Original Article

Tissue-Dependent Expression of Bitter Receptor *TAS2R38* mRNA

Jennifer E. Douglas^{1,2,*}, Cailu Lin^{2,*}, Corrine J. Mansfield²,
Charles J. Arayata², Beverly J. Cowart², Andrew I. Spielman³,
Nithin D. Adappa¹, James N. Palmer¹, Noam A. Cohen^{1,2,4} and
Danielle R. Reed²

¹Department of Otorhinolaryngology-Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA 19104, USA, ²Monell Chemical Senses Center, Philadelphia, PA 19104, USA, ³Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY 10010, USA and ⁴Philadelphia Veterans Affairs Medical Center Surgical Services, Philadelphia, PA 19104, USA

*These authors contributed equally to the work.

Correspondence to be sent to: Danielle R. Reed, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA. e-mail: reed@monell.org

Editorial Decision 13 October 2018.

Abstract

TAS2R38 is a human bitter receptor gene with a common but inactive allele; people homozygous for the inactive form cannot perceive low concentrations of certain bitter compounds. The frequency of the inactive and active forms of this receptor is nearly equal in many human populations, and heterozygotes with 1 copy of the active form and 1 copy of the inactive form have the most common diplotype. However, even though they have the same genotype, heterozygotes differ markedly in their perception of bitterness, perhaps in part because of differences in *TAS2R38* mRNA expression. Other tissues express this receptor too, including the nasal sinuses, where it contributes to pathogen defense. We, therefore, wondered whether heterozygous people had a similar wide range of *TAS2R38* mRNA in sinonasal tissue and whether those with higher *TAS2R38* mRNA expression in taste tissue were similarly high expressers in nasal tissue. To that end, we measured gene expression by quantitative PCR in taste and sinonasal tissue and found that expression abundance in one tissue was not related to the other. We confirmed the independence of expression in other tissue pairs expressing *TAS2R38* mRNA, such as pancreas and small intestine, using autopsy data from the Genotype-Tissue Expression project (although people with high expression of *TAS2R38* mRNA in colon also tended to have higher expression in the small intestine). Thus, taste tissue *TAS2R38* mRNA expression among heterozygotes is unlikely to predict expression in other tissues, perhaps reflecting tissue-dependent function, and hence regulation, of this protein.

Key words: bitter, gene, mRNA expression, sensory, taste, tissue

Introduction

Human bitter taste receptors comprise a gene family with 25 members that investigators originally discovered in taste receptor cells. Their protein products in taste tissue are the first point of

contact for bitter-tasting foods and medicines, and they initiate the signal cascade that results in the perception of bitter tastes (Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000). These receptors are also expressed in other tissues, where

they serve diverse physiological and pathophysiological roles (Lu et al. 2017). For instance, ciliated cells in the human nasal sinuses use bitter receptors to sense bacterial compounds, triggering an immune response (Lee et al. 2012). One of the bitter receptors responsible for this response (TAS2R38, encoded by the *TAS2R38* gene) also has a well-documented role in the taste system. This gene has 2 main genotypes, a sensitive and a nonsensitive form, that are almost equally common in most human populations. Most people are heterozygous (Kim et al. 2005; Wooding 2006; Risso et al. 2016) and are able to perceive the bitterness of the ligands, albeit at a reduced intensity relative to people who are homozygous for the taster form (Bufe et al. 2005). However, heterozygous people vary considerably in their sensitivity to bitter ligands (Newcomb et al. 2012), which could be due in part to variable mRNA expression of the active form of the receptor (Lipchick et al. 2013). This point may have practical importance because clinicians are exploring the use of taste testing as a means of predicting sinonasal disease risk and surgical outcomes (Adappa et al. 2016b). It would be helpful to know if heterozygous patients who experience more bitterness are also likely to express more *TAS2R38* mRNA in nasal tissue. Therefore, we sought to determine whether the range of mRNA expression in taste cells is similar to that expressed in sinonasal cells, focusing on heterozygotes. To that end, we obtained taste and sinonasal tissue from genetically characterized adults and measured mRNA expression; in addition, each person rated the bitterness of a *TAS2R38* ligand and other taste solutions. To understand whether these results generalize to other tissues, we similarly analyzed gene expression in pairs of tissues using data available from the Genotype-Tissue Expression (GTEx) Consortium (Mele et al. 2015). They provided us with gene expression data from all major tissues in the human body taken from hundreds of adults.

