


# Chronic Rhinosinusitis: *T2r38* Genotyping and Nasal Cytology in Primary Ciliary Dyskinesia

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**Objectives:** Chronic rhinosinusitis (CRS) is a major hallmark of primary ciliary dyskinesia (PCD). We investigated the possible correlation between some severity markers of CRS and several clinical features of the disease. We further studied the bitter taste receptor *TAS2R38* polymorphisms to identify the genotypes associated with more severe disease.

**Methods:** We included 39 adult PCD patients with (CRSwNP) and without nasal polyposis (CRSsNP); a sample for nasal cytology was obtained and clinical cytological grading (CCG) was determined. The SNOT-22 and Lund-Mackay scores were recorded. A sample of DNA was extracted from peripheral blood to investigate *TAS2R38* polymorphisms.

**Results:** CRSwNP patients had features of more severe disease: indeed, they had statistically significantly higher frequency of previous sinus surgery, higher SNOT-22, LM scores, and CCG than CRSsNP patients. Upon genotyping of *TAS2R38* polymorphisms, we observed that the AVI-AVI genotype, associated to homozygous nonfunctional bitter *TAS2R38* receptor, was more prevalent among CRSwNP (100%) than in CRSsNP patients (0%); furthermore, AVI-AVI subjects showed statistically significantly worse SNOT-22 and CCG scores than PAV-PAV and PAV-AVI subjects. The group of AVI-AVI patients also had more frequent respiratory exacerbations, Gram-negative infections, and *Pseudomonas aeruginosa* colonization than PAV-PAV and PAV-AVI patients.

**Conclusion:** Our findings indicate for the first time that PCD patients with CRSwNP display a more severe disease than those with CRSsNP. Genotyping of *TAS2R38* polymorphisms demonstrated that in PCD patients, the AVI-AVI genotype is strikingly more prevalent among CRSwNP than in CRSsNP, while the PAV-PAV genotype might be protective against Gram-negative infections and respiratory exacerbations.

**Key Words:** bitter taste receptors, chronic rhinosinusitis, nasal cytology, primary ciliary dyskinesia, *TAS2R38*.

**Level of Evidence:** 3

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## INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare (about 1:10,000 individuals) inherited ciliopathy (MIM 242650) characterized by recurrent upper and lower respiratory tract infections due to ciliary dysfunction. Impaired mucociliary clearance leads to oto-sino-pulmonary diseases and organ laterality defects in ~50% of cases. Symptoms usually begin early in life and include neonatal respiratory distress (NRD) (with >80% frequency in

full-term neonates), chronic nasal discharge, and wet cough. The disease typically worsens with aging and recurrent lung infections and inflammation lead to bronchiectasis and progressively decreased lung function.

Chronic rhinosinusitis (CRS) is a major hallmark of PCD, especially in adulthood, affecting more than 70% of patients, in comparison to approximately 10% in the general population. Opacified paranasal sinus on computed tomography (CT) and sinus hypoplasia or aplasia are common features.<sup>1</sup> CRS has a negative impact on the quality of life,<sup>2,3</sup> particularly because PCD patients experience more common olfactory impairment than those with non-PCD sinusitis.<sup>4</sup> Therefore, paranasal sinuses represent a crucial zone that may constitute a potential bacterial reservoir for recurrent bacterial lung infections as demonstrated by a high prevalence of simultaneous sinus and lung colonization with identical pathogens.<sup>5</sup>

The bacterial flora of PCD sinuses has been poorly investigated but *Haemophilus influenzae* seems to be the most frequent pathogen in the nasal cavity, followed by *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.<sup>6</sup> The same pathogens can contribute to lung disease in PCD and particular attention must be paid to *P. aeruginosa* because of its ability to cause lung inflammation and destruction; its presence seems indeed to correlate with increased frequency of

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respiratory exacerbations, poorer respiratory function, and globally with more severe disease.<sup>7</sup>

Whenever medical therapy fails in PCD patients with CRS (sinonasal irrigation with saline, topical steroids, and long-term antibiotics), functional endoscopic sinus surgery (FESS) with adjuvant therapy improves the quality of life; can eradicate bacteria from sinuses and lungs and ultimately stabilize lung function.<sup>8</sup>

CRS is usually subdivided into two types, that is, CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP). CRSwNP is clinically more challenging, as it tends to be more severe, recurrent, and associated with other comorbidities.

