Single Nucleotide Polymorphisms in Chemosensory Pathway Genes GNB3, TAS2R19, and TAS2R38 Are Associated with Chronic Rhinosinusitis

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Keywords

Bitterant signaling · Chronic rhinosinusitis · Genetics · Innate immune response · Single nucleotide polymorphism

Abstract

Background: Chronic rhinosinusitis (CRS) is a multifaceted disease with a significant genetic component. The importance of taste receptor signaling has recently been highlighted in CRS; single nucleotide polymorphisms (SNPs) of bitter tastant-responsive G-protein-coupled receptors have been linked with CRS and with altered innate immune responses to multiple bacterially derived signals. **Objective:** To determine in CRS the frequency of six SNPs in genes with known bitter tastant signaling function. **Methods:** Genomic DNA was isolated from 74 CRS volunteers in West Virginia, and allele frequency was determined and compared with demographically matched data from the 1,000 Genomes database. **Results:** For two SNPs in a gene recently associated with bitterant signaling regulation, RGS21, there were no associations with CRS (although the frequency of the minor allele of RGS21, rs7528947, was seen to increase with increasing Lund-Mackay CT staging score). Two TAS2R bitter

taste receptor gene variants (TAS2R19 rs10772420 and TAS2R38 rs713598), identified in prior CRS genetics studies, were found to have similar associations in this study. **Conclusion:** Unique to our study is the establishment of an association between CRS in this patient population and GNB3 SNP rs5443, a variation in an established G protein component downstream of bitterant receptor signal transduction.

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Introduction

Chronic rhinosinusitis (CRS) is a persistent inflammatory state of the sinus mucosa with symptoms of nasal obstruction or discharge with or without facial pain and changes in smell present for more than 12 weeks [1]. Prevalence estimates range from 10 to 12% of the population in the USA and Europe [2], resulting in health care costs greater than USD 770 million in the USA alone [3]. CRS patients have poor quality of life scores, similar to

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other chronic diseases, including CHF and COPD [4]. Evidence suggests a genetic component to CRS [5]. In a large population study, there was a 2.4-fold increased risk of CRS in first-degree relatives of patients [6]; this number is higher for first-degree relatives of patients with nasal polyps. Furthermore, several well-characterized genetic syndromes are associated with CRS, including cystic fibrosis [7] and primary ciliary dyskinesia [8].

An emerging concept regarding the chronic nature of CRS relates to a potential deficit in sensing and clearing bacterial infections; receptors for bitter and sweet taste have been found in the airway and are thought to play a role in sensing bacteria and regulating innate immune responses [9]. Bitter taste is initiated in the oral cavity through members of the T2R family of G protein-coupled receptors (GPCRs) [10–12]. However, T2R bitter receptors are not limited to taste bud cells; they are also found in solitary chemosensory cells and ciliated epithelial cells of the nasal and sinus cavities, functioning in these locales to “taste” secreted bacterial products, such as acyl-homoserine lactones [13]. Gram-negative bacteria, such as *Pseudomonas aeruginosa*, use acyl-homoserine lactones as quorum-sensing molecules [14]. Binding of these bacterial products to T2Rs is thought to modulate innate host immune responses, such as release of antimicrobial peptides and nitric oxide.

Genome-wide association studies (GWAS) have identified several genes potentially associated with CRS [15, 16]. Recent genetic studies have underscored the importance of taste receptor signaling in innate immunity of the upper and lower airways, and paranasal sinuses [13]; single nucleotide polymorphisms (SNPs) in taste receptor genes have since been associated with altered bacterial immune response and, thus, with CRS [17, 18]. There are ~25 type 2 taste receptors (TAS2R “bitterant receptors”), coupled to G protein signaling, that are expressed in multiple tissues. In the ciliated mucosa of the sinuses, these TAS2Rs respond to chemoirritants and bacterially produced secretions [19]. Some of us also recently showed that an inhibitor of GPCR signaling, regulator of G-protein signaling-21 (RGS21), opposes TAS2R-mediated bitterant signaling in immortalized airway epithelial cells and, given its expression in sinus mucosa and airway epithelia, may be involved in mucociliary clearance [20, 21]. Here, investigating candidate genes involved in bitterant signaling from among the *TAS2R* genes and *RGS21*, we identified three SNPs more common in West Virginia CRS patients than in healthy controls of European descent, including an SNP within *GNB3*, a downstream component of bitterant signaling previously unassociated with CRS.

