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

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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 (GPCRs) have been linked with CRS and with altered innate immune responses to multiple bacterially-derived signals.

Objective: To characterize in CRS the frequency of six SNPs in genes with known bitter tastant signaling function.

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Statement of Ethics

The study protocol has been approved by the Institutional Review Board of WVU's Office of Research Integrity and Compliance. Subjects of this study have given their written informed consent as obtained under WVU IRB 1410476782.

Disclosure Statement

The authors have no conflicts of interest to declare.

Methods: Genomic DNA was isolated from 74 CRS volunteers in West Virginia and allele frequency was determined and compared to demographically-matched data from the 1000 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 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 rs5443, a variation in an established G protein component downstream of bitterant receptor signal transduction.

Keywords

bitterant signaling; chronic rhinosinusitis; genetics; innate immune response; single nucleotide polymorphism

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 10–12% of the population in USA and Europe [2], resulting in >$770 million healthcare costs in the USA alone [3]. CRS patients have poor quality of life scores, similar to 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 [6]; this number is higher in 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 (SCCs) and ciliated epithelial cells of the nasal and sinus cavities, functioning in these locales to “taste” secreted bacterial products such as acyl-homoserine lactones (AHLs) [13]. Gram negative bacteria such as *Pseudomonas aeruginosa* use AHLs as quorum-sensing molecules [14]. Binding of these bacterial products activates innate immune responses, such as release of antimicrobial peptides and nitric oxide (NO).

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 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 TAS2Rmediated 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, including within *GNB3*, a downstream component of bitterant signaling previously unassociated with CRS.

Materials and Methods

Recruitment

Seventy-four volunteers were seen at an academic tertiary referral center in West Virginia (WV). The study was approved by the Institutional Review Board of WVU's Office of Research Integrity and Compliance. All volunteers met EPOS criteria [22] for CRS, and informed consent obtained under IRB 1410476782. Age, sex, allergy, asthma, migraine, smoking status, presence of polyps, and previous surgical history were recorded (Table 1). CT scan of the sinuses was 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 manufacturer's protocol). Purity and yield were assessed by 260/280 nm absorbance on a QIAxpert microfluidic spectrophotometer.

Genotyping

Genotyping was performed using TaqMan primer-probe sets (Table 2) and Type-it Fast SNP Probe PCR Kits (Qiagen) exactly as per 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 (*e.g.*, Figure 1 displays representative data obtained).

Statistical Analyses

Allele frequencies were compared to 1006 Europeans (1000 Genomes Project) [25], given that WV 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 1000 Genomes; its allele frequency was instead compared to 116 Europeans (CEU) from HapMap [27]. Statistical analyses of SNP frequency were performed using

Pearson's chi-square. 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 MAF in patients with (CRSwNP) or without (CRSsNP) nasal polyps, and separately based on Lund-Mackay CT score, using standard Chi-squared statistical tests (Tables 3 and 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 to CRS (*i.e.*, *TAS2R19*, *TAS2R20*, *TAS2R38*, and *GNB3*). All chosen SNPs had a minor allele frequency (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 associated with RSV infections in a genome-wide association study (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 of 0.505; Table 2). In the CRS patients in this study, this allele is even more common (MAF 0.601; p=0.024); twenty-three percent 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 phenylthiocarbamine perception [30], is expressed in sinonasal ciliated 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 1000 Genomes Project (MAF 0.423).

RGS21 rs7528947 (Regulator of G-protein signaling 21)