Besides to phenotypic distinction between CRSsNP and CRSwNP, the importance of endotyping eosinophilic and noneosinophilic mediated CRS was recently recognized because eosinophilic CRS generally shows a poor response to medical and surgical treatments in comparison to noneosinophilic mediated CRS.<sup>9</sup> Although nasal cytology is an easy method to assess nasal inflammation and disease-specific cellular features, limited studies on CRS are available. While in PCD, airway inflammations are dominated by neutrophils infiltration,<sup>10</sup> nasal cytology in these patients was never carried out previously.

In patients with CRSwNP, frequent olfactory and smell impairment occur. Nasal polyps are a physical barrier to odorant molecules reaching the olfactory epithelium, whereas edematous mucosa also contributes to nasal obstruction.<sup>11</sup> Adult PCD patients have nasal polyps in 15%–56% of cases.<sup>12</sup>

In addition to an olfactory defect, patients with CRS show decreased gustatory function, particularly pronounced for bitter taste.<sup>13,14</sup> In the CRS, T2R38 is the best-studied extraoral bitter taste receptor that seems to be involved in the regulation of innate immunity responses.<sup>15</sup>

The *TAS2R38* gene encoding T2R38 has two common haplotypes, one encoding a functional receptor and another encoding a nonfunctional receptor. The functional T2R38 receptor contains proline (P), alanine (A), and valine (V) residues, while nonfunctional T2R38 contains alanine (A), valine (V), and isoleucine (I). Loss of the valine in the AVI variant is responsible for the impairment of receptor activation. The following haplotypes influence the perception of bitter taste: PAV–PAV are “supertasters,” PAV–AVI have variable intermediate levels of taste, while AVI–AVI are “nontasters.”

Recent reports suggest that the genetic variability of the *TAS2R38* bitter taste receptor, which is expressed in the cilia of epithelial cells of the nasal–sinus mucosa, is associated with susceptibility to upper respiratory tract infections and the development of CRS.<sup>15</sup> *TAS2R38* receptor expression in tissues of patients with CRS is increased and single nucleotide polymorphisms (SNPs) in *TAS2R38* gene have been shown to correlate with Gram-negative sinusitis.<sup>16</sup> Other lines of evidence<sup>17</sup> also suggested that *TAS2R38* genotyping may predict surgical FESS outcomes in CRS.

The present study aimed at evaluating several clinical features and markers of CRS severity in a group of PCD subjects suffering from CRS with and without nasal

polyposis. Based on the hypothesis that *TAS2R38* genotype correlates with CRS disease severity, we further studied the possible correlation between *TAS2R38* genotype and CRS-related factors (comorbidities, bacterial infections and colonization, number of surgeries, nasal cytology, etc.), as well as markers of CRS severity, such as SNOT-22 and LM score, in a group of PCD subjects suffering from CRS.

## PATIENTS AND METHODS

We recruited adult PCD patients that were followed at the Center for Rare Diseases, Unit of Respiratory Diseases of the Policlinico Hospital, Milan. The cohort had a definitive PCD diagnosis based on the characteristic clinical phenotype and ciliary ultrastructural defects, abnormal ciliary function, or a genetic mutation recognized to cause PCD.

All patients were subjected to otolaryngological evaluation with nasal endoscopy; CRS was diagnosed according to the last European guidelines.<sup>18</sup>

On the basis of clinical examination, medical history, nasal endoscopy, and sinus CT (Lund-Mackay [LM] score), we included in our study two subgroups of CRS patients: CRSwNP ( $n = 23$ ) and CRSsNP ( $n = 16$ ).

