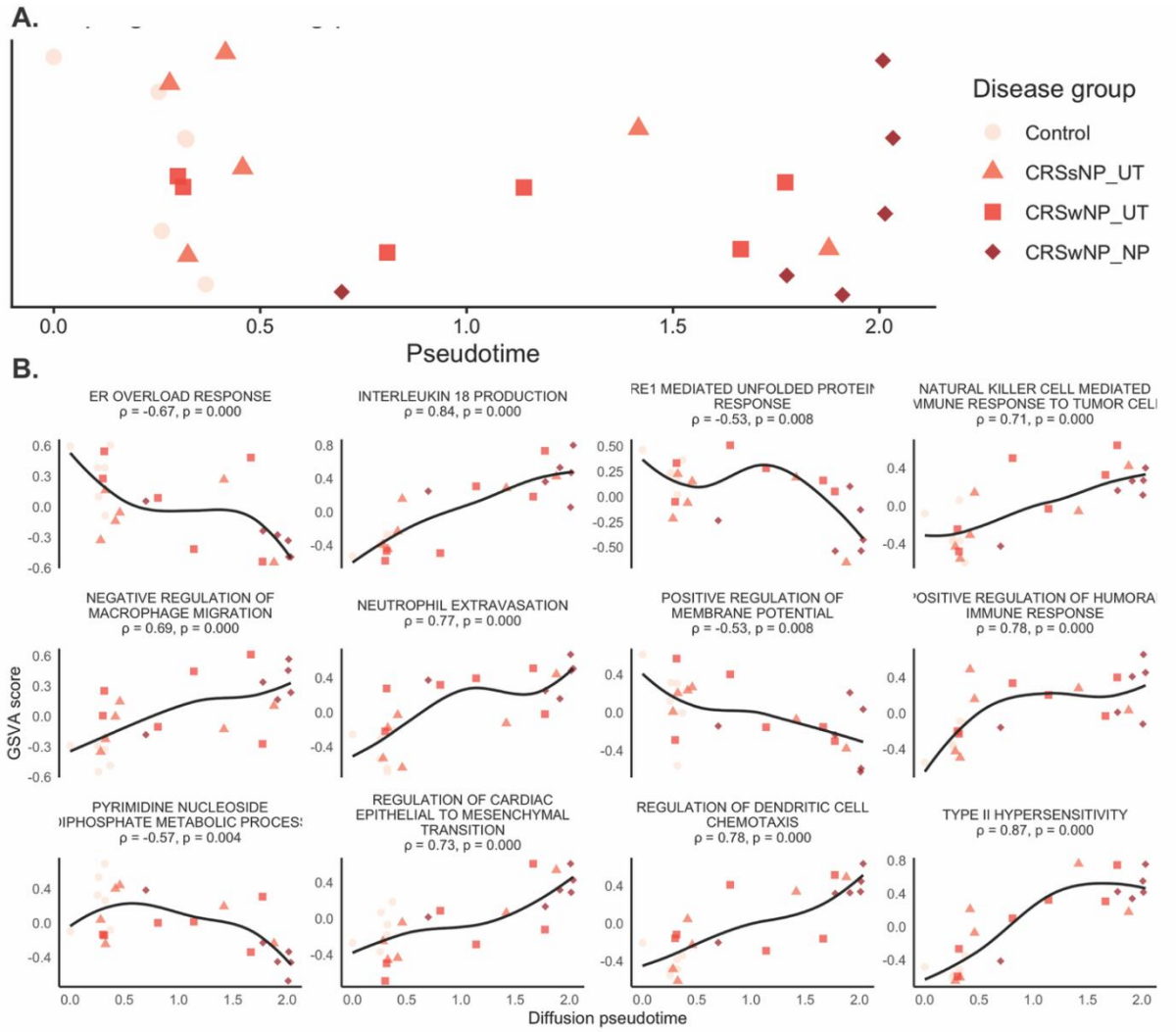

Figure 1. Pseudotime reconstruction using diffusion learning and pathway activity dynamics in chronic rhinosinusitis

A. Unsupervised diffusion pseudotime reconstruction of samples based on pathway activity scores. Each point represents an individual sample, colored and shaped by disease group. Control samples were used only to anchor the trajectory's starting point, while no other disease labels were provided to the algorithm. Pseudotime reconstruction shows control samples clustered at early positions, while CRSsNP and CRSwNP uncinate tissue samples occupy intermediate positions, and CRSwNP polyp samples cluster toward later pseudotime values. B. Several representative pathway activities are plotted along diffusion pseudotime. Scatter plots show pathway activity scores plotted against diffusion pseudotime, with points representing samples and colored by disease groups. Solid black curves indicate trends, with some pathways showing increasing activity along pseudotime, while others show decreasing activity. Spearman correlation coefficients (ρ) and associated p-values for each pathway are shown above individual panels.

