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Primary Ciliary Dyskinesia: The Impact of Taste Receptor (*TAS2R38*) Gene Polymorphisms on Disease Outcome and Severity

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Keywords

Primary ciliary dyskinesia · Bitter taste receptors · *TAS2R38* polymorphisms · Respiratory infections · Respiratory exacerbations

Abstract

Background: Primary ciliary dyskinesia (PCD) is a rare genetic disease leading to recurrent respiratory infections of upper and lower airways. Chronic rhinosinusitis (CRS) and bronchiectasis are very common in PCD patients. Recently, it has been shown the presence of taste receptors in respiratory tract and the possible involvement of bitter taste receptor *TAS2R38* gene in susceptibility to respiratory infections and rhinosinusitis. ***Objective:*** Aim of this study was to evaluate the frequency of *TAS2R38* polymorphisms in PCD patients and their possible correlations with clinical outcomes of the disease. ***Methods:*** Genetic and phenotypic data of 35 PCD patients were collected. Clinical evaluation included neonatal respiratory distress (NRD) at birth, presence of situs inversus, CRS, and bronchiectasis. We also measured the number of respiratory infections per year and the relevant pathogens, Lund-Mackay score, FEV_1, and modified Bhalla score. With regard to genetics data, 3 polymorphisms (rs1726866, rs713598, and rs10246939) within *TAS2R38* gene were analyzed and the patients were classified as PAV/PAV, PAV/AVI, and AVI/AVI. ***Results:*** A significant difference in the distribution of *TAS2R38* haplotype between patients with and without NRD emerged (p value = 0.01). A lower percentage of PAV/PAV individuals showed frequent respiratory exacerbations (≥2/year) (p value = 0.04) compared to those with AVI/AVI and AVI/PAV haplotypes. Moreover, no patients homozygous for PAV/PAV haplotype presented chronic colonization by *Pseudomonas aeruginosa,* thus supporting the possible role of *TAS2R38 gene* in susceptibility to respiratory infections. ***Conclusions:*** Here, we report, for the first time, a possible association of *TAS2R38* polymorphisms with PCD phenotype.

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Introduction

Primary ciliary dyskinesia (PCD) is a rare (estimated incidence 1:15,000–20,000) genetic disease (MIM #244400) characterized by recurrent respiratory infections of both the upper and lower airways due to alteration of cilia and flagella. Since cilia seem to play an important role in clearing fetal fluid from the lungs, many full-term neonates present with respiratory distress. Therefore, PCD patients may develop symptoms already at birth or in early childhood.

Clinical manifestations include (a) situs inversus totalis, a mirror-image reversal of all visceral organs, occurring in about 50% of cases; (b) chronic rhinosinusitis (CRS) and nasal polyposis associated with hyposmia or anosmia, very frequently observed during adult age [1, 2], altering the smell and taste perception and significantly impacting the quality of life [3]; and (c) recurrent respiratory infections and development of bronchiectasis. The impaired mucociliary clearance predisposes to recurrent respiratory exacerbations (RE) and leads to chronic lung disease, so accelerating the decline of lung function [4]. With regard to recurrent airway infections, these are caused by different microorganisms, such as *Pseudomonas aeruginosa*, whose colonization is age-dependent and associated with more severe disease [5].

Recently, taste receptors have been identified in a variety of extraoral tissues, including airways epithelia, lungs, brain, testes, and gut. Although the exact role of taste receptors in these tissues is still poorly understood, possible involvement in innate immunity or insulin production has been reported, and they may thus represent potential new targets for diseases [6]. For example, the presence of bitter taste receptors (T2Rs) in the airways might be linked to responses from inhaled foreign substances and bacteria. These receptors are expressed in airway ciliated epithelia, where their stimulation activates a calcium-dependent nitric oxide production, which directs local bactericidal defense and increases the ciliary beat [7, 8].

