

## ORIGINAL ARTICLES

## Genetic, Functional, and Phenotypic Diversity in TAS2R38-Mediated Bitter Taste Perception

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## Abstract

Mutational polymorphism in the TAS2R38 bitter taste receptor is a key determinant of threshold taste detection of isolated compounds, such as phenylthiocarbamide (PTC) and propylthiouracil (PROP), as well as complex orosensation-mediated traits such as diet choice and smoking habits. These relationships are accounted for, in part, by 2 common alleles differing in functionality, TAS2R38-PAV and TAS2R38-AVI. However, TAS2R38 harbors extensive additional polymorphism whose functional significance remains unknown. To examine this variation, we ascertained genetic diversity in 56 Caucasian subjects via whole-gene sequencing, analyzed allele-specific responses to 5 TAS2R38 agonists (PTC, PROP, goitrin, methimazole, and sinigrin) using *in vitro* assays, and assessed genotypic associations with threshold detection phenotypes. Sequencing identified 3 single-nucleotide substitutions encoding 3 amino acid changes (C145G/P49A, C785T/A262V, and A886G/I296V), which combined to form 6 haplotypes in our sample. *In vitro* assays revealed a continuous range of response across alleles, and associations with threshold were significant for all single nucleotide polymorphisms ( $P < 0.002$ ) and PAV/AVI haplotypes ( $P < 0.001$ ). Haplotypes other than PAV and AVI did not exhibit phenotypic associations in our sample, possibly as a result of their low frequencies. However, prior studies have indicated that these alleles are common in some global regions, suggesting that alleles rare in our sample may be phenotypically relevant in other populations.

**Key words:** bitter, goitrogen, isothiocyanate, phenylthiocarbamide, TAS2R, taste

## Introduction

Bitter taste perception plays key roles in human behavior and health. By shaping the attractiveness of food and other substances such as cigarette smoke before they are even ingested, it is powerfully positioned to influence intake (Duffy 2007). This has long suggested that variation in sensitivity could translate into both immediate preferences and long-term downstream effects, a hypothesis borne out in multiple reports of association between bitter responses and traits including food and alcohol choices, smoking habits, susceptibility to colon polyps, thyroid function, and

body mass index (Drewnowski and Rock 1995; Duffy and Bartoshuk 2000; Enoch et al. 2001; Tepper and Ullrich 2002; Duffy et al. 2004; Cannon et al. 2005; Dinehart et al. 2006; Hayes et al. 2011). Thus, dissecting the mechanisms underlying variability in perception is a potentially powerful means of identifying the origins of individual and group differences in both consumption and susceptibility.

Numerous complex factors including age, sex, morphology, and environment interact to produce major differences in bitter perception among individuals (Bartoshuk and Beauchamp 1994). However, mounting evidence indicates that mutations

in TAS2R receptors, a family of 25 cell-surface G protein-coupled receptors expressed in taste buds, make particularly important contributions (Drayna 2005). TAS2Rs, which control the earliest stages of bitter taste transduction, harbor extensive genetic polymorphism including numerous amino acid replacements likely to affect function (Kim et al. 2005). Studies of genetic and functional diversity in 5 receptor genes, *TAS2R9*, -16, -31, -38, and -43, have isolated alleles exhibiting divergent functionality that interact to shape taste responses to artificial sweeteners such as acesulfame K and saccharin, phytotoxins such as aloin, amygdalin, aristolochic acid, and goitrin, and a range of pharmaceuticals (Kim et al. 2003; Soranzo et al. 2005; Hinrichs et al. 2006; Pronin et al. 2007; Dotson et al. 2008; Wooding et al. 2010; Roudnitzky et al. 2011). By detecting the origins of variance in response to specific agonists, these studies have identified candidate sources of variance in related endpoint phenotypes such as preferences for artificial sweeteners and vegetables (Dinehart et al. 2006; Pronin et al. 2007; Roudnitzky et al. 2011).

TAS2R38, which mediates taste responses to thioamides including the classic markers PTC and PROP, is the most intensively investigated bitter receptor in the context of both functional and epidemiological associations. The significance of TAS2R38 to bitter perception was first demonstrated by Kim et al. (2003), who identified 2 common alleles with profoundly different functional properties associated with threshold detection of PTC, designated PAV and AVI. Subsequent investigation revealed that these alleles also associate with long-term trends in tobacco use, food preferences, and obesity (Cannon et al. 2005; Duffy 2007). Thus, TAS2R38 has been strongly implicated in connections between taste, behavior, and fitness. However, recent studies have revealed that TAS2R38 harbors extensive genetic variation beyond the PAV and AVI alleles, with 19 amino acid variants cataloged to date in worldwide populations, which could underlie unrecognized functional and phenotypic diversity (Wooding et al. 2004; Kim et al. 2005; Campbell et al. 2012). Further, associations between PAV/AVI variants and consumption of substances not known to contain thioamides have suggested that TAS2R38 may mediate taste responses to other families of compounds, but this proposition has not been tested directly (Meyerhof et al. 2010). In this study, we sought to better define the functional and phenotypic underpinnings of TAS2R38-mediated bitter responses by comprehensively examining a cohort of 56 Caucasian subjects using whole-gene sequencing, dose-response analyses of discovered haplotypes, and tests for association with threshold taste responses to thioamides and other compounds.

