Human bitter perception correlates with bitter receptor messenger RNA expression in taste cells

Sarah V Lipchock, Julie A Mennella, Andrew I Spielman, and Danielle R Reed

ABSTRACT
Background: Alleles of the receptor gene TAS2R38 are responsible in part for the variation in bitter taste perception of 6-n-propylthiouracil (PROP) and structurally similar compounds (eg, glucosinolates in cruciferous vegetables). At low concentrations, people with the PAV (“taster” amino acid sequence) form of TAS2R38 perceive these bitter compounds, whereas most with the AVI (“nontaster” amino acid sequence) form do not; heterozygotes (PAV/AVI) show the widest range of bitter perception.

Objectives: The objectives were to examine individual differences in expression of PAV-TAS2R38 messenger RNA (mRNA) among heterozygotes, to test the hypotheses that the abundance of allele-specific gene expression accounts for the variation in human bitter taste perception, and to relate to dietary intake of bitter-tasting beverages and foods.

Design: Heterozygous individuals (n = 22) provided psychophysical evaluation of the bitterness of PROP, glucosinolate-containing broccoli juice, non-glucosinolate-containing carrot juice, and several bitter non-TAS2R38 ligands as well as dietary recalls. Fungiform taste papillae were examined for allele-specific TAS2R38 expression by using quantitative polymerase chain reaction.

Results: PAV-TAS2R38 mRNA expression was measured in 18 of 22 heterozygous subjects. Relative expression varied widely and positively correlated with ratings of bitterness intensity of PROP (P = 0.007) and broccoli juice (P = 0.004) but not of the control solutions carrot juice (P = 0.26), NaCl (P = 0.68), caffeine (P = 0.24), or urea (P = 0.47). Expression amounts were related to self-reported recent and habitual caffeine intake (P = 0.060, P = 0.005); vegetable intake was too low to analyze.

Conclusions: We provide evidence that PAV-TAS2R38 expression amount correlates with individual differences in bitter sensory perception and diet. The nature of this correlation calls for additional research on the molecular mechanisms associated with some individual differences in taste perception and food intake. The trial was registered at clinicaltrials.gov as NCT01399944. Am J Clin Nutr 2013;98:1136–43.

INTRODUCTION
Bitter taste sensitivity and rejection likely evolved to prevent humans from ingesting the many chemically diverse, toxic agents in plants (1). To detect these agents, humans have a large and diverse family of taste receptors (named T2Rs), comprising 25 different bitter receptors with genes clustered primarily on chromosomes 7 and 12 (2, 3). The sequences, and thus functions, of T2Rs vary across individuals (4). Both cell-based assays and psychophysical studies have shown that single nucleotide polymorphisms (SNPs) in the genes that code for bitter receptors (TAS2Rs) result in different responses to bitter stimuli, such that individuals with one form of a gene can perceive a stimulus as intensely bitter whereas individuals with another form may not perceive its bitterness at all (5–10).

In humans, the most intensively studied TAS2R gene is TAS2R38, which is located in a cluster of bitter receptor genes on chromosome 7. This gene encodes for a bitter taste receptor that recognizes compounds with a thiourea moiety, such as 6-n-propylthiouracil (PROP) (11), and sinigrin and goitrin, found in glucosinolate-containing vegetables (12–14). Both cell-based assays and psychophysical threshold measurements confirm substantial variation resulting from 3 SNPs in the gene (A49P, V262A, and I296V) (11, 15). The most common haplotypes for TAS2R38, AVI and PAV, are associated with the most extreme phenotypes, with PAV as a “taster” amino acid sequence form and AVI as a “nontaster” amino acid sequence form (11, 15, 16). Although one would expect heterozygous PAV/AVI individuals to have thresholds between these 2 homozygous extremes, as a group they show a wide range of sensitivities to PROP (11, 16). Variations in the abundance of TAS2R38 messenger RNA (mRNA) in taste cells may be one source of this variation and may reflect the number of receptors present in taste papillae.

