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The Genetics of the Bitter Taste Receptor T2R38 in Upper Airway Innate Immunity and Implications for Chronic Rhinosinusitis

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Abstract

Introduction—Chronic rhinosinusitis (CRS) refractory to therapeutic intervention may involve a particularly resistant infection known as a bacterial biofilm. Critical to biofilm formation is the microbial process of quorum sensing, whereby microbes secrete factors that regulate the expression of microbial genes involved in biofilm formation, persistence, and virulence. Here we review recent work demonstrating that the bitter taste receptor T2R38, expressed on the apical surface of the sinonasal epithelium, serves a sentinel role in eavesdropping on microbial quorum sensing communications and regulates localized innate biocidal defenses. Furthermore, studies investigating whether cilia are necessary for T2R38 expression and function in the upper airway are presented.

Methods—Primary human sinonasal air-liquid interface cultures were used to elucidate cellular pathways responsive to quorum sensing molecules while clinical studies investigated the contribution of T2R38 polymorphisms to recalcitrant chronic rhinosinusitis.

Results—T2R38 is stimulated by acyl-homoserine lactones, gram-negative quorum sensing molecules and subsequently activates nitric oxide-dependent innate immune responses. The formation of mature cilia is necessary for T2R38 expression and function, and polymorphisms that underlie T2R38 functionality appear to be involved in susceptibility to upper respiratory infection and recalcitrant CRS.

Conclusions—Taste receptors are emerging as critical components of early phase respiratory innate immunity, detecting molecules used by microbes to communicate, and stimulating localized defenses. Genetic polymorphisms are very common within the taste receptors and recent linkage studies have demonstrated associations of taste receptor genetics with CRS. Lastly, ciliogenesis, which is often impacted in CRS is critical for the functional expression of T2R38.

Keywords

T2R38; chronic rhinosinusitis; cilia; interkingdom signaling; host-pathogen interactions

Conflict of Interest: None

Level of Evidence: NA (Basic Research and none systemic review of the literature).

Introduction

Chronic rhinosinusitis (CRS) affects more than 35 million Americans with an aggregated cost of \$8 billion annually, accounting for 1 in 5 antibiotic prescriptions in adults^{1–4}. Patients with CRS have significant decrements in quality of life both in disease-specific areas and in general health. In fact, patients requiring sinus surgery demonstrate worse scores for physical pain and social functioning than those suffering from chronic obstructive pulmonary disease, congestive heart failure, or angina⁵. Although the etiology of the disease is not known, environmental exposure to pathogens combined with an underlying genetic predisposition is proposed⁶. It remains controversial whether sinonasal infection or local inflammation is the root cause of rhinosinusitis, but there is little argument that the presence of bacteria in the sinonasal cavity contributes to the pathophysiology and that bacteria in the form of biofilms contribute to recalcitrant CRS requiring prolonged medical therapy and revision surgery^{7–10}.

Biofilms are complex sessile three-dimensional structures of bacteria that are attached to a living or an abiotic surface¹¹. They are embedded in an extracellular matrix and exhibit a distinct phenotype and genotype when compared to their planktonic counterpart¹². Biofilm formation develops in a stepwise fashion that starts with attachment to a surface. Following the attachment, there is the formation of a microbial micro-colony, and once this reaches a certain density, biofilm formation ensues. This life cycle is regulated by a process referred to as quorum sensing whereby in response to multiple environmental factors, microbes secrete chemical signals, known as autoinducers or quorum sensing molecules, which regulate the expression of microbial genes involved in biofilm formation, persistence, virulence, and other life cycle processes^{13,14}.

The sinonasal cavity is at the front line of defense of the respiratory tract^{15–17}. Host-pathogen interactions occur with every breath containing aerosolized fungal spores, bacteria, and virus particles¹⁷. However, in most individuals, the upper and lower airways remain free of infection. This is largely due to the multiple first-line innate immune mechanisms that work in concert to defend the sinonasal epithelium.

The primary physical defense is the process of mucociliary clearance^{15,16,18}. The airway surface is lined by a mucus gel made up of cross-linked glycosylated mucin macromolecules produced by airway secretory cells; the carbohydrate side-chains of the mucins create “sticky” binding sites that trap airway pathogens and particulates in the mucus^{15,16,19,20}. The spatially and temporally coordinated beating of ciliated epithelial cells then transports the debris- and pathogen-laden mucus from the upper and lower respiratory passages toward the throat, where it is cleared by swallowing or expectoration^{15,16,19}. Mucociliary clearance is complemented by the secretion of antimicrobial compounds including peptides such as lysozyme, lactoferrin, and defensins²¹, as well as the generation of reactive oxygen and nitrogen species (ROS/RNS) that can have direct antibacterial, anti-fungal, and antiviral effects^{22,23} (Fig. 1).

