活體內生物膜、格蘭氏陰性感染、及苦味接受者TAS2R38多形性在慢性鼻竇炎併息肉患者的表現

In Vivo Biofilm Formation, Gram-Negative Infections and TAS2R38 Polymorphisms in CRSw NP Patients

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Commentary

慢性鼻竇炎的成因複雜，目前包括過敏、免疫相關因素、黏膜清除能力缺陷、生物膜 (biofilm) 相關細菌感染被提出。苦味接受器 TAS2R38 與固有免疫相關, 可製造出與鈣離子相關的一氧化氮, 增加殺菌力及黏膜清除能力。鼻竇纖毛上皮的 TAS2R38 基因變異, 與上呼吸道感染的抵抗力有關。TAS2R38 多形性 (polymorphism) 造成胺基酸殘基 (residue) 的變異, 形成兩種單倍體 (haplotype): (1) 功能性的 PAV 對偶基因 (allele); (2) 無功能的 AV I 對偶基因。而這兩種單倍體會形成三種基因型: 包括 PAV/PAV、AVI/AVI、PAV/AVI。當擁有 PAV 同型 (homozygous) 或異型合子 (heterozygous) 時，則為可感覺到 PROP (苦味) 或 PTC (強烈苦味) 化合物的味覺者 (taster) 或超級味覺者 (supertaster)；若具無功能性 AVI/AVI 基因型，則為無味覺者 (nontaster)。目前未有 TAS2R38 多形性與活體生物膜形成與 G(-) 細菌感染耐受力關係的研究; 此研究將病患依 TAS2R38 基因型及表現型分組, 研究其與鼻竇炎嚴重度、呼吸道細菌感染及生物膜形成之關係。

本研究在三年內收集 100 位慢性鼻竇炎併息肉接受手術的病患; 統計 Lund-Kennedy endoscopy score (LK score), Lund-Mckay CT score (LM score), SNOT-22 score。以共軛焦顯微鏡來檢查生物膜；味覺表現型以對 PROP 的敏感度來分組，並以 VAS 主觀分數來評比，以此分數來區分病患表現型為無味覺者 (nontaster)、有味覺者 (taster)、超級味覺者 (supertaster)。TAS2R38 的基因型為 RT-PCR 檢查其中 87 位病患唾液或血液所得結果。另有 37 位復發型息肉也納入基因檢查。80 位有基因資料，其中 AVI/AVI 型 (31 位，25%) 較健康者比例高。味覺表現型與基因型具強烈相關性; 具無功能 AVI/AVI 基因者 82.4% 為無味覺者，而具 PAV 基因者 80% 為有味覺者或超級味覺者。味覺表現型與鼻竇炎嚴重度 (LK 分數、LM 分數、SNOT-22 分數) 無相關。63 位鼻竇黏膜有細菌生長，其中 53 位有基因資料者，18 位有 G(-) 細菌感染，其中只有 2 位為 PAV/PAV 基因型 (11.1%)。在 G(-) 感染中，包含 13 位 PAV/PAV 中的 2 位 (15.4%)，以及 40 位具 AVI 中的 16 位 (40%)，比例差了 2.6 倍; 但表現型則與 G(-) 感染無關。43 位鼻息肉的檢查中有 19 位被發現生物膜，分別為佔無味覺者 60%、及有味覺者 33.3%。這些病患中 5 位為 PAV/PAV，14 位具 AVI 基因，雖未達統計上差距，但本篇作者認為 AVI 基因與生物膜的形成有關。

此文獻提供了活體內生物膜的形成與味覺表現型有關，可以用味覺測試預測對 G(-) 感染的耐受性，也能夠篩選出哪些病患需接受基因測試。然而此研究並非所有病患具有完整資料；原發型及復發型疾病若分開研究也可能有不同結果；另外，生物膜檢查檢體部位若能多挑幾個 (如上頜竇底部、前篩竇), 基因檢查檢體能統一，以及增加苦味接受器與手術治療結果的調查，相信都值得進一步研究。