Medical history was focused on symptoms indicative of CRS (nasal discharge–postnasal drip, nasal congestion, and/or decreased sense of smell, with or without facial pain).<sup>19</sup> Previous sinonasal surgical procedures, allergic status, and bronchial asthma, the presence of bronchiectasis and the recurrence of infections (number of respiratory exacerbations:  $<2$  exacerbations per year or  $\geq 2$  per year) were also evaluated. Data were recorded in clinically stable patients, free from acute respiratory infections for at least 30 days.

### CRS disease severity

The more recent TC or nuclear magnetic resonance exam available were considered for LM staging; each sinus group is graded between 0 and 2 (0: no abnormality, 1: partial opacification, 2: total opacification). The ostiomeatal complex is scored as: 0 (not obstructed) or 2 (obstructed). A total score of 0–24 is possible and each side can be considered separately (0–12).<sup>20</sup>

Patients filled out SNOT-22 questionnaire that is the commonly used instrument for patient-reported CRS outcomes. It rates 22 different symptoms from 0 (no problem) to 5 (problem as bad as it can be) related to rhinological, ear, facial, general, physical, and psychological domains. The scores range from 0 to 110 with high scores indicating greater symptoms.<sup>21</sup>

A sampling for nasal cytology was performed out of respiratory exacerbations by anterior rhinoscopy using a speculum and adequate lighting. Sampling was carried out at the average portion of the lower turbinate. Samples were immediately swiped on the central area of a slide, air-dried, and smeared with May Grunwald-Giemsa.<sup>22</sup> Slides were observed at different magnification under optical microscope and not less than 50 fields were evaluated at high magnification ( $\times 1000$ ), in order to find

neutrophils, eosinophils, mast cells, bacteria, etc. Moreover, a clinical cytological grading (CCG), which is a score based on both nasal cytological findings and comorbidities, including asthma, allergy, and acetylsalicylic acid sensitivity<sup>23</sup> was calculated. A global score between 1 and 3 is considered low grade, 4 and 6 moderate, and >7 severe.<sup>24</sup>

### TAS2R38 genotyping

TAS2R38 polymorphisms were investigated on DNA extracted from peripheral blood. Briefly, DNA was obtained using the Isohelix extraction protocol-DNA isolation kit (Cell Projects, Kent, UK); genotypes of three TAS2R38 SNPs (rs1726866, rs713598, and rs10246939) were performed using the TaqMan probe-based assays

(Applied Biosystems, Foster City, CA). Participants were classified as heterozygous PAV-AVI, homozygous PAV-PAV, and homozygous AVI-AVI.

### Lung Infection Status

We considered the modified Leeds criteria to define chronic colonization when >50% sputum cultures of the preceding 12 months were positive for a specific pathogen, intermittent colonization when ≤50% sputum cultures of the preceding 12 months were positive for a specific pathogen, or free of colonization and infection when no growth occurred in the previous 12 months.<sup>25</sup>

All recruited patients signed an informed consent and the Ethical Committee of the Hospital approved the protocol.

Table I.  
Demographic and Clinical Characteristics in PCD Subjects With CRSwNP and CRSsNP.

	CRSwNP <i>n</i> = 23	CRSsNP <i>n</i> = 16	<i>p</i> -Value
Male gender, <i>n</i> (%)	11 (47.8)	9 (56.2)	0.74
Age (years, mean [SD])	39.4 (14.2)	36.9 (15.8)	0.60
Situs inversus, <i>n</i> (%)	13 (56.5)	10 (62.5)	0.75
Neonatal respiratory distress, <i>n</i> (%)	5 (21.7)	5 (31.2)	0.71
Consanguinity, <i>n</i> (%)	4 (17.4)	2 (12.5)	1.00
Allergy, <i>n</i> (%)	7 (30.4)	4 (25)	1.00
Asthma, <i>n</i> (%)	4 (17.4)	6 (37.5)	0.26
Previous sinus surgery, <i>n</i> (%)	17 (73.9)	2 (12.5)	<0.01*
SNOT-22 score, mean (SD)	55.7 (19.1)	35.5 (17.7)	<0.01*
LM score, mean (SD)	18.9 (6.1)	13 (9.9)	0.03*
Clinical cytological grading, mean (SD)	4.8 (1.3)	2.7 (1.2)	<0.01*
Respiratory exacerbations ≥2/year, <i>n</i> (%)	15 (65.2)	13 (81.2)	0.47
Gram-negative infections	15 (65.2)	6 (37.5)	0.11
PA colonization, <i>n</i> (%)	9 (39.1)	3 (18.7)	0.29

\*Statistically significant results.

CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; LM = Lund-Mackay; PA = *Pseudomonas aeruginosa*; PCD = primary ciliary dyskinesia.

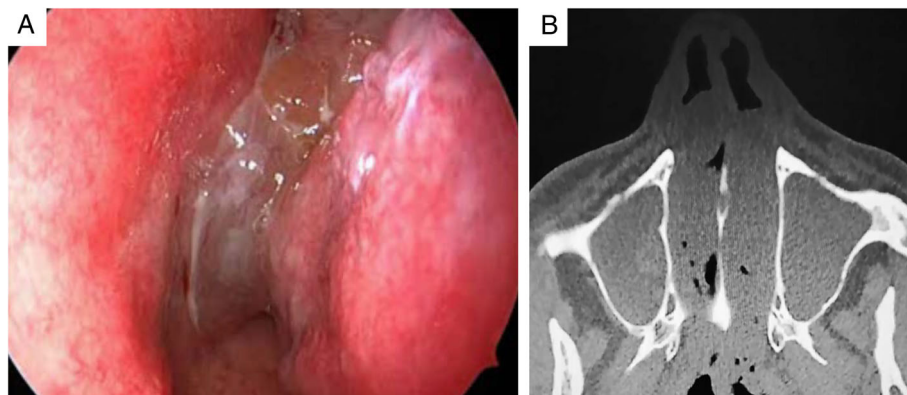


Fig. 1. (A) Typical nasal endoscopy of a PCD patient with relapsing CRSwNP where it can be observed a complete obliteration of the left middle meatus by nasal polyps covered with dense purulent secretions; (B) axial CT scan of a patient with relapsing CRSwNP documenting complete maxillary and middle meatus opacification on both sides. CT = computed tomography; CRSwNP = chronic rhinosinusitis with nasal polyps; PCD = primary ciliary dyskinesia.



## Statistical Analysis

Demographic and clinical characteristics were described by mean values with SDs for normally distributed continuous data or as absolute frequency and percentages for categorical data. Differences between percentages were tested by Fisher test while those between mean values by analysis of variance analysis; correlation analyses were performed with the Pearson's test; statistical significance was estimated for  $p < 0.05$ . Statistical analysis was performed using the R software.

## RESULTS

Thirty-nine PCD patients suffering from CRS were enrolled in the present study, subdivided into 23 cases with nasal polyposis (CRSwNP) and 16 without nasal polyposis (CRSsNP). Patients' age ranged from 19 to 70 years.

Relevant clinical data are reported in Table I. Allergic sensitization was diagnosed in 11/39 (28.2%) cases, asthma in 10/39 (25.6%), and aspirin intolerance in 2/39 (5.1%). Nineteen PCD with CRS patients (48.7%) had undergone previous sinus surgery.

No significant differences were observed between the two groups for what concerns the age and the prevalence of situs inversus, NRD, consanguinity, allergy, or asthma, but the CRSwNP group was characterized by a significant increase in the frequency of previous sinus surgery in comparison to CRSsNP group (73.9% vs. 12.5%, respectively,  $p < 0.01$ ).

CRSwNP patients had features of more severe disease: indeed, mean SNOT-22 score was significantly higher in CRSwNP relative to CRSsNP patients ( $55.7 \pm 19.1$  vs.  $35.5 \pm 17.7$ ,  $p < 0.01$ ), mean LM score was  $18.9 \pm 6.1$  and  $13 \pm 9.9$  in CRSwNP and CRSsNP ( $p = 0.03$ ), respectively.

Figure 1 shows a typical picture of nasal endoscopy and sinus CT in an adult patient with PCD.