As mentioned above multiple investigations have demonstrated an association between sinonasal bacterial biofilms and recalcitrant chronic rhinosinusitis⁷⁻⁹. While there are many challenges surrounding the diagnosis and clinical management of biofilms, one question that we have been focusing on is whether there are certain patients that are genetically predisposed to form biofilms. Recent work from our lab linking sinonasal innate immunity with bitter (T2R) taste receptors may answer this question²⁴⁻²⁹.

Bitter Taste Receptors: New Players in Airway Innate Immunity

Evidence of taste receptor expression in the airway first came in 2009, when researchers demonstrated that human bronchial cells express T2Rs that, when activated with known bitter agonists such as denatonium benzoate, stimulate calcium responses to increase ciliary beating³⁰. Interestingly, these T2Rs are found within motile cilia³⁰. While motile cilia were previously thought to be responsible only for the movement of fluid, as in the airway³¹ and during embryonic development³², this study demonstrated that motile cilia are also “chemosensory organelles” and the authors hypothesized that T2Rs protect the lower airway against inhaled noxious irritants³⁰.

Subsequent to the identification of taste receptors in lower airway epithelial cells, Lee et al reported on the expression of both bitter (T2R)²⁹ and sweet (T1R)³³ taste receptors in the human sinonasal epithelium and described robust innate immune pathways regulated by these receptors. One bitter taste receptor that was focused in this work was T2R38, which, as in the lower airway is localized to motile cilia^{29,33}. When sinonasal ciliated epithelial cells were stimulated with known T2R38-specific agonists, such as the bitter tasting compound phenylthiocarbamide (PTC), they exhibited calcium-dependent activation of nitric oxide (NO) synthase (NOS), driving robust NO production²⁹. As expected for a taste receptor, NO production depended on two important components of the canonical taste signal transduction cascade²⁹, the TRPM5 ion channel³⁴ and phospholipase C isoform $\beta 2$ (PLC $\beta 2$)³⁵, however, unexpectedly PTC stimulation of NO was independent of the canonical taste G-protein, α -Gustducin³⁶. NO production by airway epithelial cells, including ciliated cells, is believed to be important for preventing airway infection^{37,38}, because the NO radical and its derivatives can damage bacterial membranes, enzymes, and DNA^{23,39} (Fig 1). The NO produced during T2R38 activation in sinonasal epithelial cells *in vitro* was found to diffuse into the airway surface liquid (ASL) and be directly bactericidal against *P. aeruginosa*²⁹. Additionally, NO increases ciliary beat frequency by activating guanylyl cyclase and protein kinase G, which directly phosphorylates ciliary proteins³¹.

The initial T2R38 study²⁹ also identified two major *P. aeruginosa* quorum sensing molecules, N-butyryl-L-homoserine lactone (C4HSL) and N-3-oxo-dodecanoyl-L-homoserine lactone (C12HSL)⁴⁰ as physiologically relevant, endogenous T2R38 agonists²⁹. Sinonasal epithelial cells stimulated directly with these quorum sensing molecules or with conditioned medium from wild type *P. aeruginosa* produced NO in a T2R38-dependent manner. However, conditioned medium from a *P. aeruginosa* strain that lacks these quorum sensing molecules (*lasI*, *rhlI* PAO-JP2;⁴¹) did not activate NO production. Because many types of gram-negative bacteria secrete this specific type of quorum sensing molecule⁴⁰, T2R38 is likely part of a general surveillance mechanism to detect invading gram-negative

bacteria. A schematic of the T2R38 innate immune pathway elucidated by this work is illustrated in Fig. 2.