A methodology to collect human taste papillae has been developed, and the tissue collected is used to quantify TAS2R mRNA by in situ hybridization or by quantitative polymerase chain reaction (17, 18). Obtaining tissue from the back of the tongue, from the circumvallate papillae, is difficult and is presumably collected only under sedation. Biopsies of this type of papillae indicate that TAS2R mRNA was found for all 25 bitter receptors, but the amount of expression varied significantly from

1From the Monell Chemical Senses Center, Philadelphia, PA (SVL, JAM, and DRR); and the Department of Basic Science and Craniofacial Biology, College of Dentistry, New York University, New York, NY (AIS).
2Supported by NIH grants T32DC000014 and F32DC011975 (SVL), P02DC011735 (DRR), and R01DC011287 (JAM).
3Address correspondence and requests for reprints to DR Reed, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104. E-mail: reed@monell.org.
4Abbreviations used: AVI, “nontaster” amino acid sequence in TAS2R38; gLMS, general labeled magnitude scale; mRNA, messenger RNA; PAV, “taster” amino acid sequence in TAS2R38; PROP, 6-n-propylthiouracil; SNP, single nucleotide polymorphism.

Received May 16, 2013. Accepted for publication July 23, 2013.
First published online September 11, 2013; doi: 10.3945/ajcn.113.066688.
receptor to receptor (17). Fungiform papillae from the front of the tongue can be collected more easily and without sedation. Biopsies of this type of papillae from 2 PA V/AVI individuals showed that at least one bitter receptor was expressed abundantly (TAS2R38) but relative expression of the taster and nontaster forms varied from person to person (11).

In this study, we examined heterogeneity in allele-specific expression of TAS2R38 mRNA in fungiform papillae in a larger sample of PAV/AVI heterozygotes. To test the hypothesis that variations in PAV mRNA expression in fungiform papillae relate to differences in the perception of bitterness for TAS2R38-specific ligands, we measured amounts of PAV “taster” allele mRNA as well as the psychophysical perception of bitter taste. Gene expression can be related to diet, and therefore subjects provided information about recent and habitual intake of bitter-tasting foods and beverages.

SUBJECTS AND METHODS

Subjects

The study population consisted of healthy, nonsmoking adults (≤40 y of age) who were recruited from local advertisements and from a database of prior study subjects of known TAS2R38 genotype who had consented to be contacted for future studies. All procedures for the study were approved by the Office of Regulatory Affairs at the University of Pennsylvania, and informed consent was obtained from each subject. The trial was registered at clinicaltrials.gov (NCT01399944).

Testing day 1: screening and training

A timeline for the study procedures is shown in Figure 1. Seventy individuals were screened for eligibility and trained in the psychophysical methods. Carbon monoxide in breath was measured by using a Vitalograph (Lenexa) to verify nonsmoking status. Blood pressure was measured by using an automated cuff (Dinamap; GE Medical Systems) to verify that subjects were normotensive. Saliva was collected to determine TAS2R38 genotype by using SNP genotyping assays (rs713598, rs1726866, and rs10246939) with the StepOnePlus system from Applied Biosystems.

All potential subjects were trained to use the general labeled magnitude scale (gLMS), a psychophysical tool that requires subjects to rate perceived intensity along a vertical axis lined with semilogarithmically spaced adjectives (“barely detectable,” “weak,” “moderate,” “strong,” “very strong,” and “strongest imaginable”) (19, 20), by using Compusense 5 Plus software (Compusense Inc). To ensure that differences in perception were specific to taste sensations and not differences in how the scales were used, subjects were also asked to use the gLMS to rate the heaviness of 6 opaque, sand-filled jars ranging from 235 to 955 g; heaviness ratings were used to normalize taste intensity ratings as described previously (21).

Testing day 2: psychophysical evaluation and tissue collection

Genotyping results were obtained 1 wk later (n = 30 PAV/AVI, 11 PAV/PAV, 11 AVI/AVI; 7 AA/PAV, 5 AVI/AAI, 3 AAV/PAV, 2 AVI/AAV, and 1 AAV/AVI). Of the 30 PAV/AVI individuals, the 25 eligible subjects were invited to return for psychophysical testing and papilla collection, and 22 PAV/AVI subjects agreed to participate. The remaining 5 heterozygotes were deemed ineligible because they did not understand the psychophysical test (n = 2), had high blood pressure (n = 2), or smoked tobacco (n = 1). We also invited one homozygous taster (PAV/PAV) and one homozygous nontaster (AVI/AVI) to return to collect tissue for mRNA, which would serve as an internal standard for the gene expression assays.

