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Allelic Variation in TAS2R Bitter Receptor Genes Associates with Variation in Sensations from and Ingestive Behaviors toward Common Bitter Beverages in Adults

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Abstract

The 25 human bitter receptors and their respective genes (*TAS2Rs*) contain unusually high levels of allelic variation, which may influence response to bitter compounds in the food supply. Phenotypes based on the perceived bitterness of single bitter compounds were first linked to food preference over 50 years ago. The most studied phenotype is propylthiouracil bitterness, which is mediated primarily by the *TAS2R38* gene and possibly others. In a laboratory-based study, we tested for associations between *TAS2R* variants and sensations, liking, or intake of bitter beverages among healthy adults who were primarily of European ancestry. A haploblock across *TAS2R3*, *TAS2R4*, and *TAS2R5* explained some variability in the bitterness of espresso coffee. For grapefruit juice, variation at a *TAS2R19* single nucleotide polymorphism (SNP) was associated with increased bitterness and decreased liking. An association between a *TAS2R16* SNP and alcohol intake was identified, and the putative *TAS2R38*–alcohol relationship was confirmed, although these polymorphisms did not explain sensory or hedonic responses to sampled scotch whisky. In summary, *TAS2R* polymorphisms appear to influence the sensations, liking, or intake of common and nutritionally significant beverages. Studying perceptual and behavioral differences in vivo using real foods and beverages may potentially identify polymorphisms related to dietary behavior even in the absence of known ligands.

Key words: alcohol drinking, alleles, bitter taste receptors, food choice, genetics

Introduction

The 25 human bitter receptors and their respective genes (*TAS2Rs*) contain unusually high levels of allelic variation, which may indicate local adaptation for the avoidance of plant toxins (Kim et al. 2005). Work on the relationship between bitter taste phenotypes and ingestive behavior dates back to the pioneering work of Fischer, Kaplan, and Glanville a half century ago (Fischer et al. 1961; Glanville and Kaplan 1965). Of these, the most studied is differential bitter response for phenylthiocarbamide (PTC) and propylthiouracil (PROP), which is mediated by the *TAS2R38* gene (Kim et al. 2003) and possibly others (Hayes et al. 2008; Reed et al. 2010). For many years, work relating taste to food sensations focused on bitter phenotype as a monolithic entity that is high or low in an individual (e.g., Hall et al. 1975; Drewnowski et al. 1997), raising the obvious question of why individuals are particularly susceptible to the bitterness of some foods

but not others. Recent advances in molecular taste genetics (e.g., Meyerhof et al. 2010) and psychophysics (e.g., Delwiche et al. 2001) show a complex and multifaceted response to bitter stimuli. Here, in a sample of adults tested in the laboratory, we provide preliminary evidence of novel associations between *TAS2R* genes beyond *TAS2R38* and the bitterness of or ingestive behaviors toward several common bitter beverages—coffee, grapefruit juice, and alcohol—potentially explaining differential bitter response to foods across individuals. In the process, we also identify foods that may potentially contain candidate ligands for several orphan taste receptors.

The hT2R16 G protein–coupled receptor encoded by the *TAS2R16* gene (Entrez GeneID: 50833) mediates the detection of salicin and other naturally occurring bitter compounds in the beta-glucopyranoside family (Bufe et al. 2002), and polymorphisms in this locus confer differential response in vitro via functional changes in the receptor (Soranzo et al. 2005). Prevalence of these alleles vary by geographic region, suggesting allelic variation may have arisen to as an evolutionary adaptation to the local plant environment (Soranzo et al. 2005). The less sensitive K172 ancestral allele is commonly found in African populations, whereas the more sensitive N172 variant is found in almost all individuals of European or Asian origin. In the contemporary environment, the less sensitive K172 allele appears to be a risk factor for alcohol intake (Wang et al. 2007) and dependence (Hinrichs et al. 2006). Because the K172 allele is rare among European Americans (minor-allele-frequency of 0.6%), we chose to test the association between 2 alternative *TAS2R16* single nucleotide polymorphisms (SNPs) and alcohol intake among our sample of adults who are of predominantly European ancestry.

