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Do Polymorphisms in the *TAS1R1* Gene Contribute to Broader Differences in Human Taste Intensity?

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Abstract

The *TAS1R* genes encode heterodimeric receptors that mediate umami (hTAS1R1 + hTAS1R3) and sweet (hTAS1R2 + hTAS1R3) sensations. The question of interest for this study is if *TAS1R1* variation associates with differences in overall taste intensity. We leveraged an existing database of adults (n = 92, primarily European American) to test associations between 2 *TAS1R1* single nucleotide polymorphisms (SNPs) (intronic rs17492553, C/T and exonic rs34160967, G/A) and intensity of 4 prototypical tastants (NaCl, sucrose, citric acid, and quinine), applied regionally to fungiform and circumvallate loci, and sampled with the whole mouth. Both SNPs were associated with modest shifts in perceived intensities across all taste qualities. Three genotype groups were represented for the intronic SNP—minor allele homozygotes (TT) averaged 40% lower intensities than did CC homozygotes for all regionally applied tastants, as well as whole-mouth NaCl and citric acid. Similar, but less pronounced, intensity differences were seen for the exonic SNP (GG homozygotes reported greater intensities than did the AA/AG group). Our predominantly European American cohort had a low frequency of AA homozygotes, which may have attenuated the SNP-related differences in perceived intensity. These preliminary findings, if replicated, could add *TAS1R1* polymorphisms to the repertoire of genotypic and phenotypic markers of heightened taste sensation.

Key words: bitter, rs17492553, rs34160967, salty, sour, supertasting, sweet, taste genetics

Introduction

Behavioral evidence that individuals vary in the perceived intensity of tastes and oral somatosensations dates before the 20th century (Bailey and Nichols 1888). Historically, attention has been focused on characterizing this variation with the ability to taste the bitterness of phenylthiocarbamide (PTC) and chemically related propylthiouracil (PROP) (Blakeslee 1932; Guo and Reed 2001; Wooding 2006). Subsequent research has linked PTC/PROP bitter phenotypes and *TAS2R38* polymorphisms to food choice, food intake, and chronic disease risk (reviewed by Duffy 2007; Tepper 2008). Other phenotypic markers of variation in oral sensation have emerged, including thermal taste (Green and George 2004), bitterness of vanilloid receptor agonists (Green and Hayes 2004), and density of fungiform papillae (FP) (Miller and Reedy 1990; Essick et al. 2003). Likewise, additional polymorphisms in the *TAS2R* family (Hayes et al. 2011) and in other genes (Perry et al. 2007; Mandel et al. 2010; Calò et al. 2011; Pirastu et al. 2012; Dias et al. 2013) have been linked to variation in oral sensation and food liking (Duffy et al. 2009; Duffy et al. 2010; Hayes et al. 2013).

The human *TAS1R* taste receptor gene family (*TAS1R1*, *TAS1R2*, and *TAS1R3*) on chromosome 1, like the *TAS2R* family of bitter-taste receptor genes, is highly polymorphic (Kim et al. 2006; Raliou et al. 2009a). A heterodimeric G protein-coupled receptor consisting of T1R2 and T1R3 (encoded by the *TAS1R2* and *TAS1R3* genes) mediates sweet

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taste perception in humans (Li et al. 2002), whereas the T1R1/T1R3 heterodimer responds to glutamate and other 5′ ribonucleotides (Li et al. 2002; Zhao et al. 2003; Chandrashekar et al. 2006; Kim et al. 2006). Two metabotropic glutamate receptors, mGluR4 and mGluR1, respond to glutamate in rodents (Chaudhari et al. 2000; Toyono et al. 2003; San Gabriel et al. 2005), and possibly in humans (Kurihara and Kashiwayanagi 2000; Raliou et al. 2009b), with corresponding genes *GRM4* and *GRM1* located on chromosome 6 in humans. However, the T1R1/T1R3 complex appears more broadly tuned in rodents, responding to a range of amino acids (Nelson et al. 2002; Chaudhari et al. 2009). Because amino acids elicit a variety of taste qualities (e.g., in humans, glycine is sweet, phenylalanine is bitter, and glutamate is meaty), these findings in rodents suggest that *T1R1* or *T1R3* polymorphisms may be important to overall versus quality-specific differences in taste perception. Indeed, a recent study with mice (Kusuhara et al. 2013) reported that chorda tympani nerve responses to sucrose and other sweeteners, but not salty, sour, and bitter compounds, were significantly smaller in the *TAS1R1* knockout mice than in the heterozygous mice, suggesting that T1R1 is important to taste functioning beyond umami perception.

Taste findings from rodent, however, may not generalize to humans (Ishimaru et al. 2012). *TAS1R1*, in particular, is only expressed in the anterior tongue of rodents, whereas in primates (macaques), a close human relative with the same omnivorous diet, *TAS1R1* is expressed in both fungiform and circumvallate papilla, where it perhaps has different roles or is involved in different transduction mechanisms (Hevezi et al. 2009; Ishimaru et al. 2012). Intriguingly, *TAS1R1* and other *TAS1Rs* are pseudogenized in mammals with extremely narrow diets, such as bottlenose dolphins, sea lions, and common vampire bats (Jiang et al. 2012; Zhao et al. 2012). *TAS1R1* pseudogenes may play a role in the reduced or no sensitivity to sweet- and bitter-taste stimuli in dolphins and sea lions (Friedl et al. 1990; Jiang et al. 2012) or poorly developed taste in blood-feeding vampires (Thompson et al. 1982; Ratcliffe et al. 2003).

