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Anatomical Sampling Site Is Not Associated with TAS2R38 Gene Expression in Chronic Rhinosinusitis: A Cross-Sectional RT-qPCR Study

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Abstract

Background

Chronic rhinosinusitis (CRS) is a very common and complex inflammatory condition affecting the sinonasal mucosa. The bitter taste receptor T2R38, which is encoded by the TAS2R38 gene, is found on ciliated epithelial cells. These cells use the receptor to detect quorum-sensing molecules (acyl-homoserine lactones) from Gram-negative bacteria, which then triggers nitric oxide (NO) production to help speed up mucociliary clearance. However, we don't yet know if TAS2R38 transcript levels change depending on which part of the sinonasal area is sampled. This uncertainty makes it hard to decide on the best biopsy protocols for molecular airway research.

Objective

We wanted to see if mucosal TAS2R38 gene expression differs across various sinonasal locations in CRS patients undergoing endoscopic sinus surgery (ESS). We also aimed to figure out whether the specific sampling site or individual biological differences play a bigger role in these variations.

Methods

In this prospective cross-sectional molecular study, we collected 337 mucosal specimens from up to six different anatomical sites (the inferior turbinate, maxillary, ethmoid, frontal, and sphenoid sinuses, plus nasal polyps) in 67 CRS patients (following EPOS 2020 criteria). We also collected 29 non-inflammatory control tissues. We measured transcript levels using reverse transcription quantitative PCR (RT-qPCR), using GAPDH as an internal reference. We analyzed paired ΔCq values using linear mixed-effects models with patient-specific random intercepts, along with sensitivity analyses and relative expression calculations (2^-ΔΔCq).

Results

After strict quality control, we analyzed 253 valid mucosal specimens from 55 CRS patients (inferior turbinate: n = 52; maxillary: n = 50; ethmoid: n = 49; polyps: n = 44; frontal: n = 43; sphenoid: n = 15). The mean ΔCq stayed within a narrow range of 2.06 to 2.81 across all sites. One-way ANOVA showed no significant differences (F = 0.42, P = 0.834). Our linear mixed-effects model also showed that the anatomical sampling site wasn't linked to TAS2R38 expression ($\chi^2(5) = 3.58$, P = 0.611). When we looked at the variance, inter-individual differences (1.64; ICC = 0.333) were much higher than the variation caused by the anatomical site. These results held up across six sensitivity analyses and even after adjusting for age and sex (P = 0.617). We found that relative TAS2R38 expression in diseased mucosa was 2.48 times higher than in the controls.

Conclusion

TAS2R38 mRNA is expressed uniformly and widely throughout all major sinonasal areas and polyp tissues in CRS. The anatomical site doesn't really act as a confounding factor when quantifying mucosal genes. This means that sampling more accessible tissues, like the inferior turbinate, can provide a representative look at the molecular aspects of sinonasal chemosensory innate defense.

1. INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory diseases of the upper respiratory tract, affecting between 5% and 12% of adults globally [1, 2]. It is defined by key symptoms like persistent nasal blockage, thick nasal discharge, facial pressure or pain, and loss of smell lasting at least 12 weeks. CRS can seriously impact a person's quality of life and puts a huge economic strain on healthcare systems [1, 3, 4]. From a physiological standpoint, CRS is a complex syndrome caused by things like epithelial barrier issues, poor mucociliary clearance, microbial buildup, biofilms, and abnormal local immune responses [1, 5, 6].

Over the last ten years, our understanding has shifted. We now know that the sinonasal respiratory epithelium isn't just a passive barrier; it actually acts as a sentinel organ that manages local innate defense [6, 7]. A key part of this sensory surveillance involves bitter taste receptors (T2Rs), which are a family of G-protein coupled receptors first identified in the taste cells of the tongue [7, 8]. Out of the 25 known human T2R isoforms, T2R38 (encoded by the TAS2R38 gene) has become recognized as a primary pattern recognition receptor (PRR) on moving ciliated epithelial cells [7, 9]. T2R38 specifically picks up acyl-homoserine lactones (AHLs), which are signaling molecules used by Gram-negative pathogens like Pseudomonas aeruginosa [7, 9, 10]. When the receptor is activated, it triggers calcium (Ca^{2+}) movement inside the cell, which stimulates nitric oxide synthase (NOS) to produce nitric oxide (NO). This NO helps speed up the beating of cilia to clear mucus more effectively, and it also diffuses into the airway to directly attack bacterial membranes and DNA [7, 8, 9].

