

# Supertasting and PROP Bitterness Depends on More Than the *TAS2R38* Gene

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## Abstract

Polymorphisms in the *TAS2R38* gene provide insight to phenotypes long associated 6-*n*-propylthiouracil (PROP) and phenylthiocarbamide bitterness. We tested relationships between *TAS2R38* genotype, taste phenotype, and fungiform papillae (FP) number in 139 females and 59 males (age range 21–60 years), primarily of European ancestry. DNA was analyzed for 3 polymorphic sites, identifying common (alanine–valine–isoleucine [AVI/AVI]), heterozygotes, proline–alanine–valine [PAV/PAV]) and rare (proline–valine–isoleucine, alanine–alanine–valine, AAI) forms. Individuals with PROP threshold >0.15 mM were almost exclusively AVI/AVI; those with threshold <0.1 mM could have any genotype. PAV/PAVs were more difficult to identify with PROP taste measures, although perceived bitterness of moderate PROP concentrations (0.32, 1 mM) had better correspondence with genotype than did threshold. For AVI/AVIs, increases in bitterness from 1 to 3.2 mM PROP nearly paralleled those of *TAS2R38* heterozygotes and PAV/PAVs. Some bitterness gains were related to FP number sampled from a standard area on the tongue tip, yet the PROP bitterness–FP relationship differed across genotype. Among homozygotes, FP was a significant determinant of PROP bitterness; heterozygotes showed a flat relationship. Those tasting concentrated PROP as more bitter also tasted concentrated sucrose, citric acid, sodium chloride, and quinine as more intense, even after statistically controlling for *TAS2R38* genotype, FP, and intensity of tones (nonoral standard). To summarize, although PROP threshold generally exhibited single-gene complete dominance, PROP bitterness may involve additional bitter receptors as evidenced by misclassification of some nontaster homozygotes and the bitterness functions for concentrated PROP. Variability in receptor expression may explain attenuated bitterness–FP relationships. PROP bitterness does associate with heightened taste sensations (i.e., supertasting), but this is not due to *TAS2R38* polymorphisms.

**Key words:** bitter, fungiform papillae, genetics, supertasting, taste

## Introduction

Taste blindness to the bitter chemical phenylthiocarbamide (PTC) was discovered serendipitously in 1931 by Fox (1932) and was soon identified as a heritable trait (Blakeslee 1932). Historically, psychophysics has been preoccupied with characterizing the bimodal threshold response to PTC and the related thiourea compound 6-*n*-propylthiouracil (PROP). In 2003, Drayna, Kim, and coworkers found that the *TAS2R38* gene (Entrez GeneID: 5726) explains variability in PTC threshold

(Kim et al. 2003). During the intervening years, more attention has been paid to bitterness intensity and the concept of supertasting. Here, we investigate the correspondence between *TAS2R38* genotype and both PROP threshold and bitterness and ask how this genotype relates to supertasting, which has traditionally been defined in terms of PROP bitterness.

Threshold methods operationalize the PTC/PROP sensitivity phenotype by separating tasters from nontasters based

on a positive response at the antimodal concentration (Lawless 1980) or comparison of the threshold to an antimodal cutoff (Bartoshuk et al. 1994). Psychophysical advances allowed Bartoshuk et al. (1994) to identify supertasters using PROP bitterness. Ensuing research indicated that PROP supertasters also give heightened responses to a broad range of oral stimuli including sweeteners (Duffy et al. 2006), salt (Bartoshuk et al. 1998), as well as chemesthetic (Prescott and Swain-Campbell 2000; Pickering and Gordon 2006), somatosensory (Prutkin et al. 2000; Hayes and Duffy 2007), and even olfactory stimuli (Pickering et al. 2006). Recent molecular advances provide a means to dissect the supertasting phenomenon from the genetic basis of PROP bitterness.

There are 2 common forms of the *TAS2R38* gene named for single-nucleotide polymorphisms (SNPs) that result in 3 amino acid substitutions: the proline–alanine–valine (PAV) haplotype associates with tasting whereas alanine–valine–isoleucine (AVI) associates with nontasting. These haplotypes result in 3 common genotypes across the population: PAV homozygotes, heterozygotes, and AVI homozygotes. Other haplotypes and genotypes are rare (Kim et al. 2003). For PROP bitterness, Duffy, Davidson, et al. (2004) found that although PAV homozygotes taste greater bitterness than do heterozygotes, genotypic classification showed smaller intergroup differences than did phenotypically characterized groups. Subsequently, Wooding et al. (2004) suggested *TAS2R38* “accounts for up to 85% of the phenotypic variance in PTC perception,” in reference to detection threshold. Although PROP and PTC have long been treated as essentially equivalent, recent evidence suggests additional genetic factors may be involved for PROP bitterness (Bufe et al. 2005).

The question we address here is how supertasting (i.e., elevated response to taste, retronasal, somatosensory, and chemesthetic stimuli) relates to the *TAS2R38* gene. Although it was previously assumed that variation in the N–C=S binding site could not explain associations between intensity of PTC/PROP and of stimuli not containing this chemical moiety, an empirical test was not available until *TAS2R38* was identified. Some of these associations could result from differences in the density of taste receptors. Duffy, Davidson, et al. (2004) demonstrated that genotype and fungiform papillae (FP) number make independent contributions to PROP bitterness. Associations between PROP bitterness and oral burn/touch are to be expected because FP are innervated by both taste and trigeminal (touch and pain) fibers. With greater density, a stronger signal is projected centrally, analogous to spatial summation. Supertaster-like phenomena are observed using other criteria: irritant bitter tasters and thermal tasters give higher responses to other prototypical tastants (Green and George 2004; Green and Hayes 2004), resulting in the postulation that variability in oral sensation might be explained in part by some central process (Green and George 2004).