關鍵字：鼻竇炎、鼻息肉、TAS2R38、細菌生物膜、慢性細菌感染
In Vivo Biofilm Formation, Gram-Negative Infections and TAS2R38 Polymorphisms in CRSw NP Patients

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Objectives: Among the predisposing factors implicated in the immune response to airway bacterial infections, genetic variations of the bitter taste receptor TAS2R38, which is expressed in the cilia of the human sinonasal epithelial cells, seem to be associated with susceptibility to chronic rhinosinusitis (CRS) and in vitro biofilm formation. Polymorphisms in TAS2R38 generate two common haplotypes: the nonfunctional AVI (Alanine, Valine, Isoleucine) and the functional PAV (Proline, Alanine, Valine) alleles, with the latter protecting against gram-negative sinonasal infections. The aim of this study is to investigate for the first time the relevance of TAS2R38 genetic variants in the susceptibility to bacterial infections associated with in vivo biofilm formation in chronic rhinosinusitis with nasal polyps (CRSwNP) patients.

Study Design: A prospective study on 100 adult patients undergoing functional endoscopic sinus surgery (FESS) for CRSwNP.

Methods: Propylthiouracile (PROP) testing and TAS2R38 genotyping were applied to characterize patients for receptor functionality. Sinonasal mucosa samples were processed for microbiological examination and biofilm detection.

Results: The nonfunctional genotype is more frequent among CRS patients than in the general population (25% vs. 18.4%, P = 0.034). Airway gram-negative infections are primarily associated with the AVI haplotype (88.9% vs. 11.1% PAV/PAV-functional genotype, P = 0.023). Biofilm formation is prevalent in CRS patients with the AVI nontaster phenotype (62.5% vs. 33.3% PAV taster or supertaster phenotype, P = 0.05).

Conclusion: Our findings confirm an inverse correlation between TAS2R38 functionality and gram-negative infections in Italian patients with CRSwNP. In addition, for the first time we demonstrated a relationship between in vivo microbial biofilm and TAS2R38 receptor variants.

Key Words: Rhinosinusitis, nasal polyps, TAS2R38, bacterial biofilms, chronic bacterial infections.

Level of Evidence: 2b.

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INTRODUCTION

Chronic rhinosinusitis (CRS) is a multifactorial disease that affects approximately 10% of the world population. 1 Although allergy, immune-associated disorders, defects in the mucociliary clearance system, and biofilm-associated chronic bacterial infection have been demonstrated as predisposing factors, CRS pathophysiology has not been thoroughly elucidated to date. 2,3 Recently, it has been postulated that genetic factors may facilitate the onset of CRS. 4–7

For example, activation of the bitter taste receptor TAS2R38 is associated with innate bacterial defense mechanisms. These generate the calcium-dependent production of nitric oxide (NO), which is known as a bactericidal mediator and leads to increased mucociliary clearance. 8,9

Genetic variations of the bitter taste receptor TAS2R38, expressed in the cilia of the human sinonasal epithelial cells (HSEC), seem to be associated with susceptibility to upper respiratory tract infections and CRS. 10–14

Polymorphisms in the TAS2R38 gene produce changes in the amino acid residues at positions 49, 262, and 296, generating two common haplotypes: 1) the functional, “taster,” allele of the TAS2R38 encodes proline, alanine, and valine (PAV); and 2) the nonfunctional, “nontaster,” allele of the receptor encodes alanine, valine, and isoleucine (AVI), respectively. 15

These two haplotypes generate the three most frequent genotypes: PAV/PAV, AVI/AVI, and PAV/AVI, which are transmitted with quasi-Mendelian genetic inheritance, accounting for 24%, 22%, and 51% of the European population, respectively. 16

Additional supporting information may be found in the online version of this article.

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People who are homozygous or heterozygous for the functional allele PAV perceive the compounds propylthiouracile (PROP) and phenylthiocarbamide (PTC) as bitter (tasters) or intensely bitter (supertasters), whereas the same compounds will be imperceptible to individuals with the nonfunctional genotype AVI/AVI, named “nontasters.”