RGS21 is a Gα GTPase-accelerating protein expressed in 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 1000 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 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% *vs* 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 predicted by 1000 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 Gβ 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 to chronic rhinosinusitis, was found to be more highly prevalent in West Virginia CRS patients, along with two *TAS2R* variants previously identified as associated with CRS. Gβ 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 G protein-coupled receptor 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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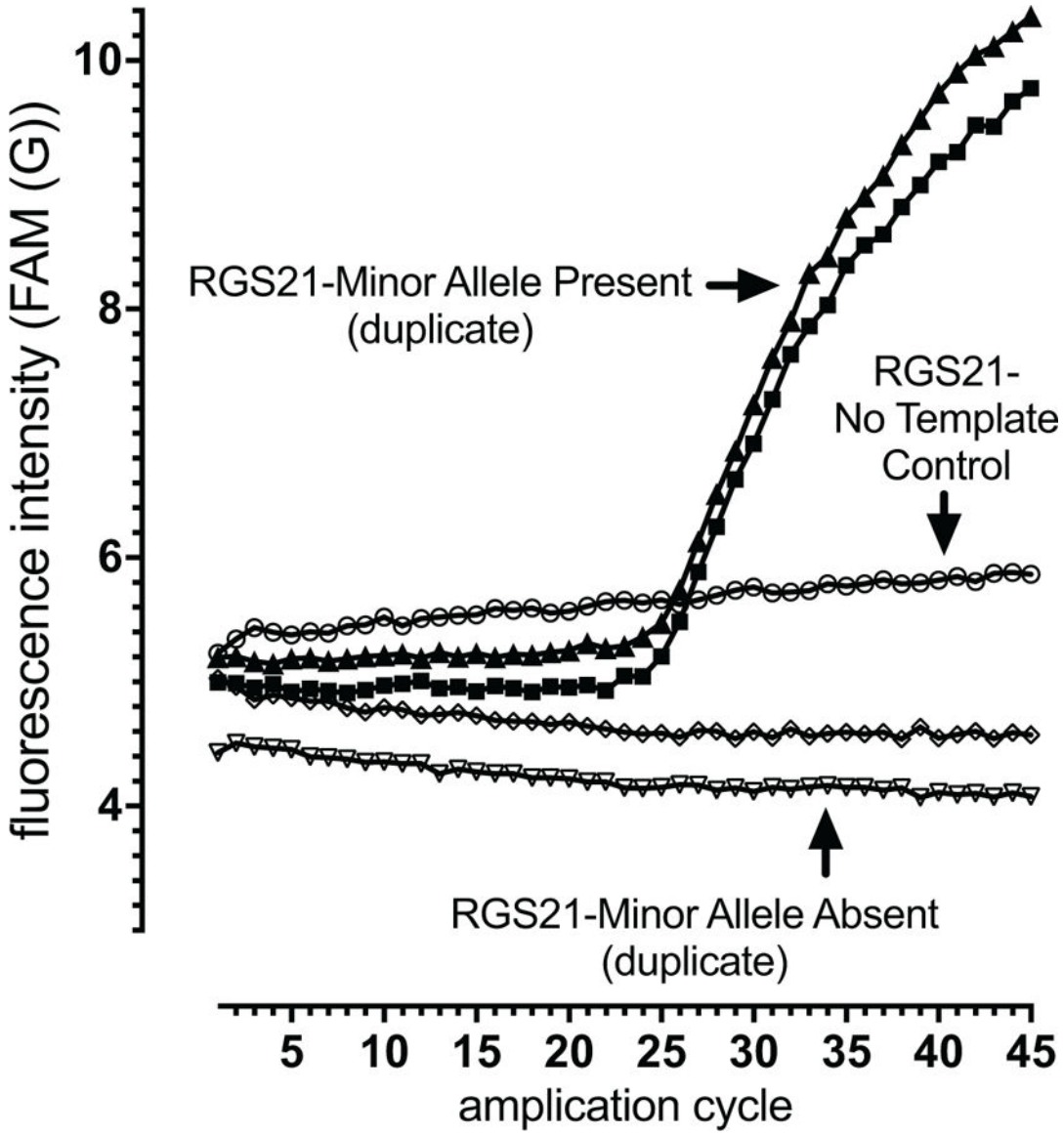

Fig. 1.

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 WV CRS patient genomic DNA using TaqMan primer-probe set C__30007846_20. Examples for probands with the minor allele (closed symbols) and lacking the minor allele (open triangle and open diamond) are shown; a separate qPCR reaction lacking input genomic DNA is also illustrated (open circles).

Table 1.

Patient Demographics

Patient variable	**N (74 total)**	**Percentage**	
Mean age	55.0	n/a	
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%	
	N (66 total)	**percentage**	
Lund-Mackay Score 1−8	22	33%	
Lund-Mackay Score 9−16	29	44%	
Lund-Mackay Score 17−24	15	23%	
		average	**CI (5%−95%)**
Lund-Mackay Score CT Score	66	11.6	10.0−13.3
	Score range	**average**	**CI (5%−95%)**
SNOT 22 (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

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‡
TAS2R38	**rs713598**	C	C___8876467_10	0.423	**0.568** ***p < 0.001***
GNB3	**rs5443**	T	C___2184734_10	0.367 (HapMap#)	**0.493** ***p = 0.014***
TAS2R19	**rs10772420**	A	C___1317426_10	0.505	**0.601** ***p = 0.024***
TAS2R20	rs12226920	T	C___1326611_10	0.383	0.458 *p = 0.062*
RGS21	rs7528947	G	C__30007846_20	0.516	0.547 *p = 0.461*
RGS21	rs1175152	A	C_____68684_10	0.433	0.439 *p = 0.875*

*Minor allele frequency reported on N = 1006 Europeans via 1000 Genomes (#except as otherwise noted: *i.e.*, 116 Europeans from HapMap); MAF comparisons between West Virginia probands and European SNP databases was previously established in Kaski, SW, *et al.* (2019) *J. Opioid Manag.* [*in press; vol. 15, issue 1*].

‡*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*"

Table 3.

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

Gene	dbSNP ID	Minor Allele	CRSwNP MAF	CRSsNP 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