Materials and methods

Subjects

Most of our subjects were patients of one of the authors (N.A.C.), an attending surgeon in the Philadelphia Veterans Affairs Medical Center and the Department of Otorhinolaryngology–Head and Neck Surgery at the University of Pennsylvania. These patients were undergoing sinonasal surgery for reasons other than chronic rhinosinusitis (e.g., reconstructive nasal surgery, tear duct surgery, or sinus surgery to access tumors in the cranial vault/skull base). We recruited additional subjects from within those departments who did not undergo surgery, likewise excluding those with a history of chronic rhinosinusitis. We did not exclude smokers because of the high rates of smoking among veterans (e.g., Brown 2010); however, we did not obtain self-report data on smoking behavior.

Sample processing

For genotyping, we collected saliva using Oragene-Discover OGR-500 collection kits (DNA Genotek), and we extracted genomic DNA following the manufacturer's directions. We genotyped alleles of the *TAS2R38* gene (accession number NM_176817) using real-time PCR single nucleotide polymorphism genotyping assays for rs713598, rs1726866, and rs10246939 (C__8876467_10, C__9506827_10, and C__9506826_10, respectively; TaqMan from Thermo Fisher) with the QuantStudio 12K Flex or StepOnePlus sequence detection system (Applied Biosystems). We assigned *TAS2R38* diplotypes (the combination of haplotypes; see Table 1) for each person, as previously described (Bufe et al. 2005).

Table 1. Common variants of *TAS2R38*

Effect	<i>TAS2R38</i> variant			Haplotype
	A49P	V262A	I296V	
Taster	PP	AA	VV	PAV
Nontaster	AA	VV	II	AVI

A, alanine; P, proline; V, valine; I, isoleucine. A49P indicates that at position +49 in the amino acid sequence, some people have an alanine and some people have a proline.

For mRNA analysis, we collected taste tissue samples (fungiform papillae [FP]) following published procedures (Spielman et al. 2010), and we collected nasal tissue from the inferior turbinate either by brush (MasterAmp Buccal Brush; Epicentre) or by biopsy (Figure 1). We noted the time of tissue collection and placed the tissue samples in RNA preservative (RNAlater; Invitrogen, Life Technologies). To isolate RNA from all tissue samples (average of 6 taste papillae and 31 mg nasal tissue per subject), we followed the manufacturer's directions (taste tissue and nasal brush samples: Quick-RNA MiniPrep R1054, Zymo Research; nasal biopsy samples: ZR-Duet kit). We evaluated RNA quality expressed as an RNA integrity number (RIN) using the Agilent 2200 TapeStation system (Agilent Technologies). We synthesized cDNA using the Ovation RNA Amplification System V2 (cat. no. 3100–12; NuGEN Technologies), including a DNase step (Zymo Research). We purified the resulting cDNA using QIAquick PCR Purification Kit (cat. no. 28104; Qiagen).

Cell-specific assays and allele-specific *TAS2R38* cDNA measures

We used allele-specific probes for the *TAS2R38* variant site from the assays listed earlier. We also measured the cell-specific markers *CFTR* (Hs00357011_m1) for motile cilia in nasal tissue (Brezillon et al. 1995; Dupuit et al. 1995; Kalin et al. 1999) and *GNAT3* (Hs01385403_m1) for type 2 taste cells in taste tissue (bitter receptors are expressed in a subset of type 2 taste cells), as well as *GAPDH* (cat. no. 4326317E) as a housekeeping gene in both tissues. The bitter taste receptor genes have a single exon, so we could not use intron-spanning primers; therefore, we verified absence of genomic DNA in the cDNA samples by size-specific primers from the *ABL1* gene (Lion 1996). We excluded cDNA samples with consistent evidence of residual genomic DNA from some analyses, as explained later.

RNA sequencing

TAS2R38 mRNA is expressed in only a small fraction of the cells within the biopsies, and measurement of this low-abundance transcript can be imprecise. Therefore, on samples that met the quality standards of the Next-Generation Sequencing Core of the University of Pennsylvania (RIN > 7), their staff performed library preparation and sequencing (100 bp single end) on the HiSeq 4000 sequencer (Illumina) using the manufacturer's sequencing protocols. We mapped reads to the reference genome (GRCh38.p10) using HISAT2 (Pertea et al. 2016) after the raw sequence data in fastq format passed Trimmomatic quality filters (Bolger et al. 2014), and we normalized the counts using the R package ballgown (Fu et al. 2016). We compared the normalized counts to the expression measured in quantitative PCR (qPCR), and we examined the abundance of associated taste signaling genes as a general validation step.