Variation in *TAS1R1* in humans has been tied with variation in sensitivity to umami, a taste exemplified by glutamate, with a fraction of individuals as monosodium glutamate (MSG) hypotasters or nontasters (Lugaz et al. 2002; Chen et al. 2009; Raliou et al. 2009b; Pepino et al. 2010; Singh et al. 2010). In vitro studies show functional variation in ability to bind MSG with amino acid substitutions in T1R1 and T1R3 receptors (Shigemura et al. 2009; Raliou et al. 2011). Recently, interest has grown in the contributions of *TAS1R1* (Entrez GeneID: 80835) and *TAS1R3* (Entrez GeneID: 83756) polymorphisms to functional differences in umami and sweet perception in vivo. Although evidence to date relates *TAS1R3* variation to sweet (Fushan et al. 2009) and umami taste perception (Chen et al. 2009; Raliou et al. 2009b; Shigemura et al. 2009), findings with *TAS1R1* polymorphisms have been mostly limited to MSG recognition or detection thresholds.

Inconsistent findings across human studies suggest the need for further investigation of *TAS1R1* in human taste perception. Two nonsynonomous single nucleotide polymorphisms (SNPs) in *TAS1R1*, A110V (rs41278020, C/T), and A372T (rs34160967, G/A) have been associated with differences in MSG taster or nontaster status (Raliou et al. 2009b). The published findings for the A372T SNP are conflicting, and homozygosity for its minor allele (genotype AA) is less frequently seen in European (3% in French [Raliou et al. 2009b]) versus Asian (18% in Japanese [Shigemura et al. 2009]) populations. In a very large sample (>3000) of adults from France (Raliou et al. 2009b), MSG nontasters were less likely to have the A allele, yet the significance in the chi-square analysis of allelic distribution was driven primarily by AG heterozygotes. In our reanalysis of the published distribution of MSG threshold categories by *TAS1R1* A372T (rs34160967, G/A) genotype from a study of adults from Japan (Shigemura et al. 2009), AG heterozygotes were significantly more likely to fall into the MSG-insensitive category than either AA or GG homozygotes.

In an unpublished human psychophysical study (Rawal et al. 2009), we leveraged an existing database to test the ability of A372T SNP (rs34160967, G/A) and another intronic SNP (rs17492553, C/T) in the *TAS1R1* gene to explain differences in liking and taste qualities from glutamate-rich foods that also are salty, sour, and bitter (soy sauce, grapefruit juice, and asparagus). The intronic SNP was investigated because of its high minor allelic frequency and its location; it lies in the third intron of the *TAS1R1* gene, 14 bp from the intron 3/exon 4 junction (IVS3-14) (NM_138697.3), and 1155 bp downstream of the A372T SNP. Although the perception of umami sensations were not assessed, we found that genotype differences in preference of these complex foods were associated with perceived sourness and sweetness.

This paper extends our preliminary findings to test if one or both of these *TAS1R1* SNPs explain differences in taste intensity, assessed in a protocol that was the foundation for the protocols in the National Institutes of Health Toolbox (Coldwell et al. 2013) and the National Health and Nutrition Examination Survey (Duffy et al. 2012). Our main finding was that homozygotes for the intronic SNP minor allele (TT) reported lower taste intensities than did CC homozygotes for all tastants, and heterozygotes for exonic SNP (AG) reported lower intensities than GG homozygotes for all regionally applied tastants except quinine. These preliminary findings, if replicated, could add *TAS1R1* polymorphisms to the repertoire of genotypic and phenotypic markers of heightened taste sensation (Green and George 2004; Green and Hayes 2004; Calò et al. 2011).

Materials and methods

Subjects

A convenience sample of reportedly healthy, nonsmoking adults was recruited from the University of Connecticut community to participate in an observational study of

variation in oral sensation, diet, and health. Exclusion criteria included pregnancy, severe food allergies, and thyroid disease. The study sample included 92 adults, primarily of European ancestry (84.8%), female (76%), and middle aged (mean 40.9 ± 12.2 SD). Other ethnicities represented in the sample were Black (5.4%), Hispanic or Latino (5.4%), Asian (3.3%), and other (1.1%). All procedures were approved by the local institutional review board; adults provided informed and written consent and were paid for their participation. All of the data were collected across 2 experimental sessions in the Duffy laboratory, followed by a visit with phlebotomist for venipuncture for DNA extraction.

