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Predominant qualities evoked by quinine, sucrose, and capsaicin associate with PROP bitterness, but not *TAS2R38* genotype

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Abstract

Genetic variability in the ability to taste thiourea compounds has been studied for 80+ years. Over the last 3 decades, many studies have reported perceived intensity of concentrated propylthiouracil (PROP) associates with greater intensity from a broad range of stimuli, including non-bitter tastants, irritants, and retronasally delivered odorants. Thus, PROP phenotype has become a common measure of individual differences in orosensation. Much, but not all, of the phenotypic variation in PROP bitterness is explained by *TAS2R38* polymorphisms. While differences in PROP bitterness are clearly due to genetic variation, mechanistically it is challenging to envision how this receptor (narrowly tuned to the N–C=S moiety) relates to overall orosensory response. Here, we report data for 200+ individuals who had been genotyped for *TAS2R38* and phenotyped for PROP in laboratory setting. Participants also reported the intensity of quinine, capsaicin, and sucrose on a general Labeled Magnitude Scale. Our data recapitulate earlier reports associating PROP bitterness with the intensity of the predominant qualities of sucrose, quinine, and capsaicin; however, we also find correlations between the intensities of sucrose, quinine, and capsaicin were much stronger with each other than with PROP. As expected, *TAS2R38* diplotype did not associate with the intensity of sucrose, quinine, or capsaicin. The strength of PROP-capsaicin and PROP-sucrose relationships increased after grouping participants by *TAS2R38* diplotype, with the greatest increases in association observed within homozygotes. Collectively, this suggests the suprathreshold intensity of PROP is a confounded phenotype that captures both genetic variation specific to N–C=S compounds and overall orosensation.

Introduction

The suprathreshold bitterness intensity of propylthiouracil (PROP) correlates with perception of sensations evoked from a variety of compounds, including prototypical tastants (Bajec and Pickering 2008, Hayes et al, 2008; Bartoshuk *et al.* 1992; Drewnowski *et al.* 1998; Hansen *et al.* 2006; Ly and Drewnowski 2001), chemesthetic agents (Bartoshuk *et al.* 1993; Campbell 2000) and real foods and beverages (Prescott *et al.* 2004; Shen *et al.* 2016). Generally, individuals who perceive greater bitterness from PROP also perceive increased intensity of sensations from these compounds and foods than do individuals reporting lower or no bitterness from PROP. This relationship is also seen with sampled alcohol or alcoholic beverage (Bartoshuk *et al.* 1993; Duffy *et al.* 2004; Intranuovo and Powers 1998) and non-nutritive sweeteners (Allen *et al.* 2013; Bartoshuk 1979; Gent and Bartoshuk 1983; Zhao and Tepper 2007). Moreover, this association has been reported for other orosensory qualities including astringency (Bajec and Pickering 2008; Pickering *et al.* 2004), sourness (Bajec and Pickering 2008), and fat (Drewnowski *et al.* 1998; Tepper and Nurse 1997; 1998). However, greater PROP bitterness does not always associate with heightened sensations of all oral stimuli (e.g., (Horne *et al.* 2002; Lim *et al.* 2008)).

Furthermore, this measure of PROP phenotype is associated with reported preference and intake of foods and beverages (Dinehart *et al.* 2006; Duffy *et al.* 2004; Keller *et al.* 2002; Mennella *et al.* 2005; Ullrich 2004) (see (Keller and Adise 2016; Tepper 2008) for reviews). For example, individuals who experience greater bitterness from PROP report consuming fewer vegetables compared to PROP non-tasters (Bell and Tepper 2006; Dinehart *et al.* 2006; Duffy *et al.* 2010; Keller *et al.* 2002). In summary, measuring PROP response is a relatively quick phenotypic measure, which can serve as

a predictor of individual variability food preference and intake, which in turn are partially driven by differences in perception of various sensory properties. However, it is only one taste phenotype, and it may not relate or generalize to other measures of taste function (see (Hayes and Keast 2011; Kalva *et al.* 2014; Webb *et al.* 2015)).

A major breakthrough in the mechanistic understanding of individual differences in the perception of thiourea (N–C=S) compounds was made in 2003 by Kim, Drayna and colleagues (Kim *et al.* 2003). They showed variable detection thresholds for phenylthiocarbamide (PTC) were due to polymorphisms in the bitter taste receptor gene *PTC*, which was subsequently renamed *TAS2R38* (HGNC: 9584). Soon thereafter, this finding was extended to suprathreshold bitterness of propylthiouracil (PROP) by multiple research groups (Bufe *et al.* 2005; Duffy *et al.* 2004). The *TAS2R38* gene contains three single nucleotide polymorphisms (SNPs) that are typically (but not always) inherited together; these 3 SNPs are named for the resulting amino acid substitutions (A49P, V262A, and I296V), and they form two common (PAV and AVI) and 4 uncommon (AAV, AAI, PAI, PVI) haplotypes (see (Boxer and Garneau 2015; Bufo *et al.* 2005; Garneau *et al.* 2014)). The *TAS2R38* genotype has been repeatedly shown to associate with PROP bitterness (e.g., (Allen *et al.* 2013; Boxer and Garneau 2015; Bufo *et al.* 2005; Duffy *et al.* 2004; Hayes *et al.* 2008). There is also evidence of *TAS2R38* genotype explaining variability in preference and intake of foods and beverages (Beckett *et al.* 2017; Duffy *et al.* 2004; Duffy *et al.* 2010; Gorovic *et al.* 2011; Hayes *et al.* 2011).

Since discovery of the functional polymorphisms in *TAS2R38* in 2003, many of the relationships originally reported between PROP intensity and other sensory properties have been attributed to genetic variation in *TAS2R38*. Critically however, it would be inaccurate to conclude that all observed relationships between the

suprathreshold bitterness of PROP (e.g., (Hayes *et al.* 2008; Hayes and Keast 2011; Webb *et al.* 2015)) and differences in food sensations, affective responses, or food intake (see reviews by (Diószegi *et al.* 2019; Keller and Adise 2016)) are due to functional variation in a bitter taste receptor that is narrowly tuned to the N–C=S moiety found on thiourea compounds (Barnicot *et al.* 1951; Wooding *et al.* 2010). For example, there is no simple biologically plausible mechanism by which *TAS2R38* genotype should influence a sensation like creaminess, yet, the absence of a simple mechanism does not preclude a reproduceable relationship between PROP bitterness (the *phenotype*) and creaminess (e.g., (Hayes and Duffy 2007; Tepper and Nurse 1998)). Indeed, prior work demonstrated that elevated responses to various stimuli (that is, supertasting) cannot be explained by *TAS2R38* genotype (Hayes *et al.* 2008). The goal of the present work was to use a large laboratory cohort to test the predictive utility of PROP bitterness on other oral sensations after controlling for *TAS2R38* genotype.

Materials and Methods

Overview

The results presented here were drawn from a larger study focused on the genetics of oral sensation. The full protocol involved four test sessions on separate days. Data presented here were obtained in the first visit, so the description of the methods will be restricted to session one. Details on the other visits have been reported elsewhere (Allen *et al.* 2014; Feeney and Hayes 2014b; Nolden *et al.* 2016). Briefly, the first test session took roughly 1 hour to complete and was conducted one-on-one with research staff. Testing included obtaining consent, completion of a food-liking questionnaire, tasting multiple chemical stimuli, measurement of anthropometric data, and collection of a salivary DNA sample. After being oriented to the scaling procedure (details below), participants sampled six compounds (both tastants and irritants) for multiple sensory qualities. Lastly, PROP phenotype was collected via a standard protocol, using both PROP, salt and auditory stimuli (e.g., Duffy, Peterson, et al. 2003; Hayes et al. 2008). Other data were also collected in session one (e.g., Byrnes & Hayes 2015) but are not described here for brevity. Participants provided written informed consent and were compensated for their time. All procedures were approved by the Pennsylvania State University Institutional Review Board (protocol number #33176), and this study complies with the Helsinki declaration.

Participants

Interested individuals were screened prior to scheduling the first visit to ensure they eligibility criteria. These included: between 18-45 years old, not pregnant or breastfeeding, non-smoker (had not smoked in the last 30 days), no known defects of

smell or taste, no lip, cheek or tongue piercings, no history of any condition involving chronic pain, not currently taking any prescription pain medication, no reported history of choking or difficulty swallowing, and no history of thyroid disease. Participants also needed to be willing to provide a salivary DNA sample. Additional demographic details on participants are provided below in the results.

Psychophysical scaling and rating of stimuli

Participants rated stimuli on a general Labeled Magnitude Scale (gLMS) (Snyder *et al.* 2004). The gLMS ranges from ‘no sensation’ (0) to ‘the strongest imaginable sensation of any kind’ (100), with intermediate descriptors at 1.4 (‘barely detectable’), 6 (‘weak’), 17 (‘moderate’), 35 (‘strong’) and 51 (‘very strong’). Each participant was oriented to the scale, first with a verbal explanation of the gLMS, followed by a practice session where participants made ratings for a list of 15 imagined or remembered sensations that included both food and non-food items (Hayes *et al.* 2013). The orientation and practice were meant to encourage participants to make ratings on the scale in a generalized context that not limited to oral sensations. They were also instructed to not let whether or not a stimulus was liked or disliked influence their intensity rating, and that they should click anywhere along the scale, not merely near the verbal labels. Participants were also told, “You may receive stimuli causing more than one quality. Please attend to all sensations on all trials.” Separate ratings for sweetness, bitterness, sourness, burning/stinging, savory/umami, and saltiness were collected for each stimulus. All ratings were collected using Compusense *five*, version 5.2 (Guelph, Ontario, Canada).

Stimuli and Tasting Procedure

Six stimuli were presented to participants in this portion of the experiment (see (Allen *et al.* 2013)), but only 3 are analyzed here: 0.41 mM quinine HCl, 0.5 M sucrose, and 25 uM capsaicin. Data for the other tastants (potassium chloride, acesulfame potassium, and an MSG/IMP blend) have been described elsewhere (Allen *et al.* 2013; Feeney and Hayes 2014a; b). All solutions were made fresh weekly with food grade reagents and stored in a refrigerator; all samples were brought to room temperature before tasting. Participants swished a 10-mL sample for 3 seconds and then expectorated prior to rating. Presentation order of all 6 stimuli was counterbalanced across participants using a Williams's design. Prior to sampling the first solution and between each solution, participants rinsed with room temperature reverse osmosis (RO) water. A minimum of break of 30 seconds was enforced between each; however, participants were encouraged to wait longer if any lingering sensation remained.

Determining an individual's PROP phenotype

PROP phenotypes were determined using a standard method described previously (e.g., (Dinehart *et al.* 2006; Duffy *et al.* 2004; Hayes *et al.* 2010)). Briefly, participants rated the intensity of 5 PROP solutions, 5 salt solutions, and 5 1-kHz tones. The salt and PROP concentrations were presented in duplicate, while the sets of tones were repeated 5 times. The presentation order of stimuli was blocked, so participants received solutions and tones in alternating series: specifically, 5 tones, 5 salt solutions, 5 tones, 5 salt solutions, 5 tones, 5 PROP solutions, 5 tones, 5 PROP solutions and 5 tones. Within a block, concentrations and tones were presented in counterbalanced orders. The concentrations of PROP solutions were made with USP grade PROP (Sigma, St

Louis MO) and had a final concentration of 0.032, 0.1, 0.32, 1, 3.2 mM, while the salt solutions were 0.01, 0.032, 0.1, 0.32 and 1M sodium chloride. All solutions were prepared with reverse osmosis (RO) water. The 1-kHz tones were generated with a Maico MA39 audiometer, which had been modified to deliver tones binaurally. The auditory stimuli were presented in a wide range, from 50-90 dB in 10 dB steps. Participants rinsed with room temperature RO water between each sample, waiting a minimum of 30 seconds before next sample. Means of the 3.2mM PROP and 80 dB tones were calculated for each participant, and used in all subsequent analyses.

Genetic Analysis

Each participant provided a salivary DNA sample using an Oragene collection kit according to manufacturer instructions (Genotek Inc, Ontario, Canada). Three single nucleotide polymorphisms (SNPS) in *TAS2R38* were determined using Sequenom MassARRAY technology (Sequenom, San Diego, CA). The three SNPs analyzed here include rs713598 (A49P), rs1726866 (V262A), and rs10246939 (I269V), which are commonly inherited together, forming three common haplotypes (PAV/PAV, PAV/AVI, or AVI/AVI). All primers were purchased from Integrated DNA Technologies (Coralville, Iowa, USA). Participant's genotypes were automatically determined using MassARRAY software (Sequenom). Additionally, two technicians independently inspected the genotypes. As a measure of reliability, a total of 15% of samples are rerun.

Statistical Analysis

Haplotypes were determined using Bayesian imputation using PHASE, and only those with a probability greater than 0.80 are reported. Data were analyzed using SAS

216 9.4 (Cary, NC). Genetic variants were analyzed via analysis of variance (ANOVA) via
217 *proc mixed*, and pairwise comparisons were adjusted for multiple comparisons using
218 the Tukey-Kramer method. Pearson correlations were conducted via *proc corr* to
219 determine the association between rated sensations. Multiple regression models were
220 created using *proc reg* to determine the association between rated sensations and dB
221 tones; information regarding the contributions of individual variables are reported as
222 semipartial correlations (sr).

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Results

TAS2R38 diplotype are associated with PROP bitterness

Of the total number of participants in the study, 89% (n=220) carried common diplotypes: PAV/PAV, PAV/AVI or AVI/AVI. The majority of these participants were common haplotype heterozygotes (PAV/AVI; n=121) with roughly balanced frequencies of PAV homozygotes (n=48) and AVI homozygotes (n=51), which is generally consistent with convenience samples in the United States (e.g., (Duffy *et al.* 2010; Garneau *et al.* 2014; Hayes *et al.* 2008; Kim *et al.* 2005)). As expected, some participants had rare diplotypes, including 8 PAV/AAV and 12 AVI/AAV individuals; the remaining 6 individuals had other rare diplotypes. Due to these low counts, individuals with rare diplotypes were dropped from further analysis to focus on the common diplotypes (i.e., PAV/PAV, PAV/AVI, AVI/AVI). Of the 220 participants whose data are reported below, 89 were men, and 131 were women, with a mean age of 25.7 (SD 7.2). Using categories provided by the 1997 OMB Directive 15 guidelines, the majority of the participants self-identified as White/Caucasian (n=152), followed by Asian (n=34), and African American (n=5).

As expected, *TAS2R38* diplotype was significantly associated with PROP bitterness ([F(2,217)=48.54; p<0.0001]). As shown in Figure 1, the PAV homozygotes rated PROP as being more bitter (55.5±2.7 SEM) than heterozygotes (43.6±1.7 SEM), or AVI homozygotes (19.4±2.6 SEM). In pairwise comparisons (Tukey-Kramer), all groups differed from each other (all p's <0.001).

Figure 1: Participants are grouped by *TAS2R38* diplotype. PROP bitterness ratings (general Labeled Magnitude Scale; gLMS) are reported on the y-axis, with labels barely detectable (BD), weak, moderate, strong, very strong, at 1.4, 6, 17, 35 and 51. In pairwise comparisons, all three diplotype groups were significantly different from each other (Tukey-Kramer; all p 's < 0.001).

The bitterness of quinine, sweetness of sucrose, and burn of capsaicin are associated with PROP bitterness, but not TAS2R38 diplotype

To determine if ratings of sampled stimuli associated with both PROP bitterness and *TAS2R38* genotype, correlations and ANOVA models were performed for the common diplotypes. As shown in Figure 2 (left), quinine bitterness was significantly correlated with PROP bitterness ($r=+0.39$; $p<0.0001$). Conversely, quinine bitterness did not differ by *TAS2R38* genotype ($[F(2,217)=1.86$; $p=0.16]$). As shown in Figure 2 (right), as expected, the bitterness of quinine showed no differences between PAV homozygotes (30.0 ± 2.78), heterozygotes (24.4 ± 1.75), or AVI homozygotes (28.8 ± 2.70).

The same analysis was then carried out for the sweetness of sucrose, where sweetness intensity was predicted separately from the bitterness of PROP and *TAS2R38* genotype. As above, the sweetness of sucrose was significantly correlated with PROP bitterness ($r=+0.23$; $p=0.0008$), as shown in Figure 3 (left). Somewhat surprisingly, *TAS2R38* genotype was marginally associated with the sweetness of sucrose ($[F(2,217)=3.25$; $p=0.04]$). However, as Figure 3 (right) shows, pairwise comparisons (Tukey-Kramer) revealed no significant differences (all p 's >0.077) between PAV homozygotes, heterozygotes, or AVI homozygotes for sweetness (mean ratings of 31.5 ± 2.45 , 26.0 ± 1.54 , and 32.2 ± 2.38 , respectively).

Finally, the same analysis was performed for the burn of capsaicin. Again, there was a positive correlation between the burn of capsaicin and PROP bitterness ($r=+0.26$; $p<0.0001$), as shown in Figure 4 (left). However, there was no relationship between the burn of capsaicin and *TAS2R38* genotype ($[F(2,217)=1.27$; $p=0.28]$), as shown in Figure 4 (right). Mean ratings of capsaicin burn for PAV homozygotes, heterozygotes, or AVI homozygotes were 37.2 ± 2.99 , 32.5 ± 1.88 , and 37.7 ± 2.90 , respectively.

Figure 2: Bitterness of quinine, as a function of bitterness of PROP bitterness (left) and *TAS2R38* diplotype (right).

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283 Figure 3: Sweetness of sucrose, as a function of bitterness of PROP bitterness (left) and *TAS2R38* diplotype (right). In the
 284 right panel, there was weak evidence of a main effect of genotype, but in pairwise comparisons (Tukey-Kramer), none of
 285 the groups differed from each other (all p 's > 0.077).

Figure 4: Burn of capsaicin, as a function of bitterness of PROP bitterness (left) and *TAS2R38* diplotype (right).

*Controlling for tone intensity ratings does not alter relationships between PROP
bitterness and the prototypical quality of each stimulus*

Because scale usage can vary across individuals (e.g., (Webb *et al.* 2015)), multiple regression models were used to partition out potential differences in scale usage that would otherwise inflate apparent relationships between PROP bitterness and the prototypical stimuli (i.e., the bitterness of quinine, the sweetness of sucrose, and the burning of capsaicin). That is, if some individuals tended to rate all stimuli as more intense, while other individuals rated all stimuli as less intense, this scaling bias would make the bitterness of PROP appear to be correlated with the intensity of quinine, sucrose, and capsaicin. By including a cross modal standard like tone intensities in the model, we can test if the elevated ratings are specific to chemosensation rather than merely being an artifact of how participants differ in their usage of the scale.

In a multiple regression model predicting the bitterness intensity of quinine, mean ratings of the 80 dB tones and PROP bitterness explained 18.9% of the variability ($p < 0.0001$); both 80dB tones ($sr = 0.21$; $p = 0.0007$) and PROP bitterness ($sr = 0.30$; $p < 0.0001$) were significant predictors. A similar result was obtained for a multiple regression model predicting the sweetness of sucrose: the overall model explained 11.4% of the variation in sucrose intensity, with both 80dB tone ratings ($sr = 0.25$, $p = 0.0001$) and PROP bitterness ($sr = 0.14$; $p = 0.031$) as significant predictors. Finally, the multiple regression model for capsaicin burn explained 10.1% of the variation in burn ratings, and again, both mean 80dB tone ratings ($sr = 0.18$; $p = 0.005$) and PROP bitterness ($sr = 0.19$; $p = 0.003$) contributed significantly to the model. In summary, mean ratings for 80 dB tones were significant predictors of quinine bitterness, sucrose sweetness, and capsaicin burn in simple regression models (not shown), but when PROP bitterness was

added to each model, additional variance was explained above and beyond what was explained by ratings of the 1-kHz tones. Collectively, this suggests that the ability of PROP bitterness to predict the burn of capsaicin, the sweetness of sucrose, and the bitterness of quinine is not merely an artifact due to differential scale usage.

Capsaicin burn is a better predictor of sucrose sweetness and quinine bitterness than PROP bitterness, but partitioning participants by genotype improves correlations between PROP bitterness and ratings for the other stimuli

As shown in Table 1, the ratings for the predominant qualities for all of the stimuli were significantly correlated with each other. In general, the ability of PROP bitterness to predict the intensity of other stimuli increased when effects of genotype were partitioned out (Table 2).

Table 1: Matrix of Pearson correlations for ratings of prototypical qualities across all 220 participants.

	Capsaicin	Quinine	Sucrose
Quinine	+0.48 <.0001	–	–
Sucrose	+0.48 <.0001	+0.51 <.0001	–
PROP	+0.26 <.0001	+0.38 <.0001	+0.23 0.0008

Table 2: Matrix of Pearson correlations for ratings of prototypical qualities, broken down by *TAS2R38* genotype group.

AVI/AVI homozygotes (n = 51)			
	Capsaicin	Quinine	Sucrose
Quinine	<i>+0.60</i> <i><.0001</i>	–	–
Sucrose	<i>+0.71</i> <i><.0001</i>	<i>+0.58</i> <i><.0001</i>	–
PROP	<i>+0.36</i> <i>.0094</i>	<i>+0.40</i> <i>.0033</i>	<i>+0.29</i> <i>.0365</i>
PAV/AVI heterozygotes (n = 121)			
	Capsaicin	Quinine	Sucrose
Quinine	<i>+0.38</i> <i><.0001</i>	–	–
Sucrose	<i>+0.33</i> <i>.0002</i>	<i>+0.47</i> <i><.0001</i>	–
PROP	<i>+0.30</i> <i>.0008</i>	<i>+0.55</i> <i><.0001</i>	<i>+0.27</i> <i>.0026</i>
PAV/PAV homozygotes (n = 48)			
	Capsaicin	Quinine	Sucrose
Quinine	<i>+0.45</i> <i>.0013</i>	–	–
Sucrose	<i>+0.36</i> <i>.0125</i>	<i>+0.44</i> <i>.0020</i>	–
PROP	<i>+0.39</i> <i>.0058</i>	<i>+0.44</i> <i>.0017</i>	<i>+0.46</i> <i>.0010</i>

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Discussion

In the data described here, variability in suprathreshold ratings of sampled quinine, sucrose, and capsaicin were associated with the bitterness of PROP but not *TAS2R38* genotype. Further, as expected, we confirm numerous prior reports that show variability in PROP bitterness associates with *TAS2R38*, with greater PROP ratings observed within PAV heterozygotes, compared to heterozygotes and AVI homozygotes (e.g., (Bufe *et al.* 2005; Duffy *et al.* 2010; Garneau *et al.* 2014; Hayes *et al.* 2008; Kim *et al.* 2003; Mennella *et al.* 2010)).

Prior work indicates bitterness, sourness, saltiness, sweetness, metallic, and astringent ratings for prototypical chemosensory stimuli are positively correlated with PROP bitterness (e.g., (Bajec and Pickering 2008; Hayes *et al.* 2008; Pickering *et al.* 2004)). Similarly, the creaminess of dairy products has been positively associated with PROP bitterness (Kirkmeyer and Tepper 2005; Tepper and Nurse 1997), although not all studies agree (cf (Hayes and Duffy 2007) and (Lim *et al.* 2008)). Likewise, the sweetness of acesulfame potassium (AceK) (but not the bitterness) associates with the bitterness of PROP (Allen *et al.* 2013). Here, we find similar effects, with significant positive relationships between quinine bitterness, sucrose sweetness, and capsaicin burn with PROP bitterness.

Notably, Lim *et al.* (Lim *et al.* 2008) reported the sweetness of sucrose was more highly correlated with ratings for other tastants, including salt, citric acid, and quinine than with PROP. Using a similar analysis here, we found evidence that the relationship between a chemesthetic agent (capsaicin) and various prototypical tastants was greater than with PROP (see Table 1), at least before controlling for genetic variation. The correlation between burn of capsaicin and the tastants (sucrose and quinine) was

greater than with the bitterness of PROP, suggesting the burn of capsaicin is a better predictor of the intensity of tastants in water than PROP bitterness.

Here, we also show that correlations between PROP bitterness and the intensity of sucrose, quinine, and capsaicin appear to be due to real differences in psychophysical responses, and are not merely an artifact of scale usage. Individuals may use the scale differently (e.g., (Webb *et al.* 2015)), with some individuals rating all stimuli as more intense, while other individuals rating all stimuli as less intense. Here, we confirm that PROP bitterness is associated with intensity ratings of sucrose, quinine, and capsaicin, even after controlling for intensity ratings of a cross modal standard (i.e., 80 dB tone). In other words, we can conclude that elevated ratings are specific to chemosensory responses and are not merely driven by differential usage of the scale.

While diverse oral sensations correlate with greater bitterness from PROP, mechanistically, it would be inaccurate to conclude these findings are driven by the *TAS2R38* genotype. Here, we illustrate that suprathreshold ratings of stimuli other than PROP were not associated with *TAS2R38* genotype, which is wholly consistent with the narrow tuning of this receptor (Meyerhof *et al.* 2010). However, these data do contradict one prior report (Laaksonen *et al.* 2013) which found a significant relationship between *TAS2R38* and astringency and sourness of berry juices in a relatively small group of participants (n=41). Specifically, PAV homozygotes (n=12) reported less astringency and sourness than AVI homozygotes (n=14) (Laaksonen *et al.* 2013). Accordingly, this may represent sampling error due to low numbers of participants. Further, while the differences were statistically significant between PAV homozygotes and AVI homozygotes, they were relatively small (~2 points on a gLMS) (Laaksonen *et al.* 2013).

On balance, current and prior data suggest *TAS2R38* genotype is not a strong predictor of intensity of suprathreshold tastants and chemesthetic compounds other than PROP.

The present data recapitulate prior reports which find no relationship between *TAS2R38* and suprathreshold ratings of other chemosensory stimuli (e.g., (Hayes *et al.* 2008). The present study emphasizes that the genotype is not the phenotype.

Frequently, PROP phenotype is evaluated as a proxy for *TAS2R38* genotype; yet, it has been demonstrated that PROP ‘taster status’ does not always conform to genotype (Bufer *et al.* 2005; Green 2013). In other words, the *TAS2R38* haplotype does not explain all the variability observed with the PROP phenotype, suggesting additional factors influence PROP perception. Some of these factors may include anatomical differences, such as fungiform papillae density (although data conflict, contrast Garneau *et al.* 2014; Feeney and Hayes, 2014a, with Hayes *et al.* 2008; Duffy *et al.* 2004), differences in relative protein expression in heterozygotes (e.g., Lipchick *et al.* 2013), salivary proteins (e.g., Melis *et al.* 2013), or polymorphisms in the gustin (CA6) gene (although again, data conflict, cf Feeney and Hayes, 2014a, with Calo *et al.* 2011; Padiglia *et al.* 2010).

Previously, Hayes and colleagues speculated the existence of second PROP receptor (i.e., another TAS2R that is also activated by PROP) could help explain the apparent recovery of function observed in some AVI homozygotes who should not otherwise respond to PROP (Hayes *et al.* 2008). Here, in Figure 1, we see a handful of AVI homozygotes who still report strong bitterness from PROP, even though the AVI variant is thought to be nonfunctional. Additional work is needed to determine if another TAS2R receptor responds to PROP, and whether polymorphisms in the corresponding *TAS2R* gene can explain additional variation in PROP phenotype.

Multiple factors have been suggested or identified as having a relationship with heightened taste responsiveness. It has been proposed that PROP response alone is not a strong predictor of heightened overall chemosensation (Webb et al 2015); indeed, other recent studies (e.g., (Kalva *et al.* 2014)) have defined supertasting using intensity ratings of multiple chemical stimuli (i.e., NaCl, sucrose, citric acid, and quinine) presented at suprathreshold concentrations. An increased chemosensory response across diverse taste qualities aligns with the central gain theory proposed previously by Green and George (Green and George 2004). Further, elevated psychophysical responses are not unique to taste and have been observed in other modalities (e.g., auditory sensations (Schneider *et al.* 2011)). The central gain theory suggests that suprathreshold responses for all chemosensory stimuli may be partly determined by differences in taste transduction mechanism in the periphery, such as nerve stimulation, but also cognitive processes (Green 2013). This view fits with other findings by Webb and colleagues (Webb *et al.* 2015), who concluded that no single measure of taste – be it detection thresholds, recognition thresholds, suprathreshold intensity ratings for PROP, or for other taste stimuli – is able to fully characterize overall taste function.

Conclusions

Present data support that PROP bitterness is a significant predictor of both taste and chemesthetic responses to diverse chemical stimuli. Participants who rated the bitterness of PROP more intensely also gave higher ratings for sucrose, quinine and capsaicin, and this was not due merely to differences in scale usage. As expected, PROP bitterness was significantly associated with the *TAS2R38* genotype. Critically however, *TAS2R38* genotype was unable to explain differences in the sweetness of sucrose,

bitterness of quinine, and burn of capsaicin. Also, ratings for sucrose, quinine, and capsaicin had higher correlations with each other than with PROP bitterness. Notably, correlations between PROP bitterness and both sucrose and quinine increased when participants were grouped by *TAS2R38* genotype. Additional work is needed to determine why the predictive value of PROP bitterness is greater when stratified by diplotype, versus looking across all participants. Present data also reinforce the view that nominal relationships between measures of PROP bitterness and other orosensory qualities cannot be attributed to *TAS2R38* genotype despite clear and robust relationships between *TAS2R38* and PROP bitterness.

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Conflict of interest

AAN and JEM have no potential conflicts to report. JEH has received speaking, travel, and consulting fees from nonprofit organizations, federal agencies, commodity boards, and corporate clients in the food industry. Additionally, the Sensory Evaluation Center at Penn State routinely conducts taste tests for industrial clients to facilitate experiential learning for students.

References

- Allen AL, McGeary JE, Hayes JE. 2014. Polymorphisms in TRPV1 and TAS2Rs Associate with Sensations from Sampled Ethanol. *Alcoholism: Clinical and Experimental Research* 38: 2550-2560.
- Allen AL, McGeary JE, Knopik VS, Hayes JE. 2013. Bitterness of the Non-nutritive Sweetener Acesulfame Potassium Varies With Polymorphisms in TAS2R9 and TAS2R31. *Chemical Senses* 38: 379-389.
- Bajec MR, Pickering GJ. 2008. Thermal taste, PROP responsiveness, and perception of oral sensations. *Physiology & Behavior* 95: 581-590.
- Barnicot N, Harris H, Kalmus H. 1951. Taste thresholds of further eighteen compounds and their correlation with PTC thresholds. *Annals of Eugenics* 16: 119-128.
- Bartoshuk L, Fast K, Karrer T, Marino S, Price R, Reed D. 1992. PROP supertasters and the perception of sweetness and bitterness. *Chem Senses* 17: 594.
- Bartoshuk LM. 1979. Bitter taste of saccharin related to the genetic ability to taste the bitter substance 6-n-propylthiouracil. *Science* 205: 934-935.
- Bartoshuk LM, Conner E, Grubin D, Karrer T, Kochenbach K, Palcso M, Snow D, Pelchat M, Danowski S. 1993. PROP supertasters and the perception of ethyl alcohol. *Chemical senses* 18: 526-527.
- Beckett EL, Duesing K, Boyd L, Yates Z, Veysey M, Lucock M. 2017. A potential sex dimorphism in the relationship between bitter taste and alcohol consumption. *Food & function* 8: 1116-1123.
- Bell KI, Tepper BJ. 2006. Short-term vegetable intake by young children classified by 6-n-propylthiouracil bitter-taste phenotype. *American Journal of Clinical Nutrition* 84: 245-251.
- Boxer EE, Garneau NL. 2015. Rare haplotypes of the gene TAS2R38 confer bitter taste sensitivity in humans. *SpringerPlus* 4: 1-4.
- Bufe 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. *Current Biology* 15: 322-327.
- Calo C, Padiglia A, Zonza A, Corrias L, Contu P, Tepper BJ, Barbarossa IT. 2011. Polymorphisms in TAS2R38 and the taste bud trophic factor, gustin gene co-operate in modulating PROP taste phenotype. *Physiol Behav.* 104(5):1065-71.
- Campbell JPaN. 2000. Responses to Repeated Oral Irritation by capsaicin, cinnamaldehyde and ethanol in PROP tasters and non-tasters. *Chemical senses* 25: 239-246.
- Dinehart M, Hayes J, Bartoshuk L, Lanier S, Duffy V. 2006. Bitter taste markers explain variability in vegetable sweetness, bitterness, and intake. *Physiology & Behavior* 87: 304-313.
- Diószegi J, Llanaj E, Adany R. 2019. Genetic background of taste perception, taste preferences and its implications: a systematic review *Frontiers in Genetics* 10: 1272.
- Drewnowski A, Ahlstrom Henderson S, Barratt-Fornell A. 1998. Genetic Sensitivity to 6-n-Propylthiouracil and Sensory Responses to Sugar and Fat Mixtures. *Physiology & behavior* 63: 771-777.
- 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)

516 Bitterness and Alcohol Intake. *Alcoholism: Clinical and Experimental Research* 28:
 517 1629-1637.
 518 Duffy VB, Hayes JE, Davidson AC, Kidd JR, Kidd KK, Bartoshuk LM. 2010. Vegetable
 519 intake in college-aged adults is explained by oral sensory phenotypes and TAS2R38
 520 genotype. *Chemosensory perception* 3: 137-148.
 521 Feeney EL, Hayes JE. 2014a. Exploring associations between taste perception, oral
 522 anatomy and polymorphisms in the carbonic anhydrase (gustin) gene CA6. *Physiology &*
 523 *behavior* 128: 148-154.
 524 Feeney EL, Hayes JE. 2014b. Regional differences in suprathreshold intensity for bitter
 525 and umami stimuli. *Chemosensory Perception* 7: 147-157.
 526 Garneau NL, Nuessle TM, Sloan MM, Santorico SA, Coughlin BC, Hayes JE. 2014.
 527 Crowdsourcing taste research: genetic and phenotypic predictors of bitter taste
 528 perception as a model. *Front Integr Neurosci* 8: 33.
 529 Gent JF, Bartoshuk LM. 1983. Sweetness of sucrose, neohesperidin dihydrochalcone,
 530 and saccharin is related to genetic ability to taste the bitter substance 6-n-
 531 propylthiouracil. *Chemical senses* 7: 265-272.
 532 Gorovic N, Afzal S, Tjv[Inn]eland A, Overvad K, Vogel U, Albrechtsen C, Poulsen HE.
 533 2011. Genetic variation in the h TAS2R38 taste receptor and brassica vegetable intake.
 534 *Scandinavian Journal of Clinical & Laboratory Investigation* 71: 274-279.
 535 Green BG. 2013. In pursuit of taste phenotypes. *Chemical senses* 38: 289-292.
 536 Green BG, George P. 2004. 'Thermal taste' predicts higher responsiveness to chemical
 537 taste and flavor. *Chemical senses* 29: 617-628.
 538 Hansen JL, Reed DR, Wright MJ, Martin NG, Breslin PAS. 2006. Heritability and
 539 genetic covariation of sensitivity to PROP, SOA, quinine HCl, and caffeine. *Chemical*
 540 *senses* 31: 403-413.
 541 Hayes JE, Allen AL, Bennett SM. 2013. Direct comparison of the generalized Visual
 542 Analog Scale (gVAS) and general Labeled Magnitude Scale (gLMS). *Food Quality and*
 543 *Preference* 28: 36-44.
 544 Hayes JE, Bartoshuk LM, Kidd JR, Duffy VB. 2008. Supertasting and PROP Bitterness
 545 Depends on More Than the TAS2R38 Gene. *Chemical Senses* 33: 255-265.
 546 Hayes JE, Duffy VB. 2007. Revisiting sugar-fat mixtures: sweetness and creaminess
 547 vary with phenotypic markers of oral sensation. *Chemical senses* 32: 225-236.
 548 Hayes JE, Keast RSJ. 2011. Two decades of supertasting: Where do we stand?
 549 *Physiology & behavior* 104: 1072-1074.
 550 Hayes JE, Sullivan BS, Duffy VB. 2010. Explaining variability in sodium intake through
 551 oral sensory phenotype, salt sensation and liking. *Physiology & Behavior* 100: 369-380.
 552 Hayes JE, Wallace MR, Knopik VS, Herbstman DM, Bartoshuk LM, Duffy VB. 2011.
 553 Allelic variation in TAS2R bitter receptor genes associates with variation in sensations
 554 from and ingestive behaviors toward common bitter beverages in adults. *Chemical*
 555 *Senses* 36: 311-319.
 556 Horne J, Lawless HT, Speirs W, Sposato D. 2002. Bitter taste of saccharin and
 557 acesulfame-K. *Chemical Senses* 27: 31-38.
 558 Intrantuovo LR, Powers AS. 1998. The Perceived Bitterness of Beer and 6-n-
 559 Propylthiouracil (PROP) Taste Sensitivity. *Annals of the New York Academy of Sciences*
 560 855: 813-815.

561 Kalva JJ, Sims CA, Puentes LA, Snyder DJ, Bartoshuk LM. 2014. Comparison of the
 562 hedonic general labeled magnitude scale with the hedonic 9-point scale. *Journal of food*
 563 *science* 79: S238-S245.
 564 Keller KL, Adise S. 2016. Variation in the ability to taste bitter thiourea compounds:
 565 implications for food acceptance, dietary intake, and obesity risk in children. *Annual*
 566 *review of nutrition* 36: 157-182.
 567 Keller KL, Steinmann L, Nurse RJ, Tepper BJ. 2002. Genetic taste sensitivity to 6-n-
 568 propylthiouracil influences food preference and reported intake in preschool children.
 569 *Appetite* 38: 3-12.
 570 Kim U, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. 2003. Positional cloning
 571 of the human quantitative trait locus underlying taste sensitivity to
 572 phenylthiocarbamide. *Science's STKE* 299: 1221.
 573 Kim U, Wooding S, Ricci D, Jorde LB, Drayna D. 2005. Worldwide haplotype diversity
 574 and coding sequence variation at human bitter taste receptor loci. *Human mutation* 26:
 575 199-204.
 576 Kirkmeyer SV, Tepper BJ. 2005. Consumer reactions to creaminess and genetic
 577 sensitivity to 6-n-propylthiouracil: A multidimensional study. *Food quality and*
 578 *preference* 16: 545-556.
 579 Laaksonen O, Ahola J, Sandell M. 2013. Explaining and predicting individually
 580 experienced liking of berry fractions by the hTAS2R38 taste receptor genotype. *Appetite*
 581 61: 85-96.
 582 Lipchock SV, Mennella, JA, Spielman AI, Reed DR. 2013. Human bitter perception
 583 correlates with bitter receptor messenger RNA expression in taste cells. *Am J Clin Nutr*
 584 98: 1136-1143.
 585 Lim J, Urban L, Green BG. 2008. Measures of individual differences in taste and
 586 creaminess perception. *Chemical senses* 33: 493-501.
 587 Ly A, Drewnowski A. 2001. PROP (6-n-propylthiouracil) tasting and sensory responses
 588 to caffeine, sucrose, neohesperidin dihydrochalcone and chocolate. *Chemical senses* 26:
 589 41-47.
 590 Melis M, Aragoni MC, Arca M, Cabras T, Caltagirone C, Castagnola M, Crnjar R,
 591 Messana I, Tepper BJ, Barbarossa IT. 2013. Marked increase in PROP taste
 592 responsiveness following oral supplementation with selected salivary proteins or their
 593 related free amino acids. *PLoS One* 8(3):e59810.
 594 Mennella J, Pepino MY, Duke F, Reed D. 2010. Age modifies the genotype-phenotype
 595 relationship for the bitter receptor TAS2R38. *BMC Genetics* 11: 60.
 596 Mennella JA, Pepino MY, Reed DR. 2005. Genetic and environmental determinants of
 597 bitter perception and sweet preferences. *Pediatrics* 115: e216-e222.
 598 Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G,
 599 Behrens M. 2010. The molecular receptive ranges of human TAS2R bitter taste
 600 receptors. *Chemical Senses* 35: 157-170.
 601 Nolden AA, McGeary JE, Hayes JE. 2016. Differential bitterness in capsaicin, piperine,
 602 and ethanol associates with polymorphisms in multiple bitter taste receptor genes.
 603 *Physiology & behavior* 156: 117-127.
 604 Padiglia A, Zonza A, Atzori E, Chillotti C, Calo C, Tepper BJ, Barbarossa IT. 2010.
 605 Sensitivity to 6-n-propylthiouracil is associated with gustin (carbonic anhydrase VI)
 606 gene polymorphism, salivary zinc, and body mass index in humans. *Am J Clin Nutr*
 607 92(3):539-45. Pickering GJ, Simunkova K, DiBattista D. 2004. Intensity of taste and

astringency sensations elicited by red wines is associated with sensitivity to PROP (6-n-propylthiouracil). *Food Quality and Preference* 15: 147-154.
 Prescott J, Soo J, Campbell H, Roberts C. 2004. Responses of PROP taster groups to variations in sensory qualities within foods and beverages. *Physiology & behavior* 82: 459-469.
 Schneider BA, Parker S, Murphy D. 2011. A model of top-down gain control in the auditory system. *Attention, Perception, & Psychophysics* 73: 1562-1578.
 Shen Y, Kennedy OB, Methven L. 2016. Exploring the effects of genotypical and phenotypical variations in bitter taste sensitivity on perception, liking and intake of brassica vegetables in the UK. *Food Quality and Preference* 50: 71-81.
 Snyder D, Fast K, Bartoshuk LM. 2004. Valid comparisons of suprathreshold sensations. *Journal of consciousness studies* 11: 7-8.
 Tepper BJ. 2008. Nutritional implications of genetic taste variation: the role of PROP sensitivity and other taste phenotypes. *Annual Review Nutrition* 28: 367-388.
 Tepper BJ, Nurse RJ. 1997. Fat perception is related to PROP taster status. *Physiology & Behaviour* 61: 949-954.
 Tepper BJ, Nurse RJ. 1998. PROP Taster Status Is Related to Fat Perception and Preference. *Annals of the New York Academy of Sciences* 855: 802-804.
 Ullrich N. 2004. PROP Taster Status and Self-Perceived Food Adventurousness Influence Food Preference. *The american diet association*.
 Webb J, Bolhuis DP, Cicerale S, Hayes JE, Keast R. 2015. The relationships between common measurements of taste function. *Chemosensory perception* 8: 11-18.
 Wooding S, Gunn H, Ramos P, Thalmann S, Xing C, Meyerhof W. 2010. Genetics and bitter taste responses to goitrin, a plant toxin found in vegetables. *Chemical senses* 35: 685-692.
 Zhao LQ, Tepper BJ. 2007. Perception and acceptance of selected high-intensity sweeteners and blends in model soft drinks by propylthiouracil (PROP) non-tasters and super-tasters. *Food Quality and Preference* 18: 531-540.