The Proline49–Alanine262–Valine296 (PAV) haplotype for *TAS2R38* (Entrez GeneID: 5726) confers the ability to taste thiourea compounds like PROP and PTC at low concentrations; these compounds are part of a structurally distinct class from the bitter beta-glucopyranosides discussed above. The ancestral PAV allele protects against the ingestion of bitter thyroid toxins; the selective pressure for the Alanine–Valine–Isoleucine (AVI) nontaster variant is unknown (Wooding 2006), although it could allow the consumption of bitter phytonutrients that confer health benefits (Duffy 2007) as this polymorphism predicts vegetable bitterness (Sandell and Breslin 2006) and intake (Duffy et al. 2010). *TAS2R38* variation also associates with alcohol intake (Duffy et al. 2004; Wang et al. 2007) but not dependence (Wang et al. 2007), and this relationship is presumably mediated through variable bitterness of alcoholic beverages (Lanier et al. 2005). We previously reported that AVI homozygotes consume more alcohol than heterozygotes, who in turn consume more alcohol than PAV homozygotes (Duffy et al. 2004). Subsequently, the Collaborative Studies on Genetics of Alcoholism (COGA) used family-based methods to link *TAS2R38* polymorphisms with alcohol intake in African-Americans (Wang et al. 2007). Because the 2 studies differ significantly in terms of method and sample, here we have tested associations between *TAS2R38* and *TAS2R16* and alcohol intake, replicating our earlier finding (Duffy et al. 2004) and demonstrating a new *TAS2R16* intake finding in a new cohort of healthy adults. Based on our finding that the endogenous sweetness and bitterness of whisky predicts alcohol intake (Lanier et al. 2005), we also tested whether *TAS2R16* and *TAS2R38* variation might explain perceived sensations from this alcoholic beverage.

PROP bitterness explains ~13% of the variance in the bitterness of sampled espresso coffee (Lanier et al. 2005), yet caffeine bitterness is only minimally correlated with the bitterness of PROP (Delwiche et al. 2001; Keast and Roper 2007). Nonetheless, the perception of caffeine and PROP appears to share a common genetic factor (Hansen et al. 2006). Here, in an exploratory analysis, we test whether variants for *TAS2R* genes might explain variation in the perceived bitterness of sampled espresso coffee. Similarly, because PROP bitterness covaries with the bitterness (Lanier et al. 2005) and liking of grapefruit juice (Drewnowski et al. 1997; Lanier et al. 2005), we test whether common bitter receptor gene SNPs explain behavioral responses to grapefruit juice.

Materials and methods

Subjects and procedure

A convenience sample of healthy adults was recruited by poster and word of mouth from the University of Connecticut community, a rural college campus. Individuals who smoked more than 9 cigarettes per week were excluded because cigarette use may alter taste perception (Sato et al. 2002) and because cigarette use is confounded with alcohol consumption (Bottoni et al. 1997). The study sample of 96 adults was mostly of European ancestry (85%), female (76%) and of middle age (mean 40.9 years ± 12.2 standard deviation). They had 2 sessions in the taste laboratory and a separate visit for venipuncture for DNA analysis. The study sample was separate from that reported previously (Duffy et al. 2004). The University of Connecticut Institutional Review Board approved all procedures; subjects gave informed written consent and were paid for their participation.

Stimuli

Approximately 10 mL samples of unsweetened grapefruit juice (Veryfine Products), instant espresso (Café Bustelo; Rowland Coffee Roasters), and a blended scotch whisky (Dewar's White Label; John Dewar and Sons) were tasted. These samples were served at room temperature in plastic medicine cups in random order under normal lighting conditions. All subjects rinsed between samples with room temperature deionized (>15 MΩ) water. Throughout the testing sessions, subjects rated the intensity of a series of 1000 Hz

tones as a cross-modal standard; they were presented in 12 dB steps (50–98 dB) as described elsewhere (Hayes and Duffy 2008).

Scaling

Intensity and hedonic scaling data were collected using the general Labeled Magnitude Scale (gLMS), a semantically labeled line scale (Green et al. 1996), anchored at the top (100) to "strongest imaginable sensation of any kind" (Bartoshuk et al. 2005). Intermediate verbal labels included "barely detectable" (1.4), "weak" (6), "moderate" (17), "strong" (35), and "very strong" (53). Subjects were oriented to the gLMS by rating the intensity of 16 remembered oral and nonoral sensations (thermal, light, and sound sensations) to encourage consistent use of the gLMS across multiple sensory domains. After scale orientation, subjects used the gLMS to rate liking/disliking and the sweetness, sourness, saltiness, and bitterness of sampled nonalcoholic and alcoholic beverages. Sourness and saltiness were not used in any analyses but were included in the tasting protocol to minimize halo-dumping response bias (Clark and Lawless 1994). When making hedonic ratings, subjects were told the bottom of the scale was neither like nor dislike, and the top was either the "strongest imaginable liking of any kind" or "strongest imaginable disliking of any kind." Subjects verbally indicated whether they liked or disliked the sample. Gene effects on the sensations and liking of the bitter beverages were assessed via analysis of covariance (ANCOVA), with covariates of age, sex, and the average intensity of 86 dB tones.