Intensity scaling

Adults used a general labeled magnitude scale (gLMS) (Bartoshuk et al. 2004) to rate the taste intensity of oral stimuli. As an intensity scale, the gLMS is a vertical scale ranging from "no sensation" (0) at the bottom to "strongest imaginable sensation of any kind" (100) at the top and with other adjectives ("barely detectable," "weak," "moderate," "strong," and "very strong") placed in a quasi-logarithmic fashion. Prior to using the gLMS to report intensities, subjects received a verbal orientation to gLMS scaling and were trained on its proper use. As part of the orientation, the subjects practiced rating intensities of recalled nonoral experiences (remembered sound and light sensations). Subjects were first asked to determine which adjective/descriptor on the scale most closely described the intensity of the sensation and then to rate it in the larger context of all sensations. They were asked to rate the sensation by clicking either near or between the adjectives that most closely approximated the strength of their sensation. Upon clicking, the computer generated a numerical value based on the distance along the scale from a zero point (no sensation), which was recorded manually by the experimenter.

Procedure

Intensity of taste stimuli on regional areas

Subjects reported the intensities for 1 M NaCl, 1 M sucrose, 32 mM citric acid, and 1 mM quinine hydrochloride painted with a cotton swab on the anterior and posterior tongue (innervated by the chorda tympani branch of cranial nerve VII and the glossopharyngeal branch of cranial nerve IX, respectively)—and sampled with the whole mouth to stimulate cranial nerves VII, IX, and X. Because applying tastants with a cotton swab may cause additional somatosensory stimulation and distort ratings, the cotton swabs were amply saturated with the taste solution prior to application on the tongue, and care was taken to "draw" the solution across the tongue tip. For the whole mouth, subjects were asked to take 10 ml of the solution into his or her mouth, rinse for approximately 5 s, and then expectorate. Between each stimulus, the subjects rinsed their mouth with deionized (>15 MΩ) water to remove any residual stimulus.

Intensity of tones as a nonoral standard

Subjects reported the intensities of a series of 1000 Hz tones ranging in 12-dB steps from 50 to 98 dB throughout the testing sessions. The tones were used as a cross-modal standard and used to statistically account for variability in intensity scale usage in the analyses.

TAS1R1 genotyping

Subjects had 15 ml of peripheral blood drawn by venipuncture, collected into ethylenediaminetetraacetic acid vacutainer tubes at the University of Connecticut. The DNA was extracted from whole blood in accordance with standard methods that followed manufacturer's instructions (Qiagen Gentra PureGene) with occasional modification for hemolyzed samples. The genomic DNA was then shipped to the University of Florida and genotyped for *TAS1R1* intronic SNP (rs17492553) and exonic SNP (rs34160967) by ABI TaqMan–automated genotyping as reported previously (Hayes et al. 2011). The assay numbers for TaqMan were C__25991161_10 (rs17492553) and C__25997001_10 (rs3416097). The polymerase chain reaction (PCR) step of the genotyping employed conditions of 58°C for 50 cycles, with genotypes subsequently identified using an ABI Prism 7900HT instrument in the University of Florida Pharmacogenomics Center. For quality control, 4 samples were genotyped in duplicate with the same result. The quality control indicators from TaqMan genotyping showed excellent separation of the 3 genotypes for the entire panel. For 3 other samples, independent PCR and sequencing verified the genotypes. The resulting allele frequencies were in Hardy–Weinberg equilibrium for all of the SNPs. Thus, the genotyping fidelity appeared excellent, as has been our experience in previous work (Hayes et al. 2011).

Additional markers of variation in oral sensation

Because variation in oral sensation has been associated with PROP-tasting phenotype, *TAS2R* genotype and number of FP, these markers were tested for potential interactions with *TAS1R1* genotypes in explaining differences in taste intensity. Established PROP-tasting *TAS2R38* genotypes and haplotypes (rs713598, rs1726866, and rs10246939) (Kim et al. 2003) were obtained by the same methods described above for *TAS1R1*. Color videomicroscopy of the tongue tip was used to assess FP density in a 6-mm-diameter circular template, using methods described previously (Duffy et al. 2004). Counts from the left and right sides of the tongue tip were averaged to obtain the mean number of FP count per standard area. Subjects also reported the intensity of 3.2 mM PROP solution sampled with the whole mouth

in standardized protocol reported previously (Duffy et al. 2004).

Data analysis

Data analyses were conducted with SPSS (version 17.0); significance criterion was set at $P \leq 0.05$. Linear regression analysis, accounting for outliers, was used to test associations between the *TAS1R1* SNPs and taste intensity. An analysis of covariance (ANCOVA), controlling for age, sex, and intensity of tones as a cross-modal standard, was used to compare rs17492553 and rs34160967 genotype group differences in the intensities of NaCl, sucrose, citric acid, and quinine hydrochloride on the FP and circumvallate papillae, and as perceived with the whole mouth. We combined AA and AG as 1 genotype group due to small numbers in the AA group, reflecting 1 or 2 copies of the minor allele. The 2 AA homozygotes were omitted in a subanalysis with no change in the findings reported. Post-hoc analyses were conducted with *t*-tests based on the error term from the overall analysis of variance (ANOVA) (Keppel 1991).