Subjects were tested individually in a closed room designed for sensory studies (psychophysics), and then tissue was collected in

![FIGURE 1. Timeline of study procedures. AVI, “nontaster” amino acid sequence in TAS2R38; BP, blood pressure; gLMS, general labeled magnitude scale; PAV, “taster” amino acid sequence in TAS2R38; PROP, 6-n-propylthiouracil.](https://academic.oup.com/ajcn/article-abstract/98/4/1136/4577144/FIGURE-1-Timeline-of-study-procedures-AVI-nontaster-amino-acid-sequence-in-TAS2R38-BP-blood-pressure-gLMS-general-labeled-magnitude-scale-PAV-taster-amino-acid-sequence-in-TAS2R38-PROP-6-n-propylthiouracil)
a surgical suite between the hours of 0830 and 1330. All subjects were instructed to eat their typical breakfast or lunch and to arrive at the center without having eaten for at least 1 h.

Psychophysics

Thresholds for PROP were determined by using a modified Harris-Kalmus recognition method (11). Subjects were then given 5 mL of each stimulus, presented in random order. The stimuli included bitter-tasting solutions that are known ligands of the TAS2R38 receptor [560 μmol PROP/L and broccoli juice (which contains glucosinolates) (22)] and those that are not (500 mmol urea/L, 8 mmol caffeine/L, 492 mmol denatonium benzoate/L, and 119 μmol quinine hydrochloride/L); these concentrations were based on previous studies (21, 23, 24). Carrot juice, which contains no known TAS2R38 ligands, was used as a control vegetable; and 100 mmol sodium chloride/L was used as a nonbitter control stimulus for general taste sensitivity. The vegetables (raw broccoli and carrots) were juiced (Panasonic Juice Extractor, model MJ-65PR) to ensure that the presence of bitter compounds, which form after breakdown of the plant cell walls (typically while chewing), was uniform for all subjects. After straining through 2 layers of cheese cloth, juices were frozen in ice cube trays with lids in 20-mL aliquots until they were thawed before testing.

Subjects swished the stimulus solution in their mouths for 5 s, expectorated, and then rated the bitterness and overall intensity by using the gLMS (19). A 1-min interval, in which subjects rinsed twice with deionized water, separated tasting of each stimulus. During testing, subjects wore nose clips to eliminate retronasally perceived volatiles, and red lights illuminated the room to eliminate the effects of appearance, allowing them to focus primarily on taste sensations.

Taste tissue collection

Before tissue collection, a dentist interviewed subjects about medical and dental history to verify that they had not undergone coumadin therapy (for bleeding risk) and inspected the tongue to confirm that each subject had no oral disease, including tongue lesions or xerostomia. Heart rate and blood pressure were measured to ensure they were in the normal range.

Under sterile conditions, 6–8 fungiform papillae were removed from the dorsal surface of the anterior tongue by using a curved spring microscissors (McPherson-Vannas type; Roboz) (see reference 18 for a detailed description of surgical methods). Each papilla was immediately transferred into Ringer buffer (130 mmol NaCl/L, 5 mmol KCl/L, 1 mmol CaCl2/L, 1 mmol MgCl2/L, 1 mmol Na-pyruvate/L, 20 mmol HEPES/L; pH 7.2) with forceps, and after 6 to 8 papillae were removed the tissue was transferred to RNAlater (Invitrogen, Life Technologies Corp) and stored at −80°C. For each subject, a map of the tongue was made to record the area from which papillae were taken (eg, right or left side, near edge or center; see Supplemental Figure 1 under “Supplemental data” in the online issue).

RNA extraction, complementary DNA preparation, and gene expression analyses

Within 72 h of papilla collection, tissue was removed from the freezer, thawed on ice, and homogenized by using Kontes disposable pellet pestles with 1.5-mL microtubes (Kimble Chase). RNA was extracted from taste tissue with the ZR-Duet DNA/RNA MiniPrep kit (Zymo Research Corp), and complementary DNA was generated by using the WT-Ovation RNA Amplification System (NuGEN Technologies Inc).