The present study examines the relationship between *TAS2R38* genotype and PROP sensitivity as well as bitter-

ness. Additional questions include 1) are other phenotypical markers independent of *TAS2R38*? 2) does the link between FP number and perceived PROP intensity differ across genotypes? and 3) does PROP bitterness still explain variability in the intensity of prototypical tastants after partitioning out *TAS2R38* receptor genetics? We also ask whether rare genotypes behave similarly to forms that are more common.

## Methods

### Participants

A sample of 198 reportedly healthy, nonsmokers were recruited via posters and word of mouth for a laboratory study of the relationship between variation in oral sensation and dietary behaviors. Potential participants were recruited from the areas surrounding the University of Connecticut campus to visit the laboratory for 2 or 3 testing sessions, typically 1 week apart, with a separate visit for venipuncture. All procedures were approved by the Institutional Review Board. Written consent was obtained, and subjects were paid for their time.

### Scaling methodology

Subjects used the general Labeled Magnitude Scale (gLMS) (Bartoshuk et al. 2003; Bartoshuk et al. 2004) to report the intensity of the samples. The gLMS ranges from “no sensation” (0) to “the strongest imaginable sensation of any kind” (100). Intermediate labels include “barely detectable” (1.4), “weak” (6), “moderate” (17), “strong” (35), and “very strong” (53). This scale generalizes the LMS (Green et al. 1993; Green et al. 1996) by broadening the context from oral sensations to all sensations of any kind. Changing the top anchor is critical because individuals do not use adjective labels to denote the same perceived intensities (Bartoshuk et al. 2003). The flawed assumption that subjects use adjective labels in a similar manner can attenuate, obfuscate, or even reverse intensity effects (Bartoshuk, Fast, et al. 2005).

### Oral sensory phenotype

Using color videomicroscopy, FP number was ascertained by staining the tongue blue and counting the number in a circular area 6 mm in diameter on the right and left tongue tip, as described previously (Bartoshuk et al. 1994; Duffy, Peterson, et al. 2004). Counts from the left and right sides of the tongue tip were averaged to obtain the number of FP per standard area.

In the first visit to the laboratory, all subjects rated the intensity of prototypical tastants: 1 M sucrose (sweet), 3.2 mM citric acid (sour), 1 M sodium chloride (salty), and 1 mM quinine (bitter). PROP threshold was determined using a modified 2-alternative-forced-choice staircase (McBurney and Collings 1977) and was the geometric mean of the 2nd through 7th reversal. Threshold was unavailable for a subset of 45 subjects. In the final testing session, a range of 5 PROP

solutions (0.032–3.2 mM) were presented in a protocol described elsewhere (Bartoshuk et al. 1994; Duffy et al. 2003; Dinehart et al. 2006). Briefly, 5 sodium chloride solutions (10 mM–1 M) and the PROP solutions were presented in duplicated blocks. Each block was preceded and separated by five 1000-kHz tones (50–96 dB); within each block, presentation order was pseudorandom. This protocol was always administered last on the final test of testing to minimize contrast and range effects (Marks 1992; Lawless et al. 2000) that may vary nonrandomly with the degree of PROP response.

### **TAS2R38 genotype**

DNA was extracted from whole blood following manufacturer's instructions (Gentra, Minneapolis, MN), with occasional modification for lysed samples. Purified DNA samples were stored at 4 °C in Tris 10 mM and EDTA 1 mM until analyzed. Haplotypes were determined from SNPs located on base pairs 145, 785, and 886 of the *TAS2R38* (formerly *PTC*) gene. DNA was amplified using TaqMan assays (Applied Biosystems, Foster City, CA). Genotypes were determined using vendor supplied assays from Applied Biosystems (hCV8876467, hCV9506827, and hCV9506826) with the plates read on an ABI Prism 7900 HT. These SNPs give rise to 3 coding substitutions—proline to alanine at residue 49, alanine to valine at residue 262, and valine to isoleucine at residue 296—resulting in 2 common (PAV, AVI) and 3 uncommon (alanine–alanine–isoleucine [AAI], proline–valine–isoleucine [PVI], alanine–alanine–valine [AAV]) haplotypes that have been observed in humans. Twenty individuals with rare genotypes were excluded from analysis unless stated otherwise, leaving 177 classified as PAV (the wildtype/ancestral homozygote), heterozygote (HET) (PAV/AVI heterozygous) and AVI (nontaster homozygote variant). We assumed that individuals heterozygous at all 3 sites (e.g., CG TC CT) were common haplotype heterozygotes (e.g., PAV/AVI rather than AAV/PVI) as the probability of having 2 rare haplotypes is extremely low (Wooding et al. 2004).

### **Statistical analysis**

Statistical analyses were conducted using SAS release 9.1.3 (SAS, Cary, NC). The test of the ability of taste measures to correctly identify genotype, the sensitivity, and specificity were calculated as follows:

$$\text{sensitivity} = TP / (TP + FN) \text{ and } \text{specificity} = TN / (TN + FP),$$

where TP, TN, FP, and FN are true positive, true negative, false positive, and false negative, respectively. Sensitivity is a measure of how well a test predicts membership in a group, whereas specificity is a measure of how well a test correctly identifies the negative case. Here, sensitivity is the measure of how well the test identifies individuals as being AVI or PAV homozygotes, whereas specificity refers to the test's ability to identify non-AVI or non-PAV homozygote individuals correctly.