The impact of *TAS2R38* diplotype, PROP bitterness phenotype, and FP number was tested as separate covariates in the ANCOVA, as an additional independent variable in 2-way ANCOVA (for example, PROP taster group by *TAS1R1* genotype for each SNP), and by removing PROP nontasters (PROP bitterness < moderate on the gLMS) or supertasters (PROP bitterness > very strong on the gLMS) in the analysis. These measures of variability in oral sensation did not influence the statistical significance or pattern of results when used as covariates. The interaction term was not significant in the individual 2-way ANOVA with *TAS1R1* genotype (for each SNP) and either taste phenotypes or *TAS2R38* genotype groups. Finally, removing PROP nontasters or supertasters from the analyses changed neither the significance nor the patterns of findings. The data from these subanalyses are not presented in the article.

Results

The genotype frequencies for both *TAS1R1* SNPs were not significantly different from those reported for Americans of northern/western European descent listed at www.ncbi.nlm.nih.gov/SNP (dbSNP 2013) (Table 1). Furthermore, both SNPs were in Hardy–Weinberg equilibrium. For rs17492553, T was the minor allele in our sample, compared with C as reported in National Center for Biotechnology Information (NCBI) although this is likely just a sampling effect because both alleles are very close in frequency and not statistically different than the NCBI reference data. As expected based on published allele frequency differences between ethnic groups, our distribution for rs34160967 genotypes differed from the Japanese cohort utilized in the Shigemura et al. study (2009) ($\chi^2 = 30.23$, $P < 0.0001$), with relatively fewer AA and AG genotypes in our cohort (Table 1).

Table 1 Genotype frequencies of *TAS1R1* intronic (rs17492553) and exonic SNP (rs34160967) in the study sample versus published dbSNP database[a] and other studies[b,c]

rs17492553	Study sample	European American population[a]		
CC	0.33	0.21		
CT	0.43	0.50		
TT	0.23	0.29		
rs34160967	Study sample	European American population[a]	Shigemura et al. (2009)[b]	Raliou et al. (2009b)[c]
AA	0.02	—	0.18	0.02
AG	0.23	0.25	0.43	0.36
GG	0.75	0.75	0.39	0.62

[a]Frequencies from NCBI dbSNP database 2013. The study sample and published dbSNP database are of similar populations: rs17492553—χ^2 (2) = 3.88, $P = 0.14$; rs34160967—Fisher's exact ($P = 0.44$).
[b,c]From Fisher's exact testing, the study sample distribution for rs34160967 was different than in adults recruited in Japan (Shigemura et al. 2009; $P < 0.01$) and not different from those recruited in France (Raliou et al. 2009b; $P = 0.13$).

Genotype variation in perceived intensities of regionally applied and whole-mouth taste sensations

The cohort showed variability in ratings for prototypical tastants applied to the FP and the circumvallate papillae and sampled with the whole mouth. Our cohort appeared to capture the usual variation in regionally applied tastants—the distribution of ratings was not statistically different from a large unpublished database ($n = 450$) comprising patients with chemosensory complaints and controls (Kolmogorov–Smirnov statistics, $P > 0.05$).

The mean intensity of tastants applied to the anterior tongue (FP) was near "moderate," ranging from "no sensation" to above "very strong." The variance across the distribution of ratings was lowest for sucrose; all other qualities had significantly higher variances (F-ratio > 2.0, $P < 0.01$). Mean posterior tongue (circumvallate) intensities were marginally larger than those for the FP; however, the magnitude varied by quality—paired *t*-tests showed significant differences by locus ($P < 0.01$), except for citric acid where a significant difference across regions was not observed ($P = 0.34$). The mean intensity ratings for the whole mouth were near "strong"; this was considerably higher than regional application on the anterior and posterior tongue (paired *t*-tests, $P < 0.01$). Again, sucrose showed the least variance out of the 4 prototypical tastants (F-ratio > 2.0, $P < 0.01$).

Mean differences across rs17492553 genotype groups for intensities of regionally applied tastants were significant in an ANCOVA model controlling for age, sex, and intensity of tones as a cross-modal standard. The TT homozygotes reported lower intensities than CC homozygotes for all 4 tastants, with heterozygotes falling between the two in most cases. This pattern was also present for whole-mouth ratings

but was significant only for NaCl and citric acid. The intronic SNP (rs17492553, C/T) had more significant comparisons with the taste intensities than the exonic SNP (rs34160967, G/A), possibly because the study cohort showed representation across all 3 intronic SNP genotypes (CC, CT, and TT).

Although not significant, the exonic SNP rs34160967 also tended to associate with differences in the mean intensity of regionally applied tastants in ANCOVA controlling for age, sex, and intensity of tones as a cross-modal standard (F (4, 262) = 2.02; P < 0.10). The AA/AG genotype group reported lower intensities than GG homozygotes for all 4 tastants (both regionally applied and sampled with whole mouth) although not all of the stimuli applied regionally showed significant differences.