Gram-negative bacteria quorum-sensing molecules that regulate the expression of genes that are engaged in life-cycle processes, such as biofilm formation, virulence, and persistence, activate the TAS2R38 receptors.9,17,18 Data in the literature shows that taste receptor-mediated immune function may decrease biofilm formation, preventing planktonic bacterial shedding from a previously established biofilm.19

A recent in vitro study showed that the PAV/PAV receptor responds to pseudomonas quorum-sensing molecules more efficiently than the heterozygous (PAV/AVI) or homozygous (AVI/AVI) hypofunctional forms.9 The hypothesis that a PAV/PAV genotype would be protective against gram-negative sinonasal infections was tested in a small pilot study, showing that the frequency of PAV/PAV genotypes was statistically lower compared to the expected distribution in a cohort of CRS patients undergoing primary functional endoscopic sinus surgery (FESS).19

Conversely, a recent study by Gallo et al. failed to show any correlation between TAS2R38 genetics and both CRS and related comorbidities in an Italian cohort of CRS patients with and without nasal polyposis.20

To date, there has been no previous study investigating in vivo biofilm formation in chronic rhinosinusitis with nasal polyps (CRSwNP) patients and determining its correlation to the TAS2R38 polymorphisms and the susceptibility to airway gram-negative infections.

We performed the first prospective study of in vivo biofilm formation in a sample of patients suffering from CRSwNP who were undergoing FESS. Patients were stratified by TAS2R38 genotypes and related phenotypes in order to correlate TAS2R8 genetics to CRS severity, airway bacterial infections, and biofilm formation.

MATERIALS AND METHODS

Population

A prospective cohort study was carried out at the University of Naples, Federico II, and at Monaldi Ospedale dei Colli–Naples, from January 2014 to September 2016 on 100 adult patients (68 males, 32 females; mean age 53 ± 14 years) undergoing FESS for CRSwNP and not responding to medical therapy.

Patients were excluded by the following criteria: age < 18 years; neurological, psychiatric, and oncological diseases; cystic fibrosis; primitive ciliary dyskinesia; traumas; hyp/oageusia or hypo/anosmia; locoregional radiotherapy; or use of drugs, including either topical nasal steroids, decongestants, antibiotics, or antihistamine, 1 month before. All participants provided their informed consent to the study, which met the approval of the local board of medical ethics (prot. n. 90/15). The study was performed according to the Declaration of Helsinki.

Information concerning age, sex, phenotype of CRS, allergies, asthma, Acetylsalicylic acid (ASA) sensitivity, and nasal microbiological data was collected for the whole cohort. Venous blood or saliva samples were harvested for DNA extraction and TAS2R38 genotyping.

The diagnosis of CRS was based on the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) diagnostic criteria and confirmed by nasal endoscopy.21 Nasal endoscopy was performed before surgery with a 2.7-mm 30° rigid endoscope (Storz, Tuttingen, Germany) without decongestant or local anesthesia, and was scored using the criteria described by Lund and Kennedy (LK-score).22

To assess the quality of life (QoL), subjects were asked to answer the Sino-Nasal Outcome Test-22 (SNOT-22),23 a validated patient self-reporting measure encompassing all major symptoms included in the diagnosis criteria set in the EPOS 2012 for CRS.24 Before surgery, all subjects underwent a sinonasal computed tomography scan, scored using the criteria described by Lund and McKay (LM-score).25

Samples for microbiological examination and for biofilm detection were collected under sterile conditions from sinonasal mucosa during the surgical procedure.

Thirty-seven CRSwNP patients (26 males, 11 females, range 19–70 years of age) who had previously undergone FESS and met the inclusion criteria of the study were also enrolled to be genotyped for TAS2R38 polymorphisms.