Human T2R38 functionality is altered by several well-studied polymorphisms in the *TAS2R38* gene^{42,43}. Two polymorphisms are common in Caucasian populations; one encodes a functional T2R38 and the other encodes a nonfunctional T2R38. These polymorphisms result in different amino acids at positions 49, 262, and 296. The functional T2R38 contains proline (P), alanine (A), and valine (V) residues and the nonfunctional T2R38 contains alanine (A), valine (V), and isoleucine (I) at these positions, respectively⁴². Loss of the V at the third position of the AVI variant likely prevents receptor activation^{42,43}. Homozygous AVI/AVI individuals (~30% frequency in Caucasians) are “non-tasters” for T2R38-specific agonists such as PTC and 6-propyl-2-thiouracil (PROP)⁴². Homozygous PAV/PAV individuals (~20% frequency in Caucasians⁴²) are “supertasters” that perceive PTC and PROP as intensely bitter. Heterozygote PAV/AVI individuals have varying intermediate taste levels^{42,43}. These natural T2R38 variants were experimentally exploited by growing primary sinonasal cells from genotyped PAV/PAV “supertasters” and AVI/AVI “nontasters.” NO-dependent responses correlated with these polymorphisms. PAV/PAV “supertaster” cells had much higher levels of NO production, increased mucociliary clearance, and more efficient bacterial killing in response to either PTC or AHLs compared with cells from AVI/PAV heterozygote or AVI/AVI “nontaster” individuals²⁹. *TAS2R38* polymorphisms alter how efficiently sinonasal epithelial cells respond to gram-negative bacteria^{29,33}. These findings with regard to T2R38 in human sinonasal innate immunity are summarized in Table 1.

This initial study also found that PAV/PAV “supertasters” had a lower frequency of gram-negative sinonasal infection than PAV/AVI or AVI/AVI patients with lower levels of T2R38 function²⁹. Further clinical studies by our group as well as others have validated the relevance of *TAS2R38* genotype to sinonasal disease^{25,44,45}. A retrospective study²⁵ was first carried out in which *TAS2R38* genotyping was performed on sinonasal tissue samples from 28 patients who had undergone primary functional endoscopic sinus surgery (FESS). Out of these patients, 3.6% were PAV/PAV, 50% were PAV/AVI, and 46% were AVI/AVI, which was significantly different from the expected distributions of 20% PAV/PAV, 50% PAV/AVI, and 30% AVI/AVI ($P < 0.043$ by χ^2 analysis)²⁵. Only one patient out of 28 was a PAV/PAV “supertaster,” suggesting that “supertasters” are less likely to need surgical intervention for CRS. In a subsequent prospective study of *TAS2R38* genotype in 70 patients undergoing primary FESS at the same institution⁴⁴, there was a statistically significant difference in the frequency of AVI and PAV alleles compared with the general population. Distributions of AVI/AVI, AVI/PAV, and PAV/PAV alleles were 37%, 54%, and 8.5%, respectively, in the CRS patients compared with 29%, 51%, and 20%, respectively, in the general regional population ($P < 0.0383$ by χ^2 analysis)⁴⁴. No significant differences were found in the allele distribution with respect to allergies, asthma, nasal polyposis, aspirin sensitivity, diabetes, smoking exposure, or other risk factors, suggesting that *TAS2R38* is an independent risk factor for CRS requiring FESS.

Another study was carried out with the goal of verifying if taste receptor polymorphisms correlate with CRS. Two separate Canadian CRS populations were compared with control

populations⁴⁵, using previously collected pooling-based genome wide association data that was screened for single-nucleotide polymorphisms (SNPs) in taste receptors, including the *TAS2R38* I296V (*rs10246939*) thought to underlie the difference in functionality between PAV and AVI variants. A difference in I296V frequency of ~11% and ~15% in the two CRS groups compared with control individuals was found, confirming that this SNP is associated with CRS. Three other missense variants in *TAS2R* genes were associated with CRS (frequency difference 10% between CRS and control), one in *TAS2R14* (*rs1015443*) and two in *TAS2R49* (*rs12226920* and *rs12226919*)⁴⁵. Roles for T2R14 or T2R49 in sinonasal immunity require further research.

Current study hypothesis

Multiple studies have demonstrated that patients with Chronic Rhinosinusitis have disrupted sinonasal epithelium with goblet cell hyperplasia and decreased ciliation (Fig 3). Prior work has shown that environmental insults such as exposure to tobacco smoke as well as inflammation impairs cilia formation and disrupts cilia function which may contribute to CRS^{46–48}. The hypothesis tested here is that cilia are not only critical to drive mucociliary clearance, but are needed for T2R38 expression and function. To test this hypothesis primary human sinonasal air-liquid interface cultures were utilized. These cultures are initially established in submersion conditions where the epithelial cells are not ciliated⁴⁹. Once tight junctions are formed the cultures are transitioned to an air-liquid interface and over the ensuing 2 weeks cilia form, typically first appearing around day 10.

