dead space observed in the simulated N_2 washout curves (Figure 1) likely also contributes to the increased CO_2 dead space, which appears to play a pivotal role in dyspnea in COPD (9). Although our simulations were aimed at explaining the Sacin increases in smokers, over and above the effect of age (Figure 1), it is tempting to suggest that the normal increase in Sacin with age (6) may be at least partly a result of a reduced patency of a number of terminal bronchioles (10). Obviously, it is impossible to systematically use micro–computed tomography for information about the number of patent terminal bronchioles in smokers or in normal aging subjects (1). However, the combination of new CT imaging analyses (11) and noninvasive tests of ventilation heterogeneity may constitute a promising early detection tool to monitor potential loss of patent airways in smokers. ■

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Impact of T2R38 Receptor Polymorphisms on *Pseudomonas aeruginosa* Infection in Cystic Fibrosis

To the Editor:

The T2R38 (taste receptor 2 member 38) bitter taste receptor on respiratory epithelia detects *Pseudomonas aeruginosa N*-acyl-L-homoserine lactones (AHLs). *In vitro*, T2R38 activation by AHLs initiates calcium-mediated increases in nitric oxide production and ciliary beat frequency, dependent on polymorphisms in the *TAS2R38* gene (1). In patients with chronic rhinosinusitis, the *TAS2R38* genotype is proposed to modify mucosal responses to *P. aeruginosa* (1).

Polymorphisms in the *TAS2R38* gene result in two high-frequency haplotypes associated with taste perception of the bitter compound phenylthiocarbamide (2). The "taster" haplotype codes proline-alanine-valine (PAV), and the "nontaster" haplotype codes alanine-valine-isoleucine (AVI) at positions 49, 262, and 296 in the receptor protein. Responses to AHLs *in vitro* are greatest in PAV/PAV epithelial cells, and this genotype is reported to be protective against *P. aeruginosa* in the sinonasal airway (1).

P. aeruginosa is the most frequently isolated respiratory pathogen in cystic fibrosis (CF), and chronic infection is associated with accelerated rates of disease progression. Determining the impact of *TAS2R38* polymorphisms on *P. aeruginosa* infection in CF could have implications for patient risk stratification and, as naturally occurring and synthetic agonists to T2R38 are already in clinical use (3), could identify promising therapeutic targets.

We characterized T2R38 localization in the CF airway and investigated the hypothesis that *TAS2R38* polymorphisms would

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modify the prevalence and impact of *P. aeruginosa* infection in CF. Some of the results of these studies have previously been reported in the form of abstracts (4, 5).

Methods

Nasal and/or bronchial brushings were obtained from four children with CF undergoing bronchoscopy and four healthy adult controls. T2R38 localization was evaluated by immunocytochemistry with antibodies to T2R38 and ciliary proteins as described previously (6). Slides were imaged with a Zeiss LSM-510 confocal microscope, and colocalization was quantified using the JACoP plug-in for ImageJ (7).

DNA was extracted from blood from 271 subjects with CF (>6 yr old) and subjected to PCR for the common *TAS2R38* polymorphisms rs713598, rs1726866, and rs10246939. *P. aeruginosa* infection status was categorized in patients with three or more respiratory cultures during 2014, according to Leeds criteria (8), as chronic (>50% positive), intermittent (≤50% positive), free (previous *P. aeruginosa* but none for >12 mo), or never. Clinical data were obtained from each patient's 2014 annual assessment.

Cryopreserved *P. aeruginosa* isolates from *TAS2R38*-genotyped patients (matched for age and FEV_1) were revived in Luria-Bertani broth in triplicate and filter sterilized. Quantitative analysis of *N*-butanoyl-L-homoserine lactone (C4-HSL) and *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) was performed by liquid chromatography with tandem mass spectrometry. The limits of detection and limits of quantification were defined as signal:noise ratios of 3:1 and 10:1, respectively, as previously described (9).

Power calculations predicted that 250 patients would provide 80% power to detect a difference in chronic *P. aeruginosa* infection of ⩾20% in PAV/PAV compared with other genotypes at an α of 5%. *P. aeruginosa* infection by *TAS2R38* genotype was analyzed by chi-squared analysis and logistic regression. Graphpad Prism 7 and SPSS 23 were used, and the null hypothesis was rejected at $P < 0.05$.