## Materials and methods

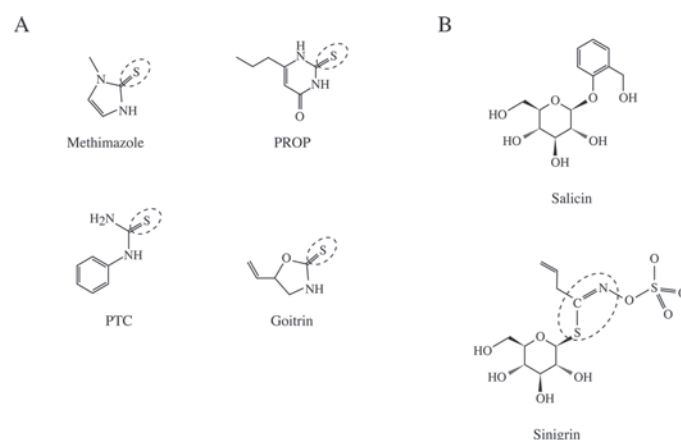
### Subjects

Subjects were studied under protocols following Helsinki Declaration guidelines for ethical research, which were approved by the Institutional Review Board (IRB) at the University of Texas Southwestern Medical Center. Following

initial recruitment, participants providing informed consent were screened via self-report to identify individuals with prior clinically diagnosed taste deficits or other significant health problems. Candidates meeting selection criteria were invited to take part in 6 taste tests and a blood draw for DNA extraction and genetic analysis. Twenty-eight subjects were participants in an earlier study of goitrin perception in the laboratory of SPW (Wooding et al. 2010). In total, 56 subjects completed the full course of the project (38 female, 18 male, mean age = 41.6).

### Taste phenotypes

Detection threshold measures were used to examine taste responses to 6 compounds potentially informative about phenotypic variation and functional polymorphism in TAS2R38: 5 known agonists of TAS2R38 (PTC, Sigma Aldrich P7629; PROP, Sigma Aldrich P3755; goitrin, Alfa Aesar L02639; methimazole, Sigma Aldrich M8506; sinigrin, Sigma Aldrich 85440) and a known nonagonist used as a control and comparative baseline (salicin, a  $\beta$ -glucoside, Sigma Aldrich S0625) (Figure 1). Phenotypes were assessed using the blind sorting method of Harris and Kalmus (1949), which estimates the lowest concentration at which a subject can distinguish tastant and control solutions in a stepwise dilution series, or perception threshold. In our study, we performed testing with aqueous solutions of PTC, PROP, goitrin, methimazole, sinigrin, and salicin dissolved in deionized water at room temperature, with plain deionized water used as a control. Threshold estimation for each tastant was performed in 14 stages, with each stage requiring the subject to distinguish 3 portion cups containing 25 ml aliquots of the tastant solution from 3 containing only the control. For PTC, PROP, methimazole, and salicin, test solutions started at a concentration of 1 micromole ( $\mu$ M) and increased by 100% (i.e., doubled) at each step to a maximum



**Figure 1** Chemical structures of tested agonists. (A) Thioamides, which harbor the NCS moiety present in all known TAS2R38 agonists examined to date (circled). (B) Salicin, a  $\beta$ -glucoside, and sinigrin, a glucosinolate (with NCS component circled).

of 8192  $\mu\text{M}$ . For goitrin, concentrations started at a concentration of 14  $\mu\text{M}$  and increased by 50% at each step to a maximum of 2731  $\mu\text{M}$ . Sinigrin concentrations started at a concentration of 297  $\mu\text{M}$  and increased by 33% at each step to a maximum of 12 500  $\mu\text{M}$ . Threshold responses to 5 of these compounds, salicin, PTC, PROP, goitrin, and methimazole, were successfully collected from the complete subject pool ( $n = 56$ ). Responses to sinigrin were collected from 25 of the 56 subjects (15 female, 10 male, mean age = 48.9).

### Genetic variation in TAS2R38

DNA samples were extracted from whole blood, drawn during each subject's first visit for taste testing, by the Human Genetics Clinical Laboratory (HGCL) core facility at the University of Texas Southwestern Medical Center. To comprehensively ascertain mutational polymorphism, the *TAS2R38* gene was sequenced in its entirety (1002 bp) in all subjects using standard methods. Polymerase chain reaction (PCR) amplification was performed with oligonucleotide primers designed by Kim et al. (2003) using Eppendorf Mastercycler thermal cyclers. After being tested for size and mass by gel electrophoresis, PCR products and primers were submitted to the Southwestern Medical Center's DNA Sequencing and Genotyping core facility for processing with ABI 3730xl instrumentation. ABI-generated chromatograms were analyzed using the PHRED, PHRAP, and Consed computer programs. Following automated analysis and a manual data inspection and cleanup, in-house software was used to identify single nucleotide polymorphisms (SNPs) and determine individual genotypes. Haplotypes were inferred using the PHASE computer program, which examines SNP genotypes to determine the most likely configuration of SNP alleles along a single chromosome (Stephens et al. 2001).

### Functional analysis

The functional characteristics of TAS2R38 alleles identified through sequencing were examined using *in vitro* assays developed by Bufer et al. (2002; 2005a; b). The cDNA constructs of the TAS2R38 haplotypes were available from previous studies (Bufer et al. 2005a; Wooding et al. 2010). In addition to the receptor cDNA cloned in pcDNA5FRT vectors (Invitrogen), they were composed of an N-terminal element consisting of amino acids 1–45 of the rat somatostatin type 3 receptor to facilitate cell-surface transport and a C-terminal herpes simplex virus glycoprotein D epitope to allow immunocytochemical localization (Bufer et al. 2002). Dose–response analyses were then performed by transiently transfecting allelic constructs into a human embryonic kidney HEK293T cell line expressing the chimeric G-protein subunit  $G_{16-Gust44}$  using Lipofectamine 2000 (Invitrogen), exposing transfected cells to serial dilutions of tastants dissolved in C1 solution (130 mM NaCl, 5 mM KCl, 10 mM HEPES, 2 mM  $\text{CaCl}_2$ , and 10 mM glucose, at a pH of 7.4), and quantifying stimulation

with an automated Molecular Devices fluorometric imaging plate reader (FLIPR). Each experiment was carried out in duplicate, and data were processed with SigmaPlot (SPSS), normalizing peak responses relative to background fluorescence ( $\Delta F/F = (F - F_0)/F_0$ ) and subtracting baseline noise. Statistical differences in allele-specific  $\text{EC}_{50}$  and  $\Delta F/F$  values were assessed using ANOVA and Tukey *post-hoc* tests.