Methods

Sinonasal ALI cultures

Patients were recruited from two institutions with full approval of both Institutional Review Boards. Informed consent was obtained during the pre-operative clinic visit or in the pre-operative waiting room. Selection criteria for recruitment were patients undergoing sinonasal surgery. Exclusion criteria included a history of systemic diseases such as Wegner's, Sarcoid, Cystic fibrosis, immunodeficiencies, and use of antibiotics, oral corticosteroids, or anti-biologics (e.g. Xolair) within one month of surgery. Sinonasal mucosal specimens were acquired from residual clinical material obtained during sinonasal surgery and transported to the laboratory in saline placed on ice. ALI cultures were established from human sinonasal epithelial cells (HSEC) enzymatically dissociated human tissue as previously described^{29,33} and grown to confluence in tissue culture flasks (75 cm²) with proliferation medium consisting of DMEM/Ham's F-12 and bronchial epithelial basal medium (BEBM; Clonetics, Cambrex, East, NJ) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin for 7 days. Cells were then trypsinized and seeded on porous polyester membranes (6~7×10⁴ cells per membrane), in cell culture inserts (Transwell-clear, diameter 12 mm, 0.4 µm pores; Corning, Acton, MA) coated with 100µl of coating solution [BSA (0.1 mg/ml; Sigma-Aldrich), type I bovine collagen (30 µg/ml; BD), fibronectin (10µg/ml; BD) in LHC basal medium (Invitrogen)] and left in a tissue culture laminar flow hood overnight. Five days later the culture medium was removed from the upper compartment and the epithelium was allowed to differentiate by using the differentiation medium consisting of 1:1 DMEM (Invitrogen, Grand Island, NY) and BEBM (Clonetics, Cambrex, East Rutherford, NJ) with

the Clonetics complements for hEGF (0.5 ng/ml), epinephrine (5 g/ml), BPE (0.13 mg/ml), hydrocortisone (0.5 g/ml), insulin (5 g/ml), triiodothyronine (6.5 g/ml), and transferrin (0.5 g/ml), supplemented with 100 UI/ml penicillin, 100 g/ml streptomycin, 0.1 nM retinoic acid (Sigma-Aldrich), and 10% FBS (Sigma- Aldrich) in the basal compartment.

Immunofluorescent Staining and Confocal-Microscopy

As previously described²⁹, primary human sinonasal epithelial ALI cultures were grown to maturity (described above), washed 3 times with PBS, and fixed in 4% paraformaldehyde for 20 min at room temperature. The transwell permeable support was again washed 3 times with PBS prior to excision of the membrane containing the cells from its plastic supports. The membrane was immersed in Tris-buffered saline (TBS) with 0.3% Triton X-100, 5% normal donkey serum, and 1% bovine serum albumin for 60 min at room temperature to permeabilize the plasma membrane and block non-specific binding sites. Two primary antibodies raised from different hosts were chosen for the double immunofluorescent staining: mouse monoclonal anti- β -Tubulin IV (1:1000; Abcam, Cambridge, MA) and rabbit polyclonal anti-T2R38 (1:500; Santa Cruz Biotech, Santa Cruz, CA). Visualization was carried out with Alex Fluora® 488 (green)-conjugated donkey anti-mouse IgG for tubulin IV and Alex Fluora® 594 (red)-conjugated donkey anti-rabbit IgG for T2R38. Both secondary antibodies (Invitrogen, Grand Island, NY) were diluted at 1:500. The incubation time was overnight at 4°C for primary antibodies and 75 min at room temperature for secondary antibodies, respectively. Counterstaining was done with Hoechst (blue), a nuclear dye. Confocal images were acquired with Olympus Fluoview System at the Z-axis step of 0.5 μ m. Sequential scanning module was used to prevent bleed-through of fluorophores into other channels.

Live-cell imaging of DAF-FM in human ALI cultures

As previously described²⁹ DAF-FM imaging was performed according to standard methods using the 488 nm argon laser line of a Fluoview FV1000 laser scanning confocal system and IX-81 microscope (10 $\times$, 0.3 NA UPlanFLN objective; Olympus). Cells were loaded with DAF-FM by incubation in DPBS containing 10 μ M DAF-FM diacetate in PBS containing 5 μ M carboxy PTIO, a cell permeant NO scavenger (on the apical side only). After 30 min, cultures were copiously washed (at least 5 times) with PBS to remove all traces of unloaded DAF-FM. Cultures were then incubated for ~15 min to allow for dye retention before imaging was performed. DAF-FM fluorescence images were acquired at 5 second intervals. Because the magnitudes of DAF-FM fluorescence changes are used to approximate NO production, care was taken to follow the loading protocol strictly to normalize dye loading and microscope/software settings were identical for each experiment.