Tissue Preparation and Microbial Identification

Nasal polyps were processed within 30 minutes after collection. Each polyp was cut into two pieces to perform bacterial identification and biofilm detection, respectively. The specimens were pretreated with 5 mL of 0.1% (weight/volume) dithiothreitol in sterile saline and vortexed briefly to make bacteria accessible for microbiological examinations. The specimens were later incu-bated for 15 minutes at room temperature and again vortexed thoroughly. The supernatant was spun down at 4,200 rpm for 15 minutes, and then the pellet was visually analyzed after gram staining and seeded on selective media for gram-positive and gram-negative bacteria and fungi. Microbial identification and antimicrobial susceptibility testing was performed by automatic systems (Vitek II, BioMerieux, Marcy l’Etoile, France; Phoenix, Becton Dickinson, Franklin Lakes, NJ).

Confocal Laser Scanning Microscopy Assay

On untreated specimens, evidence of biofilm was obtained by confocal laser scanning microscopy (CLSM) using phalloidin, a cytoskeletal stain specific for filamentous actin, and propidium iodide (PI) to visualize bacteria and host cell nuclei. The samples were gently washed twice in phosphate-buffered saline to remove only planktonic bacteria and then were fixed in neutral buffered formalin (pH 7.2–7.4). The samples were later permeabilized with 0.1% Triton X-100 (Sigma-Alrich srl, Milano, Italy) and stained with Alexa Fluor 488 Phalloidin (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions, and PI. Thereafter, the samples were rinsed and visualized with a LSM 700 confocal laser-scanning microscope (Zeiss, Arese, Milano, Italy). Signals were recorded in the green channel for phalloidin (excitation 495 nm, emission 518 nm) and in red channel for PI (excitation 535 nm, emission 617 nm). Images from three randomly selected areas were acquired for each sample. Sequential optical sections of 0.1 μm in thickness were collected in sequence along the z-axis over the whole sample. The investiga-tor evaluating the presence of biofilm was blinded to the CRS severity, patient genotype, and bacterial identification data.

Propylthiouracile Tasting

To characterize individuals by genetic taste sensitivity to PROP, we used the suprathreshold procedure. Subjects rated

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Due to the strong TAS2R38 genotype-phenotype relationship, we performed further analysis by considering the phenotype, as well as the genotype, as representative of the receptor functionality. It should be noted that the complete absence of the functional PAV haplotype does produce hyposensitivity to PROP, whereas the presence of just one functional PAV haplotype is sufficient to restore function.

No relationship was observed between TAS2R38 and CRS severity. To evaluate the association between TAS2R38 functionality, QoL, and severity of the sinonasal disease, we compared SNOT-22, LK, and LM scores between PROP-sensitive and PROP-insensitive patients. Using Student t test, we observed no differences between tasters and nontasters (Table II). Furthermore, no correlation between PROP sensitivity and SNOT-22, LK, and LM was found by Pearson correlation test (Supporting Fig. S2).

We further compared the prevalence of asthma, allergies, and ASA syndrome (aspirin sensitivity, asthma and CRSwNP), the more commonly coexisting comorbidities in CRSwNP, among TAS2R38 genotypes in 84 patients. Chi-square analysis showed no statistically significant difference between PAV taster and AVI nontaster, suggesting no association between receptor functionality and CRS-related comorbidities ($\chi^2$: 6.113; $P = 0.295$).

Among the samples collected from the sinonasal mucosa, 63 were found positive for bacterial growth. Gram-negative bacteria were found in 20 of 63 (32%) patients, gram-positive bacteria in 36 of 63 (57%) patients, and both in 22 of 63 (24%) patients. In only one sample, we observed a mixed flora composed by Candida albicans together with S. aureus. The most common bacterium was Staphylococcus epidermidis, isolated in 20 of
63 (32%) patients. Fifteen cultures (24%) were positive for S. aureus, and 22 (35%) for Enterobacteriaceae (Klebsiella spp., Citrobacter koseri, Serratia marcescens).

**Nineteen polyps were found biofilm-positive.** Forty-three samples of nasal mucosa were tested for the presence of bacterial biofilms. Presence of biofilm was defined as evidence of immobile, attached bacterial cells, visible as clusters of microcolonies on the mucosal surface.29

Biofilms were associated with cultures positive for gram-negative organisms as Klebsiella, Citrobacter, Haemophilus, as well as with cultures positive for gram-positive organisms of the genus Kocuria or Staphylococcus (aureus and epidermidis).