Genetic variations in TAS2R38 (like the functional PAV and non-functional AVI types) and changes in receptor transcript levels have been linked to how susceptible people are to sinonasal infections, the severity of their symptoms, and how well they respond to surgery [9, 10, 11, 12]. However, there is still a big question we haven't answered: does TAS2R38 expression change depending on which part of the sinonasal area you look at (like the turbinates, sinuses, or polyps)? Because different areas have different airflow, different amounts of pathogens, and different tissue structures, regional differences could lead to sampling bias in molecular rhinology studies [12, 13]. So, this study aimed to measure mucosal TAS2R38 mRNA expression across six different anatomical locations in surgical CRS patients. We also wanted to see if the location or the individual patient's biology had a bigger impact using linear mixed-effects modeling.

2. MATERIALS AND METHODS

2.1. Study Design and Ethical Approval

This cross-sectional molecular study took place at the Center of Otorhinolaryngology and Cochlear Implant Surgery, Hanoi Medical University Hospital, Vietnam, from April 2025 to June 2026. The protocol was reviewed and approved by the Institutional Review Board of Hanoi Medical University (Approval No. 1671/GCN-HMUIRB). All participants gave written informed consent before joining the study.

2.2. Patient Selection and Clinical Phenotyping

We prospectively enrolled adult patients (18 years or older) who were diagnosed with CRS based on EPOS 2020 criteria and were scheduled for functional endoscopic sinus surgery (ESS) [1]. We excluded anyone with an acute upper airway flare-up, sinonasal cancers, previous radiation therapy, cystic fibrosis, or severe systemic immune deficiencies. We carefully recorded baseline clinical details, PTC taste sensitivity, SNOT-22 scores, and preoperative Lund-Mackay CT scores [1, 3]. Additionally, we collected 29 non-inflammatory tissue samples (mostly from nasal septal reconstructive surgeries) to serve as a baseline for non-CRS cases.

2.3. Intraoperative Tissue Sampling

During the ESS procedures, we took mucosal biopsies from up to six different sinonasal sites per patient: (1) inferior turbinate, (2) maxillary sinus, (3) ethmoid sinus, (4) frontal sinus, (5) sphenoid sinus, and (6) nasal polyp tissue if it was present. We initially collected 337 surgical specimens, which were immediately snap-frozen and stored at -80°C.

2.4. RNA Extraction, cDNA Synthesis, and RT-qPCR Quantification

We isolated total RNA using the easy-spin™ Total RNA Extraction Kit (DNA-free; iNtRON Biotechnology) following the manufacturer's instructions. We checked the RNA concentration and purity (A260/A280 ratio between 1.8 and 2.1) using a NanoDrop™ spectrophotometer. We then synthesized complementary DNA (cDNA) using the Maxime™ RT PreMix Kit. Quantitative real-time PCR was done using RealMOD™ Green W2 2× qPCR Mix on an automated thermal cycler. We used Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the steady internal control [12]. We measured target transcript abundance by determining the quantification cycle (Cq). Relative expression was calculated as $\Delta Cq = Cq(TAS2R38) - Cq(GAPDH)$, where a lower ΔCq means there is more TAS2R38 mRNA. We estimated fold differences compared to the non-CRS control using the 2^-ΔΔCq method.

2.5. Quality Control and Statistical Modeling

We followed strict quality control (QC) rules: any samples that didn't show valid amplification ($Cq > 35$ or no amplification curve) for either TAS2R38 or GAPDH were left out. To account for the fact that we took multiple biopsies from the same person, we used a linear mixed-effects model: $\Delta Cq_{ij} = \beta 0 + \beta k(\text{Anatomical Site}_{ij}) + u0i + \varepsilon_{ij}$, where $u0i$ represents the patient-specific random intercept and ε_{ij} is the residual error. We used a likelihood-ratio test to check the overall significance of the anatomical site.

We also calculated the intraclass correlation coefficient (ICC) as ICC = $\sigma_u^2 / (\sigma_u^2 + \sigma_e^2)$. We checked the model using residual-versus-fitted and normal Q-Q plots. We also ran sensitivity analyses and adjusted for age and sex. All analyses were done in R (v4.3) and SPSS (v26.0), with statistical significance set at $P < 0.05$.

3. RESULTS

3.1. Clinical Characteristics and Specimen Flow

Out of the 67 patients enrolled, 55 met all the QC requirements with both target and reference measurements, giving us 253 valid tissue specimens (84 specimens were excluded during QC because they failed to amplify). The demographic and disease severity data were similar between the full group and the group used for analysis (Table 1). The mean preoperative Lund-Mackay CT score was 15.1 ± 4.9 and the baseline SNOT-22 was 41 ± 14, which shows moderate-to-severe mucosal inflammation. SNOT-22 scores improved significantly to 20 ± 9 one month after surgery.