Salt

As shown in Figure 1, among rs17492553 genotype groups, TT homozygotes reported significantly lower intensities

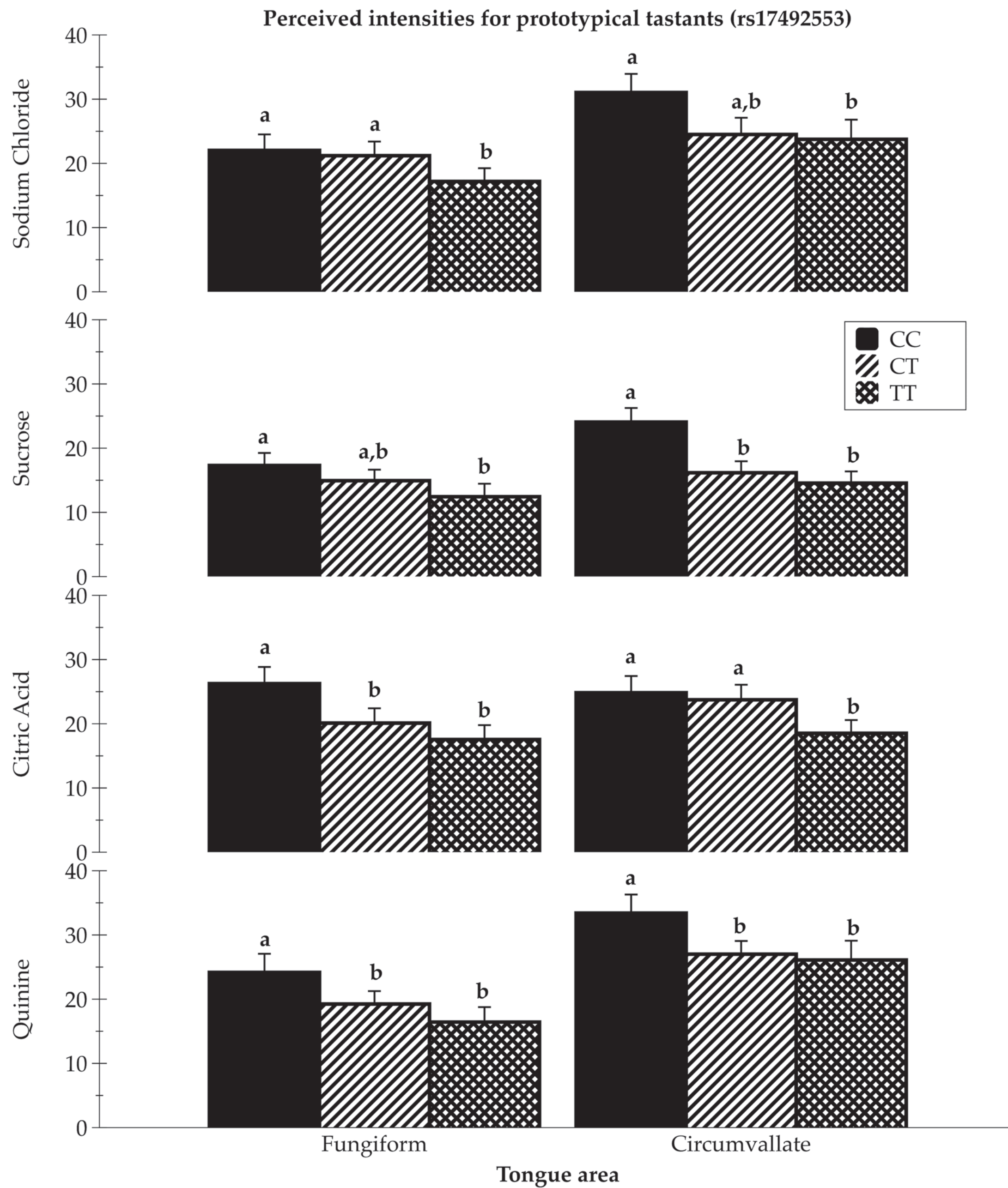

Figure 1 Intensity ratings for 1 M sodium chloride (mean ± SEM), 1 M sucrose (mean ± SEM), 32 mM citric acid (mean ± SEM), and 1 mM quinine hydrochloride (mean ± SEM), applied bilaterally to the tongue tip (fungiform papillae) and to the back of the tongue (circumvallate papillae) among 3 rs17492553 genotype groups (0.33 CC, 0.43 CT, and 0.23 TT), controlling for age, sex, and intensity of tones as a cross-modal standard; [F (6, 261) = 2.18, P < 0.04], with significant pairwise comparisons shown. Different superscript letters a, b indicate significant differences at P < 0.05.

than did CC and CT genotypes for the regionally applied 1 M NaCl on the FP. TT homozygotes also reported significantly lower intensities than did CC homozygotes for the regional application of 1 M NaCl on the circumvallate papillae. Consistent with fungiform and circumvallate ratings, TT homozygotes reported significantly lower intensities than CC homozygotes for 1 M NaCl sampled with the whole mouth ($P < 0.02$; data not shown).

For rs34160967 (G > A, exon 3), the AA/AG group reported significantly lower intensities than did GG homozygotes for the regionally applied 1 M NaCl on the circumvallate papillae (Figure 2; $P < 0.05$).