Among bitter taste receptors, T2R38 receptor is the most studied one. DNA variations in *TAS2R38* gene encoding for T2R38 receptor are responsible for individual differences in bitter taste perception. Three single-nucleotide polymorphisms (SNPs) result in relevant amino acid substitutions at specific protein positions (i.e., A49P, A262V, and V296I), giving rise to PAV (the taster variant) and AVI (the non-taster variant). Individuals with PROP sensitivity to T2R38 agonists (i.e., phenylthiocarbamide or 6-propyl-2-thiouracil-PROP) carry 1 or 2 dominant alleles (PAV/PAV or PAV/AVI), whereas those without PROP sensitivity are mainly AVI/AVI [9, 10]. Genetic variation in *TAS2R38* gene may also play a role in the susceptibility to some respiratory infections, diseases, and their clinical outcomes [6].

It has been demonstrated that T2R38 receptor in airway cilia might be activated by molecules (acyl-homoserine lactone quorum-sensing) secreted by *P. aeruginosa* and other gram-negative bacteria, playing a role in innate antimicrobial effects and killing the opportunistic respiratory pathogens [8]. Moreover, PAV/PAV subjects show an increased ability to detect, clear, and kill gram-negative bacteria as compared to PAV/AVI and AVI/AVI ones. The final outcome is a reduction of gram-negative bacterial infections in PAV/PAV individuals.

More recently, a relationship between *TAS2R38* gene and CRS has been described. In particular, AVI/AVI homozygous patients show a higher prevalence of biofilm-forming sinonasal bacteria and a greater risk for CRS requiring surgical intervention [11–14]. Moreover, a study on cystic fibrosis' patients showed that rhinological symptoms are less severe in PAV/PAV patients as compared to PAV/AVI and AVI/AVI ones [15].

Up to now, the association of *TAS2R38* polymorphisms with PCD phenotype has never been tested and reported. In this study, for the first time, we look at the distribution of *TAS2R38* genotypes in a cohort of PCD patients and verify the possible correlations between these genotypes and clinical features.

Patients and Methods

Patients Recruitment

This study included 35 subjects with a diagnosis of PCD attending the Regional Center for Rare Diseases, Unit of Bronchopneumology at the Policlinico Hospital located in Milan. The ethical committee of the hospital approved the protocol, and informed consent was obtained from all patients; parental consent was obtained for patients <18 years of age.

Genetic analysis (i.e., *TAS2R38* polymorphisms) has been carried out on DNA extracted from peripheral blood. Briefly, DNA was obtained using Isohelix extraction protocol-DNA isolation kit (Cell Projects, Kent, UK); genotypes of 3 *TAS2R38* SNPs (rs1726866, rs713598, and rs10246939) were defined using the TaqMan probe-based assays (Applied Biosystems, Foster City, CA, USA). Participants were classified as PAV/AVI heterozygous, PAV/PAV homozygous, and AVI/AVI homozygous. Spirometry data were obtained in patients older than 5 years of age, according to the ATS/ERS guidelines [16].

Table 1. Clinical features and parameters of PCD patients enrolled in the study divided according to *TAS2R38* taste receptor gene polymorphisms

	TAS2R38 haplotype			*p* value
	PAV/PAV	PAV/AVI	AVI/AVI	
Females, *n* (%)	3 (16)	9 (47)	7 (37)	0.06
Age, mean (SD)	27.3 (16.2)	28.3 (15.9)	37.7 (20.3)	0.36
Clinical features				
NRD, *n* (%)	0 (0)	6 (55)	5 (45)	0.01[a]
Situs inversus, *n* (% yes)	7 (33)	11 (53)	3 (14)	0.30
CRS, *n* (% yes)	5 (24)	10 (47)	6 (29)	0.89
Bronchiectasis, *n* (% yes)	5 (21)	12 (50)	7 (29)	0.51
Asthma, *n* (% yes)	0 (0)	4 (50)	4 (50)	0.09
RE ≥2/year, *n* (% yes)	4 (17)	11 (48)	8 (35)	0.04[a]
PA colonization, *n* (% yes)	0 (0)	6 (60)	4 (40)	0.08
Clinical parameters				
Lund-Mackay score, mean (SD)	12.6 (9.4)	15.3 (9.3)	17.5 (7.0)	0.58
FEV_1, mean (SD)	92.2 (17.7)	78.5 (17.9)	78.4 (19.1)	0.33
Modified Bhalla score, mean (SD)	9.8 (9.8)	12.3 (11.4)	15.2 (9.4)	0.58