Ethics review committees approved the protocol (02-019 and 10/H0504/9), and written consent was obtained from the subjects or their parent/guardian.

Results

T2R38 immunostaining was present in all nasal ($n = 3$) and bronchial ($n = 3$) samples from patients with CF, and in all nasal samples ($n = 4$) from healthy controls. T2R38 stained proximally to acetylated α-tubulin (ciliary microtubules) and γ-tubulin (ciliary basal bodies), and colocalized with rootletin (ciliary rootlets) in CF and control cells (Figure 1). The thresholded Manders' correlation coefficients (mean ± SD of four cells) for T2R38 and rootletin were 0.91 ± 0.07, 0.90 ± 0.08, and 0.90 ± 0.04 for control nasal, CF nasal, and CF bronchial cells, respectively, indicating that ⩾90% of green (rootletin) pixels were positive for red (T2R38).

Of 271 patients with CF, 225 had the common AVI/AVI ($n = 74$), AVI/PAV ($n = 110$), or PAV/PAV ($n = 41$) genotypes and three or more respiratory cultures during 2014. Between *TAS2R38* genotype groups there was no significant difference in median age, sex, or proportion of p.Phe508del *CFTR* (cystic fibrosis transmembrane conductance regulator) mutations. There was no association between *TAS2R38* genotype and *P. aeruginosa* infection