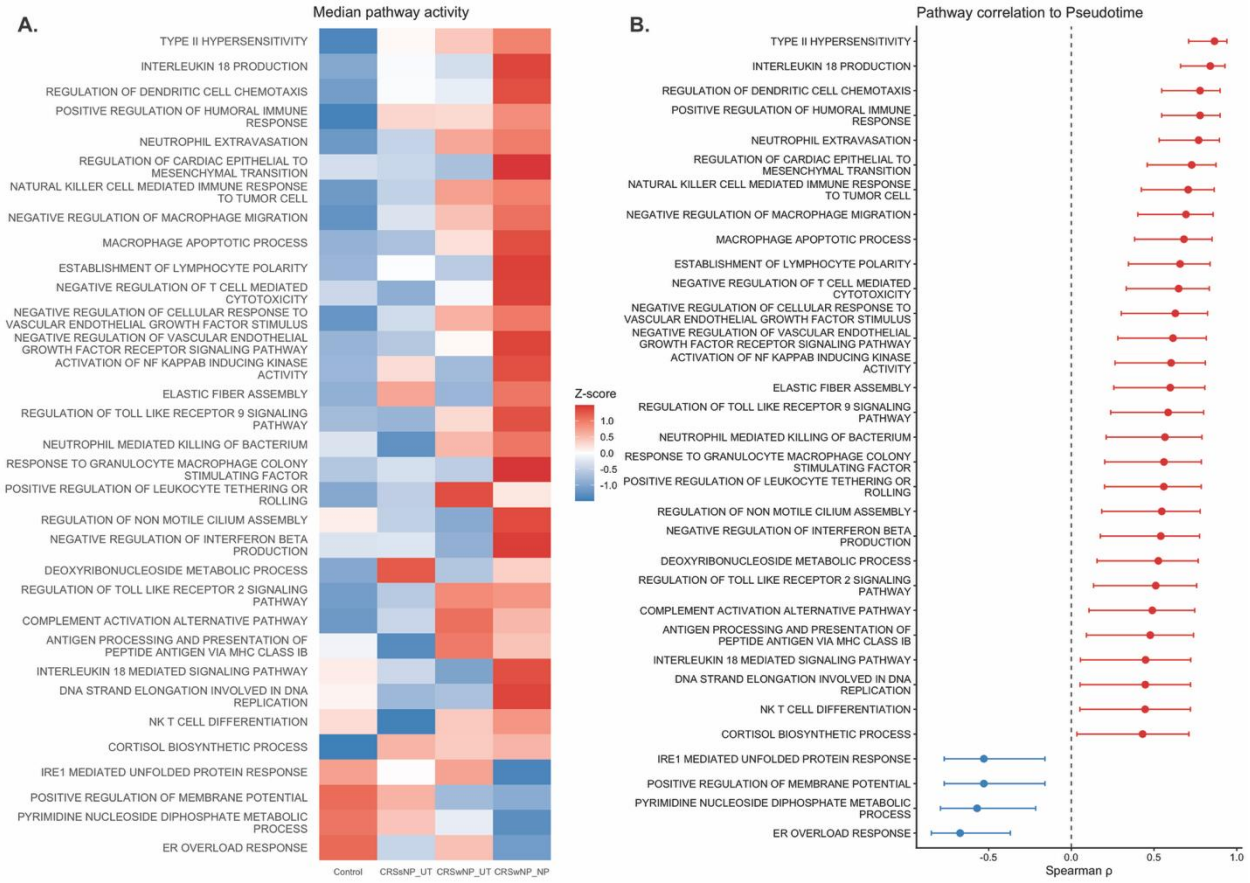

Figure 2. Pathway activity patterns across disease groups and along diffusion pseudotime.
A. Heatmap showing median pathway activity scores across disease groups. Pathways with positive associations with disease progression display a stepwise increase in activity. In contrast, several pathways show higher activity in Control samples with progressive decrease toward later disease states.
B. Forest plot summarizing Spearman correlations between pathway activity scores and pseudotime. Pathways with positive correlations reflect increasing activity along pseudotime, whereas negatively correlated pathways reflect decreasing activity with progression. The direction and magnitude of correlations support the median patterns observed in panel A. Points represent correlation coefficients (ρ), with horizontal bars indicating confidence intervals; the dashed vertical line denotes zero correlation.

These findings reflect gains or losses in pathway activity as samples progressed along the trajectory. For example, pathways related to immune activation and inflammatory responses, including interleukin-18 production, NK-cell mediated immune response, and type II hypersensitivity, showed increasing activity toward later pseudotime values. In contrast, other pathways, such as ER overload response, RE1-mediated unfolded protein response, and pyrimidine nucleoside diphosphate metabolic process, showed decreasing activity as the disease progresses.