Table 1
Demographic and baseline clinical characteristics of the study cohort

Characteristic	All Enrolled Patients (n = 67)	Analytical Cohort (n = 55)
Age (years), mean ± SD (range)	44.5 ± 13.0 (18–74)	44.7 ± 13.3 (18–74)
Sex: Male / Female, n (%)	37 (55.2%) / 30 (44.8%)	31 (56.4%) / 24 (43.6%)
PTC Taste Test: Non-taster (0) / Taster (1), n (%)	44 (66.0%) / 23 (34.0%)	36 (65.5%) / 19 (34.5%)
Preoperative Lund-Mackay CT Score, mean ± SD	15.1 ± 4.9 (3.0–24.0)	15.3 ± 4.8 (4.0–24.0)
Preoperative SNOT-22 Score, mean ± SD	41 ± 14 (17–76)	42 ± 13 (18–76)
Postoperative 1-Month SNOT-22 Score, mean ± SD	20 ± 9 (7–40)	19 ± 8 (7–38)

3.2. Uniform TAS2R38 Gene Expression Across Anatomical Sites

We looked at 253 valid CRS specimens taken from six different anatomical sites: inferior turbinate (n = 52), maxillary sinus (n = 50), ethmoid sinus (n = 49), nasal polyps (n = 44), frontal sinus (n = 43), and sphenoid sinus (n = 15). When we checked the summary statistics for ΔCq values, they were remarkably consistent across all the different sub-sites (Table 2).

Table 2
Summary of TAS2R38 expression (ΔCq) across sinonasal anatomical sampling sites

Anatomical Sampling Site	Valid Specimens (n)	Mean ΔCq	SD	Median ΔCq	IQR
Inferior Turbinate (Reference)	52	2.48	2.03	2.38	2.52
Maxillary Sinus	50	2.06	2.15	1.79	2.91
Ethmoid Sinus	49	2.36	2.11	2.50	2.90
Nasal Polyp	44	2.13	2.78	2.49	3.45
Frontal Sinus	43	2.16	2.13	1.59	2.79
Sphenoid Sinus	15	2.81	2.15	2.95	2.27

One-way ANOVA test: F = 0.42, P = 0.8344, indicating no statistically significant difference among anatomical sites.

3.3. Linear Mixed-Effects Modeling and Variance Decomposition

In our primary linear mixed-effects model, which accounted for intra-subject clustering, the anatomical location didn't significantly affect TAS2R38 expression (likelihood-ratio test, $\chi2(5) = 3.58$, P = 0.611). We also ran pairwise comparisons using the inferior turbinate as a baseline, and found no significant differences in any sinus subsite or polyp tissue (Table 3).

Table 3
Linear mixed-effects model evaluating the association between anatomical sampling site and TAS2R38 ΔCq

Comparison vs. Inferior Turbinate	Coefficient β (ΔCq)	95% Confidence Interval	Standard Error	P Value
Maxillary Sinus	−0.383	−1.091 to 0.325	0.361	0.289
Ethmoid Sinus	−0.065	−0.778 to 0.648	0.364	0.858
Nasal Polyp	−0.395	−1.130 to 0.340	0.375	0.293
Frontal Sinus	−0.344	−1.085 to 0.396	0.378	0.362
Sphenoid Sinus	0.373	−0.712 to 1.457	0.553	0.501

Global anatomical site effect: Likelihood-ratio $\chi^2(5) = 3.58$, P = 0.611. Estimated between-patient variance $\sigma_{u}^2 = 1.64$; residual variance $\sigma_{e}^2 = 3.29$; ICC = 0.333.

When we broke down the variance, we found that inter-individual variance ($\sigma_{u2} = 1.64$) made up a large part of the overall variance (ICC = 0.333). This suggests that about one-third of the total transcript

variability comes from biological differences between patients, while the differences within a single patient across different anatomical regions are actually quite small.

3.4. Sensitivity Analyses, Model Diagnostics, and Adjusted Models

To make sure our results were solid, we tested six different sensitivity models (Table 4). In every single scenario, the global anatomical site effect wasn't significant ($P > 0.50$). Even when we used a multivariable mixed-effects model adjusted for age and sex, the anatomical location still didn't matter ($\chi2(5) = 3.54$, $P = 0.617$), and neither age ($P = 0.613$) nor sex ($P = 0.527$) had any independent effect.

Table 4
Prespecified sensitivity analyses assessing model robustness

Analytical Scenario	Included Specimens (n)	Global P Value (Likelihood-Ratio Test)
1. Primary analytical model (Full dataset)	253	0.611
2. Exclusion of nasal polyp specimens	209	0.540
3. Exclusion of sphenoid sinus specimens	238	0.715
4. Restriction to patients with ≥ 4 valid specimens	230	0.581
5. Patient-level averaged anatomical site data	253	0.611
6. Exclusion of extreme residuals (|residual| > 3)	251	0.771

3.5. Relative Expression Compared with Healthy Controls

In the non-CRS control mucosa ($n = 29$), the median ΔCq was 2.62 (IQR: 1.80 to 4.04). Using this as a baseline, we found that TAS2R38 mRNA in diseased CRS mucosa was, on average, 2.48 times higher than in the non-inflammatory control mucosa.