Gene expression for GAPDH (glyceraldehyde 3-phosphate dehydrogenase; a general housekeeping gene) and GNAT3 (gustducin α-3 chain; a gene associated with bitter taste signaling) was measured by using TaqMan quantitative polymerase chain reaction gene expression assays (4326317E, Hs01385403_m1; Invitrogen). GNAT3 was used as a positive control for the presence of type 2 taste receptor cells; any complementary DNA samples that were negative for GNAT3 were excluded from all analyses. Expression of TAS2R38 alleles was measured by using TaqMan SNP genotyping assays (rs713598, rs1726868, and rs10246939). Expression of other bitter receptor genes was also measured and will be reported elsewhere. Gene expression of GNAT3 and all TAS2R38 alleles was compared with GAPDH by using the following equation:

\[
\text{Expression of gene} = 2^{\left(\frac{Ct_{\text{Gene}} - Ct_{\text{GAPDH}}}{(\text{AvgCtgene} - \text{AvgCtGAPDH})}\right)}
\]

(1)

Assays were repeated in triplicate, and the median value was calculated for each SNP. The log of gene expression relative to the control gene was used in all analyses, with values of 0.0 changed to 0.001 before log transformation. The average expression of the taster form of the gene from each of the 3 variant sites (P49, A262, and V296) was computed and used in all subsequent analyses.

Reported dietary habits

Data on both recent and habitual dietary intake were obtained from each subject. On testing day 2, subjects used the Automated Self-Administered 24-Hour Recall system (version 2009) developed by the National Cancer Institute (25) to record dietary intake the day before testing (hereafter referred to as recent dietary intake). Intake during the previous year was assessed by using a modified version of the Harvard Service food-frequency questionnaire, a semiquantitative dietary assessment tool (26). Collard greens were not included in the original questionnaire but were added because they contain glucosinolates. Reported intake (servings/mo) for food and beverage items was determined by using the following scoring system: “never” = 0, “less than once per month” = 0.5, “1–3 times per month” = 2, “once per week” = 4, “2–4 times per week” = 12, “5–6 times per week” = 22, and “1 or more times per day” = 30 servings/mo (27, 28).

We focused on glucosinolate-containing (eg, broccoli, cauliflower, Brussels sprouts, collard greens, and kale) (22, 29) and non–glucosinolate-containing (eg, carrots, spinach, peas, corn, tomatoes, and green beans) vegetables (22, 29) as well as beverages that contain the bitter agent caffeine (eg, coffee, tea, and carbonated beverages). From the survey data, we determined whether subjects had consumed glucosinolate- and non–glucosinolate-containing vegetables in general, and broccoli and carrots specifically, and, if so, the number of servings the day before testing as well as the number of servings per month for the past year. We also determined daily caloric (kcal/d) and sodium (mg/d) intake and...
whether or not subjects drank caffeinated beverages and, if so, their daily caffeine intake (mg/d).

Statistical analyses
All analyses were conducted by using Statistica version 10 (StatSoft Inc). Values are presented as means ± SEs. Pearson’s correlation analyses were used to determine relations between PAV-TAS2R38 expression and bitterness intensity ratings and self-reported dietary intake. Drawing on results of these analyses, hypotheses were formed about the relative contribution of self-reported dietary intake. To explore these relations, several multivariate general linear models were evaluated.

RESULTS
Characteristics of the study population
The final study population consisted of 22 subjects who were heterozygous for PAV/AVI alleles of TAS2R38. The population was 55% female, 19–40 y of age (29.9 ± 1.3 y), and represented the ethnic diversity of Philadelphia (32% black, 55% white, and 14% other/mixed race). Two homozygous subjects donated tissue for mRNA assay standards: the AVI/AVI control was a 27-y-old white woman and the PAV/PAV control was a 32-y-old white man.

Gene expression
All participants except for 4 PAV/AVI individuals expressed GNAT3, indicating that taste cells were present in the tissue sample. Perhaps the samples of these 4 individuals did not contain any type 2 cells, or alternatively, their low mRNA levels were below the sensitivity of our assay. PAV-TAS2R38 expression (ie, mRNA from the taster form of the TAS2R38 gene) was measured in the taste tissue of 18 heterozygous subjects [reported as log(PAV-TAS2R38/GAPDH); see Table 1], and detectable amounts were found in 11 of the 18 subjects. Expression amounts spanned 4 orders of magnitude, ranging from −3 (whereby the level of expression was below the limit of detection) to 1.01, with a mean of −1.74 ± 0.37. The PAV/PAV control showed no AVI expression, and the AVI/AVI control showed no PAV expression, confirming that the SNP assays were specific to the appropriate genetic variants. Among PAV/AVI heterozygotes, PAV-TAS2R38 and AVI-TAS2R38 expression amounts were positively correlated [r(16) = 0.76, P < 0.001].