To test for differences in intensity across genotypes, replicated intensity ratings for the PROP concentration series were averaged for each subject; the 5 average ratings for the concentration series were then tested via repeated-measures analysis of variance (ANOVA). As controls, similar ANOVAs were conducted with salt and sound intensities as dependent variables; the salt and sound series did not differ across genotype (i.e., the 2-way genotype by salt concentration [ $F_{8,684} = 0.50, P = 0.85$ ] and the genotype by sound pressure level [ $F_{8,684} = 1.05, P = 0.40$ ] interactions were not significant). The relationship between PROP bitterness and FP number was assessed using multiple regression analysis. Outliers and high-leverage individuals were identified—by standardized residual  $>2.5$  or  $<-2.5$  and leverage plots of hat values versus studentized residual, respectively—and removed ( $n < 5$ ). Hat value is an alternate formulation of the Mahalanobis criterion used previously. Semipartial regression (sr) coefficients are reported. The mean intensity of the 86-dB tone across the sessions was included in the regression models (Duffy et al. 2006; Hayes and Duffy 2007). This approach partitions the variance due to PROP tasting from that of a nonoral sensory standard, as a control for idiosyncratic scale usage.

A regression interaction model for continuous and 3-level categorical variables (Chen et al. 2005) was used to test the contribution of PROP bitterness to the intensity of prototypical tastants after controlling for genotype. Dummy codes for the genotypes and interaction terms in the model allowed the effect of PROP on perceived intensity to differ across genotype using the following equation:

$$Y\text{-hat} = \beta_0 + \beta_1 \text{PROP} + \beta_2 \text{AVI} + \beta_3 \text{HET} + \beta_4 (\text{AVI} \times \text{PROP}) + \beta_5 (\text{HET} \times \text{PROP}) + \text{error},$$

where AVI and HET were coded as 0/1 and PROP was the bitterness of 3.2 mM PROP. The omitted group—the PAVs—acted as the control group with  $\beta_1$  as the effect of PROP on tastant intensity for the PAVs;  $\beta_4$  and  $\beta_5$  (the coefficients for the interaction terms) were the differential effects of PROP for the AVIs and HETS, respectively. Likewise, the intercept ( $\beta_0$ ) represented the mean bitterness for the PAV group and  $\beta_2$  and  $\beta_3$  differential means for the AVI and HET groups, respectively. Determining if  $\beta_4$  and  $\beta_5$  differed from zero tested the assumption of homogenous slopes (e.g., PROP effects on the prototypical tastants were uniform across genotypes). Unstandardized regression coefficients—the amount the dependent variable increases with a 1 unit increase in the predictor—are reported.

## **Results**

### **Sample diversity**

The present sample was primarily of European ancestry but diverse in haplotype and genotype (Tables 1 and 2) with distributions similar to other samples for Americans of

**Table 1** Haplotype diversity in the sample

	Self identified ethnic group						Total	European ancestry frequencies	
	Asian	Black	Caucasian	Hispanic	Native American	Other		COGA <sup>a</sup>	Kim <sup>b</sup>
PAV	12	5	151 (0.452)	12	—	1	181	0.42	0.49
PVI	—	—	1 (0.003)	—	—	—	1	—	—
AAV	—	—	13 (0.039)	—	1	—	14	0.04	0.03
AAI	—	4	1 (0.003)	—	—	—	5	—	—
AVI	6	5	168 (0.502)	10	3	1	193	0.53	0.47
Total	18	14	334	22	4	2			

<sup>a</sup>References from Wang et al. 2007.

<sup>b</sup>References from Kim et al. 2003.

COGA, Collaborative Studies on Genetics of Alcoholism.

**Table 2** Genotype diversity in the sample

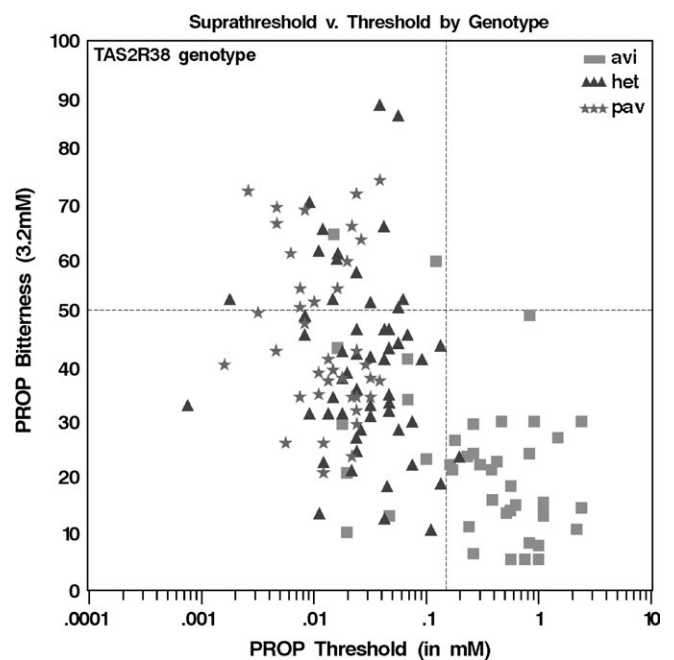
	All subjects	Women/men	Caucasian
AVI/AVI	52 (26%)	31/16	43
HET (AVI/PAV)	75 (38%)	50/25	69
PAV/PAV	50 (25%)	36/14	39
Rare	20 (10%)	16/4	16
Incomplete	1 (<1%)	1/0	1
Total	198	139/59	168

European ancestry (Wang et al. 2007). Mean age was 38 years ( $\pm 13$  standard deviation), ranging from 21 to 60.