Self-reported intake

Subjects reported the quantity and frequency of consuming beer, wine, and liquor, where a standard drink equaled 12, 5, and 1.5 oz, respectively on a validated semiquantitative food frequency survey (www.nutritionquest.com). We tested *TAS2R* associations with frequency of alcohol consumption (number of times per year) and total alcohol intake (frequency multiplied by servings per drinking episode), relying on frequency as the primary intake measure for 2 reasons: 1) taste gene effects may become attenuated as a drinking bout progresses and 2) if taste genotype is systematically related to beverage choice, using standard drinks might bias intake estimates given that mixed drinks are potentially more variable in alcohol content. Genetic effects on alcohol intake were assessed via ANCOVA, with age as a covariate.

DNA extraction and genetic analysis

DNA was extracted from whole blood following manufacturer's instructions (Gentra), with occasional modification for lysed samples. Purified DNA samples were stored at 4 °C in Tris 10 mM and EDTA acid 1 mM until analyzed. When necessary, DNA was amplified using the Illustra GenomiPhi V2 D amplification kit from GE Lifesciences. Genotypes were determined using vendor-supplied assays from Applied Biosystems (Supplement Table 1) with the plates read on an ABI Prism 7900 HT (Applied Biosystems). For *TAS2R38*, we assumed that individuals heterozygous at all 3 SNPs (e.g., genotypes 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). SNP frequencies in our sample were similar to values seen in reference data sets, and none of the SNPs showed significant deviation from Hardy–Weinberg equilibrium (Supplementary Table S2).

We present unadjusted and Bonferroni adjusted P values here for the coffee and grapefruit hypotheses as there is an active debate as to whether multiple comparison adjustments are appropriate for exploratory studies (see Bender and Lange 2001). Here, the P values were adjusted by multiplying observed P value by 10 (2 haplotypes plus 7 unrelated SNPs on chromosome 7 and 1 SNP on chromosome 12). For alcohol, tests related to *TAS2R16* and *TAS2R38* were separate a priori hypotheses to replicate prior work (Duffy et al. 2004; Wang et al. 2007), so no multiple comparisons adjustments were made. As population stratification may potentially cause false negatives and false positives in gene association studies (Hamer and Sirota 2000), we also analyzed our data excluding those individuals who were not of European ancestry; because none of the results were substantively different in this subset, results for the entire sample are presented here.

Results

Confirmation that *TAS2R16* and *TAS2R38* polymorphisms explained differences in alcohol intake

Over the last year, the self-reported frequency of consuming all types of alcoholic beverages was 102 ± 13 (mean ± standard error of the mean), with good variability in intake frequency across the sample (interquartile range [IQR]: 15–141). In terms of total beverage intake (quantity times frequency), the mean intake was 188 ± 28 standard drinks per year (IQR: 18–268). Out of total drinks reported by the sample, 45% were beer, 40% were wine, and 15% were spirits (neat or with mixers).

The *TAS2R16* C>A (rs846672) SNP was significantly associated with frequency of consuming alcoholic beverages ($F_{2,65}$ = 8.30, P = 0.0006); the minor allele homozygotes (AA carriers) consumed alcoholic beverages twice as frequently as the heterozygotes or major allele homozygotes, who did not differ in drinking frequency (Figure 1). A parallel analysis accounting for total beverage intake also indicated that the AA homozygotes drank more ($F_{2,65}$ = 3.74 P = 0.029) than did the AG (P = 0.007) or GG (P = 0.039) carriers.

For the C>G SNP in *TAS2R16* (rs1308724), there was a trend for CC homozygotes to consume alcohol less frequently (48 times/year) than did the heterozygotes (88 times/year), who consumed less frequently than did the GG homozygotes