Data are shown as *n* (%) or mean (SD). SD, Fisher test, and ANOVA were used to test differences between percentages and means. PCD, primary ciliary dyskinesia; NRD, neonatal respiratory distress; CRS, chronic rhinosinusitis; RE, respiratory exacerbations; PA, *Pseudomonas aeruginosa*. [a] Statistically significant results.

Medical History

A detailed anamnesis was collected focusing on the recurrence of infectious episodes and the mean number of exacerbations that resulted, on average, 2 per year. Thus, patients were grouped into 2 classes: those with <2 exacerbations per year and those with ≥2 exacerbations per year (frequent exacerbations). Moreover, additional clinical features such as CRS, previous sinonasal surgical procedures, allergic status and bronchial asthma, and the presence of bronchiectasis were also recorded.

Clinical variables tested were grouped as follows: 7 clinical characteristics, including neonatal respiratory distress (NRD), situs inversus, CRS, bronchiectasis, asthma, frequent RE (≥2 per year), and *P. aeruginosa* colonization, and 3 measured clinical parameters, including the Lund-Mackay score used for radiological staging of CRS [17], FEV_1 as a parameter of the lung function, and the modified Bhalla score widely used to calculate the radiological severity of lung disease [18, 19].

All available microbiological sputum cultures were analyzed: for each patient, at least 3 sputum bacteriology has been yearly collected. Chronic bronchial infection was defined as the presence of at least 2 respiratory isolates episodes of the same pathogen during the previous 12 months (3 months apart). Similarly, regarding *P. aeruginosa* colonization, patients have been classified as "non-colonized" if they resulted negative or, only once positive, to this pathogen, and as "colonized" if they showed at least 2 positive sputum cultures during the previous 12 months (3 months apart).

Continuous baseline characteristics of the patients were presented as the mean and SD or percentages. Differences between the percentages were tested by the Fisher test, while those from means by ANOVA and considered $p = 0.05$ to be statistically significant. Statistical analysis was performed using R software.

Results

Thirty-five PCD patients were enrolled in the study. The median age was 28 years (range: 4–62 years) and 54% (19/35) were females. After *TAS2R38* polymorphisms genotyping, the prevalence of PAV/PAV individuals was 28.6%, while that of PAV/AVI and AVI/AVI was 48.6 and 22.8%, respectively. The main features of patients enrolled in the study, described according to *TAS2R38* haplotypes, are given in Table 1. Among the analyzed clinical features, a significant difference in the distribution of *TAS2R38* haplotype was found for the NRD and the frequent RE (≥2/year) phenotypes. A significant association among patients presenting with or without NRD has been detected (p value = 0.01), in particular, no PAV/PAV genotype was detected among patients with NRD (Table 1). A significant difference has been also observed among patients suffering from frequent RE: among the 3 TAS2R38 haplotypes, a lower percentage of PAV/PAV subjects showed frequent RE (p value = 0.04). Moreover, looking at the chronic colonization by *P. aeruginosa* pathogen in PCD patients, a p value threshold of 0.05 emerged between the PAV/PAV individuals and the non-PAV/PAV ones (i.e., PAV/AVI and AVI/AVI).

Additional interesting results include a suggestive trend for other clinical variables, although the difference

between the 3 genotypes did not reach statistical significance. Interestingly, it is worth to note that (1) the Lund-Mackay score was higher in both AVI/AVI and PAV/AVI patients as compared to PAV/PAV ones, (2) no PAV/PAV patients had bronchial asthma, and (3) when considering respiratory function parameter, AVI/AVI and PAV/AVI patients showed lower values of FEV_1, and when considering the severity of the disease, higher values of pulmonary CT scores were observed in AVI/AVI and PAV/AVI patients than in PAV/PAV (modified Bhalla score).