Airway gram-negative infections are mainly associated with AVI haplotype. Eighteen of the 53 patients with available genotypes were affected by airway gram-negative infections. Of those, only two (11.1%) were PAV homozygous. The remaining (88.9%) patients were AVI homo- and heterozygous. Concerning the recurrence of gram-negative infections among the PAV/PAV patients, two of 13 (15.4%) were infected versus 16 of 40 (40%) with other genotypes, 2.6 times more (Table III). We did not identify a statistically significant correlation between PROP phenotype and infection by gram-negative bacteria.

**Biofilm formation is prevalent in CRS patients with nontaster phenotype.** As Table IV shows, we detected the presence of biofilms in the sinonasal mucosa of more than 60% (10 of 16) of patients with a nontaster phenotype but only in one-third (9 of 27) of the patients with a functional TAS2R38 phenotype (tasters or supertasters).

Among these patients, five were PAV/PAV patients and only one with sinonasal gram-negative bacteria (Fig. 1). Conversely, we found evidence of biofilm in 14

<table>
<thead>
<tr>
<th>TAS2R38 Genotype</th>
<th>Infection by Microorganisms</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Gram</td>
<td>Gram+</td>
</tr>
<tr>
<td>PAV/PAV</td>
<td>2 (15.4)†</td>
<td>7(53.8)</td>
</tr>
<tr>
<td>AVI/other</td>
<td>16 (40.0)</td>
<td>22 (55.0)</td>
</tr>
<tr>
<td>Total</td>
<td>18 (34.0)</td>
<td>29 (54.7)</td>
</tr>
</tbody>
</table>

*n(%) χ² = 7.508; P = 0.023.

patients with the AVI haplotype (homo- or heterozygous) among five having sinonasal gram-negative bacteria (Fig. 2). Although the difference is not statistically significant, the biofilm-associated infections are prevalent.

**Table III.** Comparison of Bacterial Infections Between TAS2R38 PAV Homozygous and Clustered AVI Hom- and Heterozygous (AVI/#)

<table>
<thead>
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<th>TAS2R38 Genotype</th>
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*n(%) χ² = 7.508; P = 0.023.

CRSwNP = chronic rhinosinusitis with nasal polyps.

**Table IV.** Detection of Bacterial Biofilm on Sinonasal Mucosa of CRSwNP Patients Stratified by TAS2R38 Phenotype.

<table>
<thead>
<tr>
<th>TAS2R38 Phenotype</th>
<th>Biofilm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nontasters</td>
<td>10 (62.5)*</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Tasters/Supertasters</td>
<td>9 (33.3)</td>
<td>18 (66.7)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (44.2)</td>
<td>24 (55.8)</td>
</tr>
</tbody>
</table>

*n(%) χ² = 3.485; P = 0.05.

CRSwNP = chronic rhinosinusitis with nasal polyps.
in patients with the AVI-nonfunctional haplotype (Supporting Table SI).

DISCUSSION
CRS is a multifactorial disease arising from the contribution of different etiological factors, although most of them are not definitively demonstrated. Recent in vitro research showed that the functional bitter taste receptor TAS2R38, expressed in the airway and blood cells, has a significantly increased response to quorum-sensing AHLs compared with the nonfunctional receptor. The hypothesis that a PAV/PAV genotype would be protective against gram-negative infections was supported by recent studies showing a statistically significant paucity of PAV/PAV genotypes compared to the expected distribution in CRS patients undergoing primary FESS. We also found a higher prevalence of the AVI/AVI at risk diplotype among the CRS patients, compared to the healthy population of the same geographic area. The association with PROP phenotypes is obviously less stringent because individuals carrying the AVI haplotype may be nontasters (AVI homozygous) or tasters (PAV/AVI heterozygous).