The sample was also diverse in phenotypic measures. From gLMS ratings, 24% were nontasters (3.2 mM PROP bitterness <22), 54% medium tasters (>22 but <51), and 22% supertasters (>51). In a subsample ( $n = 154$ ; 53 men) for which detection threshold was available, 41 were nontasters compared with 113 tasters, which is comparable to population norms. Sex differences for PROP threshold were not observed as 15 of 41 nontasters and 38 of 113 tasters were men ( $\chi^2_1 = 0.12$ ,  $P = 0.73$ ), in contrast to the small but significant sex effect for PTC threshold reported elsewhere (Kim et al. 2003). When the distributions of PROP bitterness were compared for men and women using a 2-sample K-S (Kolmogorov–Smirnov) test—a distribution-free statistic that detects any difference between the 2 distributions (e.g., central tendency, dispersion or skew)—no differences were found ( $D = 0.10$ , nonsignificant). A K-S test for FP number revealed the distributions were significantly different ( $D = 0.26$ ,  $P < 0.01$ ) with women having more papillae.

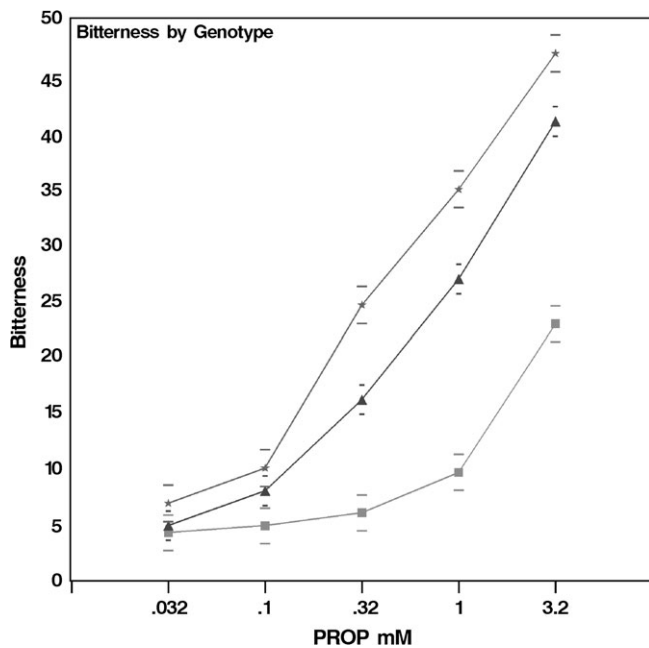
#### Identifying *TAS2R38* genotype using taste tests

PROP suprathreshold bitterness showed the typical negative association with threshold (Figure 1); in multiple regression, log threshold ( $sr = -0.60$ ,  $P < 0.0001$ ) and 86-dB tone



**Figure 1** PROP bitterness versus PROP sensitivity by genotype. Stars are PAV homozygotes, triangles are heterozygotes, and squares are AVI homozygotes. Dashed lines indicate cutoffs for threshold (0.15 mM) and supertasting (51 on gLMS).

( $sr = 0.27$ ,  $P = 0.0001$ ) but not age ( $sr = 0.09$ ,  $P = 0.16$ ) were significant predictors of the bitterness of 3.2 mM PROP. Although threshold separated AVI homozygotes from HETs and PAV homozygotes, there was substantial overlap between the 2 taster genotypes. Figure 1 also illustrates that 2 copies of the PAV allele were not needed to be a PROP supertaster (3.2 mM PROP > 51). The PROP functions differed by genotype (Figure 2)—in repeated-measures ANOVA, the 2-way concentration by genotype interaction was significant ( $F_{8,688} = 22.8$ ,  $P < 0.0001$ ). The psychophysical functions for the PAV and HET groups were



**Figure 2** PROP bitterness by *TAS2R38* genotype. Stars are PAV homozygotes, triangles are heterozygotes, and squares are AVI homozygotes

roughly parallel, whereas the function for the AVI group was relatively flat until an inflection point at 1 mM. In Tukey HSD pairwise comparisons, group differences were not seen at 0.032 or 0.1 mM (all  $P$ 's > 0.6), except for the PAVs who tended to be higher than the HET at 0.1 mM ( $P = 0.089$ ). For the 0.32, 1, and 3.2 mM PROP, PAV > HET > AVI (all  $P$ 's < 0.005).

Measures of test accuracy were used to provide information on the utility of threshold as well as several suprathreshold criteria for identifying *TAS2R38* genotype. The PROP functions in Figure 2 suggested testing the 3 top concentrations for the best sensitivity (i.e., test identifies group membership correctly) and specificity (i.e., test identifies group nonmembership correctly). The AVI homozygotes were easier to identify than PAV homozygotes using taste tests. Threshold misclassified 23% of the AVI homozygotes (77% sensitivity); a single HET and no PAVs were misidentified as being AVI (99% specificity). For the PAV homozygotes, threshold appeared to be very good at identifying people who were PAVs (100% sensitivity), yet a large number of HETs and some AVIs also had taster thresholds, resulting in a specificity of only 34%. (A test identifying everyone as PAV regardless of true status would have a 100% sensitivity and 0% specificity.) To achieve the best balance between sensitivity and specificity (i.e., lowest number of false positives and false negatives), 1 mM PROP bitterness was best at discriminating AVIs (80.4% sensitivity, 91.1% specificity) whereas 0.32 mM PROP bitterness was best for identifying PAVs (58.3% sensitivity, 83.3% specificity; Table 3).

### Independence of FP number and *TAS2R38* genotype

The distribution of FP number was essentially independent of *TAS2R38* genotype ( $\chi^2_2 = 4.4$ ,  $P = 0.11$ ), as shown in Figure 3. In 1-way ANOVA, the mean number of FP did not differ across genotype ( $F_{2,170} = 0.90$ ,  $P = 0.41$ ). Because of the sex differences in FP number (reported above), we confirmed that FP remained independent of *TAS2R38* genotype after controlling for sex: the overall 2-way ANOVA was not significant ( $F_{5,167} = 1.66$ ,  $P = 0.14$ ).