Table 1. Patient demographics (total *n* = 74 patients)

Mean age, years	55.0
Male	38 (52)
Female	36 (48)
Allergy	42 (57)
Asthma	28 (38)
Migraine	8 (11)
Current smoker	9 (11)
History of smoking	19 (26)
Polyps	45 (61)
Previous surgery	29 (39)
Lund-Mackay score (<i>n</i> = 66 patients)	
1–8	22 (33)
9–16	29 (44)
17–24	15 (23)
Average score	11.6 [10.0–13.3]
SNOT22	
Total (0–110)	49.0 [43.5–54.6]
Rhinologic symptoms (0–30)	13.0 [11.4–14.5]
Extranasal rhinologic symptoms (0–15)	7.0 [6.1–8.0]
Ear/facial symptoms (0–25)	9.7 [8.4–11.1]
Psychological dysfunction (0–35)	17.5 [15.1–19.8]
Sleep dysfunction (0–25)	12.4 [10.8–14.0]

Data are presented as *n* (%) or average score [CI: 5–95%]. In SNOT22, the score range is indicated in parentheses.

Materials and Methods

Recruitment

Seventy-four volunteers were seen at an academic tertiary referral center in West Virginia. The study was approved by the Institutional Review Board of West Virginia University (WVU) Office of Research Integrity and Compliance. All volunteers met EPOS criteria [22] for CRS, and informed consent was obtained under IRB 1410476782. Age, sex, allergy, asthma, migraine, smoking status, presence of polyps, and previous surgical history were recorded (Table 1). CT scans of the sinuses were available for 66 of the 74 patients. Lund-Mackay CT score was calculated based on the standard radiologic staging of CRS for these patients [23]. Sino-nasal Outcome Test-22 (SNOT22) scores were obtained from all patients; total SNOT22 and the five standard quality of life domain scores were calculated (Table 1) [24]. Patients with cystic fibrosis, primary ciliary dyskinesia, known immunodeficiency, and craniofacial abnormalities were excluded from the study population.

Tissue Collection and DNA Extraction

Volunteers provided two buccal swabs, which were each placed in a 15-mL polystyrene conical centrifuge tube and stored at –80°C until DNA extraction using QIAamp DNA Mini Kits (exactly as per the manufacturer’s protocol). Purity and yield were assessed by 260/280 nm absorbance on a QIAxport microfluidic spectrophotometer.

Table 2. Gene variant minor allele frequency (MAF) data obtained from West Virginia CRS clinic patients ($n = 74$) and public databases with demographically matched cohorts

Gene	dbSNP ID	Minor allele	TaqMan probe	European MAF*	MAF in CRS probands [‡]
<i>TAS2R38</i>	rs713598	C	C_8876467_10	0.423	0.568 $p < 0.001$
<i>GNB3</i>	rs5443	T	C_2184734_10	0.367 (HapMap [#])	0.493 $p = 0.014$
<i>TAS2R19</i>	rs10772420	A	C_1317426_10	0.505	0.601 $p = 0.024$
<i>TAS2R20</i>	rs12226920	T	C_1326611_10	0.383	0.458 $p = 0.062$
<i>RGS21</i>	rs7528947	G	C_30007846_20	0.516	0.547 $p = 0.461$
<i>RGS21</i>	rs1175152	A	C_68684_10	0.433	0.439 $p = 0.875$

* Minor allele frequency reported on $n = 1,006$ Europeans via 1,000 Genomes ([#] except as otherwise noted: i.e., 116 Europeans from HapMap); MAF comparisons between West Virginia probands and European SNP databases were previously established in Kaski et al. [44] (2019). [‡] p value denotes significance of difference between MAFs derived by comparison between CRS probands and European MAF using binomial test (null hypothesis is “no difference between CRS probands and Europeans”; alternative is “there is difference between CRS probands and Europeans.”)

Fig. 1. Sample real-time quantitative polymerase chain reaction (qPCR) results for the detection of the absence or presence of RGS21 SNP rs7528947 (minor allele G) within West Virginia CRS patient genomic DNA using TaqMan primer-probe set C_30007846_20. Examples of probands with the minor allele (closed symbols) and lacking the minor allele (open triangles and open diamonds) are shown; a separate qPCR reaction lacking input genomic DNA is also illustrated (open circles).

Genotyping

Genotyping was performed using TaqMan primer-probe sets (Table 2) and Type-it Fast SNP Probe PCR Kits (Qiagen) exactly as per the manufacturer's protocols. Reactions were run on a Qiagen Rotor-Gene Q in duplicate; no-DNA negative control reactions were also performed for each primer-probe set (representative data obtained are displayed in Fig. 1).