Figure 1. Overview of tissue collection, processing, and qPCR analysis.

FP density, taste testing, and dietary measures

Using published methods (Nuessle et al. 2015), we scored FP density by counting each subject's number of taste papillae within a 10 mm circular cutout. Prior to biopsy, we asked subjects to rate the intensity of several taste solutions, including phenylthiocarbamide (PTC), a ligand of the TAS2R38 receptor (Bufe et al. 2005). Each subject rated 6 taste solutions twice using a labeled category scale ranging from 0 (No Intensity At All) to 12 (Extremely Intense): water, PTC (180 μ M), denatonium benzoate (1.8 μ M), quinine (56 μ M), sodium chloride (0.25 M), and sucrose (0.35 M). People also chose a quality descriptor for each solution from the following list: salty, sour, bitter, sweet, and no flavor. (Detailed taste methods are available online at <https://osf.io/hn87s/>.) We also asked subjects to quantify their habitual caffeine consumption using a modified version of the Harvard Food Frequency Questionnaire, a semiquantitative dietary assessment tool (Sutor et al. 1989; Lipchok et al. 2017) (available online at <https://osf.io/j3suy/>). We included questions about caffeine

consumption because prior studies suggested that subjects who consumed more caffeine have higher expression of bitter taste receptors in taste papillae (Lipchok et al. 2013, 2017).

Analysis

The abundance of each *TAS2R38* allele was expressed relative to the housekeeping gene *GAPDH* (Livak and Schmittgen 2001) using the median of 3 reporters as input values, with the 3 variant sites averaged as previously described (Lipchok et al. 2013). If we detected no expression, we used a cycle threshold (C_t) value of 40. We excluded from some analyses samples that did not contain the target cell type, and we evaluated the correlation of gene expression in taste versus nasal tissue using a Spearman rank correlation coefficient; we also used this statistical method to compute the correlation between qPCR and RNA sequencing (RNAseq) values. For all analyses listed earlier and later, we computed the statistical tests with R (version 3.4.2) and RStudio (version 1.1.383).

Ethics and reproducibility

The institutional review boards of the Philadelphia Veterans Affairs Medical Center and the University of Pennsylvania approved these protocols. In advance of data collection, we registered our hypothesis at the Center for Open Science (<https://osf.io/q4a3u/>). All data and analysis scripts are available for download (<https://github.com/DanielleReed/TAS2R38>). This study was registered at ClinicalTrials.gov (#NCT02766959).

GTEX data

After we obtained appropriate approvals, administrators from the GTEx project provided RNAseq data from postmortem tissue samples from 738 people (51 tissues; GTEx #12732—Bitter receptor gene expression; patterns across tissues). We extracted the normalized RPKM (reads per kilobase of transcript, per million mapped reads) values for the *TAS2R38* gene across the tissues. We classified samples as expressing *TAS2R38* mRNA if the RPKM was above 0 and computed the percentage of samples that expressed *TAS2R38* mRNA by tissue. For samples where this gene was expressed, we tested whether it was affected by *TAS2R38* genotype with a Kruskal-Wallis test. We examined whether people expressing higher amounts in one tissue were likely to express higher amounts in other tissues with Spearman rank correlational analysis.

Results

Screening and subjects

Forty-five adult subjects provided samples for *TAS2R38* genotyping during a preoperative or other clinic visit, from which we identified a sample enriched for heterozygotes (AVI/PAV; $N = 19$) to provide nasal epithelial tissue and taste tissue from the FP. (We chose to study people of the same genotype to reduce the impact of genetic variation on mRNA expression, and we chose to study heterozygotes because they have a wide range of perceptual responses compared with homozygotes; Newcomb et al. 2012.) We also recruited 4 PAV/PAV and 4 AVI/AVI subjects for comparison. In all, we obtained tissue and performed sensory testing on 27 subjects: 23 males and 4 females. Sixteen subjects (59%) were of European descent, and the remaining were racially diverse, which reflects the metropolitan area from which the sample was collected (Philadelphia, PA) (Pew Charitable Trust 2014). Supplementary Table 1 lists individual subjects and their characteristics. The median age of the subjects was 35 years (range, 23–71 years). Figure 2 depicts the subjects and procedures.