### FP number did not predict PROP bitterness in heterozygotes

Across the 3 common genotypes, FP number ( $sr = 0.17$ ,  $P = 0.027$ ) was a significant predictor of 3.2 mM PROP bitterness, controlling for tone intensity. However, comparing the PAV and AVI homozygotes with the heterozygotes revealed differences (Figure 4). That is, FP was a stronger predictor of bitterness across the 2 homozygous groups ( $sr = 0.33$ ,  $P = 0.001$ ), whereas no relationship was observed ( $sr = 0$ ,  $P = 0.99$ ) in the heterozygotes. Separate analyses for PAVs ( $sr = 0.30$ ,  $P = 0.035$ ) and AVIs ( $sr = 0.32$ ,  $P = 0.024$ ) revealed similar slopes but an overall shift in bitterness intensity between the 2 groups of homozygotes.

### Individuals with rare genotypes generally behaved as expected

PROP thresholds were generally lower for those with a PAV or PVI haplotype (e.g., AA\*/PAV or PVI/AVI) than for AA\*/AVI individuals, but as with the common genotypes the suprathreshold bitterness was more varied (not shown). The AA\*/AVI subjects exhibited a strong FP–intensity effect: when split at 26 FP/6 mm<sup>2</sup>, 7 of 8 of the low-FP individuals had bitterness below 20, compared with 0 of 5 in the high-FP group (Fisher's exact test,  $P = 0.004$ ), suggesting AA\*/AVI individuals are behaviorally similar to AVI homozygotes with regard to suprathreshold PROP bitterness.

### PROP bitterness associated with tastant intensity even after controlling for genotype

There was no apparent relationship between *TAS2R38* genotype and any of the tastants. In 1-way ANOVA, mean quinine bitterness was not different across genotype ( $F_{2,167} = 0.75$ ,  $P = 0.47$ ). Similarly, ANOVAs for salt ( $F_{2,167} = 1.27$ ,  $P = 0.28$ ), sucrose ( $F_{2,167} = 1.30$ ,  $P = 0.28$ ), and citric acid ( $F_{2,167} = 1.14$ ,  $P = 0.32$ ) were not significant. For example, although there was a correlation between quinine and PROP bitterness ( $r = 0.32$ ,  $P < 0.0001$ ), all 3 common genotypes were observed across the entire range of quinine bitterness, as shown in Figure 5.

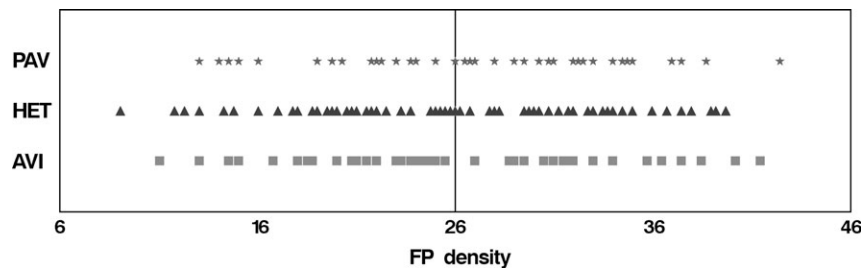
To test our contention that PROP bitterness captures multiple sources of variability in oral sensation, we used regression interaction models for continuous and 3-level categorical variables to predict the intensity of concentrated

**Table 3** Identification of *TAS2R38* homozygotes by PROP psychophysics

	AVI	Taste test	Sensitivity <sup>a</sup> of psychophysical test (%)	Not AVI	Taste test	Specificity <sup>b</sup> of psychophysical test (%)
Threshold	43	33	76.7	96	95	99.0
0.32 mM bitterness	51	35	68.6	106	123	86.2
<b>1 mM bitterness</b>	<b>51</b>	<b>41</b>	<b>80.4</b>	<b>112</b>	<b>123</b>	<b>91.1</b>
3.2 mM bitterness	51	34	66.7	105	123	85.4
PROP ratio	51	37	72.5	108	123	87.8
	PAV	Taste test	Sensitivity <sup>a</sup> of psychophysical test (%)	Not PAV	Taste test	Specificity <sup>b</sup> of psychophysical test (%)
Threshold	40	40	100.0	34	99	34.3
<b>0.32 mM bitterness</b>	<b>48</b>	<b>28</b>	<b>58.3</b>	<b>105</b>	<b>126</b>	<b>83.3</b>
1 mM bitterness	48	21	43.8	99	126	78.6
3.2 mM bitterness	48	21	43.8	99	126	78.6
PROP ratio	48	21	43.8	99	126	78.6

<sup>a</sup>Sensitivity is the ability to correctly predict group membership.

<sup>b</sup>Specificity is the ability to correctly predict group nonmembership. The optimal measure, set in bold face for AVIs (top) and PAVs (bottom), simultaneously maximizes sensitivity and specificity.



**Figure 3** When split at the sample median (26 FP/6 mm<sup>2</sup>), the proportion of individuals with FP number above or below the median did not differ by genotype.

sucrose, citric acid, sodium chloride, and quinine with 3.2 mM PROP bitterness while controlling for genotype. Because the relationship between PROP and prototypical tastant intensity did not differ across genotype (i.e., assumption of homogenous slopes was met), we analyzed a simplified model. Even after using the 86-dB tone as a sensory standard and accounting for genotype, PROP bitterness was still a significant predictor of tastant intensity. The unstandardized coefficients for PROP were 0.43 for quinine ( $P < 0.0001$ ), 0.33 for citric acid ( $P < 0.0001$ ), 0.24 for sodium chloride ( $P = 0.0038$ ), and 0.19 for sucrose ( $P = 0.0127$ ). Adding FP or age to the regression equation did not alter the contribution of PROP bitterness to the tastant intensity.