### Association tests

Genotype–phenotype associations were analyzed using linear regression. To take into account evidence from previous studies that the 2 common alleles of TAS2R38, PAV and AVI, contribute additive phenotypic effects, genotypes were coded under a codominant model with subjects carrying 0, 1, or 2 copies of the major (i.e., most common) allele. Haplotype contributions to threshold responses were first examined in diplotype-phenotype analyses focused on PAV and AVI, which together accounted for ~85% of the sample. In these analyses, only subjects carrying PAV/PAV, PAV/AVI, and AVI/AVI diplotypes (i.e., haplotype pairs), which scored as carrying 0, 1, or 2 copies of the PAV allele, were included.

To examine the possible contributions of alleles other than PAV and AVI to associations between TAS2R38 variation and threshold, we designed an exploratory diplotype-phenotype analysis, incorporating measures of receptor function derived from *in vitro* function assays, which we termed Function Adjusted Association (FAA). Here, each observed haplotype was assigned an adjusted score weighted according to its  $\Delta F/F$  value at the maximum agonist concentration not showing evidence of functional inhibition in dose–response curves. Thus, highly responsive alleles were assigned high scores, nonresponsive alleles assigned low scores, and intermediate-function alleles received intermediate scores. Subject genotypes were then coded under a codominant model as the sum of function scores of carried haplotypes.

## Results

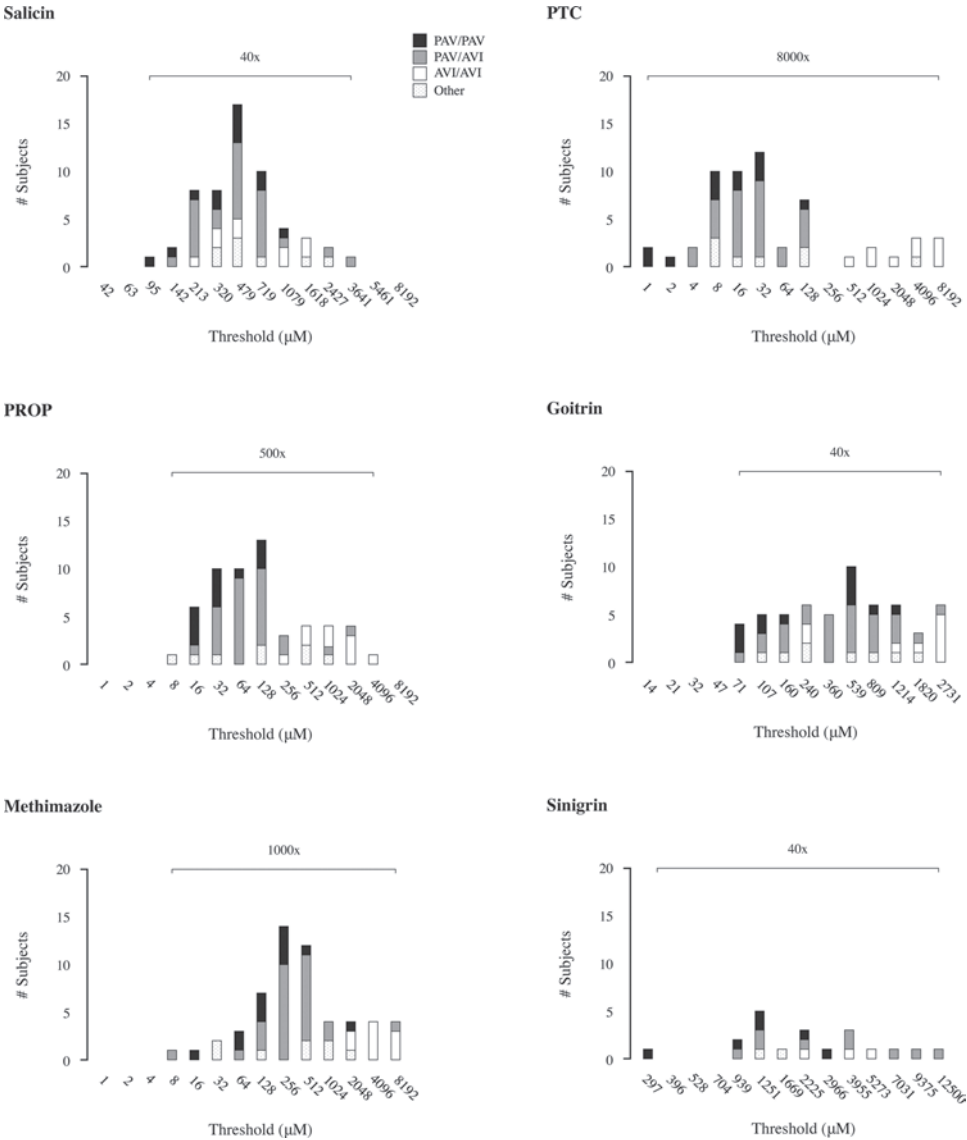
Threshold responses in subjects varied substantially among both individuals and compounds (Figure 2). Distributions closely matched prior observations for all 4 substances examined in previous studies (salicin, PTC, PROP, and goitrin), with salicin displaying a nearly unimodal distribution, PTC a strongly bimodal distribution, PROP a weakly bimodal distribution, and goitrin having no conspicuous modality pattern (Wooding et al. 2010). Methimazole thresholds, which have not been studied previously, exhibited a pattern most similar to that of PROP and resembling those of PTC and goitrin, consistent with their shared chemical composition (Figure 1). Sinigrin thresholds, which have also not been studied previously, were highly dispersed with no clear modality. As with overall distributions, distribution ranges and means reflected similarities and differences

among tastants. Mean thresholds varied more than 40-fold among compounds, from a minimum of ~50  $\mu\text{M}$  (PTC) to a maximum of ~2000  $\mu\text{M}$  (sinigrin), whereas within-compound threshold ranges varied from 40-fold (salicin, goitrin, and sinigrin) to 8000-fold (PTC) (Figure 2).