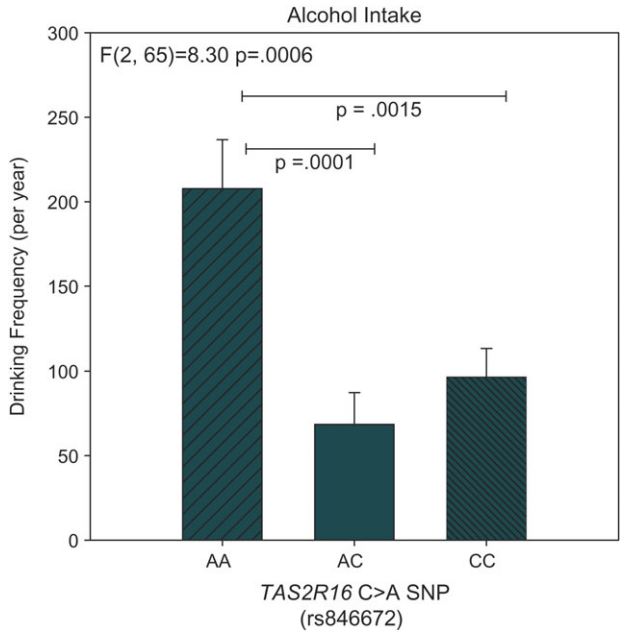

Figure 1 Self-reported alcohol intake frequency in 68 adults. Effects of *TAS2R16* SNPs on intake were assessed via ANCOVA, controlling for age (see text). Data represent means ± standard errors. Pairwise comparisons between groups (Fisher least significant difference) are indicated with horizontal lines.

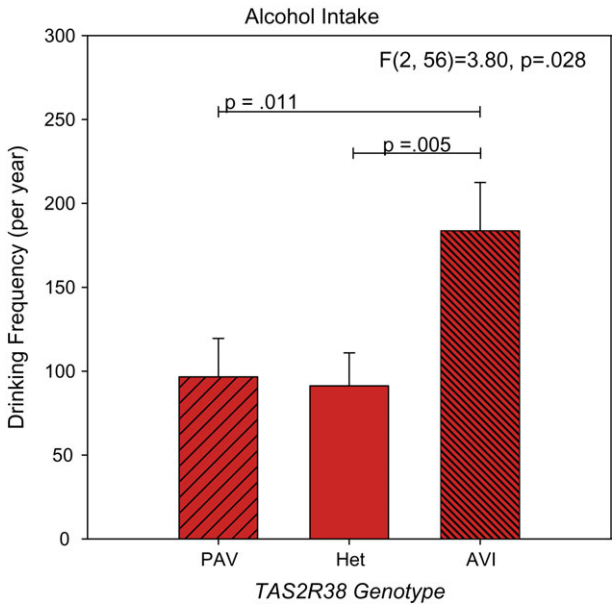

Figure 2 Self-reported alcohol intake frequency in 60 adults. Effects of *TAS2R38* on intake were assessed via ANCOVA, controlling for age. Genotypes are PAV = PAV/PAV; Het = PAV/AVI; AVI = AVI/AVI; individuals with rare haplotypes were excluded. Data represent means ± standard errors. Pairwise comparisons between groups (Fisher least significant difference) are indicated with horizontal lines. These data are from a new sample of subjects, providing independent confirmation of our earlier report (Duffy et al. 2004).

(122 times/year); however, this pattern was not significant for frequency ($F_{2,64}$ = 2.05 P = 0.137) or total intake ($F_{2,64}$ = 1.93 P = 0.15). Removing alcohol abstainers (those who reported no alcohol consumption over the year) improved the association between the C>G SNP and the 2 alcohol measures (P = 0.05 and 0.07). Because the 2 SNPs are not in linkage disequilibrium (LD; Figure 5), a larger sample is needed to rule out whether the C>G allelic variant (rs1308724) makes a separate contribution after accounting for the C>A (rs846672) effects.

Here, we reaffirm the prior finding (Duffy et al. 2004) that AVI homozygotes drink alcohol more frequently than do PAV homozygotes (Figure 2). However, the heterozygotes here did not show an intermediate level of intake between the AVI and PAV homozygotes; it appears having one copy of the taster allele may be sufficient to depress intake ($F_{2,56}$ = 3.80 P = 0.028). As above, using total beverage intake as the consumption variable showed consistent findings; AVI homozygotes drank more ($F_{2,56}$ = 4.32 P = 0.018) than either heterozygotes (P = 0.004) or PAV homozygotes (P = 0.008).

As expected given the lack of LD between *TAS2R16* and *TAS2R38* (Figure 5), each contributed independently to the alcohol consumption measures in 2-way analysis of variance ($p < 0.05$). The small numbers in the subgroups do not warrant pairwise comparisons.

Polymorphisms in *TAS2R16* or *TAS2R38* failed to explain variability in taste sensations (sweetness, sourness, saltiness, and bitterness) elicited by the scotch whisky nor did they explain variation in liking (not shown).