4. DISCUSSION

To the best of our knowledge, this is one of the most thorough cross-sectional studies to date that systematically measures TAS2R38 gene expression across various sinonasal sites using mixed-effects modeling [12]. Our main finding is that mucosal TAS2R38 transcript levels don't differ significantly whether they come from the inferior turbinate, the maxillary, ethmoid, frontal, or sphenoid sinuses, or nasal polyps ($P = 0.611$).

Looking at it from a mucosal biology standpoint, our results support the idea that T2R38 is a widespread innate immune chemosensor found throughout the entire upper respiratory epithelium [7, 8, 9]. As Takemoto et al. (2023) and Jeruzal-Swiatecka et al. (2022) have shown, T2R38 expression is mostly found on the ciliated apical membranes [12, 13]. In cases of severe CRS that need surgery (which usually involves high CT opacification and widespread mucosal remodeling), inflammatory signals and pathogens seem to affect the whole mucosa rather than just staying in specific spots [1, 6]. Because of this, the epithelial transcription of these chemosensory tools stays uniform across different anatomical areas.

Importantly, our variance decomposition analysis showed that the differences between individuals (ICC = 0.333) are much larger than the differences between anatomical sites within the same person. This tells us that a person's own biology is what really drives TAS2R38 expression levels. These factors probably include things like genetic variations (like PAV/AVI haplotype status), the type of inflammation (eosinophilic Type 2 vs. non-Type 2), whether they have asthma, and the makeup of their sinonasal microbiome [9, 10, 12, 13]. In fact, previous studies have shown that CRS patients with asthma or different endotypes have different TAS2R38 transcription levels in their polyp tissues [12, 13].

The 2.48-fold increase in TAS2R38 mRNA we saw in CRS patients compared to controls might be happening for two different, but not necessarily mutually exclusive, reasons [12]:

1. Compensatory upregulation: When there is chronic microbial growth or biofilm stress, respiratory epithelial cells might increase TAS2R38 transcription to become more sensitive to quorum-sensing. This would boost NO production to help fight off pathogens and speed up mucociliary transit [7, 9, 12].

2. Functional uncoupling: Just because there is a lot of mRNA doesn't mean there are actually functional receptor proteins on the surface [12, 13]. In people who are non-tasters (AVI/AVI homozygotes) or in mucosa that has been heavily remodeled with goblet cell metaplasia and loss of cilia, the high mRNA levels might just be a failed attempt by the body to compensate for a broken NO defense system [9, 10, 12].

Clinical and Methodological Implications

Our findings offer some important reassurance for airway research. Since TAS2R38 expression is stable across different sinonasal locations, researchers and clinicians can reliably use easy-to-reach mucosal biopsies, like the inferior turbinate or anteriorethmoid, as a stand-in for the whole paranasal sinus system. This means future trials can avoid the need for more invasive biopsies in delicate sinus areas without losing the quality of their molecular data.

Study Strengths and Limitations

The strengths of this study include our large cohort of 253 valid specimens from 55 subjects, our strict quality control, and the use of rigorous linear mixed-effects modeling. However, there are some limitations: (1) we measured mRNA transcripts via RT-qPCR but didn't perform protein quantification or

functional tests like fractional exhaled NO or ciliary beat frequency [12, 13]; (2) we didn't include TAS2R38 genotyping (PAV/AVI sequencing) in this part of the study; and (3) most of our control tissues came from the nasal septum rather than from matched sites in multiple sinuses [12].

5. CONCLUSIONS AND RECOMMENDATIONS

To wrap things up, TAS2R38 mRNA is expressed uniformly across the major paranasal sinuses, inferior turbinates, and nasal polyps in CRS patients undergoing surgery. Where you take the sample doesn't significantly change the results. Instead, transcript levels are mostly driven by the patient's own biology and are about 2.5 times higher than in non-inflammatory controls. Because of this, inferior turbinate biopsies can serve as a reliable and easy way to represent TAS2R38 expression in the sinonasal area. Future studies should look into how genotype relates to phenotype, where the protein is actually located, and how these levels change over time clinically.

Declarations

Ethics approval and consent to participate: Approved by the Institutional Review Board of Hanoi Medical University (Approval No. 1671/GCN-HMUIRB). All participants gave written informed consent.

Consent for publication: Not applicable.

Availability of data and materials: Datasets analyzed during this study are available from the corresponding author upon reasonable academic request.

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