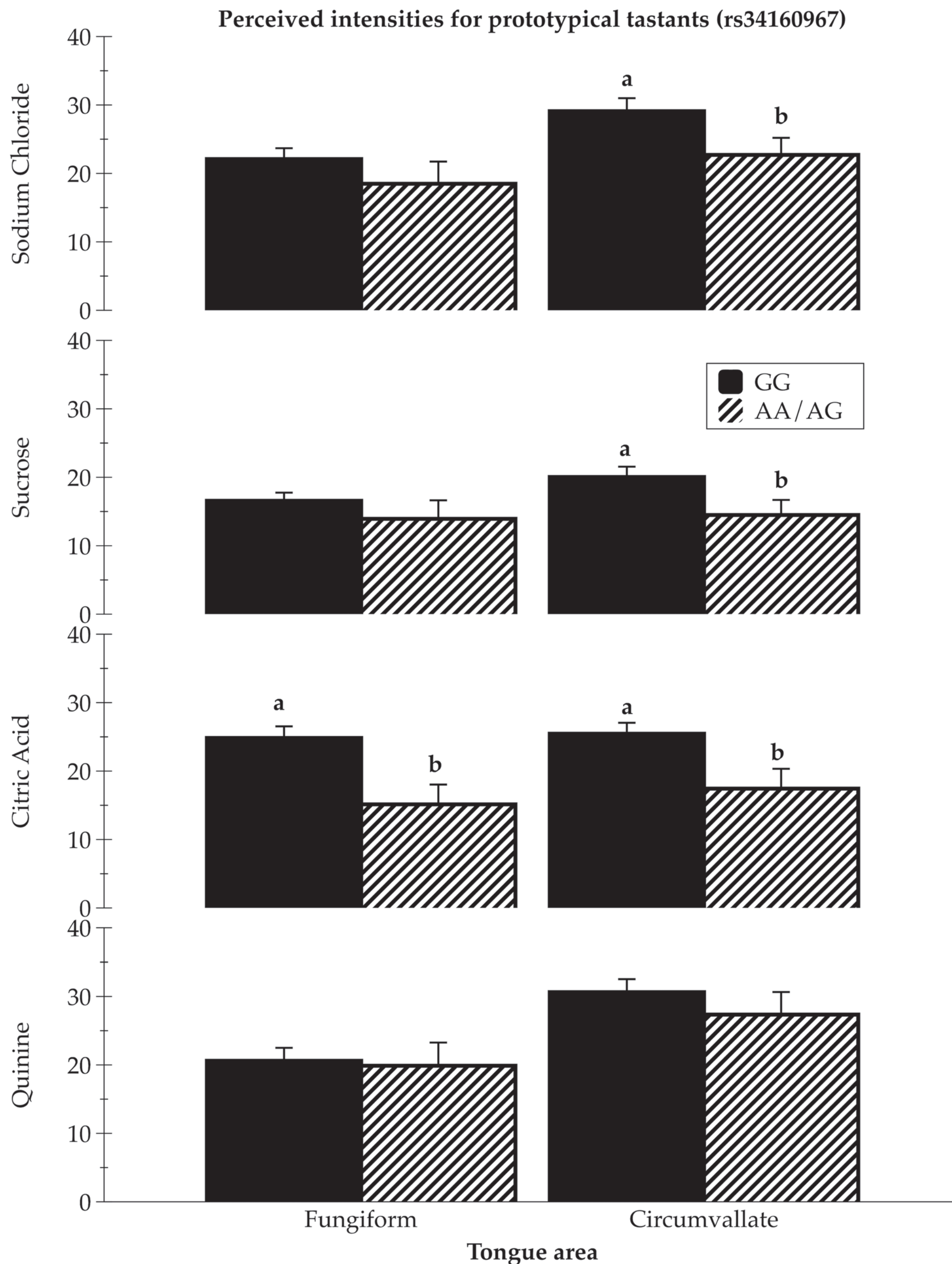

Figure 2 Intensity ratings for 1 M sodium chloride (mean ± SEM), 1 M sucrose (mean ± SEM), 32 mM citric acid (mean ± SEM), and 1 mM quinine hydrochloride (mean ± SEM), applied bilaterally to the tongue tip (fungiform papillae) and to the back of the tongue (circumvallate papillae) among 2 rs34160967 genotype groups (0.25 AA/AG and 0.75 GG), controlling for age, sex, and intensity of tones as a cross-modal standard; [F (4, 262) = 2.02, $P < 0.10$], with significant pairwise comparisons shown. Different superscript letters a, b indicate significant differences at least $P < 0.05$.

Sweet

As shown in Figure 1, among rs17492553 genotype groups, TT homozygotes reported significantly lower intensities than did CC homozygotes for the regional application of 1 M sucrose on the FP. On the circumvallate papillae, both heterozygotes and TT homozygotes reported significantly lower intensities than did CC homozygotes for 1 M sucrose. For the whole-mouth ratings, a similar, albeit nonsignificant, trend was seen for 1 M sucrose, with TT homozygotes tending to report lower intensities than both CC and CT groups (data not shown).