SNPs in *TAS2R3*, *TAS2R4*, and *TAS2R5* formed a haploblock that explained coffee bitterness

Four SNPs in *TAS2R3*, *TAS2R4*, and *TAS2R5* each explained variation in the bitterness of coffee (not shown). Given this consistent pattern, and because these genes are located near each other on chromosome 7, we determined whether these SNPs formed a haplotype block (Figure 5) and found that 89% of our sample possessed one of 3 common haplotypes as shown below (consistent with unpublished data from Alarcon et al. 2008). Analyzed as a haplotype, allelic variation across *TAS2R3*, *-R4*, and *-R5* explained variability in coffee bitterness ($F_{2,63}$ = 6.32, P = 0.003, adjusted P = 0.03); individuals with 1 or 2 copies of the more responsive

TAS2R3	TAS2R4	TAS2R5		% Of sample
rs765007	rs2234001	rs2234012	rs2227264	
C	C	G	T	26%
Y	S	R	K	41%
T	G	A	G	22%
5′UTR	Val96Leu	5′UTR	Ser26Ile	

Y = C/T, S = C/G, R = A/G, K = G/T

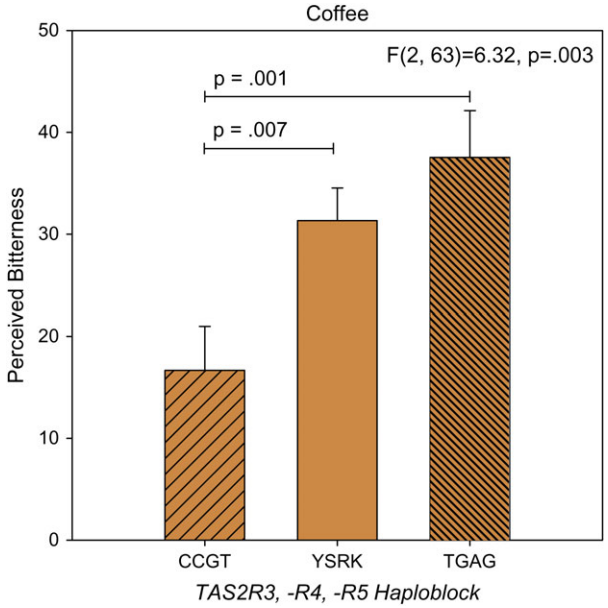

Figure 3 Bitterness intensity ratings of espresso coffee, collected using the gLMS, a psychophysical scale with ratio properties (i.e., a 40 is twice as great as a 20). Effects of the *TAS2R3/R4/R5* haploblock were assessed via ANOVA. Genotypes are CCGT = CC,CC,GG,TT; YSRK = C or T, C or G, A or G, G or T; TGAG = TT, GG, AA, GG. Data represent means ± standard errors. Pairwise comparisons between groups (Fisher least significant difference) are indicated with horizontal lines.

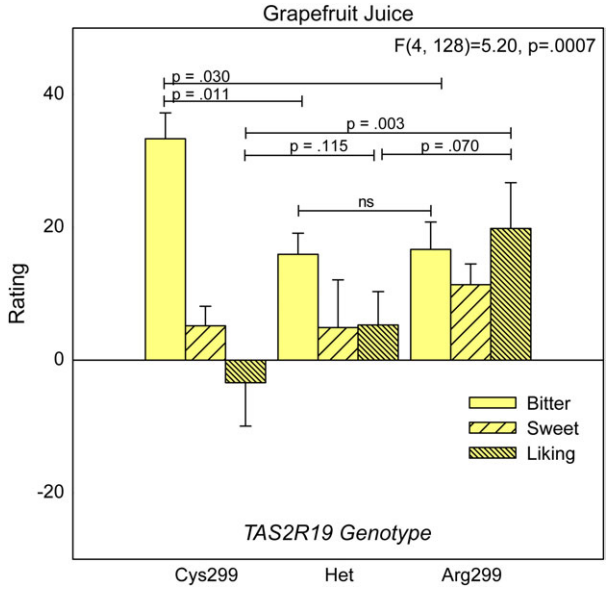

Figure 4 Bitterness, sweetness, and liking ratings of unsweetened white grapefruit juice. Effects of the *TAS2R19* (rs10772420) SNP were assessed via ANOVA. Data represent means ± standard errors. Pairwise comparisons between groups (Fisher least significant difference) are indicated with horizontal lines.

haplotype (TGAG) experienced twice as much bitterness compared with individuals homozygous for the less responsive haplotype (CCGT) (Figure 3). Notably however, the haploblock did not predict coffee liking ($F_{2,63}$ = 1.31, P = 0.278).