Tasting phenotypes, which are an expression of the receptor functionality, were strongly related to genotypes; however, as expected, the correlation between the genotype and the predicted phenotype is less than 100%. This finding is observed because the PROP phenotype is a complex genetic trait linked to more than a single gene. Indeed, whereas polymorphisms in the bitter taste receptor TAS2R38 explain the majority of phenotypic variation in the PROP sensitivity, other “modifier” genes could contribute to the responsiveness of thiourea compounds. This is demonstrated by misclassification of some nontaster homozygous individuals and variability in PROP scoring among individuals with the PAV haplotype. It has been shown that polymorphisms in both TAS2R38 and CA VI/Gustin genes accounted for up to 60% of the phenotypic variance in PROP bitterness and up to 40% in threshold value. Moreover, the responsiveness to PROP is associated with salivary levels of II-2 peptide and Ps-1 protein, which are products of the PRB1 gene.

Thus far, data in the literature has demonstrated a correlation between TAS2R38 genotype and biofilm formation in CRS patients only in vitro. To the best of our knowledge, this is the first study evaluating the role of TAS2R38 polymorphisms in the susceptibility of airway gram-negative infections, severity of CRSwNP, and sinonasal in vivo biofilm formation. Among known CRS contributing factors, bacteria significantly contribute to chronic mucosal inflammation. Although the role of bacteria in CRS is still unclear, infections by Staphylococcus aureus, coagulase-negative staphylococci, and anaerobes seem to dominate to various extents in different studies.

Gram-negative aerobic and facultative anaerobic bacteria are more frequently isolated from CRSwNP patients. Previous studies demonstrated that individuals with a nonfunctional T2R38 allele having significantly blunted NO and ciliary responses following exposure to gram-negative quorum-sensing molecules are infected with gram-negative bacteria, such as Pseudomonas aeruginosa, and develop CRS. Therefore these individuals are more likely to need surgery than those with functional receptor alleles.

Based on this hypothesis, we compared the microbiology of sinonasal mucosa of PAV/PAV patients to those with other genotypes. Our findings confirmed data in the literature in which gram-negative infections are mainly associated with the AVI haplotype.

Bacterial biofilms have been implicated as important features of sinonasal infections both in chronic rhinosinusitis sine nasal polyps (CRSsNP) and CRSwNP, with detection rates reaching 75% of cases, providing support to the proposed role in either the pathogenesis or persistence of CRS. Bacteria growing in slime-enclosed aggregates are much more resistant to antibiotics and immune response effectors when compared with planktonic cells promoting persistence of the infection. Moreover, biofilm might act as a reservoir of bacterial cells and/or products, causing an acute infection and/or a sustained stimulation for the host immune system, resulting in persistence of sinonasal mucosal inflammation seen in CRS.

Among hypotheses based on the concept that CRS results from a dysfunctional host–pathogen interaction, the biofilm hypothesis takes into consideration the most prominent environmental factors in CRS, namely, fungal and bacterial infections, whereas the immune barrier hypothesis focuses on specific host factors.

In particular, the biofilm hypothesis postulates that whereas biofilms are present in both healthy and inflamed subjects, the establishment of a pathogenic condition could be due to environmental and individual factors. The nature and extent of biofilm could be key factors for its pathogenic role. Under this point of view, polymicrobial biofilms in which the absolute quantity of each species is low may be balanced and compatible with the healthy state; instead, biofilms with lower microbial diversity may be related to chronic inflammation. The concept of microbial dysbiosis has been implicated in the development of various human diseases.

Conversely, the immune barrier hypothesis suggests that defects in the physical barrier, opposed at the mucosal interface against foreign pathogens or in the humoral immune response, might account for the susceptibility to CRS.

As previously mentioned, TAS2R38 receptors, which are expressed on the membrane of the ciliated cells, activate the innate immune defenses in response to bacterial quorum-sensing molecules.

The relevance of the T2R genetics in the individual’s susceptibility to CRS is further enhanced by the genome-wide association study of Mufana Endam et al. The authors found that the TAS2R38 polymorphism, I296V (the third SNP of the AVI/PAV haplotypes), as well as three other missense variants in TAS2R13 and
TAS2R49 genes, were associated both with CRS, with and without nasal polyps.