Nasal cytology also showed a statistically significant difference between CRSwNP and CRSsNP: CCG was  $4.8 \pm 1.3$  in CRSwNP versus  $2.7 \pm 1.2$  in CRSsNP,  $p < 0.01$ . Altogether, the predominant inflammatory cells at nasal cytology were neutrophils (38/39 patients corresponding to 97.4%); eosinophils were observed in 11/39 patients (28.2%), while in some cases (10/39,

corresponding to 25.6%), a mixed inflammation (neutrophils and eosinophils) was found; bacteria were also frequently observed in association to neutrophils (15/39, corresponding to 38.5%).

Most CRSsNP patients had low-grade CCG, while CRSwNP had medium or high CCG score (see Table II).

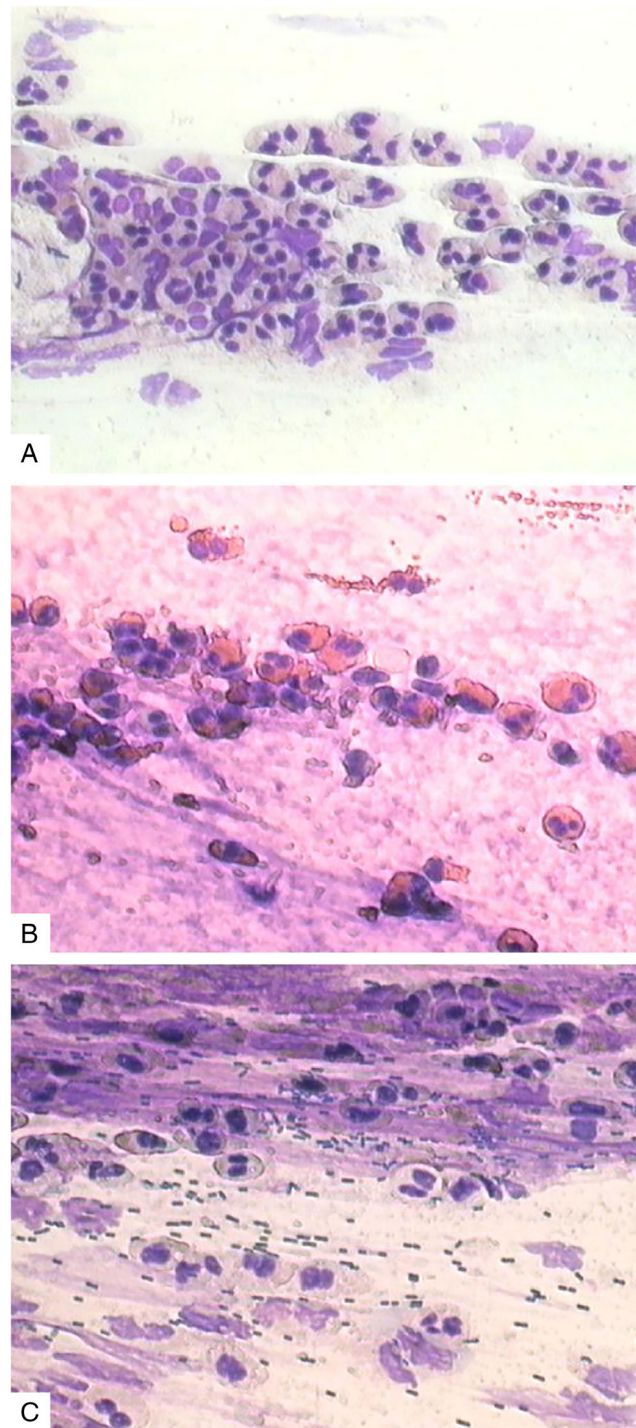


Fig. 2. Examples of nasal cytology in PCD: (A) the inflammatory picture is dominated by neutrophils; (B) a mixed inflammation with neutrophils and eosinophils; (C) neutrophils and associated bacteria. PCD = primary ciliary dyskinesia.