TAS2R19 variation influenced grapefruit juice bitterness and liking

The rs10772420 A>G coding single nucleotide polymorphism (cSNP) in *TAS2R19* results in the substitution of Arg for a Cys at amino acid position 299 (Cys299Arg), and this polymorphism appeared to influence grapefruit juice bitterness and thus sweetness and liking ($F_{4,128}$ = 5.20, P = 0.0007, adjusted P = 0.007). Individuals who were homozygous for the Cys299 allele rated grapefruit juice twice as bitter as Arg299 homozygotes (P = 0.030) or heterozygotes (P = 0.011) (Figure 4). We found that the more sensitive Cys299 homozygotes (P = 0.003) and the heterozygotes (P = 0.070) liked the grapefruit juice less than the less sensitive Arg299 homozygotes. For the rs4595035 SNP in *TAS2R60*, similar patterns were observed for bitterness and liking ($F_{4,128}$ = 2.49, P = 0.0469), but the effect was no longer significant after multiple comparisons adjustment (P = 0.47) suggesting this finding in particular requires independent confirmation in a larger sample to disentangle it from *TAS2R19* effects.

Discussion

In sharp contrast to earlier work that viewed bitter response as a monolithic entity within in an individual (e.g., Hall et al. 1975; Drewnowski et al. 1997), present findings are wholly consistent with the modern view that bitter taste transduction occurs via a broad repertoire of T2R receptors, each of which are, for the most part, specific to particular classes of ligands (e.g., Bufe et al. 2002; Kuhn et al. 2004; Behrens et al. 2007; Pronin et al. 2007). To date, specific ligands have been identified for over half of the 25 hTAS2Rs (Ley 2008; Meyerhof et al. 2010), although only a handful are ecologically relevant ligands found in foods or beverages (e.g., Brockhoff et al. 2007; Intelmann et al. 2009). Here, we find preliminary evidence that polymorphisms in or near multiple *TAS2R* genes may influence the sensations, liking, or intake of common beverages that contain phytochemicals and other pharmacologically active ingredients linked to chronic diseases such as cardiovascular disease and cancer. Specifically, it appears that bitterness of alcoholic beverages, coffee, and grapefruit juice are mediated by multiple T2R receptor genes, and each of these genes appear to contain functional polymorphisms both within and across individuals, with the potential to influence intake.

Alcohol

Previously, Wang et al. (2007) reported an association between *TAS2R38* haplotype and the maximum number of drinks in a 24-h period among Africans Americans but not European Americans. In contrast, present data generally

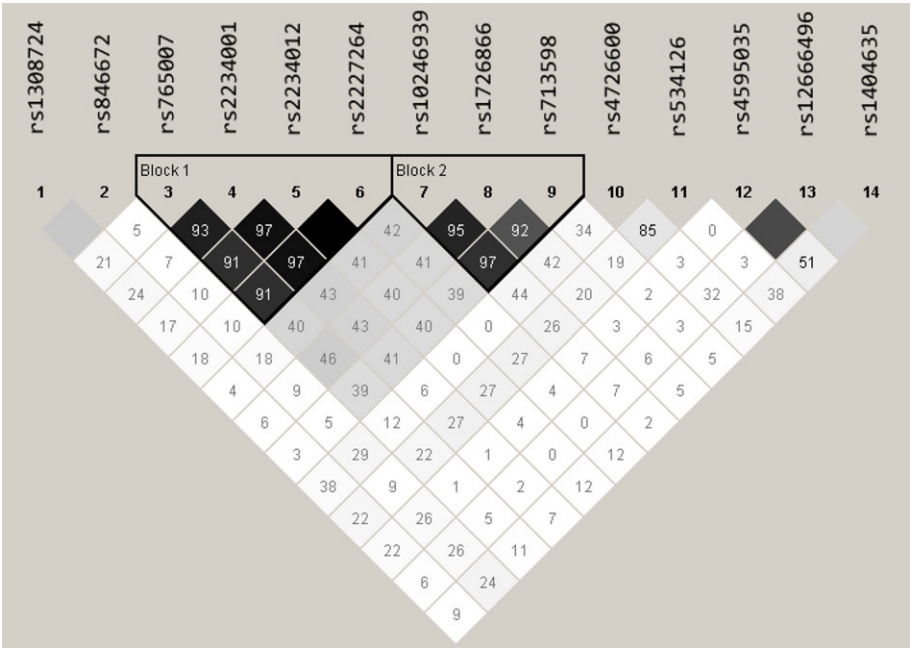

Figure 5 LD Plot for *TAS2R* SNPs on chromosome 7 showing R2 values generated via Haploview. Block 1 indicates a haploblock across *TAS2R3*, *TAS2R4*, and *TAS2R5* (see text for details), and block 2 indicates the expected PAV/AVI haploblock for *TAS2R38*.