Statistical Analyses

Allele frequencies were compared with 1,006 Europeans (1,000 Genomes Project) [25], given that West Virginia is demographically homogenous [26], i.e., 94% White European ancestry (<http://censusviewer.com/state/WV>). Given no information for the frequency of the *GNB3* SNP rs5443 in 1,000 Genomes, its allele frequency was instead compared with 116 Europeans (CEU) from HapMap [27]. Statistical analyses of SNP frequencies were performed using Pearson's χ^2 . A p value < 0.05 was used to infer that the allele frequency from CRS patients is significantly different from demographically matched, public genome data. Subgroup analyses were performed comparing the SNP minor allele frequency (MAF) in patients with (CRS_{swNP}) or without (CRS_{snNP}) nasal polyps, and separately based on Lund-Mackay CT score, using standard χ^2 statistical tests (Tables 3, 4).

Results

Given our recent findings that a negative regulator of bitterant GPCR signaling, RGS21, is expressed in sinus mucosa and airway epithelia [20, 21], we interrogated the status within a CRS patient population of two SNPs within *RGS21*, as well as four SNPs within other bitterant signaling genes with established or unknown association with CRS (i.e., *TAS2R19*, *TAS2R20*, *TAS2R38*, and *GNB3*).

Table 3. Gene variant minor allele frequency (MAF) data from CRS patients with and without nasal polyps ($n = 74$).

Gene	dbSNP ID	Minor allele	CRS wNP MAF	CRS sNP MAF	p value
TAS2R38	rs713598	C	0.567	0.569	0.978
GNB3	rs5443	T	0.522	0.448	0.380
TAS2R19	rs10772420	A	0.589	0.621	0.700
TAS2R20	rs12226920	T	0.456	0.466	0.906
RGS21	rs7528947	G	0.567	0.517	0.555
RGS21	rs1175152	A	0.389	0.517	0.125

Table 4. Gene variant minor allele frequency (MAF) data from CRS based on Lund-Mackay (LM) CT score ($n = 66$).

Gene	dbSNP ID	Minor allele	LM 1–8 MAF	LM 9–16 MAF	LM 17–24 MAF	p value
TAS2R38	rs713598	C	0.614	0.500	0.600	0.459
GNB3	rs5443	T	0.568	0.517	0.467	0.688
TAS2R19	rs10772420	A	0.591	0.603	0.600	0.992
TAS2R20	rs12226920	T	0.523	0.362	0.533	0.167
RGS21	rs7528947	G	0.386	0.603	0.700	0.017
RGS21	rs1175152	A	0.500	0.431	0.333	0.364

All chosen SNPs had an MAF greater than 30% so they would be identifiable in this patient population. Buccal swabs from CRS patients (Table 1) were used for genomic DNA isolation and genotyping.

TAS2R19 rs10772420 (Type 2 Taste Receptor 19)

TAS2R19 (previously known as TAS2R48) is a GPCR with bitterant taste receptor activity located on chromosome 12. SNP rs10772420 within *TAS2R19* was previously identified as being associated with RSV infections in a GWAS [28] and also identified in a GWAS of Canadian patients with sinusitis [15]. The minor allele (A) causes a missense mutation (arginine 299 to cysteine) in the encoded protein and is associated with intense quinine perception [29]. The “minor” allele is actually more prominent in Europeans (MAF 0.505; Table 2). In the CRS patients in this study, this allele is even more common (MAF 0.601; $p = 0.024$); 23% of the CRS patients were homozygous (A/A) for the minor allele.

TAS2R38 rs713598 (Type 2 Taste Receptor 38)

TAS2R38, a bitterant receptor key to phenylthiocarbamide perception [30], is expressed in sinonasal cili-

ated epithelium [31] and upper airways [32]. TAS2R38 is implicated in innate immunity and the response to *Pseudomonas* [31]. rs713598 is a common missense SNP in *TAS2R38* [30], associated with quinine intensity [29] and other taste preferences [33–35]. rs713598 is one of the most common SNPs in the *TAS2R38* gene, causing a missense mutation to the encoded GPCR (alanine 49 to proline) [30]. In the CRS patients in this study, this allele is more common (MAF 0.568; $p < 0.001$) than in Europeans of the 1,000 Genomes Project (MAF 0.423).