TABLE 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>log(GNAT3/GAPDH)</td>
<td>0.33 ± 0.17 (−1.12 to 1.32)</td>
</tr>
<tr>
<td>log(PAV-TAS2R38/GAPDH)</td>
<td>−1.74 ± 0.37 (−3.00 to 1.01)</td>
</tr>
<tr>
<td>log(AVI-TAS2R38/GAPDH)</td>
<td>−1.91 ± 0.31 (−3.00 to 0.65)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs; ranges in parentheses. n = 18. AVI, “non-taster” amino acid sequence in TAS2R38; PAV, “taster” amino acid sequence in TAS2R38.

Self-reported dietary intake
On average, subjects reported consuming 1815 ± 179 kcal and 3386 ± 428 mg sodium on the day before testing day 2 (Table 2). Average total vegetable consumption was reported as 1.2 ± 0.2 servings on the day before testing day 2 and 40.0 ± 7.4 servings/mo in the previous year. Only 4 of the 18 subjects ate glucosinolate-containing vegetables the day before testing, and most (83%) reported consuming <1 serving/d during the previous year. Few subjects reported ingesting either of the test vegetables, broccoli and carrots, the day before testing. In contrast, caffeine was consumed by most (89%) of the subjects, averaging 133.3 ± 22.9 mg the day before testing and 29.1 ± 4.3 servings/mo of caffeinated beverages during the previous year. Reported caffeine and vegetable consumption the day before testing correlated with reported ingestion during the previous year [caffeine: r(16) = 0.74, P < 0.001; vegetables: r(16) = 0.62, P = 0.006].

Gene expression, perception, and diet
Bitterness and intensity ratings for each taste solution were highly and positively correlated, so we focused on bitterness for simplicity. PAV-TAS2R38 expression amounts positively correlated with psychophysical ratings of bitterness for PROP [r(16) = 0.62, P = 0.007; Figure 2A] and broccoli juice [r(16) = 0.64, P = 0.004; Figure 2B], as well as the non-TAS2R38 ligands quinine [r(16) = 0.47, P = 0.051] and denatonium benzoate [r(16) = −0.61; P = 0.007]. No such relation was observed for the control solutions carrot juice [bitterness: r(16) = −0.28, P = 0.26] and NaCl [saltiness: r(16) = 0.10, P = 0.68] or for the other 2 non-TAS2R38 bitter stimuli [area: r(16) = 0.18, P = 0.47; caffeine: r(16) = 0.29, P = 0.24]. PROP recognition thresholds ranged from 12 to 1540 μmol/L and averaged 263 ± 94 μmol/L. However, no relation was observed between PAV-TAS2R38 expression amounts and PROP recognition thresholds [r(16) = −0.17, P = 0.51].

PAV-TAS2R38 expression amounts did not relate to recent intake of calories [r(16) = 0.12, P = 0.63] or sodium intake [r(16) = 0.20, P = 0.44]. Although we intended to determine whether PAV-TAS2R38 expression was related to vegetable intake, specifically vegetables containing glucosinolates, both recent and habitual intakes for glucosinolate-containing vegetables were very low (Table 2 and Figure 3). This limitation in the data violated assumptions of normality and homogeneity of variance and prevented comparison of PAV-TAS2R38 expression amounts with recent vegetable intake. Low habitual intake of vegetables resulted in a lack of power to find correlations with PAV-TAS2R38 expression amounts [glucosinolate-containing vegetables: r(16) = −0.016, P = 0.95; non–glucosinolate-containing vegetables: r(16) = 0.012, P = 0.96]. There was a trend for PAV-TAS2R38 expression amounts to be positively correlated with amount of caffeine consumed the day before testing [r(16) = 0.45, P = 0.060; Figure 4]. Consistent with this finding, PAV-TAS2R38 expression amounts significantly related to reported habitual caffeine intake [r(16) = 0.59, P = 0.010; Figure 4].

General linear modeling was conducted with 2 types of predictor variables for ratings of PROP bitterness: TAS2R38 gene expression and caffeine intake, defined categorically (yes or no: drank coffee the day before the test and habitually drinks...
1 cup of coffee and continuously (in mg: how much caffeine the subject ate or drank the day before the test and habitually consumes per day). We first assessed the influence of TAS2R38 gene expression and caffeine intake separately and then included both in the same model. The models were conducted for the perception of broccoli juice and broccoli intake, which were exploratory because of the low dietary intake of broccoli.