Discussion

PCD is a rare genetic disease characterized by altered ciliary structure and function: the defective mucociliary clearance predisposes affected individuals to recurrent upper and lower respiratory tract infections. Here, for the first time, we describe the distribution of *TAS2R38* haplotype in PCD patients showing an association with some clinical features and/or parameters related to the disease. In particular, despite the overall prevalence of *TAS2R38* haplotype detected in the 35 investigated patients, overlaps with that reported in European populations [9, 20], some significant differences in the distribution of AVI/AVI, PAV/AVI, and PAV/PAV individuals and the relevant clinical phenotype have been found. In particular, the proportion of PAV/PAV subjects showing NRD, frequent RE, and chronic colonization by *P. aeruginosa* is significantly lower than AVI/AVI and AVI/PAV ones.

Recurrent lung infections and RE are responsible for the progression of the PCD disease, leading to bronchiectasis development, irreversible lung damage, and function decline. Often, the same pathogens colonize sinus and lung simultaneously, indicating that the sinus may constitute a potential bacterial reservoir for recurrent lung infections [21]. There are several pathogens involved in RE (e.g., *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catharralis*, *Staphylococcus pneumoniae,* and nontuberculous mycobacteria) [21], and one of the major clinically relevant bacteria is *P. aeruginosa*, with an overall prevalence of about 27% of PCD patients (both children and adults) [5]. In this light, our data further support the emerging role of *TAS2R38* gene in the susceptibility to upper respiratory gram-negative bacterial infections [22]. In particular, despite focusing on a specific subset of patients' population (i.e., PCD cases), our data supported literature studies, thus confirming that AVI/AVI subjects are more susceptible to *P. aeruginosa* colonization as compared to the PAV/PAV ones [8, 13, 14]. In addition, recent studies highlighted that AVI haplotype is associated with gram-negative bacterial infections in patients suffering from CRS, a common disease characterized by chronic inflammation of nasal mucosa and paranasal sinuses [14, 23]. Moreover, *TAS2R38* genotype was reported as an independent risk factor for CRS patients failing medical therapy and requiring surgical intervention [12]. Furthermore, in cystic fibrosis patients, PAV/PAV patients showed significantly lower CRS symptoms [15].

In addition, our study is intriguing that no PAV/PAV subjects have been found among PCD cases suffering from bronchial asthma: this finding is in agreement with previous studies showing the presence of T2R receptors in human airway smooth muscle where they seem to play a role in the airway relaxation [24].

Both taste and smell are chemosensors belonging to a chemical sensing system. Interestingly, it has been recently demonstrated significant olfactory impairment in PCD patients as compared with no PCD, as measured by the Sniffin' Sticks Extended Test [25]: this finding might be due to primary ciliopathy affecting the olfactory cilia, suggesting an additional cause for loss of smell in PCD patients. In this light, together with the TAS2R genotype evaluation, PCD patients should be tested for both taste and smell ability with the final goal of better understanding the role of these senses in the disease.

Overall, preliminary findings from our study, despite obtained in a small series of subjects, suggest that the genotypic characterization of polymorphisms of T2R38 receptor could be useful to better classify PCD patients and to predict those at greater risk of respiratory infections. Additional studies on larger series of PCD cases should be carried out to further confirm present findings.

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Statement of Ethics

The research was carried out ethically in accordance with the World Medical Association Declaration of Helsinki. The Ethical Committee of the Policlinico Hospital (Milan) approved the protocol for the diagnosis and treatment of PCD patients including genetic testing of patients and relatives (No. 16 19/3/2007 and following revisions).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

G.P.: conception and design, data analysis and interpretation, drafting and revising the manuscript, and final approval; U.A.: conception and design, acquisition of data, and final approval; A.R.: sample analysis, interpretation of data, drafting and revising the manuscript, and final approval; G.G.: sample analysis, revising the manuscript, and final approval; and P.G.: revising the manuscript and final approval.

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