Whole-gene sequencing detected 3 SNPs (C145G, C785T, and A886G) encoding 3 amino acid substitutions (P49A, A262V, and V296I) in our sample (Figure 3). All 3 have been identified in previous studies and alternate alleles at each site are common worldwide, with C145, C785, and A886 having mean frequencies of 0.54, 0.59, and 0.55, respectively, across African, Asian, and Caucasian populations (Wooding et al. 2004; Kim et al. 2005). Rare TAS2R38 variants are also known to exist, but these were not found in our subjects (Kim et al. 2003; Wooding et al.

2004; Kim et al. 2005; Campbell et al. 2012). To date, a total of 21 SNPs have been identified at *TAS2R38* in humans, 19 (90%) of which encode amino acid substitutions—an extremely high rate attributable to pressure from natural selection (Campbell et al. 2012). Most of these variants have been discovered in African populations, with Asians and Caucasians having lower diversity. Thus, although representative of only a small portion of global diversity, the presence of only the 3 most common variants of TAS2R38 in our sample is consistent with the Caucasian composition of our subject pool.

Together, the C104G/P49A, C785T/A262V, and A886G/V296I variants defined 6 haplotypes, denoted according to amino acid composition, which have also been described previously (Figure 3) (Kim et al. 2003; Bufer et al. 2005a).



**Figure 2** Phenotypic threshold distributions. Shading indicates diplotype with respect to haplotypes outlined in Figure 3. Bars above each distribution indicate the range between minimum and maximum observed measures.



A

Haplotype	Nucleotide			Amino Acid			Occ.	Freq.
	145	785	886	49	262	296		
AAI	G	C	A	A	A	I	1	0.01
AAV	G	C	G	A	A	V	5	0.05
AVI	G	T	A	A	V	I	48	0.43
PAI	C	C	A	P	A	I	1	0.01
PAV	C	C	G	P	A	V	55	0.49
PVI	C	C	A	P	V	I	2	0.01
							112	1.00

B

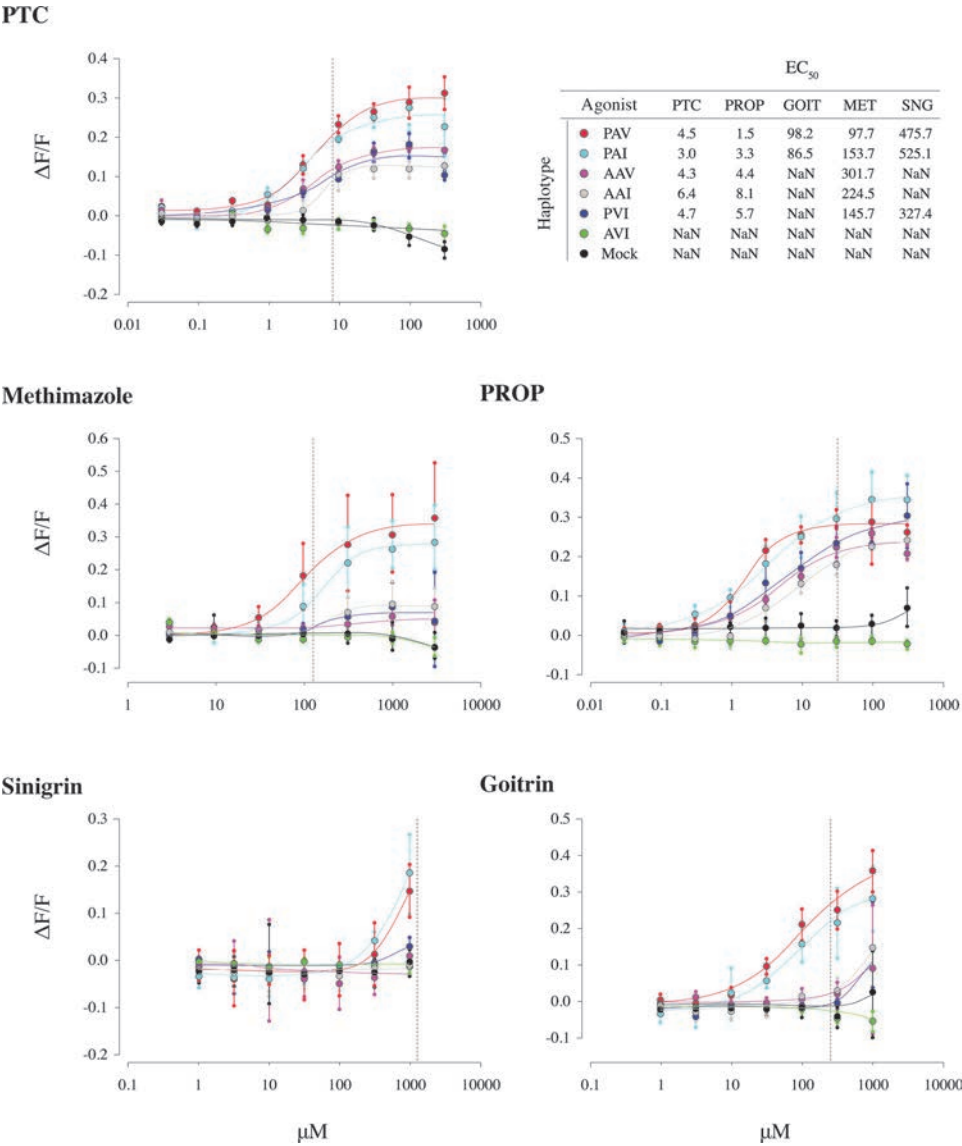
Diploptype	Occ.	Freq.
AAI/PAV	1	0.02
AAV/AVI	2	0.04
AVI/AVI	9	0.16
PAI/PVI	1	0.02
PAV/AAV	3	0.05
PAV/AVI	27	0.48
PAV/PAV	12	0.21
PAV/PVI	1	0.02
	56	1.00