We then visualized pathway activity patterns across groups and their correlation with pseudotime (Figure 2A & 2B). Figure 2A shows the median GSVA scores of significant pathways correlated with disease progression (pseudotime). Several pathways showed a stepwise increase in median activity from control to CRSwNP polyp samples, particularly the inflammatory pathways. These were also represented by the positive correlations shown in Figure 2B. Example pathways include type 2 hypersensitivity, with a strong positive correlation and progressively increasing activity that peaks in polyp tissue. In contrast, a subset of pathways showed decreasing median activity throughout disease progression. Pathways such as endoplasmic reticulum overload response, IRE1-mediated unfolded protein response, pyrimidine nucleoside diphosphate metabolic process, and positive regulation of membrane potential showed higher activity in Control samples and progressively lower activity toward CRSwNP, particularly in polyp tissue. This pattern indicates an early dominance of

cellular stress and metabolism-associated programs that diminishes as the disease progresses. Figure 2B further quantifies these trends by showing a Spearman correlation between pathway activity and pseudotime. Pathways with significant positive correlations correspond to late-gain pathways, whose activity increased with disease progression. However, pathways with significant negative correlations correspond to early-loss pathways. The direction and magnitude of these correlations mirrored the group-wise median patterns observed in Figure 2A and provided evidence that pathway activity changes across discrete disease groups are statistically significant and reflect the evolution of their activity as the disease progresses. Taken together, our analyses showed that chronic rhinosinusitis progression is characterized by the evolution of several key pathways, with progressive activation of immune pathways and deactivation of stress- and metabolism-related pathways. The combination of these pathways culminates in a specific pattern of activation associated with nasal polyp tissue.

DISCUSSION

In this study, we showed that chronic rhinosinusitis represents a continuous progression of disease with overlapping molecular activations ending in nasal polyps. Using a diffusion-based pseudotime approach, samples were shown to be organized along a trajectory extending from control tissue through CRSsNP to CRSwNP. The strong association between pseudotime and disease category (Spearman ρ=0.743) provides support for this progression. This finding supports current evidence that CRS is best understood as a heterogeneous and progressive disorder driven by inflammatory pathways.[31] Previous transcriptomic studies have similarly suggested overlap among CRS phenotypes. However, they did not aim to construct a temporal association to explain disease evolution.[24] The strong correlation between pseudotime and disease stage observed in our study showed that, given only transcriptomic data, our approach successfully constructed a temporal model. These approaches provide evidence that the molecular pathway changes are arranged to support a progressive disease model.

The pseudotime trajectory revealed a gradual transition from control tissue through CRSsNP to CRSwNP, with nasal polyp samples forming a distinct terminal cluster. This suggests that CRSsNP may represent an intermediate state. Previous studies have reported overlapping molecular signatures between CRSsNP and CRSwNP but did not try to reconstruct progression.[24,25] Our findings extend these observations by demonstrating that polyp tissue represents a distinct, end-stage molecular state characterized by a unique activation of biological pathways that differs from CRSsNP. This is consistent with clinical observations that CRSwNP exhibits more aggressive inflammation, increased recurrence, and differential response to therapy.[32]

Pathway-level analysis demonstrated progressive activation of immune-related pathways along pseudotime. The result is particularly associated with type 2 inflammation, IL-18 signaling, NK cell-mediated immunity, and hypersensitivity responses. These pathways showed increasing activity from control samples to CRSwNP polyp tissue, consistent with current knowledge of the immunologic profile of advanced CRS. Type 2 inflammation, driven by IL-4, IL-5, and IL-13, was involved in eosinophilic inflammation, tissue edema, and polyp formation. IL-4 and IL-13 can disrupt epithelial tight junctions, increase immunoglobulin E (IgE) production, and stimulate goblet cell proliferation. These changes further promote susceptibility to allergens, immune reactions, and inflammation. IL-5 primarily recruits eosinophils and prolongs eosinophil survival, leading to chronic, persistent inflammation and the release of substances that further damage the mucosa and promote polyp growth. [33] Our findings provide further evidence from previous studies, demonstrating that these immune pathways emerge gradually. This progressive immune activation likely represents one of the main drivers of disease severity.

Pathways related to epithelial stress responses and cellular metabolism showed progressive downregulation along pseudotime, unlike those related to immune activation. Pathways such as endoplasmic reticulum stress, including the IRE1-mediated unfolded protein response and pyrimidine metabolism, were most active in control and early-stage disease but declined as disease progressed toward CRSwNP. These pathways are critical for maintaining epithelial integrity and cellular homeostasis under environmental stress.[34,35] Previous research has shown that epithelial barrier dysfunction and

impaired stress responses contribute to CRS susceptibility and persistence.[36] The observed decline in these protective pathways suggests a loss of epithelial resilience as inflammation becomes dominant, further increasing the nasal mucosa's susceptibility.