Results

ALI cultures at day 0, 3, 6, 9, and 12 after transitioning to apical exposure to air were subjected to double immunofluorescence staining with type IV β -tubulin, a marker for motile cilia (green), and T2R38 (red). Merger of the images (yellow) confirms the localization of T2R38 to sinonasal cilia demonstrating that expression of T2R38 is directly

linked to ciliogenesis, as no T2R38 expression is evident prior to cilia formation (days 0, 3, and 6) (Fig. 4).

Further experiments were performed to investigate whether cilia are necessary for T2R38 mediated production of NO. Sinonasal ALI cultures derived from PAV/PAV patients were stimulated with the T2R38 agonists PTC and the quorum sensing molecule C12HSL at various time points during maturation (Day 0, 3, 6, 9, and 12). As expected from the results above (Fig. 4.), T2R38-mediated nitric oxide production was not evident prior to cilia formation (Fig. 5).

Discussion

This study is additive to the broader body of work generated by our lab with regard to the role of the T2R38 receptor in CRS. Our findings indicate that T2R38, originally identified as a bitter taste receptor, is expressed in human sinonasal epithelial cells where it triggers a rapid inter-kingdom signaling response bridging microbial secretions and the human upper airway epithelium, making it an integral component of upper respiratory early response defenses, acting well before Toll-like or Nod-like receptors⁵⁰. When stimulated by acyl-homoserine lactones (AHLs), T2R38 elicits calcium-dependent nitric oxide (NO) production that increases ciliary beat frequency and mucus clearance. Additionally, this NO diffuses into the airway and contributes to innate antimicrobial effects. Furthermore, we demonstrated that upper airway epithelial cells from individuals with one or two nonfunctional T2R38 alleles (AVI) have significantly blunted nitric oxide and ciliary responses following exposure to gram-negative quorum sensing molecules and that these individuals are more likely to be infected with gram-negative bacteria such as *Pseudomonas aeruginosa* than those with two functional receptor alleles²⁹ and develop CRS necessitating surgical intervention²⁵.

The finding of this study demonstrating that cilia formation is necessary for T2R38 expression and function has broad implications in chronic rhinosinusitis where sinonasal ciliary integrity is compromised (Fig 3). This stresses the need for mucosal preservation surgery and aggressive post-operative therapies to normalize the sinonasal mucosa⁵¹.

A perplexing observation from this data is that many heterozygotes (PAV/AVI) behaved more like non-functional homozygotes (AVI/AVI) with regard to sinonasal defense and manifestation of CRS^{25,29,44}. The range of PTC taste sensitivity within the heterozygote (PAV/AVI) population has been a scientific conundrum as well. The taste puzzle has been at least partially solved by the observation that heterozygous people differ in the amount of functional (PAV) mRNA they express, and those who express little or no functional forms are phenotypically similar to people with a nontaster (AVI/AVI) genotype⁵², a phenomenon known as allele specific expression. Thus, the heterozygotes in the original data (Table 1) may have been skewed and were poor PAV expressers, which would explain both their low production of NO and susceptibility to CRS. The possibility of allele specific expression in the heterozygote population as an explanation for this finding is under investigation utilizing allele specific and tissue specific quantitative PCR.

Further prospective clinical studies of *TAS2R38* genotype and CRS susceptibility, including the influence of *TAS2R38* genotype on patient outcomes, are currently ongoing. However, the T2R38 pathway is already a potential therapeutic target to promote endogenous immune responses in patients with upper respiratory infections. One caveat, though, is that there would likely be a large subset of patients that would be sub-optimally responsive to treatment with T2R38 agonists (ie, PAV/AVI and AVI/AVI individuals). It is thus still necessary to further define T2R38 signaling mechanisms in airway cells as well as to identify other T2Rs that activate similar responses.

It is also likely that other T2R isoforms expressed in other airway cell types may have important clinical relevance. In particular, the genetics of T2Rs in nasal solitary chemosensory cells (SCCs) may play an important role in CRS susceptibility. As has recently been shown by our lab, taste receptors expressed in SCCs control antimicrobial peptide secretion in humans³³ and others have shown that in the mouse nose SCC control both breath-holding⁵³ and inflammation⁵⁴ in mice. Clinical consequences of taste receptor genetics on SCCs remain to be determined. Additionally, the middle ear is lined by a ciliated epithelium and preliminary studies suggest expression of T2Rs localizing to cilia in human middle ear mucosa. Thus, similar taste receptor genetics that are emerging in chronic rhinosinusitis may similarly be contributing to otitis media.