**Figure 3** TAS2R38 SNP, haplotype, and diplotype frequencies. **(A)** Six haplotypes defined by 3 SNPs were observed in our sample. The composition of each haplotype with respect to nucleotide positions 145, 785, and 886 is shown, along with composition with respect to amino acid positions 49, 262, and 296. The number of occurrences of each haplotype indicates the total number of observations in the sample, which includes 2 haplotypes for each of the 56 subjects, for a total of 112 observations. **(B)** The number of occurrences and frequency of each observed diplotype (i.e., haplotype pairing in an individual).

Two, PAV and AVI, were most common, accounting for 103 of 112 (90%) observations in our sample, with the remaining 4 (AAI, AAV, PAI, and PVI) observed 1 to 5 times (1–5%) (Figure 3). As with the SNP alleles, this pattern is similar to previous findings in Caucasians but differs from patterns in Africa, where PAV and AVI account for a smaller fraction of overall variation (~80%), and other haplotypes are found at higher frequencies. The largest difference in haplotype frequency occurred with the AAI haplotype, which was observed only once in our sample (<1%), but Campbell et al. (2012) found at frequencies of 8% and 19% in West Central and East Africans, respectively. Diplotype frequencies in our sample were consistent with expectations given haplotype frequencies, with PAV/PAV, PAV/AVI, and AVI/AVI accounting for 48 subjects (86% of the sample) and rare types accounting for the remaining 8 (14%) (Figure 2). Again, although representative of patterns in Caucasians, diplotype frequencies in our sample differed from trends in other populations, particularly African groups, in which AAI-containing diplotypes are common.

Dose–response assays *in vitro* detected extensive variability in receptor function with respect to both  $EC_{50}$ , the agonist concentration across which response changes most rapidly, and concentration-specific response as measured by  $\Delta F/F$

(Figures 4 and 5).  $EC_{50}$  values varied primarily across agonists. Differences were most apparent for PAV, which exhibited responses differing more than 100-fold between PTC and sinigrin, and other alleles exhibiting smaller but still broad ranges. However, allelic differences in  $EC_{50}$  within agonists were not statistically significant. In contrast, within-agonist comparisons revealed highly divergent functionality among alleles as measured by  $\Delta F/F$ . This was most apparent for the 2 common alleles, PAV and AVI, whose differences in  $\Delta F/F$  were statistically significant for all TAS2R38 agonists (Figure 5). Although PAV was highly responsive, AVI showed no response above baseline to any. This finding is consistent with previous studies examining PAV- and AVI-mediated perception of PTC, PROP, and goitrin (Wooding et al., 2010) and demonstrates that methimazole and sinigrin, which have not been previously studied in detail for their activation of TAS2R38 receptor variants, evoke analogous receptor activity. PAI exhibited responses not significantly different from those of PAV, responding to every tested TAS2R38 agonist, thus appearing to be a rare secondary taster allele, underscoring previous observations (Bufe et al. 2005a). These patterns fit with expectations under the classic “lock-and-key” model of receptor-agonist interactions, which predicts that structurally similar ligands (e.g., thioamides) will elicit similar responses