	CRSwNP $n = 23$	CRSsNP $n = 16$	$p$ -Value
CCG, mean $\pm$ SD	$4.8 \pm 1.3$	$2.7 \pm 1.2$	$<0.01^*$
CCG score			
Low ( $\leq 3$ )	5 (33.3)	10 (66.7)	0.02*
Medium <sup>4-6</sup>	16 (76.2)	5 (23.8)	$<0.01^*$
High ( $\geq 7$ )	2 (100)	0 (0)	0.5

\*Statistically significant results.

CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; PCD = primary ciliary dyskinesia.

Figure 2 shows an example of nasal cytology in a patient with PCD.

Concerning the prevalence of frequent respiratory exacerbations ( $\geq 2$ /year), Gram-negative infections or PA colonization, no statistical differences between CRSwNP and CRSsNP subjects were found, albeit a trend of greater prevalence of Gram-negative infections and PA colonization was observed in CRSwNP compared to CRSsNP (65.2% vs. 37.5% and 39.1% vs. 18.7%, respectively).

Genetic data on *TAS2R38* polymorphisms were available for 27 PCD patients. Upon genotyping of *TAS2R38* polymorphisms, the prevalence of PAV–PAV subjects with PCD was 25.9%, while that of PAV–AVI and AVI–AVI was 51.9% and 22.2%, respectively. The AVI–AVI genotype was strikingly more prevalent among CRSwNP (100%) than among CRSsNP patients (0%).

Table III shows the prevalence of clinical characteristics within the three groups: PAV–PAV patients were less likely to need surgical intervention for CRS compared to AVI–AVI and PAV–AVI: indeed, 83.3% of AVI–AVI and 50% of PAV–AVI underwent previous sinus surgery, compared to 42.8% only of PAV–PAV subjects, although these values did not reach statistical significance ( $p = 0.35$ ).

Significant statistical differences were observed concerning SNOT-22 and CCG ( $p = 0.03$  and  $p = 0.02$ , respectively), with homozygotes for the functional *TAS2R38* having a significantly better rhinological quality of life as assessed by SNOT-22 than PAV–AVI or AVI–AVI individuals. In addition, AVI–AVI subjects showed worse CCG than PAV–PAV and PAV–AVI.

The whole group of AVI–AVI patients (100%) had more than two respiratory exacerbations–year.

Similarly, Gram-negative infections were mainly associated with AVI–AVI or PAV–AVI genotypes: only 2 out of 14 patients (14.3%) were PAV–PAV homozygous; the remaining 12 patients were AVI homo- and heterozygous (85.7%).

None of the patients with the PAV–PAV genotype had PA colonization.

Through correlation analyses, a significant relationship was found between SNOT-22 and LM score ( $p < 0.01$ ,  $r = 0.59$ ), between SNOT-22 and nasal CCG ( $p < 0.01$ ,  $r = 0.74$ ), and between LM score and nasal CCG ( $p < 0.001$ ,  $r = 0.57$ ).

CRS severity was not correlated to any characteristics of PCD (i.e., nNO, CBF, and ultrastructural phenotype).

We did not find any correlations between parameters of CRS severity, such as SNOT-22 or LM score, and age ( $p = 0.75$  and  $p = 0.17$ , respectively), or between frequent respiratory exacerbations and age ( $p = 0.16$ ). Frequent respiratory exacerbations correlated neither with SNOT-22 nor with LM score ( $p = 0.43$  and  $p = 0.5$ , respectively).

## DISCUSSION

CRS is recognized as a multifactorial disorder where several factors, such as mucociliary function, anatomical features, immune response to infections, and respiratory allergies may be involved.

Several well-characterized genetic syndromes are associated with CRS, including the PCD,<sup>26,27</sup> a rare hereditary disease due to a malfunctioning of respiratory ciliated cells. Impaired mucociliary clearance in paranasal sinuses leads to bacterial colonization, recurrent infections, inflammation, and development of CRS. Inflammation of the upper airways may facilitate infections of the lower airways as well, with development of bronchiectasis and decreased lung function.