confirm our prior finding that alcohol intake in European Americans is related to *TAS2R38* genotype, although the question of whether these effects are additive (Duffy et al. 2004) or dominant (present data) are unresolved. The discrepancy in findings between our studies and Wang et al. could arise from using very different alcohol consumption measures (maximum drinks in 24 h vs. usual yearly consumption), the study sample (high-risk vs. reportedly healthy adults), or the analysis strategy (family based methods vs. explaining variance in intake among unrelated individuals).

Polymorphisms in *TAS2R16* or *TAS2R38* neither explained variability in taste sensations (sweetness, sourness, saltiness, and bitterness) elicited by blended scotch whisky nor liking for this alcoholic beverage. The genotype effects on alcohol consumption may be a generalized response to alcoholic beverages as a whole and not a sensory response to the particular specific alcoholic beverage tasted here. This suggests that any protective effect of *TAS2R16* and *TAS2R38* on alcohol intake may be unrelated to the bitterness of ethanol per se and could be due to other bitter ligands in other alcoholic beverages. Presumably, protective effects against intake generalize to all types of beverages via associative learning during early exposure. That is, heightened bitter response to ligands commonly found in some alcoholic beverages may depress the acquisition of preference for alcohol as a whole when an individual is first learning to consume alcohol. The most obvious candidate beverage would be beer, although this interpretation is complicated by the observation that hop-derived bitter tastants also fail to activate *T2R16* and *T2R38* expressing HEK cells in culture (Intelmann et al. 2009). Nonetheless, the present study is logically consistent with prior reports that alcohol behaviors in humans associate with certain bitter receptor alleles (e.g., Duffy et al. 2004; Wang et al. 2007) or bitter phenotypes (Intranuovo and Powers 1998; Lanier et al. 2005). This implies that taste receptor genes and bitter phenotypes may serve as useful biomarkers to study predisposition to chronic diseases associated with dietary behaviors (Sacerdote et al. 2007; Hayes 2010).

Coffee

The 4 SNPs across *TAS2R3*, *-R4*, and *-R5* are located close to each other on chromosome 7, forming a haplotype block in our sample. The TGAG homozygotes perceived twice as much bitterness as CCGT homozygotes, with the heterozygotes falling in the middle. Within the CCGT/TGAG haploblock, 2 of the polymorphisms encode amino acid substitutions in *TAS2R4* and *-R5*, whereas the other 2 occur in the 5′ untranslated region (5'UTR) of *TAS2R3* and *-R4*. The C>G cSNP in *TAS2R4* (rs2234001) results in Leu being substituted for Val at amino acid 96 (Val96Leu), whereas the T>G cSNP in T*AS2R5* (rs2227264) results in Ile being substituted for Ser at amino acid 26 (Ser26Ile). The rs765007 SNP in *TAS2R3* and the rs2234012 SNP in *TAS2R5* are located in the 5'UTR, a region that typically contains sequences that regulate translation efficiency (or messenger RNA stability, although that is less likely to influence phenotype). Whether the variation in coffee bitterness seen for this haplotype results from functional alteration of T2R4 or T2R5 or influences on transcription that alter the expression of T2R3 or T2R4 is unclear from

present data. As with all association data, these significant SNPs may simply be linked to other unmeasured polymorphisms that confer the functional effect. Regardless, the significant results indicate that there is a polymorphic property of that genomic region that affects the phenotype.