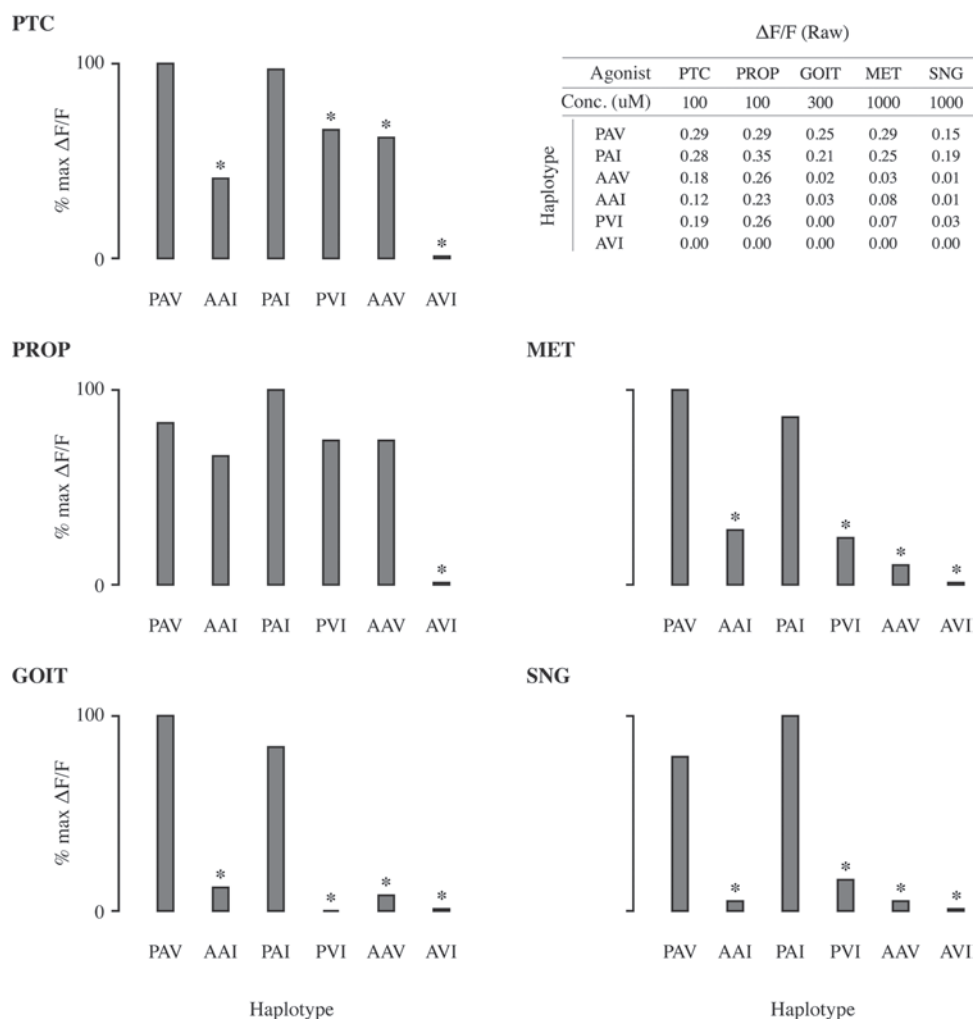


**Figure 4** Results of *in vitro* functional assays. Curves indicate dose–response profiles of each TAS2R38 variant to each tastant, with color key and EC<sub>50</sub> values at upper right.

from a given receptor. However, 3 alleles, PVI, AAI, and AAV, showed unexpectedly heterogeneous response profiles. These 3 exhibited  $\Delta F/F$  values significantly different from PAV to methimazole, goitrin, sinigrin, and PTC, but not to PROP. This result is unexpected under the basic lock-and-key model for TAS2R38, suggesting instead that minor differences in agonist structure may translate into major differences in receptor affinity (Kobilka and Deupi 2007).

Regression analyses revealed highly significant associations between common alleles in TAS2R38 and phenotypic variation in threshold responses (Figure 6). Associations of SNPs with thioamide thresholds were strong, with all 3 (C145G, C785T, and G886A) having *P* values below 0.005 and  $\beta$  exceeding 1.85. This pattern recapitulates previous studies of PTC, PROP, and goitrin thresholds, which also

have found strong associations, and demonstrates that variation in TAS2R38 makes similar contributions to methimazole thresholds. As with thioamides, associations with sinigrin threshold were statistically significant for all 3 SNPs and were characterized by large effect sizes ( $\beta > 2.10$ ). These had higher *P* values (0.04, 0.03, and 0.03, respectively); however, they were derived from a very small subject panel (*N* = 25), providing little statistical power and thus likely comparable to those of thioamides. No SNP showed evidence of association with salicin thresholds (*P* > 0.15 for all SNPs). Diplootype-phenotype analyses restricted to the 2 common TAS2R38 alleles, PAV and AVI, yielded results similar to SNP-by-SNP analyses, with PAV/PAV homozygotes having the lowest mean thresholds (i.e., greatest sensitivity), PAV/AVI heterozygotes having intermediate thresholds, and AVI/



**Figure 5** Comparison of functional responses. Graphs indicate relative  $\Delta F/F$  values for each agonist and TAS2R38 variant at the maximum agonist concentration not showing evidence of functional inhibition in Figure 4. 100  $\mu\text{M}$  PTC, 100  $\mu\text{M}$  PROP, 300  $\mu\text{M}$  goitrin, 1000  $\mu\text{M}$  methimazole, and 1000  $\mu\text{M}$  sinigrin. Upper right panel shows raw values. Asterisks indicate statistically significant differences from  $\Delta F/F$  value for PAV.

AVI homozygotes having the highest thresholds (Figure 7). Like the SNP associations, these were highly significant and strong for all thioamide responses,  $P < 0.005$  and  $\beta > 1.5$ , and marginally significant ( $P = 0.045$ ) but equally strong ( $\beta = 2.20$ ) for sinigrin.

FAA analyses, which incorporated *in vitro* measures of allelic function, yielded results similar to those of the standard regression approach in our sample (Figure 8). Dose–response curves revealed that the maximum concentrations with no evidence of agonist inhibition occurred at 100  $\mu\text{M}$  PTC, 100  $\mu\text{M}$  PROP, 300  $\mu\text{M}$  goitrin, 1000  $\mu\text{M}$  methimazole, and 1000  $\mu\text{M}$  sinigrin (Figures 4 and 5). At these concentrations, functional weights largely matched predictions based on overall similarities and differences among dose–response curves. While  $\Delta F/F$  was high for PAV across all agonists it was low for AVI, and other alleles exhibited response patterns varying across tastants. Associations with threshold were highly significant ( $P < 1.5 \times 10^{-3}$ ) for all thioamides and

marginally significant for sinigrin ( $P = 0.04$ ) with  $\beta$  exceeding 1.5 in all cases, indicating overall strong genetic effects. However, in spite of the 15% increase in sample size (from 48 subjects to 56) allowed by FAA,  $P$  values in weighted analyses were similar to those in unweighted analyses. Thus, although they exhibited substantial functional variability *in vitro*, effects from alleles other than PAV and AVI did not appreciably influence the correlations observed in our sample, possibly as a result of the low power of our study design to detect effects from rare variants. We anticipate that investigating the phenotypic contributions of these alleles will be better accomplished with larger, more genetically diverse population samples to provide increased statistical power.