A better understanding of the T2R isoforms expressed in ciliated cells will speed identification of potential compounds that stimulate innate defenses. The role of T2Rs as global mediators of innate immunity was recently supported by a report demonstrating that T2R-expressing chemosensory cells in the rodent urethra respond to the bitter compounds as well as heat-inactivated uropathogenic *E. coli*; activation of these cells causes release of ACh to activate the bladder detrusor muscle⁵⁵. Further investigation of T2Rs in innate immunity will likely result in significant clinical impact in the airway and other organ systems.

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Figure 1. Mucociliary clearance (MCC) and respiratory epithelial innate immunity

Inhaled pathogens, such as viruses, bacteria, and fungal spores, are trapped by mucus secreted by goblet cells of the respiratory epithelium. These trapped microbes are then removed from the airway by the process of mucociliary transport, the airway's primary physical innate defense against inhaled pathogens and irritants. Constant beating of motile cilia drives the pathogen-laden mucus toward the oropharynx, where it is then cleared out of the airway by expectoration or swallowing. Mucociliary transport is complemented by more direct mechanisms of pathogen inactivation and/or killing, including the generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) and the production of antimicrobial peptides.

Figure 2. The role of the bitter taste receptor T2R38 in human sinonasal epithelial innate immunity

Reading from left to right, gram-negative bacteria secrete acyl-homoserine lactone (AHL) molecules to regulate quorum sensing. AHLs activate T2R38 in sinonasal cilia, initiating a calcium (Ca^{2+}) signal that activates nitric oxide synthase (NOS)-dependent nitric oxide (NO) production. NO production has 2 distinct effects: an increase in ciliary beating and mucociliary transport, and NO diffuses directly into the airway surface liquid, where it directly permeabilizes and kills bacteria.

Figure 3. Decreased cilia in sinonasal mucosa from a patient with chronic rhinosinusitis
Scanning electron micrograph of sinonasal mucosa obtained from the maxillary sinus from a patient with no CRS (A) compared to a patient with CRS (B). In the control patient the lining of the sinus has the appearance of “shag carpet” completely covered by cilia while in the CRS patient the surface has few ciliated cells.

Figure 4. Expression of T2R38 is localized to cilia

Prior to ciliogenesis human sinonasal epithelial cells do not demonstrate expression of T2R38. Air-liquid interface cultures were subjected to dual label immunofluorescence for type IV β -Tubulin (a marker for motile cilia) (green) and T2R38 (red). During the first week of differentiation (A–C), ALI cultures are non-ciliated, but by day 9 (D) and more so on day 12 (E) cilia are present. Expression of T2R38 mirrors the appearance of cilia (yellow).

Figure 5. Cilia are needed to T2R38 function in respiratory innate immunity

Intracellular NO was assessed in primary human sinonasal air-liquid interface cultures (PAV/PAV) using the intracellular dye DAF-FM following apical stimulation of the cells with the bitter molecule PTC as well as the gram-negative quorum sensing molecule C12HSL. (A–C) Prior to cilia formation (Fig. 4.) PTC nor C12HSL induced NO production. (D) On the ninth day of ALI culture maturation, as cilia begin to form, both PTC (left) and C12HSL (right) induced NO production which was also apparent on day 12 (E). Direct comparison of NO produced at day 9 and day 12 (F) demonstrates statistically significant more NO produced on day 12 than day 9 (1-way ANOVA with Bonferroni post-test; $P < 0.05$ was considered statistically significant). Sodium Nitroprusside (SNAP) liberates NO and is used as a control to assure equal loading of cells with DAF-FM.

Table 1

Sinonasal epithelial function in response to secreted bacterial AHLs and clinical sequelae influenced by the bitter receptor *TAS2R38* genotype (PAV=active haplotype, AVI=inactive haplotype)

BIOLOGICAL OUTCOME	TAS2R38 GENOTYPE		
	PAV/PAV	PAV/AVI	AVI/AVI
Increased intracellular Ca ²⁺	+	–	–
Intracellular NO production	+	–	–
Extracellular NO diffusion	+	–	–
Increased ciliary beat frequency	+	–	–
Increased mucociliary clearance	+	–	–
Bacteriocidal activity	+	–	–
Gram-negative sinonasal infection	–	+	+