In addition, further T2R genes may play a role in sinonasal immune defense. T2R4 and T2R16 receptors, expressed in the cilia of human epithelial cells of the sinonasal mucosa, are able to trigger NO production by using the canonical taste-signaling pathway, as well as TAS2R38.14

Recent research provides evidence through a variety of methods for the expression of functional TAS2R38 receptors on neutrophils, monocytes, and myeloid cell lines.17 In particular, this research shows an appreciable variation of receptor expression among individuals. Expression of TAS2R38 on neutrophils indicates that the cells “sense” bacterial infection and up-regulate defense-relevant actions as a result. Therefore, T2R38 qualifies as a pathogen-associated molecular pattern-recognition receptor functioning as a receptor for AHLs on neutrophils. Nevertheless, the efficacy of neutrophils response to stimulation by AHLs could be quite different among individuals, even in the presence of a fully functional isoform, due to individual variation in the protein expression.

With this finding in mind, it is not surprising that T2R38 could contribute to the defense of sinonasal mucosa against bacteria with a tendency for biofilm formation. Adappa et al.19 demonstrated an inverse correlation between PTC (phenylthiocarbamide) taste sensitivity of CRS patients and in vitro biofilm-forming capacity of bacterial strains recovered from the patient’s nasal swabs. In our study, we have directly visualized bacterial biofilm formation in vivo on the sinonasal mucosa of CRSwNP patients. In agreement with Adappa et al.,19 CRSsNP patients with a PTC/PROP nontaster phenotype most frequently showed evidence of biofilm on sinonasal mucosa in comparison to taster and super-taster patients.

CONCLUSION
Despite the remarkable efforts invested in the past decades, CRS pathophysiology has not been thoroughly elucidated to date. In particular, we still do not have answers to fundamental questions of disease pathophysiology, such as why certain individuals are more susceptible to develop CRS than others, and why some individuals respond well to therapy, whereas others manifest a therapeutically recalcitrant disease or frequent bacterial exacerbations. Thus, if biomarkers can be identified that correlate with therapeutic success rates, they could be used to counsel patients toward improved outcomes.

We demonstrated a relationship between the presence of microbial biofilm in the sinonasal mucosa of CRSwNP patients and TAS2R38 receptor variants. In fact, by using CLSM imaging we detected bacterial biofilms more frequently in sinonasal specimens from TAS2R38 nontasters than in tasters or supertasters. The prevalence of biofilm-associated infections in nontaster patients confirms in vivo the negative association previously showed in vitro by Adappa et al.19 (i.e., an increased susceptibility for infections by biofilm-forming bacteria in individuals with the nonfunctional TAS2R38 phenotype). This gives further support to use the phenotypic test of oral sensitivity to PROP/PTC to predict the susceptibility to gram-negative infections, in particular to screen patients for genetic testing. Indeed, whereas the insensitivity to PROP is linked to AVI-nonfunctional homozygosity, the taster phenotype may deserve further discrimination between individuals with two copies of the PAV functional haplotype (potentially protected) and patients with any other genotype who are more susceptible to gram-negative infections.

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Authorship: E.C. has been involved in patient enrollment and FESS surgical procedure at the University Federico II, and also carried out the analysis of clinical data and wrote the manuscript. R.N. performed genetic analysis and was involved in acquisition, analysis, and interpretation of data and the writing of the manuscript. E.R. carried out the study of biofilms. M.R.C. has been involved in biofilm study and revision of the manuscript. E.G. enrolled the patients and was involved in sinus surgery at Monaldi-Ospedale dei Colli. P.C. and M.M. enrolled the patients and performed the phenotypic test of bitter sensitivity at Monaldi-Ospedale dei Colli. C.A.L. has made contributions to the conception and design of the study and carried out FESS sinus surgery at Monaldi-Ospedale dei Colli. C.A.L. contributed to study design. A.A.S. performed the microbiological analysis. L.G. provided substantial contributions to study design and statistical analysis of data, revised critically the manuscript, and gave final approval of the version to be published. E.C. and R.N. should be considered joint first author.

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