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Impact of T2R38 receptor polymorphisms on *Pseudomonas aeruginosa* infection in cystic fibrosis

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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); the ‘non-taster’ 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 modify prevalence and impact of *P. aeruginosa* infection in CF. Some of the results of these studies have previously been reported as abstracts (4, 5).

Methods

Nasal and/or bronchial brushings were obtained from 4 CF children undergoing bronchoscopy and 4 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 aged >6yrs and subjected to PCR for the common *TAS2R38* polymorphisms (rs713598, rs1726866, and rs10246939). *P. aeruginosa* infection status was categorised in patients with ≥ 3 respiratory cultures during 2014, according to Leeds criteria (8), as chronic (>50% positive), intermittent ($\leq 50\%$ positive), free (previous *P. aeruginosa* but none for >12 months), or never. Clinical data was obtained from each patient's 2014 annual assessment.

Cryo-preserved *P. aeruginosa* isolates from *TAS2R38*-genotyped patients (matched for age and FEV₁) 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. 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 250 patients would provide 80% power to detect a difference in chronic *P. aeruginosa* infection of $\geq 20\%$ in PAV/PAV compared to other genotypes at α of 5%. Analysis of *P. aeruginosa* infection by *TAS2R38* genotype was by Chi-squared analysis and logistic regression. Graphpad Prism 7 and SPSS 23 were used and the null hypothesis was rejected at $p < 0.05$.

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

Results

T2R38 immunostaining was present in all nasal ($n=3$) and bronchial ($n=3$) samples from CF patients and 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). Thresholded Manders' correlation coefficients (mean \pm SD of 4 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 $\geq 90\%$ of green (rootletin) pixels were positive for red (T2R38).

Of 271 CF patients, 225 had the common AVI/AVI (74), AVI/PAV (110) or PAV/PAV (41) genotypes and ≥ 3 respiratory cultures during 2014. Between *TAS2R38* genotype groups there was no significant difference in median age, sex, or

proportion of p.Phe508del *CFTR* mutations. There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status ($P=0.46$) (table). 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% CI 1.03-1.07). There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status when the PAV/PAV genotype was compared against AVI/AVI or AVI/PAV genotypes.

Among patients with intermittent or chronic *P. aeruginosa* infection ($n=141$) there was no difference by *TAS2R38* genotype in median percent-predicted FEV₁ (AVI/AVI 54.0%, AVI/PAV 62.0%, PAV/PAV 53.5%, $p=0.3$) or in the proportion of patients who isolated mucoid *P. aeruginosa* (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 were 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 in the same distribution as in non-CF epithelia. Previous studies report T2R38 localization ranging from the ciliary tip (10) to below the ciliary base (1, 11). Our experiments demonstrate that in fresh, non-cultured 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 have found no association between *TAS2R38* genotype and *P. aeruginosa* infection status, within the range of difference that our study was powered to detect. Our results show only age to be 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* adds further evidence to the lack of a protective effect of the PAV/PAV genotype. Finally, in a small sample of clinical isolates we observed no relationship between *TAS2R38* genotype and AHL profiles, suggesting that polymorphisms in this receptor are not exerting 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 there to be no prognostic value of *TAS2R38* genotyping in patients with CF, nor do our findings indicate the T2R38 receptor to be a promising drug target in CF mucosal immunity.

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Figure. Confocal microscopy images of nasal epithelial cells from a subject with CF. Cells were stained with antibodies to T2R38 (red), and acetylated α -tubulin (A) or rootletin (B) (both stained green). Nuclei were stained with 4',6-diamidino-2-phenylindole (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). Antibodies used in these immunocytochemistry assays were: T2R38 (AB130503, Abcam, UK), acetylated α -tubulin (T6793, Sigma, UK), and rootletin (SC-374056, Santa Cruz Biotechnology, USA).

Table. *P. 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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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.

Impact of T2R38 receptor polymorphisms on *Pseudomonas aeruginosa* infection in cystic fibrosis

Figure

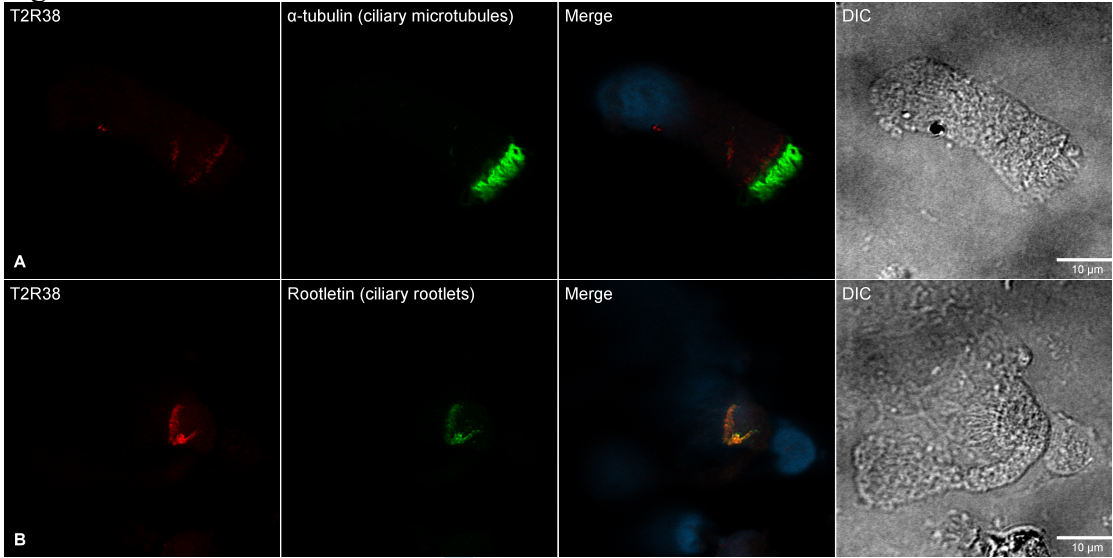


Table 2. Odds ratios for 'intermittent or chronic' *P. aeruginosa* infection by logistic regression

	Univariate		
	Odds ratio	Lower 95% CI	Upper 95% CI
Age	1.051	1.029	1.073
Sex			
Male*	1		
Female	1.352	0.785	2.330
CFTR			
F508/F508*	1		
F508/other	1.285	0.723	2.281
Other/other	1.465	0.521	4.121
T2R38			
PAV/PAV*	1		
PAV/AVI	1.511	0.731	3.125
AVI/AVI	1.693	0.776	3.694

- Baseline group for comparison of odds ratios by logistic regression.