All subjects provided FP by biopsy, and they also provided nasal tissue, 15 by nasal brush and 12 by biopsy. The RINs for the taste samples were noticeably higher than those for the nasal samples [$t(26) = 5.06$, $P = 2.857 \times 10^{-5}$]; Median RINs were 6.4 for the taste samples and 2.2 for nasal samples overall, with an advantage for the

biopsy (RIN = 2.6) over the brush method (RIN = 1.4; Supplementary Table 2). However, people providing samples that produced the highest-quality taste RNA were not more likely to provide high-quality nasal samples (Spearman $r = 0.10$, $P = 0.78$; Supplementary Figure 1). Despite the difference in RNA quality between taste and nasal tissues, observed C_t for the housekeeping gene *GAPDH* did not differ between these 2 tissues [$t(26) = 1.10$, $P = 0.2794$], indicating the RNA was of sufficient quality to measure in both tissues the abundance of a house keeping gene. Cycle threshold ranges from 0 to 40 and is an indirect measure of gene abundance.

Of the 27 taste samples collected, 13 had detectable amounts of the cell-specific marker *GNAT3*; of the 27 nasal samples, 19 had detectable amounts of the cell-specific marker *CFTR*. Nine subjects had samples with appropriate cell-specific markers in both tissues. Eight of these 9 samples were from people who were *TAS2R38* heterozygotes, but we excluded 2 additional samples because we consistently detected residual genomic DNA (Supplementary Table 2). Among these 6 people, there was no relationship between *TAS2R38* PAV mRNA abundance from taste versus nasal tissue ($r = 0.2$, $P = 0.70$, Spearman; Figure 3A). The pattern of results was similar when we included all 19 heterozygous subjects in the analysis (Supplementary Figure 2A). Thus, our preregistered hypothesis, that heterozygotes with higher *TAS2R38* mRNA in taste tissue would also have higher expression in nasal tissue, was not confirmed.

For RNAseq analysis, 6 samples, all of which were from taste tissue and none from nasal tissue, were of sufficient quality for sequencing (RIN > 7; Supplementary Table 2). Although there were too few samples to draw meaningful statistical comparisons, we noted that the expression of *TAS2R38* mRNA was similar between the 2 methods commonly used to measure gene expression, RNAseq and qPCR ($r = 0.71$, $P = 0.11$, Spearman; Supplementary Figure 3). As expected, we also detected gene expression for markers of taste receptor cells, *TRPM5* and *GNAT3*, albeit at a low abundance (Supplementary Table 3).

People with 1 or more PAV haplotypes rated PTC as more intense than did those homozygous for the inactive form [$F(2, 24) = 7.13$, $P = 0.0037$; Supplementary Figure 4]. There was no relationship between *TAS2R38* PAV mRNA abundance from fungiform or nasal tissue and ratings of PTC bitterness intensity (fungiform, $r = -0.20$, $P = 0.70$; nasal, $r = 0.31$, $P = 0.54$; Spearman; Figure 3B, C). For the 19 heterozygous subjects (which include those with no cell-specific expression), we observed a similar result (fungiform, $r = -0.43$, $P = 0.06$; nasal, $r = -0.04$, $P = 0.88$; Spearman; Figure 3B, C). The negative correlation between taste tissue mRNA and sensory measures (-0.43) is in the opposite direction of that we observed in an earlier study (Lipchick et al. 2013).

The amount of caffeine consumed by the 6 heterozygous subjects that met the most stringent inclusion criteria (see Figure 3A; Supplementary Table 2) was also unrelated to *TAS2R38* PAV mRNA

Figure 2. Experimental flowchart.

Figure 3. Correlation between abundance of the *TAS2R38* PAV mRNA in nasal and taste tissue (A) and between PTC taste intensity ratings and abundance of the *TAS2R38* PAV mRNA in taste tissue (B), or nasal tissue (C) ($n = 6$). Each dot represents data from 1 of 6 heterozygous subjects that provided samples in which we could detect appropriate cell-specific markers and no residual genomic DNA. The solid lines show linear correlation; the gray shading shows confidence intervals. Scales at the x-axis are the log of the relative abundance of the *TAS2R38* PAV mRNA relative to *GAPDH*.

abundance ($r = 0.37$, $P = 0.47$, Spearman). Other taste ratings were as expected, but with a few anomalies (Supplementary Table 4). All subjects described sucrose as sweet, and all but one subject described sodium chloride as salty. A number of subjects reported the bitter stimuli to be sour, but this is a common error (O'Mahony et al. 1979). However, a few people rated the water as having a flavor (Supplementary Table 5). In most cases, this incorrect rating was for the second but not first tasting of water and may reflect carryover effects from preceding taste stimuli.