## Discussion

Phenotypic and functional associations in our data reiterate the well-established significance of TAS2R38-PAV and -AVI

	SNP	Amino Acid	Salicin	PTC	PROP	Goitrin	Methimazole	Sinigrin
<i>P</i>	C145G	P49A	0.26	$7.98 \times 10^{-13}$	$6.33 \times 10^{-12}$	$2.30 \times 10^{-4}$	$8.05 \times 10^{-9}$	0.044
	C785T	A262V	0.19	$1.01 \times 10^{-10}$	$2.32 \times 10^{-7}$	$6.09 \times 10^{-4}$	$2.33 \times 10^{-8}$	0.026
	G886A	V269I	0.20	$1.34 \times 10^{-9}$	$3.50 \times 10^{-6}$	0.0020	$9.04 \times 10^{-7}$	0.026
<i>r</i> <sup>2</sup>	C145G	P49A	0.024	0.62	0.59	0.22	0.46	0.21
	C785T	A262V	0.032	0.54	0.39	0.20	0.44	0.25
	G886A	V269I	0.030	0.50	0.33	0.17	0.36	0.25
$\beta$	C145G	P49A	0.43	3.90	2.54	1.93	2.32	2.12
	C785T	A262V	0.51	3.75	2.14	1.87	2.33	2.18
	G886A	V269I	0.48	3.51	1.92	1.67	2.06	2.18

**Figure 6** SNP–phenotype associations. Results of association tests between genotype at each variable position are shown for each tastant. *P* indicates probability of no association, *r*<sup>2</sup> indicates coefficient of determination, and  $\beta$  indicates effect size.

in mediating threshold responses to thioamides and likely responses to foods and other substances containing them. Across the spectrum of genetic variation in TAS2R38, which in our sample included a total of 6 haplotypes, PAV and AVI together accounted for 103 of 112 sequences (>90%). Also consistent with previous functional studies, we found that PAV and AVI exhibited major differences in response to thioamide agonists *in vitro*, were strongly associated with threshold detection of PTC, PROP, goitrin, and the previously unexamined compound methimazole, and exhibited heterozygote effects, with PAV/AVI diplotypes having intermediate phenotypes. Although these associations were highly significant, however, substantial phenotypic variance remained unexplained, as evidenced by their *r*<sup>2</sup> values (Figure 6). This result reinforces previous reports that factors beyond the function of TAS2R38 make major contributions to threshold phenotypes (Hayes et al. 2008).

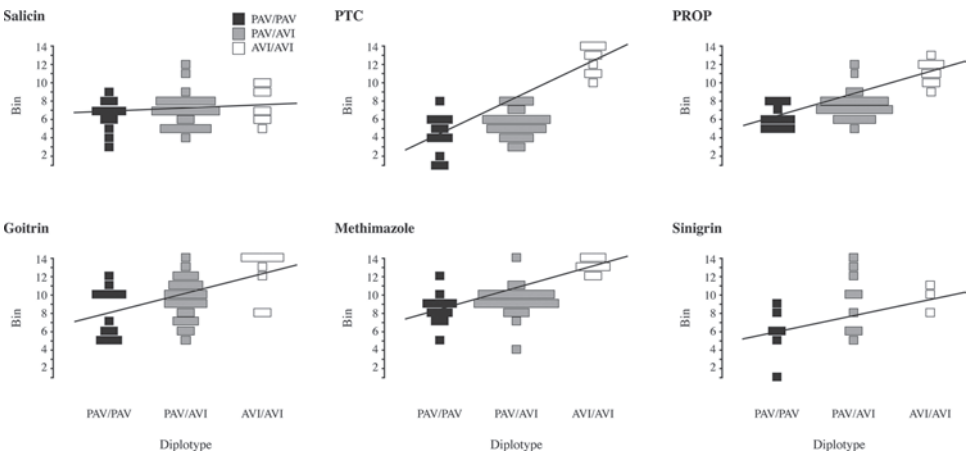
Our findings also provide explicit evidence that polymorphism in TAS2R38 shapes threshold responses to the glucosinolate sinigrin, a nonthioamide, which has previously been hypothesized but never tested directly. The possibility of an allele-dependent TAS2R38–sinigrin relationship was first recognized by Sandell and Breslin (2006), who demonstrated

		<i>P</i> -value		<i>r</i> <sup>2</sup>		$\beta$	
		PAV/AVI	Weighted	PAV/AVI	Weighted	PAV/AVI	Weighted
Tastant	PTC	2.22 × 10 <sup>-10</sup>	1.36 × 10 <sup>-11</sup>	0.59	0.57	3.83	3.85
	PROP	6.44 × 10 <sup>-9</sup>	6.55 × 10 <sup>-9</sup>	0.52	0.46	2.34	2.70
	Goitrin	2.92 × 10 <sup>-4</sup>	1.30 × 10 <sup>-3</sup>	0.25	0.16	2.07	1.76
	Methimazole	3.53 × 10 <sup>-7</sup>	1.91 × 10 <sup>-7</sup>	0.43	0.39	2.23	2.20
	Sinigrin	0.045	0.041	0.23	0.17	2.20	2.20

**Figure 8** Comparison of weighted association analyses (*N* = 56 subjects) with analyses restricted to PAV/PAV, PAV/AVI, and AVI/AVI diplotypes (*N* = 48 subjects). *P* indicates probability of no association, *r*<sup>2</sup> indicates coefficient of determination, and  $\beta$  indicates effect size.

that the perceived bitterness of sinigrin-containing vegetables is correlated with TAS2R38 genotype; however, those results faced the confound that plants containing sinigrin nearly always contain goitrin, an established TAS2R38 agonist, leaving the specific contribution of sinigrin unclear. The role of TAS2R38 in sinigrin perception was partly confirmed by Meyerhof et al. (2010), who demonstrated that sinigrin is an agonist of TAS2R38–PAV, but the functional significance of substitutional variants has remained unresolved. Thus, our results represent the first unambiguous illustration that polymorphism in TAS2R38 drives variable responses to compounds other than thioamides and that threshold detection varies as predicted by functional measures. The extent to which this trend extends to other glucosinolates, a large family of compounds including numerous sinigrin analogs found in foods, stands out as a line of future investigation.