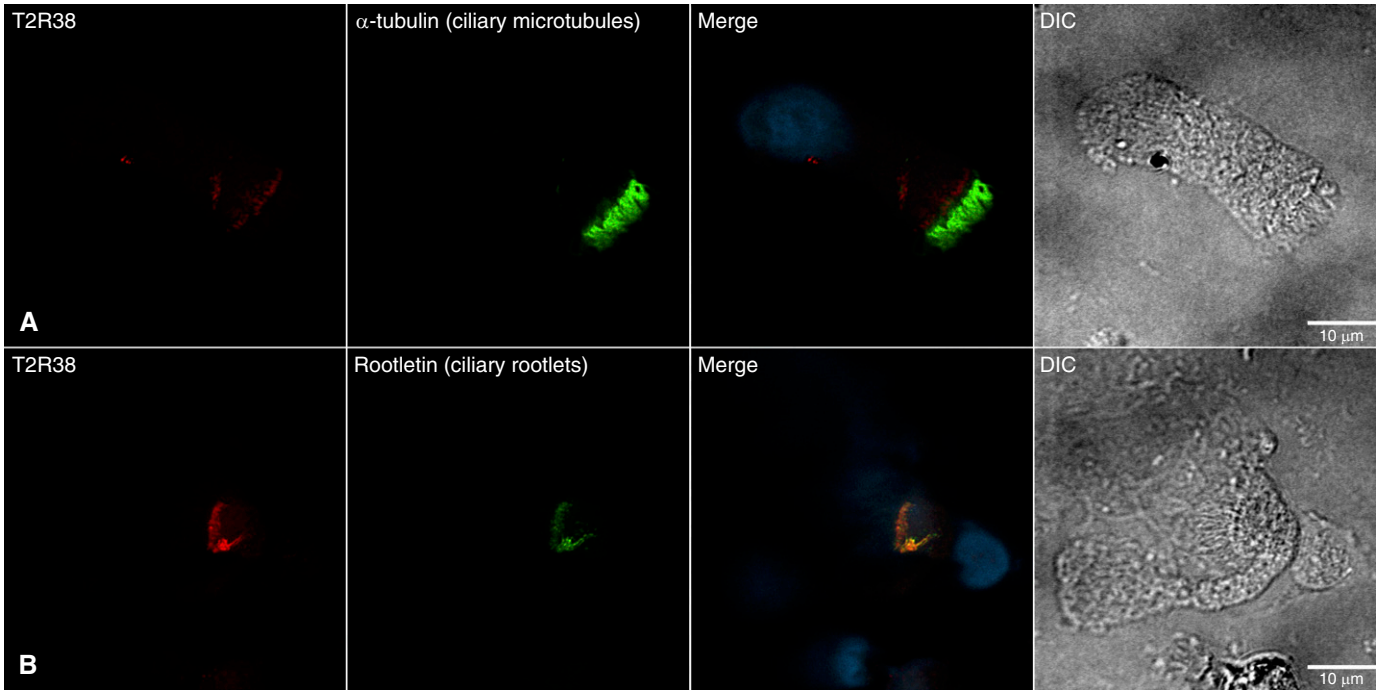

Figure 1. Confocal microscopy images of nasal epithelial cells from a subject with cystic fibrosis. (*A* and *B*) Cells were stained with antibodies to T2R38 (red) and acetylated α-tubulin (*A*) or rootletin (*B*) (both stained green). Nuclei were stained with DAPI (blue). Colocalized antibodies appear yellow in the merged images. Epithelial cell morphology is shown by differential interference contrast (DIC) images. T2R38 stains proximally to acetylated α-tubulin (ciliary microtubules) and colocalizes with rootletin (ciliary rootlets). The antibodies used in these immunocytochemistry assays were T2R38 (AB130503, Abcam), acetylated α-tubulin (T6793, Sigma), and rootletin (SC-374056, Santa Cruz Biotechnology).

status ($P = 0.46$; Table 1). In the logistic regression model with "intermittent and chronic" and "never and free" groups as dependent variables, and age, sex, *CFTR* genotype, and *TAS2R38* genotype as independent variables, only age was associated with intermittent or chronic *P. aeruginosa* infection (odds ratio, 1.05; 95% confidence interval, 1.03–1.07). There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status when the PAV/PAV genotype was compared against the AVI/AVI or AVI/PAV genotype.

Among patients with intermittent or chronic *P. aeruginosa* infection ($n = 141$), there was no difference by *TAS2R38* genotype in median FEV_1% predicted (AVI/AVI, 54.0%; AVI/PAV, 62.0%; PAV/PAV, 53.5%; $P = 0.3$) or in the proportion of patients from whom mucoid *P. aeruginosa* was isolated (AVI/AVI, 69%; AVI/PAV, 60%; PAV/PAV, 68%; $P = 0.5$). In 18 *P. aeruginosa* isolates from *TAS2R38*-genotyped patients, there was no difference by genotype in the proportion of isolates in which C4-HSL or 3-oxo-C12-HSL was below the limit of quantification ($P = 0.8$).

Discussion

We have identified T2R38 in CF nasal and bronchial epithelium, where it localizes to the ciliary rootlet with the same distribution as in non-CF epithelia. Previous studies reported T2R38 localization ranging from the ciliary tip (10) to below the ciliary base (1, 11). Our experiments demonstrate that in fresh, noncultured cells, T2R38 colocalizes with rootletin, a structural component of the ciliary rootlet, originating from the ciliary basal body and extending toward the nucleus (12).

In this study of 225 children and adults with CF, we found no association between the *TAS2R38* genotype and *P. aeruginosa* infection status within the range of differences that our study was powered to detect. Our results show that only age was associated with intermittent or chronic infection, consistent with CF registry data (13). Among patients with intermittent or chronic infection, the lack of any difference in spirometry or prevalence of mucoid *P. aeruginosa* further supports the lack of a protective effect of the PAV/PAV genotype. Finally, in a small sample of clinical isolates, we observed no relationship between the *TAS2R38* genotype and AHL profiles, suggesting that polymorphisms in this receptor do not exert a selective pressure on *P. aeruginosa* in the CF lung.

Our results indicate that *TAS2R38*-related differences in sinonasal immunity do not translate to clinically relevant changes in the CF airway, where mucociliary clearance is significantly impaired. We suggest that *TAS2R38* genotyping has no prognostic value in patients with CF, nor do our findings indicate that the T2R38 receptor is a promising drug target for CF mucosal immunity. ■

Table 1. *Pseudomonas aeruginosa* Infection Category by *TAS2R38* Genotype

	AVI/AVI ($n = 74$)	AVI/PAV ($n = 110$)	PAV/PAV ($n = 41$)
Never, *n* (%)	4 (5)	4 (4)	3 (7)
Free, *n* (%)	21 (28)	36 (32)	16 (39)
Intermittent, *n* (%)	11 (15)	26 (24)	8 (20)
Chronic, *n* (%)	38 (51)	44 (40)	14 (34)

Definition of abbreviations: AVI = alanine-valine-isoleucine; PAV = proline-alanine-valine.