Subjects differed in FP density, with scores ranging from 0 to 6, but those with a higher density did not rate PTC as more bitter than did those with a lower density ($r = -0.05$, $P = 0.81$, Spearman). For the RNAseq data, the mRNA expression of *TAS2R38* in taste tissue was not correlated with that of *TRPM5* ($r = -0.03$, $P = 0.96$, Spearman) or *GNAT3* ($r = 0.029$, $P = 1.0$, Spearman), indicating that bitter receptor expression and downstream signaling are not correlated within a given taste tissue sample.

In the GTEx data set, *TAS2R38* mRNA could be detected in as few as 2.5% of the samples for some tissues (e.g., muscle) and as many as 77% in the small intestine, although not all tissues had complete data, and thus were less informative than others (Supplementary Table 6). Samples that expressed detectable amounts of *TAS2R38* mRNA had a >300-fold range of RPKM values (0.0036–1.159), with the highest RPKM values in small intestine, testis, pancreas, and

transverse colon (Supplementary Figure 5 and 6). *TAS2R38* mRNA expression in these tissues did not differ by diplotype (Kruskal-Wallis, $P > 0.05$; Supplementary Figure 6), so all GTEx donors in the data set were included in the remaining analyses. Using the tissues with higher *TAS2R38* mRNA expression, we learned that the abundance of expression in 1 tissue was unrelated to abundance in the other tissues (e.g., small intestine vs. pancreas: $r = 0.08$, $P = 0.57$; Figure 4; Supplementary Figure 7), except for the small intestine and colon (Supplementary Figure 8). We omitted bladder tissue from these correlational analyses because there were too few tissue pairs for meaningful comparisons (e.g., $n = 2-3$).

Discussion

The *TAS2R38* gene codes for the bitter taste receptor protein *TAS2R38* and has 2 common forms that predict whether people can taste the ligand PTC at a low concentration. However, the majority of people are heterozygotes, and their ability to taste PTC ranges widely. This observation is important medically because *TAS2R38* is expressed in extraoral tissues, including nasal/sinus tissue, where it contributes to pathogen defense. Thus, PTC sensitivity may predict susceptibility to and surgical outcome for sinonasal disease (Lee et al. 2012; Adappa et al. 2013, 2014, 2016a). This bitter receptor may act as a pathogen defense system in other tissues, for instance,

Figure 4. Correlation of *TAS2R38* mRNA expression between small intestine and pancreas from the GTEx database. Each dot represents data from 1 GTEx donor. (RNAseq data expressed as RPKM). The solid line shows linear correlation; the gray shading shows confidence intervals. Scales at the x-axis are the log of the relative abundance of the *TAS2R8* PAV mRNA relative to *GAPDH*.

for the teeth and gums, and the ability to taste PTC predicts other conditions such as dental caries (Chung et al. 1964; Lin 2003; Rupesh and Nayak 2006; Oter et al. 2011; Pidamale et al. 2012). Previously, we demonstrated that *TAS2R38* heterozygous people with higher expression of the taster allele in taste tissue rated the bitterness of a *TAS2R38* ligand (propylthiouracil) as more intense than did those with lower expression (Lipchock et al. 2013). We were motivated to conduct the current research in part by the practical usefulness of knowing whether taste ratings by heterozygotes would predict the abundance of functional *TAS2R38* (PAV) mRNA in the sinonasal epithelium and thus a more vigorous immune response. This information would in turn help us understand their disease risk and to counsel them regarding expected surgical outcomes. However, our results in this study showed that this was not the case—higher gene expression in taste tissue did not predict higher expression in sinonasal tissue. This result was also true more generally. That is, we also found that tissue-specific expression levels appear to be characteristic of other tissues that express *TAS2R38* mRNA as well, although there are exceptions to this generalization. This result is broadly consistent with the pattern of gene expression for many other genes, where abundance depends on the particular tissue more than on the particular individual from which it comes (Mele et al. 2015). Coupled with our recent observation that PTC taste test results predicted ex vivo biofilm formation (Adappa et al. 2016b), these findings lead us to conclude that, although taste testing for PTC bitterness remains a highly effective way to estimate *TAS2R38* genotype and related outcomes, *TAS2R38* mRNA gene expression is a less informative outcome measure.