Although we did not investigate receptor–agonist interactions directly, variable patterns of response across the 5 structurally divergent TAS2R38 agonists and 6 alleles in our study shed new light on the structure–function relationships underlying bitter taste. Recent reports addressing the effects of mutational polymorphism have yielded disparate results (Floriano et al. 2006; Miguet et al. 2006; Biarnés et al. 2010; Tan et al. 2012). Whereas some have concluded that variable positions 49, 262, and 296 are not directly involved



**Figure 7** Phenotypic associations with PAV/PAV, PAV/AVI, and AVI/AVI diplotypes (*N* = 48 subjects). The relative number of observations at each threshold is indicated by bar width. Raw values are given in Figures 2 and 3.



in agonist-receptor interaction but affect receptor activation properties (Biarnés et al. 2010), others have suggested that direct contacts may occur (Tan et al. 2012). Our finding that the rank order of potency among the tested agonists (PTC/PROP > methimazole/goitrin > sinigrin) was similar for all analyzed receptor variants indicates that none preferentially interacted or, conversely, failed to interact with any of the receptor variants. However, evidence that methimazole and goitrin, like PROP and PTC, represent full agonists for the PAV and PAI allele, but exert weaker stimulatory effects on AAV, AAI, and PVI than do PROP and PTC, indicates that receptor efficacy is somewhat agonist dependent. This may indicate that the activation properties of the individual TAS2R38 variants are not purely receptor intrinsic.

Previous studies have demonstrated that much of the functional differentiation between TAS2R38 taster and nontaster alleles is attributable to the P49A and A262V variants (Bufer et al. 2005a). However, these variants do not have uniform effects across ligands. In our *in vitro* experiments, differences among functional TAS2R38 alleles were smaller for PROP than for other agonists. This may indicate that the receptor binding site for PROP is somewhat shifted compared with the other agonists used in this study. In addition, although the responses of alleles harboring different combinations of residues in positions 49 (P or A) and 262 (A or V) in our sample do not definitively point to a differential, agonist-selective contribution to receptor function, the fact that they are spaced far apart and in different structural domains (position 49 in intracellular loop 1 and position 262 in transmembrane domain 6) suggests that they may influence receptor activation by different mechanisms. Transmembrane domain 6, which harbors position 262, is thought to tilt upon receptor activation (Biarnés et al. 2010). Thus, it may be that bound agonists interfere with this movement, particularly if a bulky valine residue is present instead of the small alanine. Singh et al. (2011) proposed an interhelical hydrogen-bond network between transmembrane domains 1, 2, and 7 important for receptor activation of the bitter taste receptor TAS2R1. Position 49, placed in the intracellular loop 1 between transmembrane domains 1 and 2, could interfere with the formation of such a network and, as a consequence, with receptor activation. Because both positions seem not to interfere with agonist binding per se, we hypothesize that they contribute to receptor responsiveness independently and additively, which would fit to previous data on the relative contribution of these residues (Bufer et al. 2005a).

In addition to shedding light on the basic mechanisms of receptor-agonist interactions, the continuous range of functionality observed across TAS2R38 alleles illustrates the potential diversity of receptor contributions to threshold responses. Early studies inferred that the heritability of PTC perception is explained by the presence of simple dominant and recessive “taster” and “nontaster” alleles. However, in the first successful attempt to map the locus underlying the trait, Kim et al. (2003) discovered that TAS2R38 harbors at least 5

alleles, and subsequent studies have identified 19 polymorphic amino acid positions to date, suggesting that alleles other than PAV and AVI likely make independent, differential contributions to phenotype (Campbell et al. 2012). Our results confirm that this genetic diversity has the potential to drive substantial functional variation and provide a practical basis for dissecting their phenotypic contributions. Additionally, they confirm that although threshold responses to PTC and related compounds are driven in large part by a simple “taster” (PAV) and “nontaster” (AVI) dichotomy, a range of “intermediate-taster” alleles are also found, indicating that threshold responses to these substances are in fact complex traits.

Patterns of genetic diversity on population levels also have implications for TAS2R38-driven variation in thresholds. Although alleles exhibited diverse functionality *in vitro*, most were so rare that our study design did not provide sufficient statistical power to ascertain their phenotypic effects. However, TAS2R38 allele frequencies vary substantially among human populations raising the possibility that alleles rare in our sample, which was of European ancestry, could make important phenotypic contributions in other groups. For instance, Wooding et al. (2004) found that the AAI allele, which was rare in our sample and not associated with PTC thresholds, occurs at a frequency of ~15% in African populations, which could drive significant associations. This prediction is consistent with the recent results of Campbell et al. (2012) in an analysis of PTC perception in Central and West African population, which harbored AAI at frequencies of 10–20%. Association tests revealed that in those populations, AAI/AI homozygotes exhibited thresholds ~16-fold greater than AVI/AVI homozygotes but ~4-fold lower than PAV/PAV homozygotes, exactly the pattern predicted by our functional assays. Similar differences in function, allele frequency, and phenotypic variability could be found with other alleles in worldwide populations. Moreover, because some such variants exhibit activation properties deviating appreciably from the major PAV and AVI alleles, they will likely affect threshold phenotypes in individuals harboring such alleles.

The high levels of genetic diversity found in TAS2R38 are not unique among TAS2Rs. As a group, the TAS2R family harbors exceptionally high levels of variations relative to other human genes, and mutational variants affecting bitter taste responses have been directly documented at 4 positions, TAS2R16, -43, -44, and -38. Further, levels of population differentiation vary widely among TAS2R loci, indicating that if functional variants are present, they could underlie substantial between-group differences in perception and, hence, complex downstream phenotypes. We anticipate that joint function- and association-based approaches will be highly effective in dissecting these complex effects.

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