In the rs34160967 (exon 3 SNP) analysis, the AA/AG group reported significantly lower intensities than did GG homozygotes for the regionally applied 1 M sucrose on the circumvallate papillae (Figure 2; $P < 0.05$).

Sour

Similar to the trend seen with intensity ratings of 1 M sucrose, among rs17492553 genotype groups, both TT and CT genotypes reported significantly lower intensities than CC homozygotes on the anterior tongue for 32 mM citric acid (Figure 1). TT homozygotes also reported significantly lower perceived intensities than both CT and CC genotypes on the posterior tongue for citric acid. For the whole-mouth ratings, individuals with the TT genotype also reported significantly lower intensities than both CT ($P < 0.01$) and CC genotypes ($P < 0.05$) for 32 mM citric acid (data not shown).

As shown in Figure 2, among rs34160967 genotypes, the AA/AG group reported significantly lower intensities than did GG homozygotes for 32 mM citric acid on the anterior ($P < 0.01$) and posterior tongue ($P < 0.01$). The whole-mouth ratings for 32 mM citric acid tended to show the same pattern, with AA/AG group reporting lower intensities than GG homozygotes ($P < 0.07$) (data not shown).

Bitter

Quinine intensity also differed across rs17492553 genotype groups with regional application (Figure 1). TT homozygotes and heterozygotes reported significantly lower intensities than CC homozygotes for 1 mM quinine applied to the FP. Similar patterns were also observed for quinine applied to the circumvallate papillae, with TT homozygotes and heterozygotes reporting significantly lower intensities than CC homozygotes. Although not significant, a consistent trend was seen with whole-mouth ratings for 1 mM quinine, with TT homozygotes reporting lower intensities than both CC and CT groups (data not shown).

Among rs34160967 genotypes, there was no evidence of differences in bitterness perceived from whole-mouth sampled and regionally applied 1 mM quinine.

Discussion

Polymorphisms in the *TAS2R38* gene and others in the *TAS2R* bitter receptors family are associated with variation in oral sensation, preference, and dietary behaviors. Although *TAS1R1* polymorphisms have been tied to differences in threshold and intensity of umami stimuli, data from rodents (Nelson et al. 2002; Kusuhara et al. 2013) and mammals (Thompson et al. 1982; Friedl et al. 1990; Ratcliffe et al. 2003; Jiang et al. 2012), as well as from our laboratory (Rawal et al. 2009), suggest that these polymorphisms also associate with variation in taste perception beyond umami. In this study, we report from an existing database that an intronic (rs1749255) and exonic (rs34160967) SNP in *TAS1R1* were associated with modest intensity differences of concentrated aqueous solutions across 4 prototypical tastants (sucrose, NaCl, citric acid, and quinine hydrochloride) applied regionally and/or with intensity differences of NaCl and citric acid perceived with the whole mouth. These differences were statistically independent of the effects of PROP taster status and density of FP on perceived taste intensity. If confirmed, this study's findings provide impetus for future research to assess if *TAS1R1* polymorphisms have an indirect or direct influence on overall taste functioning or are merely a genetic marker for differences in taste intensity.

Of the 2 *TAS1R1* SNPs examined, all 3 genotype groups were represented for the intronic SNP (rs17492553, C/T) (Table 1), and CC homozygotes reported higher intensity than the TT homozygotes across all tastants applied to the tongue tip and posterior tongue and, in some cases, sampled with the whole mouth. The magnitude of difference averaged approximately 40%, ranging from about 28% to 61%. The exonic SNP (rs34160967, G/A, p.A372T) showed a similar association with taste intensity although the study cohort had only 2 AA homozygotes (Table 1). Individuals with one or more copies of the A minor allele reported lower intensities than GG homozygotes, significant only for some tastants (not quinine) and mostly at the circumvallate region. If the exonic SNP showed the same taste effects as the intronic SNP, the AA homozygotes would report the lowest taste intensity, which could increase the number of significant SNP–taste associations. However, in our reanalysis of the Shigemura et al. (2009) data, AG heterozygotes had higher MSG thresholds than either AA or GG homozygotes. Thus, we are uncertain about the impact of a greater frequency of AA homozygotes on the strength of association between the exonic SNP (rs34160967) and taste intensity. The taste intensity associations did not extend to other umami-related gene polymorphisms (unpublished analyses). Using the same database and analysis strategy, we failed to find significant associations between 3 *GRM4* gene polymorphisms (rs2228623, rs963733, and rs2229901) and perceived intensities of the 4 prototypical tastants used here.

There is some agreement between the present findings in adult humans and reported literature on taste associations with *TAS1R1*. Our data parallel those in mammals who have very limited diets, some reduced taste functioning, and *TAS1R1* psuedogenes (Thompson et al. 1982; Friedl et al. 1990; Ratcliffe et al. 2003; Jiang et al. 2012). Although rodents

may not be a good model for human *TAS1R1* (Ishimaru et al. 2012), our data were consistent with that of Kusuhara and colleagues (2013) who found that the *TAS1R1* knockout mice, compared with the heterozygous mice, had smaller nerve responses to sucrose and other sweeteners on their anterior tongue. In contrast, they did not see any significant difference between the 2 mice for nerve responses to salty, sour, and bitter compounds, which may relate to differential expression pattern and functioning of *TAS1R1* in rodents versus humans (Hevezi et al. 2009; Ishimaru et al. 2012).

There may be multiple explanations for why *TAS1R1* SNPs were associated with taste intensity. One or both SNPs may have a direct mechanistic impact (apart or together) on taste intensity or an indirect impact through strong linkage disequilibrium with other, unmeasured polymorphisms in *TAS1R1* or other genes. We are unaware of prior data regarding the functional significance of either SNP tested here. Because the exon 3 amino acid substitution (alanine to threonine) in the exonic rs34160967 SNP is a nonconservative amino acid change that also is not conserved across species, we are uncertain whether the amino acid substitution would affect T1R1 function. Interestingly, the rs34160967 SNP lies in exon 3 that is alternatively spliced, suggesting that part of the peptide encoded by this exon is less critical for T1R1 receptor functioning and instead is involved in other ways to influence taste functioning. Although unpublished, the alternative isoform due to the skip of exon 3 was reported to NCBI in 2008 by a Japanese group and sequenced from a human spleen full-length cDNA library (NCBI accession number AK2922014). The exonic SNP also could affect splicing (it appears to be immediately adjacent to an exonic enhancer identified by the bioinformatics program ESEfinder [Cartegni et al. 2003]) or the frequency of alternative splicing of exon 3.

Although difficult to interpret possible functions of most intronic SNPs, we can speculate a possible effect of rs17492553 SNP on alternative splicing of exon 3. The rs17492553 SNP is 1155 bp downstream of SNP rs34160967, in the polypyrimidine tract of intron 3, which is important for recognition of branch point and lariat formation in normal splicing (Ast 2004). The tract is already somewhat weak, with no stretches of thymines longer than 3, and the first base of exon 4 is "weak" (C, found only 10–15% of the time; G is most frequent in the consensus). The first base of exon 3 also is weak (A), further enabling its alternative splicing. Together, our phenotype–genotype observations are consistent with the speculation that one or both SNPs impact the alternative splicing of exon 3, and that the relative expression of this exon may influence taste. Our findings suggest the need for functional analyses of each SNP and haplotypes, as well as deep sequencing of the haploblock region.

We also should consider that *TAS1R1* may have minimal impact on taste intensity—the observed effects may have occurred via interaction with other gene(s) that impact taste function and intensity. For example, the *GNAT3* gene is highly coexpressed with *TAS1R1* (Ishimaru et al. 2012) and encodes G protein alpha subunit gustducin, a taste signaling molecule involved in transduction of sweet, bitter, and umami tastes (Glendinning et al. 2005). Genetic variations occurring at *GNAT3* gene have been associated with sucrose sensitivity (Fushan et al. 2010), and alpha-gustducin knockout mice show diminished behavorial and gustatory nerve responses to sweet, bitter, umami, and even highly concentrated salty but not sour substances (Glendinning et al. 2005).

Our study had a number of limitations. The database included a relatively homogenous sample of adults of European ancestry and did not capture the variation in *TAS1R1* genes seen in Asians (Shigemura et al. 2009), which restricts the generalizability of present findings. A prototypical umami stimulus like MSG also was not tested, which could have tested parallels between *TAS1R1* variation and the intensity of salty, sweet, sour, bitter, and umami tastes. Future research should be extended to pure and simple glutamate stimuli like MSG or MSG + IMP/GMP solutions. We were also unable to perform haplotype analysis of the intronic and exonic SNP together because grouping of the 4 potential haplotypes resulted in some group sizes that were too small for meaningful analyses (data not shown). Preliminarily, the 2 SNPs exhibit a high degree of linkage disequilibrium ($D' = 0.91$; G and C tending to be on the same DNA strand). Haplotype analysis of a larger data set is a logical next approach because it is feasible that some haplotypes may affect transcription (e.g., exon 3 splicing) more than others.

In conclusion, we provide human psychophysical evidence that *TAS1R1* polymorphisms associate with modest differences in overall taste intensity, which were independent from other well-studied markers of variation in oral sensation. To date, the phenomenon of heightened taste sensations or "supertasting" has been consistently defined by the bitterness of PROP and density of FP although additional phenotypes and markers continue to emerge (Hayes and Keast 2011), including bitterness from capsaicin and other irritants (Green and Hayes 2004), perception of taste from thermal stimulation (Green and George 2004), and polymorphism in the gustin gene (Calò et al. 2011). The intronic SNP *TAS1R1* C > T (rs17492553), alone or in combination with the exonic SNP, could add to the growing list of genetic markers for "supertasting." Finally, the National Health and Nutrition Examination Survey for the first time includes a taste exam involving regional application of concentrated NaCl and quinine to the tongue tip (Duffy et al. 2012). Findings from this study suggest that observed variability in tongue tip taste intensity may result from taste gene polymorphisms and exposure to insults that may alter chorda tympani nerve taste (Bartoshuk et al. 2012).

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