However, there are several limitations to the present study that arise from RNA quality and low expression of the *TAS2R38* gene. We found marked differences in RNA quality between sinonasal and taste tissues. RNA quality was low in a few taste samples, but it was low or very low in nearly all nasal samples. Other investigators have observed that some tissues have consistently lower RNA quality than others, but it appears that nasal tissue is at the low extreme, possibly due to the presence of RNA-degrading enzymes within the nasal mucus (Knowles and Boucher 2002; Tomazic et al. 2014). Furthermore, only a few cells within human taste (Behrens et al. 2012) and nasal (Lee et al. 2012) tissues express the *TAS2R38*

bitter taste receptor gene, so abundance in the tissue overall is very low, near the level of detection for qPCR. However, the results from RNAseq (which can detect a greater range of expression than qPCR) partially offset this limitation, and the results from these 2 methods agreed, although we were able to obtain RNAseq data from only a subset of samples.

To determine if this independence of *TAS2R38* mRNA in taste and nasal tissues followed a general rule or was an exception, we also examined data from the GTEx Consortium (Mele et al. 2015). Consortium investigators collected and measured RNA expression in tissues from many people after death. *TAS2R38* mRNA expression was undetectable in most tissues, so we focused on tissues with higher expression (e.g., small intestine and pancreas). Extrapolating from observations in the mouse, in the small intestine, ligands of *TAS2R38* initiate hormone release from enteroendocrine cells that slow nutrient absorption (Jeon et al. 2008). We learned here that the small intestine and colon have similarly high or low *TAS2R38* expression within a person, implying that *TAS2R38* has the same or similar functions in these 2 tissues. We know little about pancreatic *TAS2R38* mRNA expression or its *TAS2R38* function except that, according to the Human Protein Atlas, the protein is present in exocrine glandular cells.

It appears that tissues that express *TAS2R38* mRNA do so largely independently, and higher expression in 1 tissue does not predict higher expression in a different tissue, similar to our observation in taste and nasal tissues. We also note a mismatch between low *TAS2R38* mRNA patterns in the GTEx data set and more abundant protein in the Human Protein Atlas data set. This mismatch between mRNA and protein abundance (albeit from 2 different large-scale data repositories) points to translation of mRNA to protein as an uncharacterized but potentially important regulatory step.

In contrast to our earlier research outcomes (Lipchock et al. 2013, 2017), in this study we did not observe a relationship between ratings of PTC bitterness intensity and *TAS2R38* mRNA abundance or between gene expression and caffeine consumption. We have no explanation for these discrepancies. One possibility is that the RNA quality may have been lower among the samples obtained in this study than in the previous studies. Another explanation is that the difference may arise from the subjects investigated, which in the former cases

were healthy nonsmoking young adults whereas in this study, subjects were older, under medical care, and drawn from a subject population with high rates of alcohol use and smoking. Also, in the case of the caffeine measures, the intake was self-reported, which can be an imperfect measure of consumption. Likewise, in contrast to previous studies, we found no correlation between taste papillae density and bitterness perception (Bartoshuk et al. 1994; Delwiche et al. 2001); this negative result may be explained by a restriction of range due to TAS2R38 genotype (most of our subjects were heterozygotes and would not be expected to give the most extreme ratings of intensity). As we mentioned earlier, the subjects themselves were markedly different too; healthy young subjects were tested by Lipchick et al. (2013) compared with older patients in this study. In addition, we used the TAS2R38 ligand PTC for sensory testing rather than 6-*n*-propylthiouracil (PROP). However, the relationship we found between TAS2R38 genotype and PTC bitterness was clear and is consistent with many other sensory studies of TAS2R38 ligands (Kim et al. 2003; Duffy et al. 2004; Bufer et al. 2005; Timpson et al. 2007; Newcomb et al. 2012); thus, even with these caveats, the results clearly show that deficiencies in the sensory testing, if present, were not large.

Supplementary material

Supplementary material can be found at <http://www.chemse.oxfordjournals.org/>

Funding

This work was supported by the National Institutes of Health (NIH) [grant number R21DC013886 to N.A.C. and D.R.R.]. The National Institute on Deafness and Other Communication Disorders Administrative Research Supplement to Promote Emergence of Clinician-Scientists in Chemosensory Research provided support for J.E.D. We collected genotype data from equipment purchased in part with funds from NIH [grant number OD018125].

Acknowledgements

The authors thank John Edward Tordoff at the Center for Open Science for assistance with hypothesis pre-registration.

Conflict of Interest

None declared.