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H2 Receptor Antagonist Use and Mortality in Pulmonary Hypertension: Insight from the VA-CART Program

To the Editor:

Pulmonary hypertension (PH) is common and predicts or mediates poor outcomes in many medical disorders (1). Independent of the underlying etiology, persons affected by PH have increased right-ventricular load and often develop right-heart failure. Pulmonary vasodilators are beneficial in some forms of PH (e.g., pulmonary arterial hypertension), but they can be harmful or have no impact in other forms (2).

Histamine H2 receptor antagonism may be relevant in the myocardial stress response and beneficial in right-heart dysfunction and right-heart failure (3–6). To explore this possibility, we examined relationships between H2 receptor antagonist (H2RA) use and mortality in a cohort of veterans with PH confirmed at right-heart catheterization (RHC).

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Author Contributions: All authors participated in the conception and design of the research. E.H. and A.E.B. conducted the statistical analysis. G.C., B.A.M., R.T.Z., and T.L. developed the right-heart catheterization cohort of the VA-CART program. P.J.L. and T.L. interpreted the data and drafted the report. All authors reviewed, revised, and approved the final version of the manuscript.

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Methods

We included veterans from the Veterans Affairs (VA) Clinical Assessment, Reporting, and Tracking (VA-CART) program who received RHC at a VA center between 2008 and 2014, had a mean pulmonary artery pressure (mPAP) of ⩾25 mm Hg, and had a pulmonary artery wedge pressure (PAWP) recorded. The Colorado Multiple Institutional Review Board approved this study (#14-1649).

Exposure. Participants were considered to have used an H2RA if an outpatient prescription was filled within 90 days of the RHC. Participants were excluded if they died within 90 days or had a hospitalization lasting longer than 60 days after catheterization. This provided at least 30 days to detect outpatient medication use.

Outcome. The outcome was the rate of all-cause mortality determined using the combined VA vital status file (97.6% exact agreement with the National Death Index) (7). Risk time accrued after the 90-day window that was used to establish exposure status. Exposure and outcome assessment did not temporally overlap in an effort to avoid immortal time bias.

Statistical Analysis

We used Cox proportional hazards models to estimate associations between H2RA use and mortality. In limited models, we adjusted for age, sex, race, and body mass index. In fully adjusted models, we also accounted for participants' markers of socioeconomic status and health behaviors. In separate models, we further adjusted for comorbid medical conditions or comedication use.

To account for confounding by indication, analyses were repeated in a restricted cohort comparing participants who used H2RAs with those who used proton pump inhibitors. In a second restricted cohort, propensity scores were used to match H2RA users with nonusers. Analyses were repeated in cohorts limited to participants with a diagnosis of heart failure or chronic obstructive pulmonary disease (COPD).

Careful phenotyping by World Health Organization group was not feasible; however, PAWP was available for all participants. PAWP was evaluated as an effect modifier of the relationship between H2RA use and mortality. A typical cutoff of 15 mm Hg and a more stringent cutoff of 12 mm Hg to exclude left-heart disease were used.

Analyses were performed using SAS 9.4 (SAS Institute) and R 3.3.1 (R Project for Statistical Computing).

Results

Participant characteristics are included in Table 1. A total of 589 H2RA users died in 4,719 person-years (12.5 deaths per 100 person-years) and 6,341 nonusers died in 46,129 person-years (13.7 deaths per 100 person-years). H2RA use within 90 days of RHC was associated with a 10% lower risk for all-cause mortality (adjusted hazard ratio, 0.90; 95% confidence interval, 0.83–0.98; $P = 0.02$; the proportional hazard assumption was not violated). This relationship was slightly stronger when we accounted for comedication use, accounted for comorbidity, compared H2RA users with users of proton pump inhibitors, and when we compared H2RA users with propensity-matched nonusers (Table 2). When associations were evaluated in cohorts limited to veterans with COPD or heart failure, estimates were similar to those obtained in the full cohort but less precise.

There was no evidence that PAWP modified relationships between H2RAs and mortality using either conventional (15 mm Hg;