In the separate models, TAS2R38 gene expression amounts accounted for 38.0% \[F(1,16) = 9.77, P = 0.006\] of the variance in PROP bitterness intensity (Figure 5). In the combined model, both predictors accounted for 64.6% of the variation overall \[F(6,11) = 3.36, P = 0.039\], indicating that the relation between gene expression and caffeine intake largely overlaps. The perceived bitterness of broccoli was related to gene expression \[F(1,16)=11.07, P = 0.004\], which accounted for 41% of the variation in individual ratings of its bitterness but was unrelated to broccoli intake \[F(2,15) = 1.51, P = 0.25\]. Given the lack of effect of broccoli consumption on its perceived

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Summary of reported dietary intake(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent dietary intake (day before testing)</td>
<td>Mean ± SEM (range)</td>
</tr>
<tr>
<td>Calories (kcal/d)</td>
<td>1815 ± 179 (632–3550)</td>
</tr>
<tr>
<td>Vegetables (servings/d)</td>
<td></td>
</tr>
<tr>
<td>Glucosinolate-containing</td>
<td>0.2 ± 0.1 (0–0.5)</td>
</tr>
<tr>
<td>Broccoli</td>
<td>0.05 ± 0.03 (0–0.5)</td>
</tr>
<tr>
<td>Non-glucosinolate-containing</td>
<td>1.1 ± 0.2 (0–3.3)</td>
</tr>
<tr>
<td>Carrots</td>
<td>0.04 ± 0.03 (0–0.38)</td>
</tr>
<tr>
<td>Caffeine (mg/d)</td>
<td>133.3 ± 22.9 (0–302.8)</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>3386 ± 428 (901–7179)</td>
</tr>
<tr>
<td>Habitual dietary intake (servings/ mo)</td>
<td></td>
</tr>
<tr>
<td>Glucosinolate-containing vegetables</td>
<td>13.4 ± 4.0 (0–60)</td>
</tr>
<tr>
<td>Broccoli</td>
<td>6.9 ± 2.2 (0–30)</td>
</tr>
<tr>
<td>Non-glucosinolate-containing vegetables</td>
<td>26.6 ± 4.1 (6–70)</td>
</tr>
<tr>
<td>Carrots</td>
<td>5.3 ± 0 (0.5–12)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>29.1 ± 4.3 (2–60)</td>
</tr>
</tbody>
</table>

\(^1n = 18. Dashes indicate that all subjects consumed calories and sodium.\)

\[\approx 1\text{ cup of coffee/d}\) and continuously (in mg: how much caffeine the subject ate or drank the day before the test and habitually consumes per day). We first assessed the influence of TAS2R38 gene expression and caffeine intake separately and then included both in the same model. The models were conducted for the perception of broccoli juice and broccoli intake, which were exploratory because of the low dietary intake of broccoli.

In the separate models, TAS2R38 gene expression amounts accounted for 38.0% \[F(1,16) = 9.77, P = 0.006\] and caffeine intake accounted for 56.6% \[F(5,12) = 3.13, P = 0.04\] of the variance in PROP bitterness intensity (Figure 5). In the combined model, both predictors accounted for 64.6% of the variation overall \[F(6,11) = 3.36, P = 0.039\], indicating that the relation between gene expression and caffeine intake largely overlaps. The perceived bitterness of broccoli was related to gene expression \[F(1,16)=11.07, P = 0.004\], which accounted for 41% of the variation in individual ratings of its bitterness but was unrelated to broccoli intake \[F(2,15) = 1.51, P = 0.25\]. Given the lack of effect of broccoli consumption on its perceived
intensity, it was not combined with gene expression in a final model.

DISCUSSION

We provide the first experimental evidence that mRNA expression amounts of the PAV allele of the \(\text{TAS2R38}\) taste receptor gene correlate with sensory perception of bitterness and with dietary habits in humans. PAV-TAS2R38 expression was detected in the majority of PAV/AVI heterozygotes (61%), with substantial variation in expression that related to variations in psychophysical perception of known TAS2R38 ligands PROP and broccoli juice: the greater the gene expression, the greater the ratings of bitterness. Expression was unrelated to bitterness ratings of nonbitter solutions (ie, NaCl and carrot juice). To our knowledge, this is also the first study to relate caffeine intake to mRNA expression amounts of a taste receptor gene: caffeine consumption showed substantial variation that was positively correlated with PAV-TAS2R38 expression amounts. We acknowledge that the small number of subjects limits our ability to generalize these results to all human populations, but they do provide impetus for larger future studies.