References

- Adappa ND, Farquhar D, Palmer JN, Kennedy DW, Doghramji L, Morris SA, Owens D, Mansfield C, Lysenko A, Lee RJ, et al. 2016a. TAS2R38 genotype predicts surgical outcome in nonpolypoid chronic rhinosinusitis. *Int Forum Allergy Rhinol.* 6:25–33.
- Adappa ND, Howland TJ, Palmer JN, Kennedy DW, Doghramji L, Lysenko A, Reed DR, Lee RJ, Cohen NA. 2013. Genetics of the taste receptor T2R38 correlates with chronic rhinosinusitis necessitating surgical intervention. *Int Forum Allergy Rhinol.* 3:184–187.
- Adappa ND, Truesdale CM, Workman AD, Doghramji L, Mansfield C, Kennedy DW, Palmer JN, Cowart BJ, Cohen NA. 2016b. Correlation of T2R38 taste phenotype and in vitro biofilm formation from non-polypoid chronic rhinosinusitis patients. *Int Forum Allergy Rhinol.* 6:783–791.
- Adappa ND, Zhang Z, Palmer JN, Kennedy DW, Doghramji L, Lysenko A, Reed DR, Scott T, Zhao NW, Owens D, et al. 2014. The bitter taste receptor T2R38 is an independent risk factor for chronic rhinosinusitis requiring sinus surgery. *Int Forum Allergy Rhinol.* 4:3–7.
- Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. 2000. A novel family of mammalian taste receptors. *Cell.* 100:693–702.
- Bartoshuk LM, Duffy VB, Miller IJ. 1994. PTC/PROP tasting: anatomy, psychophysics, and sex effects. *Physiol Behav.* 56:1165–1171.
- Behrens M, Born S, Redel U, Voigt N, Schuh V, Raguse JD, Meyerhof W. 2012. Immunohistochemical detection of TAS2R38 protein in human taste cells. *PLoS One.* 7:e40304.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* 30:2114–2120.
- Brezillon S, Dupuit F, Hinnrasky J, Marchand V, Kälin N, Tümmeler B, Puchelle E. 1995. Decreased expression of the CFTR protein in remodeled human nasal epithelium from non-cystic fibrosis patients. *Lab Invest.* 72:191–200.
- Brown DW. 2010. Smoking prevalence among US veterans. *J Gen Intern Med.* 25:147–149.
- Bufer B, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, Kim UK, Drayna D, Meyerhof W. 2005. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr Biol.* 15:322–327.
- Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ. 2000. T2Rs function as bitter taste receptors. *Cell.* 100:703–711.
- Chung CS, Witkop CJ, Henry JL. 1964. A genetic study of dental caries with special reference to PTC taste sensitivity. *Am J Hum Genet.* 16:231–245.
- Delwiche JF, Buletic Z, Breslin PA. 2001. Relationship of papillae number to bitter intensity of quinine and PROP within and between individuals. *Physiol Behav.* 74:329–337.
- Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, Reed DR, Snyder DJ, Bartoshuk LM. 2004. Bitter receptor gene (TAS2R38), 6-*n*-propylthiouracil (PROP) bitterness and alcohol intake. *Alcohol Clin Exp Res.* 28:1629–1637.
- Dupuit F, Kälin N, Brezillon S, Hinnrasky J, Tümmeler B, Puchelle E. 1995. CFTR and differentiation markers expression in non-CF and delta F 508 homozygous CF nasal epithelium. *J Clin Invest.* 96:1601–1611.
- Fu J, Frazee AC, Collado-Torres L, Jaffe AE and Leek JT. 2016. *Ballgown: Flexible, isoform-level differential expression analysis.* R package version 2.4.3.
- Jeon TI, Zhu B, Larson JL, Osborne TF. 2008. SREBP-2 regulates gut peptide secretion through intestinal bitter taste receptor signaling in mice. *J Clin Invest.* 118:3693–3700.
- Kälin N, Claass A, Sommer M, Puchelle E, Tümmeler B. 1999. DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. *J Clin Invest.* 103:1379–1389.
- Kim UK, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. 2003. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science.* 299:1221–1225.
- Kim U, Wooding S, Ricci D, Jorde LB, Drayna D. 2005. Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci. *Hum Mutat.* 26:199–204.
- Knowles MR, Boucher RC. 2002. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest.* 109:571–577.
- Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Abraham V, Doghramji L, Adappa ND, Palmer JN, et al. 2012. T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest.* 122:4145–4159.
- Lin BP. 2003. Caries experience in children with various genetic sensitivity levels to the bitter taste of 6-*n*-propylthiouracil (PROP): a pilot study. *Pediatr Dent.* 25:37–42.
- Lion T. 1996. Appropriate controls for RT-PCR. *Leukemia.* 10:1843.
- Lipchick SV, Mennella JA, Spielman AI, Reed DR. 2013. Human bitter perception correlates with bitter receptor messenger RNA expression in taste cells. *Am J Clin Nutr.* 98:1136–1143.
- Lipchick SV, Spielman AI, Mennella JA, Mansfield CJ, Hwang LD, Douglas JE, Reed DR. 2017. Caffeine bitterness is related to daily caffeine intake and bitter receptor mRNA abundance in human taste tissue. *Perception.* 46:245–256.

- Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta C(T)) method. *Methods*. 25:402–408.
- Lu P, Zhang CH, Lifshitz LM, ZhuGe R. 2017. Extraoral bitter taste receptors in health and disease. *J Gen Physiol*. 149:181–197.
- Matsunami H, Montmayeur JP, Buck LB. 2000. A family of candidate taste receptors in human and mouse. *Nature*. 404:601–604.
- Mele M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, Young TR, Goldmann JM, Pervouchine DD, Sullivan TJ, et al.; GTEx Consortium. 2015. Human genomics. The human transcriptome across tissues and individuals. *Science*. 348:660–665.
- Newcomb RD, Xia MB, Reed DR. 2012. Heritable differences in chemosensory ability among humans. *Flavour*. 1:9.
- Nuessle TM, Garneau NL, Sloan MM, Santorico SA. 2015. Denver papillae protocol for objective analysis of fungiform papillae. *J Vis Exp*. Jun 8;(100):e52860.
- O'Mahony M, Goldenberg M, Stedmon J, Alford J. 1979. Confusion in the use of the taste adjectives "sour" and "bitter." *Chem. Senses Flavour*. 4:301–318.
- Oter B, Ulukapı I, Ulukapı H, Topçuoğlu N, Cildir S. 2011. The relation between 6-*n*-propylthiouracil sensitivity and caries activity in schoolchildren. *Caries Res*. 45:556–560.
- Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. 2016. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc*. 11:1650–1667.
- Pew Charitable Trust. 2014. Philadelphia: the state of the city. A 2014 update [Internet]. Available from: <http://www.pewtrusts.org/en/research-and-analysis/reports/2014/04/05/philadelphia-the-state-of-the-city-a-2014-update>
- Pidamale R, Sowmya B, Thomas A, Jose T. 2012. Genetic sensitivity to bitter taste of 6-*n* Propylthiouracil: a useful diagnostic aid to detect early childhood caries in pre-school children. *Indian J Hum Genet*. 18:101–105.
- Risso DS, Mezzavilla M, Pagani L, Robino A, Morini G, Tofanelli S, Carrai M, Campa D, Barale R, Caradonna F, et al. 2016. Global diversity in the TAS2R38 bitter taste receptor: revisiting a classic evolutionary PROPosal. *Sci Rep*. 6:25506.
- Rupesh S, Nayak UA. 2006. Genetic sensitivity to the bitter taste of 6-*n* propylthiouracil: a new risk determinant for dental caries in children. *J Indian Soc Pedod Prev Dent*. 24:63–68.
- Spielman AI, Pepino MY, Feldman R, Brand JG. 2010. Technique to collect fungiform (taste) papillae from human tongue. *J Vis Exp*. 18:2201.
- Suitor CJ, Gardner J, Willett WC. 1989. A comparison of food frequency and diet recall methods in studies of nutrient intake of low-income pregnant women. *J Am Diet Assoc*. 89:1786–1794.
- The Human Protein Atlas [Internet]. Available from: <https://www.proteinatlas.org/ENSG00000257138-TAS2R38/tissue/pancreas>.
- Timpson NJ, Heron J, Day IN, Ring SM, Bartoshuk LM, Horwood J, Emmett P, Davey-Smith G. 2007. Refining associations between TAS2R38 diplotypes and the 6-*n*-propylthiouracil (PROP) taste test: findings from the Avon Longitudinal Study of parents and children. *BMC Genet*. 8:51.
- Tomazic PV, Birner-Gruenberger R, Leitner A, Obrist B, Spoerk S, Lang-Loidolt D. 2014. Nasal mucus proteomic changes reflect altered immune responses and epithelial permeability in patients with allergic rhinitis. *J Allergy Clin Immunol*. 133:741–750.
- Wooding S. 2006. Phenylthiocarbamide: a 75-year adventure in genetics and natural selection. *Genetics*. 172:2015–2023.