Nearly all PCD patients have otorhinolaryngological symptoms since childhood: a rhinitis with chronic nasal congestion can be observed in almost 100% of patients, out of which  $\geq 70\%$  are affected by CRS. Nasal polyposis in patients with PCD is more common compared with the general population; it is rare in childhood (approximately

Table III.  
Distribution of *TAS2R38* Haplotypes Polymorphisms in PCD Patients,  $n$  (%).

	PAV/PAV $n = 7$	PAV/AVI $n = 14$	AVI/AVI $n = 6$	$p$ -Value
CRSwNP	5 (71.4)	5 (35.7)	6 (100)	0.02*
CRSsNP	2 (28.6)	9 (64.3)	0 (0)	0.02*
Situs inversus	5 (71.4)	9 (64.3)	2 (33.3)	0.4
Neonatal respiratory distress	0 (0)	4 (28.6)	3 (50)	0.13
Previous sinus surgery	3 (42.8)	7 (50)	5 (83.3)	0.35
SNOT-22 score, mean (SD)	44.3 $\pm$ 14.5	44.5 $\pm$ 19.7	67.7 $\pm$ 17.2	0.03*
LM score, mean (SD)	14.9 $\pm$ 7.4	15.2 $\pm$ 7.9	18.2 $\pm$ 6.8	0.68
Clinical cytological grading, mean (SD)	3.4 $\pm$ 1.5	3.6 $\pm$ 1.6	5.7 $\pm$ 1.4	0.02*
Respiratory exacerbations $\geq 2$ /year	4 (57.1)	9 (64.3)	6 (100)	0.24
Gram-negative infections	2 (28.6)	7 (50)	5 (83.3)	0.2
PA colonization	0 (0)	4 (28.6)	2 (33.3)	0.34

\*Statistically significant results.

CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; LM = Lund-Mackay; PA = *Pseudomonas aeruginosa*; PCD = primary ciliary dyskinesia.

15%),<sup>28</sup> but in adulthood, it ramps up to 56% of subjects.<sup>12</sup> Sinonasal disease may persist and worsen in adulthood.<sup>29</sup> A recent study<sup>30</sup> identifies sinonasal disease as a primary cause of decreased quality of life in these patients. Effective treatment of CRS starting from childhood is crucial to delay lung disease.

The present study reveals that CRSwNP patients with PCD have significantly higher SNOT-22 and LM scores than CRSsNP patients, in agreement with the idea that inflammatory nasal polyps are seen as an end point of inflammation.<sup>31</sup> The CRSwNP group had also undergone an elevated number of previous sinus surgery in comparison to the CRSsNP group. Altogether, these findings indicate that in PCD CRwNP is a more severe disease than CRSsNP.

Other Authors<sup>2,32</sup> have already emphasized that CRSwNP is clinically more severe than CRSsNP, unresponsive to currently established treatments or endoscopic sinus surgery and is associated with other comorbidities. However, here we demonstrate, for the first time that clinical CCG, which is a composite score based on nasal cytology and comorbidities, is higher in PCD patients with CRSwNP than in CRSsNP patients.

Although nasal cytology is not performed routinely to study cellular profiling of CRS because of its overall low estimated accuracy,<sup>33</sup> it represents a useful tool to assess the CRS endotypes (eosinophilic and non-eosinophilic CRS). In fact, in addition to the phenotypic distinction between CRSwNP and CRSsNP, it is currently important to distinguish between eosinophilic and noneosinophilic mediated CRS, because eosinophilic CRS is generally associated with a poor response to medical and surgical therapies. The CCG has been proposed to better define the management strategy, identify a prognostic index of relapse, and adopt a personalized medical approach.<sup>24</sup> A CCG threshold >4 has been proposed to suspect an olfactory impairment in CRSwNP patients.<sup>23</sup> In PCD patients the most frequently inflammatory cells found in nasal cytology were neutrophils, but a considerable percentage of patients showed eosinophils or a mixed inflammation with both eosinophils and neutrophils. These endotypes could have different responses toward drugs (e.g., new biological drugs) and to surgery for CRS, so nasal cytology could be a useful easy method to personalize treatments.