PROP and other structurally related compounds are primarily recognized by the TAS2R38 bitter receptor (11), but there is a range in individual bitterness perception, even among those with the same genotype (16, 30). The relation we found between PAV-TAS2R38 mRNA expression amounts and bitterness intensity ratings for PROP and broccoli juice suggests that mRNA expression is a proxy for the density of these receptors within the papillae, which in turn may affect transduction of the bitter taste intensity signal. The lack of a relation between gene expression and ratings for control taste stimuli (ie, carrot juice and NaCl) indicates that the effect was not a result of an overall increase in taste sensitivity. We also found a lack of relation between PAV-TAS2R38 expression and recognition thresholds for PROP [the values of which were consistent with previously published results (11)]. This may be a result of the method used to measure recognition thresholds (31) or it may indicate that expression affects suprathreshold perception, not sensitivity. Perhaps at higher stimulus concentrations, most or all receptors are activated and the higher the number, the more intense the perception; whereas at low concentrations, there are so few bitter molecules, each will find a receptor, even at low density. This is an important area for future research.

Two of the bitter non-TAS2R38 ligands that were tested (caffeine and urea) were not related to PAV-TAS2R38 expression, suggesting that the relation between expression and PROP and broccoli juice is not a result of an increase in bitterness sensitivity overall but is specific to TAS2R38. However, subjects’ ratings of the bitterness of the non-TAS2R38 ligands quinine and denatonium benzoate did significantly correlate with PAV-TAS2R38 expression. Both of these ligands bind to TAS2R4 and TAS2R39 (13), which reside on either side of TAS2R38 on chromosome 7 (15). Expression of the bitter receptors on chromosome 7 may be regulated by shared control elements, causing TAS2R38 expression to correlate with expression of other receptors in that region. The direct relation between PAV-TAS2R38 expression and AVI-TAS2R38 expression suggests that expression at the TAS2R38 locus may also share common control mechanisms for both alleles. These possibilities are currently under investigation.

PAV-TAS2R38 expression amounts varied substantially among individuals, spanning 4 orders of magnitude. Behrens et al’s (17) study on TAS2R gene expression in human circumvallate papillae reported expression amounts ~2 orders of magnitude lower than
the average PAV-TAS2R38 expression we measured in fungiform papillae. This difference may relate to dissimilarities in expression between human papilla types, which are currently unknown, the number of subjects studied, or methodologic differences between the studies.

Caffeine was widely consumed and in highly variable amounts in our study participants, and this variation was positively correlated with PAV-TAS2R38 expression amounts. There have been reports of a common genetic factor for PROP and caffeine (32); however, a genomewide association study of habitual caffeine intake found no association with TAS2R38 SNPs (33). Whether caffeine intake drives bitter receptor expression or vice versa remains unknown. That there was no relation between receptor expression amounts and bitterness ratings of caffeine suggests that bitter taste is not the driving force in intake here. The most commonly consumed caffeinated beverages—sodas, tea, and coffee—are often sweetened and hence the bitter taste is masked. In addition, caffeine produces mild psychostimulant effects that are thought to underlie its widespread use (34).

Unlike caffeine, glucosinolate-containing vegetables were not widely consumed by our study participants and therefore we could not test whether a relation existed with expression amounts. These findings raise important issues about the nature of bitter perception in the short and long term. In addition, because “taste” receptors are found in other tissues, such as nasal epithelium, where they affect susceptibility to chronic sinusitis (38), studies of regulation of gene expression could have implications beyond taste and diet.

We acknowledge the expert technical assistance of Sara Castor, Laura Lukasewycz, Loma Inamdar, Kristi Roberts, Christina Furia, Anna Lysenko, Liang-Dar (Daniel) Hwang, Rebecca James, and Brad Fesi and the editorial assistance of Patricia Watson. The authors’ responsibilities were as follows—SVL, JAM, and DRR: designed the research, analyzed data, performed statistical analysis, and wrote the manuscript; SVL and AIS: conducted the research; and JAM and DRR: share primary responsibility for the final content. All authors read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES