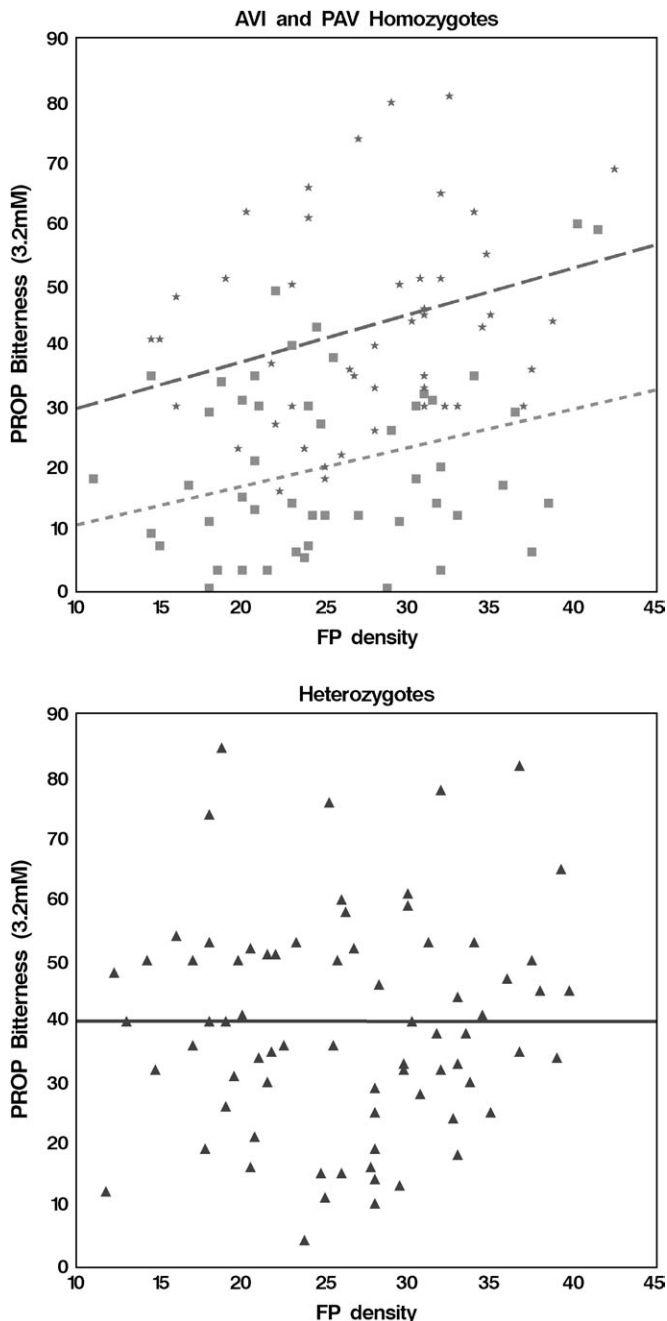
## Discussion

The present paper advances our understanding of the relationship between PROP phenotype, tastant intensity, and the *TAS2R38* gene in 3 major ways. First, we found that

PROP suprathreshold responses provided more information to understand gene–taste relationships than did thresholds. At higher PROP concentrations, the nontaster genotype (AVI/AVI) showed increases in bitterness that paralleled the taster genotypes (HETs, PAV/PAV), suggesting other receptors may be involved in tasting high concentrations of PROP. Second, we uncovered that the relationship between FP number and suprathreshold bitterness was attenuated in *TAS2R38* heterozygotes, although FP had a strong influence on bitterness intensity in PAV and AVI homozygotes. Finally, we found that suprathreshold PROP bitterness, but not *TAS2R38* genotype, predicted the intensity of prototypical tastants, suggesting continued utility for PROP bitterness as a marker of variability in oral sensation.

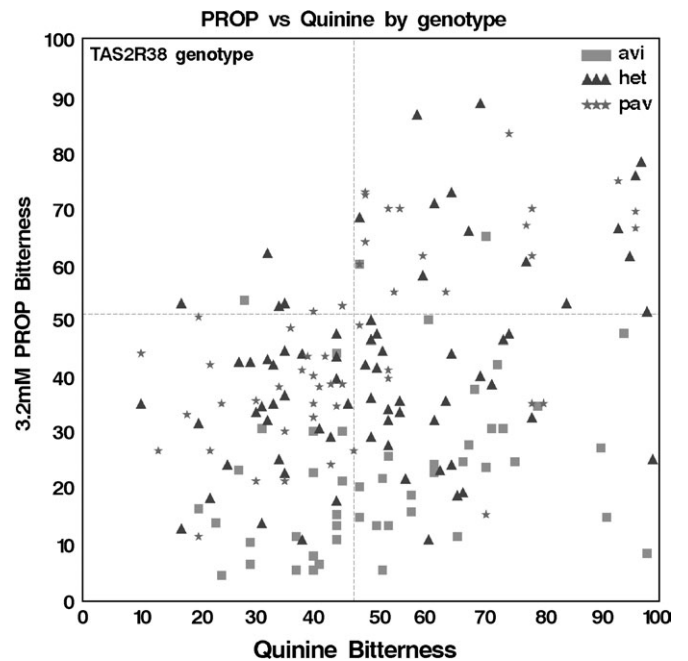
## Correspondence between *TAS2R38* genotype and PROP tasting

The present data suggested that although information about receptor function was gleaned from threshold responses,



**Figure 4** Scatterplots with sr lines for the relationship between FP number and 3.2 mM PROP bitterness for *TAS2R38* homozygotes (top) and heterozygotes (bottom). Stars are PAV homozygotes, triangles are heterozygotes, and squares are AVI homozygotes.

perceived intensity of suprathreshold concentrations provided a more accurate picture of receptor genotype as well as additional information about response to the tastant at highest concentrations. Suprathreshold ratings of 0.32 and 1 mM PROP were better at minimizing both false positives and false negatives in predicting PAV and AVI homozygosity than was threshold. Bitterness of the highest PROP concentration (3.2 mM) suggested that PROP bitterness is not



**Figure 5** PROP and quinine bitterness are related, but quinine is independent of *TAS2R38* genotype.

entirely explained by *TAS2R38* genotype—a PAV homozygote was not always a PROP supertaster and an AVI homozygote with a high number of FP may be a medium taster. Thus, supertasting is not a dominant trait in the classical Mendelian sense. In contrast, threshold is closer to a simple dominant trait for *TAS2R38*, consistent with previous work. That is, 1 or 2 copies of the PAV allele are sufficient to shift the detection threshold from a nontaster to a taster threshold. The inability to separate heterozygotes from homozygote tasters via threshold recapitulates what Blakeslee reported in his seminal paper 75 years ago (Blakeslee 1932).

For those with rare haplotypes, having a PAV or PVI allele conferred a lower detection threshold irrespective of the second allele. Previously, AAV has been reported as an “intermediate taster” haplotype either explicitly (Kim et al. 2003; Timpson et al. 2007) or implicitly (Cannon et al. 2005). Here, we found limited evidence to support these views. Of 8 AAV/AVI individuals, 3 had thresholds below 0.2 mM, but only 1 fell clearly in the taster range. More cannot be said given our small sampling of rare genotypes.

Here, we found the increases in intensity for AVI homozygotes paralleled those for heterozygotes and PAV homozygotes at higher PROP concentrations. The simplest explanation is that the AVI receptor variant is less functional—whether via binding affinity, inhibition of G-protein activation, or some other mechanism. This view would account for a gap between heterozygotes and the PAV homozygotes; 2 fully functional copies of the receptor should produce a stronger signal than 1 fully functional and 1 less functional copy. However, in silico modeling by Floriano et al. (2006)

indicates the amino acid substitutions for the nontaster variant do not alter binding affinity—instead, the substitutions occur in transmembrane regions that would inhibit or prevent G-protein activation. If steric hindrance prevents G-protein activation, merely increasing concentration still would not trigger any response; if so, the AVI homozygote response seen here would require a second PROP receptor.

We also report that some AVI homozygotes had taster thresholds (<0.15 mM), which could occur if a second receptor restored response in those with nonfunctional hT2R38 receptors. A second receptor hypothesis is also buttressed by several other lines of evidence. First, PTC (and presumably PROP) heritability is greater in AVI homozygotes, leading Kim et al. (2003) to suggest other genetic factors may restore response in these individuals. Second, genomic scanning implies the existence of another PROP receptor on chromosome 5 (Reed et al. 1999). Third, cultured cells expressing hT2R4 respond to high PROP concentrations (Chandrashekar et al. 2000) and hT2R4 contains 4 coding SNPs that may result in functional variability in bitter taste response (Ueda et al. 2001). Before the second receptor hypothesis is accepted, more work is needed to 1) confirm the *in silico* findings of Floriano et al. (2006) *in vitro* or *in vivo* and 2) determine if the steric hindrance from the AVI amino acid substitutions results in reduced function or complete loss of function *in vivo*. Whereas the single receptor reduced-function hypothesis can explain the AVI homozygote response at higher PROP concentrations, data here and elsewhere are also consistent with the existence of a second lower affinity PROP receptor.

#### Relationship between FP number and PROP bitterness differed by *TAS2R38* genotype

In 1994, Bartoshuk et al. (1994) reported that FP number was significantly lower for nontasters. At that time, subjects were classified as nontasters using both PROP threshold and PROP ratio (the bitterness of PROP to the saltiness of NaCl). Critically, those subjects who had nontaster thresholds but PROP ratios indicating the ability to taste PROP as quite bitter were labeled as “unclassifiable” and excluded from the analyses. This exclusion likely removed nontasters with the most FP. The present study finds that PROP bitterness increased with FP number for AVI homozygotes as well as PAV homozygotes, yet that FP number did not vary systematically across *TAS2R38* genotype.

Present data suggested that heterozygotes had a minimal association of bitterness with FP number, whereas in homozygotes, FP number was a significant determinant of PROP bitterness irrespective of receptor genotype. Although DNA sequence mediates hT2R38 function, variation in gene expression also drives phenotype. Bufe et al. (2005) biopsied papillae from 2 heterozygous individuals and found allele-specific mRNA levels varied greatly: in one individual the mRNA levels for each allele were roughly equal whereas

in the other individual, more than 85% of the mRNA expressed was for the PAV allele. Thus, psychophysically, a heterozygote might be expected to be more like a PAV or AVI homozygote depending on whether they express more of the PAV- or AVI-type receptor. In a larger sample, we would expect the observed FP–intensity association to be merely attenuated rather than absent among the heterozygotes. Expression variability also potentially explains our overlapping threshold data for the heterozygotes and the PAV homozygotes. Heterozygotes who express more of the AVI allele would be expected to shift toward higher thresholds and those who express more of the PAV allele to shift lower. Patterns consistent with this can be seen in the Figure 1: below 0.007 mM, all but 2 of 10 subjects are PAV homozygotes, whereas above 0.04 mM, all 23 tasters were heterozygotes. Recently, Timpson et al. (2007) reported that even within genotypic subgroups, children show considerable variability in PROP bitterness, and this variability appears to result from physiological differentiation rather than psychosocial factors that influence rated bitterness; our present work provided several mechanisms in support of this view.

Indirect evidence suggests that environmental factors influence the regulation of receptor expression. Pilot data from Mennella et al. (2007) found threshold varied across age groups, but only in *TAS2R38* heterozygotes, leading them to speculate about a role for puberty in PROP sensitivity. The implicit role for hormones in modulation of bitter response is consistent with data on pregnancy (Duffy et al. 1998) and menstruation (Glanville and Kaplan 1965; Prutkin et al. 2000). A heterozygote advantage could result if heterozygotes are able to downregulate PAV expression in a low-energy environment to reduce food rejection or upregulate PAV during pregnancy or positive energy balance. This could help explain the existence of the AVI variant of *TAS2R38*. The ancestral form is the PAV taster variant, which is thought to prevent plant toxin ingestion (e.g., the natural goitrogen 1-5-vinyl-2-thio-oxazolidone and PTC have identical thresholds (Boyd 1950). The AVI nontaster variant may not have a ligand as the mutations are not in the binding pocket. Alternatively, the taster and nontaster alleles may be preserved by balancing selection (Wooding et al. 2004) or random genetic drift (Kidd et al. 2004). Stability of *TAS2R38* expression across the life span is currently unknown; future work should explore potential changes with diet or hormonal status.

#### PROP–taste intensity relationships were separate from *TAS2R38* genotype

The present study found relationships between the intensity of PROP and prototypical tastants that persisted even after statistically removing *TAS2R38* genotypic variability. PROP supertasting is thought to result from increase peripheral signals to the central nervous system due to the increased



innervation density (Bartoshuk et al. 1994), analogous to spatial summation. Here, we failed to find that FP explained the heightened whole-mouth taste intensity of prototypical tastants for those whom PROP was most bitter. Although FP number explains some variability in taste response (Zuniga et al. 1993; Bartoshuk et al. 1994; Delwiche et al. 2001), particularly in discrete regions (Doty et al. 2001), it may be that FP number does not fully capture differences in peripheral innervation and mechanisms to produce whole-mouth taste intensity differences between PROP non-tasters and supertasters. Two individuals with comparable numbers of FP may still have considerable variation in taste bud density (Bartoshuk et al. 1994) and the whole-mouth taste intensity–papillae number relationship may become decoupled with taste damage (Bartoshuk, Snyder, et al. 2005). Other morphological differences in FP may explain normal variation in orosensation such as epithelial differences (Just et al. 2005). Rodent models suggest that environmental exposure may influence taste bud size or levels of transduction-related proteins (e.g., Tomassini et al. 2007), even if FP number does not vary. Thus, although FP number has proven utility in predicting elevated taste response (i.e., supertasting) and dietary behaviors, it cannot be the sole explanation for orosensory variation.

Heightened orosensory response may also result from a central process as well as peripheral contributions. Supertasting-like phenomena have been documented using oral stimuli unrelated to PROP, including bitter response to irritants on the posterior tongue (Green and Hayes 2004) and thermal taste on the anterior tongue (Cruz and Green 2000). Supertasters might exhibit greater central gain as speculated by Green and George (2004), or there may be an another overall factor (e.g., Lawless 1979; Olson et al. 1989). Individual differences in the second messenger cascade within taste receptor cells could also explain elevated oral sensations. Although TRPM5, a member of the transient receptor potential protein subfamily (TRPM), might explain thermal taste (Talavera et al. 2005), it cannot explain supertasting; knocking out TRPM5 eliminates responses mediated by G-protein coupled receptors yet does not affect those mediated by ion channels (Zhang et al. 2003). Whether supertasting results from a developmental process that drives anatomical differences or differences in central or peripheral amplification is unknown.

Limitations of the present study should be noted. The sample was primarily composed of individuals of European ancestry—how these results may generalize to other groups, particularly those with greater frequencies of rare haplotypes (i.e., individuals of African descent [Kim et al. 2003], is unclear. The present analyses only considered a single gene found on chromosome 7—genome-wide scans imply loci on chromosomes 5 (Reed et al. 1999) and 16 (Drayna et al. 2003) also have roles in PROP/PTC tasting. The role of T2R4 (Chandrashekar et al. 2000) in PROP tasting remains to be determined in vivo. The estimates of FP number used

here were based on standard videomicroscopy techniques—it is possible that newer methods (e.g., Shahbake et al. 2005) may better quantify FP and their contribution to taste intensity.

## Conclusions

Here, hT2R38 polymorphisms were insufficient to explain suprathreshold bitterness elicited by concentrated PROP, providing support for additional receptors or regulatory mechanisms in tasting PROP beyond threshold levels. Number of FP did not differ across *TAS2R38* genotypes, but the relationship between the bitterness of concentrated PROP and FP differed across genotype; FP explained heightened bitterness in homozygotes (AVI/AVI or PAV/PAV) but not heterozygotes. PROP bitterness, the historical probe for supertasting, was associated with heightened intensity of prototypical tastants (NaCl, sucrose, citric acid, quinine), even after statistically removing the contributions of *TAS2R38* genotype, FP number, and the intensity of tones as a nonoral sensory standard, which supports its utility as a phenotypic marker of oral sensation. Other behavioral probes of heightened oral sensation have emerged recently, so it may be wise to decouple the concept of supertasting from an operational definition dependent on PROP, as PROP bitterness may only be one marker of supertasting. More work is needed to characterize interrelationships between emerging orosensory phenotypes.

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