RGS21 rs7528947 (Regulator of G-Protein Signaling-21)

RGS21 is a Gα GTPase-accelerating protein expressed in the lingual epithelium, lung, and gastrointestinal tissues [21]; ablation of *Rgs21* in mice blunts bitterant signaling [36]. rs7528947 is a 3' untranslated region variant of *RGS21* with no known disease associations. The rs7528947 MAF in CRS patients is 0.547, similar to the European MAF (0.516; $p = 0.461$). No difference in rs7528947 MAF was observed when stratified between CRS patients with or without nasal polyps (Table 3); however, a significant difference ($p = 0.017$) was observed across the spectrum of Lund-Mackay CT staging scores (Table 4), with the minor allele appearing more frequently with higher Lund-Mackay scores.

RGS21 Intergenic Region rs1175152 (Regulator of G-Protein Signaling-21)

A second *RGS21*-associated SNP, rs1175152, is located in the intergenic region between *RGS18* and *RGS21* and is also of unknown consequence. To date, there have been no known studies identifying this marker with any known phenotype or in association with any disease. Its MAF among Europeans of the 1,000 Genomes Project is 0.433, similar to the frequency observed in the CRS patients of this study (MAF 0.439; $p = 0.875$).

TAS2R20 rs12226920 (Type 2 Taste Receptor 20)

TAS2R20 (also known as TAS2R49) is a bitterant receptor expressed in multiple tissue types [37]. Its missense SNP rs12226920 (histidine 143 to glutamine) was previously associated with CRS, with a biallelic difference of 16% versus controls, suggesting a possible role for variations in this GPCR in CRS pathogenesis [15]. In the CRS patients of this study, the MAF (0.458) was higher than that predicted by 1,000 Genomes (European MAF 0.383); however, this difference did not meet our statistical significance threshold ($p = 0.062$).

GNB3 rs5443 (*G Protein Subunit Beta 3*)

GNB3 encodes a G β subunit of the G protein heterotrimer. Its SNP, rs5443, has been studied extensively in other contexts and is associated with numerous metabolic-related diseases, including obesity [38], coronary artery disease, and hypertension [39], as well as the increased efficacy of antidepressants in major depressive disorder [40]. All of these associations are with minor allele (T) carriers. The HapMap European MAF is 0.367, whereas the CRS patients of this study showed an increased MAF of 0.493 ($p = 0.014$).

Discussion

While *RGS21* is functionally linked to bitterant GPCR signaling [20, 21, 36], neither *RGS21* SNP tested was found in this study to be associated with CRS (although the minor allele of *RGS21* SNP rs7528947 was seen more frequently in CRS patients with higher Lund-Mackay CT staging scores). Conversely, *GNB3* SNP rs5443, a gene variation in an established component of bitterant GPCR signaling [41] but without a previous association with CRS, was found to be more highly prevalent in West Virginia CRS patients, along with two *TAS2R* variants previously identified as being associated with CRS. G β subunits of G protein heterotrimers, such as that encoded by *GNB3*, are known to assemble with E3 ubiquitin ligase complexes responsible for degradation of GRK2, a GPCR kinase [42]. Recent work has shown that *GNB3* SNP rs5443, identified more commonly in the CRS patients of this study, is associated with decreased GRK2 ubiquitination [43]. Further studies are therefore required to clarify the role of altered *GNB3* function (and potentially GRK2 function) in bitterant signaling, innate immunity activation/regulation, and CRS.

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Statement of Ethics

The study protocol was approved by the Institutional Review Board of WVU Office of Research Integrity and Compliance. The subjects of this study gave their written informed consent as obtained under WVU IRB 1410476782.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

David P. Siderovski: conception and design, data analysis and interpretation, revising the manuscript, final approval, and agreement to be accountable. Phillip R. Purnell: conception and design, acquisition of data, data analysis and interpretation, drafting and revising the article, final approval, and agreement to be accountable. Benjamin L. Addicks: conception and design, acquisition of data, data analysis and interpretation, revising the manuscript, final approval, and agreement to be accountable. Habib G. Zalzal: conception and design, acquisition of data, revising the manuscript, final approval, and agreement to be accountable. Scott Shapiro: conception and design, acquisition of data, revising the manuscript, final approval, and agreement to be accountable. Sijin Wen: data analysis and interpretation, revising the manuscript, final approval, and agreement to be accountable. Hassan H. Ramadan: conception and design, revising the manuscript, final approval, and agreement to be accountable. Vincent Setola: conception and design, data interpretation, revising the manuscript, final approval, and agreement to be accountable.

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