Notably, none of these polymorphisms were predictive of coffee liking. That variation in bitter receptor genes was unable to explain variation in liking is unsurprising: We have previously reported that coffee bitterness ratings do not predict liking (Lanier et al. 2005). Coffee flavor is a complex percept that depends on far more than bitterness (~30 different odorants make critical contributions to its flavor (Czerny et al. 1999; Maeztu et al. 2001), and caffeine plays only a minor role in eliciting coffee bitterness (Frank et al. 2006). Moreover, liking for coffee flavor is altered by variable pharmacological effects of caffeine and prior experience (see Cines and Rozin 1982). As with alcohol, for some individuals, desirable pharmacological effects may override innate dislike of bitterness, regardless of intensity. Likewise, epistatic interactions may attenuate relationships between taste receptor polymorphisms and liking. For example, individuals may learn to dislike coffee flavor due to caffeine's anxiogenic properties in spite of carrying the less responsive haploblock found here. Because the anxiety elicited by caffeine varies with polymorphisms in the *ADORA2A* gene (Alsene et al. 2003; Childs et al. 2008), it would be prudent to stratify by the A2a allele in future work assessing the impact of the CCGT/TGAG haploblock on coffee liking and intake. Additionally, present findings may inform in vitro efforts to identify naturally occurring ligands for hT2R3 and hT2R5 as our findings suggest novel candidate ligands might be isolated from coffee.

Grapefruit juice

Individuals who carry the Arg299 allele for *TAS2R19* found grapefruit juice to be less bitter. Reed et al. (2010) recently provided independent evidence that this polymorphism is functional in humans. Bitterness and sweetness suppress each other (Keast and Breslin 2003) and such mixture suppression occurs centrally not peripherally (Lawless 1979), suggesting that functional differences in bitter taste receptors may thus affect perceived sweetness, at least in beverages or foods that are perceptual mixtures. Consistent with this, we previously reported that individual differences in perceived sweetness and bitterness independently predict variation in grapefruit juice liking (Lanier et al. 2005). Here, we find that the more sensitive Cys299 carriers liked the grapefruit juice less than the less sensitive Arg299 homozygotes. Likewise, we found somewhat equivocal evidence of similar patterns of bitterness and liking for the *TAS2R60* SNP. This is intriguing as the rs4595035 C>T substitution in *TAS2R60* is a silent (synonymous) polymorphism. It was recently demonstrated that natural variation in "synonymous" codons may result in proteins with the same amino acid sequences that are functionally distinct (Kimchi-Sarfaty et al. 2007). Whether this is true for the highly polymorphic T2R family remains to be determined. Additionally, no ligand has been identified for *TAS2R60* to date (Meyerhof et al. 2010), and *TAS2R19* was recently implicated as a possible quinine receptor (Reed et al. 2010). Our findings suggest it may be worthwhile challenging cells expressing *TAS2R19* (and possibly *TAS2R60*) receptors with taste-active compounds isolated from grapefruit juice to identify additional naturally occurring ligands. Notably, using dietary behavior as a rapid screen to identify potential ligands need not be limited to sampled stimuli. Previously, we analyzed self-reported grapefruit juice liking data obtained from a pencil and paper questionnaire and found similar effects of the Arg299Cys polymorphism in *TAS2R19* (nee *TAS2R48*) (Duffy et al. 2009). This suggests that rapid hedonic surveys could help focus receptor-ligand discovery work toward ecologically relevant compounds found in specific foods. Additionally, as recently noted by Wooding et al. (2010), it seems likely that interactions between whole foods and bitter receptors involve multiple ligands simultaneously. Thus, using human behavioral response, in particular hedonics, complements functional assays that test single ligands in vitro by providing a more holistic view.

Conclusions

Collectively, we interpret these results to suggest that variation in perceived bitterness of alcoholic beverages, coffee, and grapefruit juice (e.g., regular foods and not pharmaceuticals like quinine or PROP) may be explained by common polymorphisms in *TAS2R* bitter receptor genes. Of course, as with any exploratory data on putatively functional polymorphisms, these associations need to be confirmed in an independent sample. Additionally, attempts to identify ecologically relevant receptor ligands may be informed by human behavioral data by narrowing the field of candidates to be surveyed. For example, present data suggest that the *TAS2R19* and *TAS2R60* gene products (the hT2R19 and hT2R60 receptors) should be interrogated in vitro with bitter taste active compounds found in grapefruit. In light of present findings, studies that attempt to link food choice, diet, and health to chemosensory variation need to take advantage of contemporary advances in molecular taste genetics and move beyond the historically narrow focus on thiourea (PROP and PTC) phenotypes and genetics, to include newly identified genetic variation. Additionally, more attention needs to be paid to other biological factors that influence dietary behaviors. For example, recent work indicates that fungiform papilla density (a proxy for receptor density) influences vegetable intake independently from *TAS2R* variation (Duffy et al. 2010). Given the wide diversity of *TAS2R*s, single studies that fail to uncover links between specific bitter taste markers and liking for or intake of specific foods do not invalidate the broader hypothesis that chemosensory variation drives ingestive behaviors, with the

potential to impact health and wellness. Cataloging these effects will require extensive effort to move forward.

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