In agreement with other studies,<sup>12</sup> the most frequently found bacteria in sputum samples were *Haemophilus influenzae* and *Staphylococcus aureus*, followed by *Streptococcus pneumoniae* and *Escherichia coli*. PA colonization was found in 30.8% of subjects; this prevalence is similar to that found by Wijers et al.<sup>34</sup> A trend of greater prevalence of Gram-negative infections and *Pseudomonas aeruginosa* colonization has emerged in CRSwNP in comparison to CRSsNP patients, even if it did not reach statistical significance.

Furthermore, similarly to previously reported by Barbato et al.,<sup>35</sup> the severity of CRS symptoms did not correlate with ultrastructural defects in PCD, nasal nitric oxide, or genotype.

The taste is frequently compromised in CRS; recent evidences showed that extraoral bitter taste receptors

(T2R) may play a role in the pathophysiology of chronic airway inflammation, such as CRSwNP and CRSsNP. Genetic differences and individual's sensitivity to bitter taste may affect development of CRS and susceptibility to infections. Immunochemical analyses using anti-TAS2R38 antibodies showed statistically significantly higher *TAS2R38* expression in nasal mucosa of patients with CRS in comparison to control subjects, as well as its close correlation with the severity of LM score.<sup>36</sup>

The TAS2R38 bitter taste receptors are not only expressed within the cilia of the respiratory epithelium but they are also present on the extracellular membrane of peripheral blood neutrophils, monocytes, and lymphocytes.<sup>37,38</sup> TAS2R38 would act as an immune sentinel active in detecting pathogens, in modulating immune mechanisms and in regulating the balance between commensalism and pathogenicity of bacteria.

An SNP in the *TAS2R38* gene has been recently shown to correlate with Gram-negative sinusitis<sup>16</sup>: in particular, *TAS2R38* AVI–AVI patients in which the receptor is nonfunctional are more susceptible to Gram-negative bacterial infections and have a higher prevalence of biofilm-forming sinonasal bacteria.<sup>17,39</sup> Gram-negative bacteria quorum sensing molecules, such as *N*-(3-oxododecanoyl)-L-homoserine lactone (AHL-12), activate TAS2R38 receptors and PAV–PAV receptor responds to *Pseudomonas* quorum sensing molecules more efficiently than PAV–AVI or AVI–AVI in an in vitro study.<sup>15</sup> These genotypic differences in the bitter taste receptors that influences the response toward bacterial infections may have a crucial importance, particularly in PCD patients, who are already prone to recurrent respiratory infections because of deficient–absent mucociliary clearance as a protective mechanism.

In the present study, genotyping of *TAS2R38* polymorphisms in PCD patients demonstrated a distribution of prevalence comparable to the literature data, showing that in Caucasian populations the known distribution of PAV–PAV, PAV–AVI, and AVI–AVI genotypes is approximately 20%, 50%, and 30%, respectively.<sup>40</sup>

The AVI–AVI genotype turns out to be more prevalent among CRSwNP than in CRSsNP patients. PAV–AVI and AVI–AVI patients previously underwent more frequent sinus surgery than PAV–PAV subjects and showed higher SNOT-22 and CCG scores than PAV–PAV, thus demonstrating that these genotypes are associated with more severe CRS.

The whole group of AVI–AVI patients had more than two exacerbations–year and underwent more frequent Gram-negative infections and PA colonization than PAV–PAV, demonstrating that PAV–PAV genotype might be protective against Gram-negative infections, in agreement with previous studies.<sup>15,16</sup>

A limitation of our study is the small number of patients that were able to recruit; failure to correlate sinus disease severity with PCD features (ciliary ultrastructure, nasal nitric oxide, and genotype) also likely reflects our small sample size. Another limitation of our study is the lack of a control group. Nevertheless, it can be considered that PCD is a rare disease. Thus, only large-scale, multicenter studies may allow further



information on the possible correlations between clinical features of CRS and phenotypes–genotypes of PCD.

## CONCLUSION

Our study emphasizes the need for better stratification of patients with PCD that could allow direct efforts and intensive care toward individuals that require more intensive treatments.

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