Our findings showed that CRS pathogenesis might occur in two stages. In the early stage, epithelial dysfunction and metabolic stress are the main drivers. These changes are potentially caused by environmental exposure or host susceptibility. As the disease further progresses, immune-mediated pathways become the main drivers. This immune activation leads to a state characterized by type 2 inflammation, tissue remodeling, and nasal polyp formation. Our findings also reconcile previous studies that have suggested that CRS is either an epithelial or an immune disorder by integrating both processes into a temporal continuum.[37] Nasal polyps are, in themselves, a separate disease entity caused by sustained inflammatory and remodeling pathways.[38] This research showed that targeting these inflammation pathways might only solve one main driver of nasal polyps in CRS.

Several limitations should be acknowledged. First, this analysis was based on cross-sectional transcriptomic data, which limits the ability to infer the true temporal progression hence it is called a pseudotime reconstruction. Prospective longitudinal sampling is required to validate whether the transitions reflect true disease evolution within individual patients. Second, the use of bulk tissue prevents precise attribution of pathway activity to specific cellular populations. Third, the analysis is restricted to the groups available in GSE36830. The dataset is limited to 24 samples and does not include paired nasal mucosa (the data used paired uncinate tissues) or recurrent-CRSwNP patients, which limits further elaboration and analysis.

Clinically, framing CRS as a molecular continuum rather than as discrete phenotypes carries several implications. Pseudotime position could serve as a quantitative staging biomarker to identify patients progressing toward a polypoid, type 2–high end state who may benefit from earlier endotype-guided or biologic therapy, whereas patients at earlier trajectory positions with preserved epithelial function may be managed with less aggressive, mucosa-directed treatment. A continuum framework may thus support patient stratification and the timing of intervention before irreversible remodeling occurs, as patients with nasal polyps have prolonged immune system activation and may be more likely to exhibit refractory disease and to benefit from biologic therapies targeting type 2 inflammation. This aligns with clinical trials demonstrating improved outcomes with biologic agents such as mepolizumab, omalizumab,[39] and dupilumab.[17] in CRSwNP. However, patients with early-stage disease characterized by preserved epithelial function may not benefit as much from aggressive immune therapy. Nevertheless, they represent a window for intervention before irreversible remodeling occurs.[40] These findings support a precision medicine approach in approaching CRS.

Future studies should integrate longitudinal sampling with single-cell or spatial transcriptomic profiling to better resolve cell-type-specific dynamics along the CRS continuum. Validation in independent cohorts and correlation with clinical outcomes, including treatment response and disease recurrence, will be essential to establish the clinical utility of these findings.

CONCLUSION

This study demonstrates that chronic rhinosinusitis represents a continuous disease spectrum. By integrating pathway-level analysis with diffusion-based pseudotime modeling, we show that CRS follows an ordered biological trajectory from healthy mucosa to CRSsNP and then to CRSwNP. The changes are characterized by a progressive shift from epithelial stress and metabolic dysregulation toward immune and inflammatory signaling. The emergence of a distinct molecular state in nasal polyp tissue supports the concept that CRSwNP represents rather a terminal disease phenotype. These findings provide a mechanistic framework that merges epithelial dysfunction and immune activation as components of CRS progression. Importantly, this finding suggests that advanced disease may benefit most from targeted biologic therapies. Together, these results support a precision medicine approach to CRS and provide a foundation for future studies linking its pathophysiology and progression to guide individualized treatment strategies.

Conflict of Interest

The authors declare no conflict of interest.

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Data Availability Statement

The datasets analyzed in the current study are publicly available in the Gene Expression Omnibus (GEO) repository under accession GSE36830. All data used in this study can be accessed without restriction at: https://www.ncbi.nlm.nih.gov/geo/. All computational analyses were performed using publicly available tools and packages, and the analytic workflow can be reproduced based on the methods described in this manuscript.

Authors' Contributions

EE conceptualized and designed the study, curated the data, supervised and wrote the manuscript, including drafting and critical revision. RS and RM contributed to methodological development, data interpretation, validation, and manuscript review and editing. AS contributed to the implementation of the methodology, formal statistical analysis, data visualization, and manuscript writing, including drafting and revision. All authors reviewed and approved the final manuscript.

Declaration of AI Usage in Scientific Writing

Artificial intelligence tools (ChatGPT v5.2; Grammarly v1.150.0.0) were used during the preparation of this manuscript to support language refinement, correct grammatical errors, and improve clarity of expression. No scientific content was generated with the use of artificial intelligence tools. The authors reviewed, edited, and verified all content and take full responsibility for the accuracy, originality, and integrity of the final manuscript.

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