Polymorphisms in *TRPV1* and *TAS2Rs* Associate with Sensations from Sampled Ethanol

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Background: Genetic variation in chemosensory genes can explain variability in individual's perception of and preference for many foods and beverages. To gain insight into variable preference and intake of alcoholic beverages, we explored individual variability in the responses to sampled ethanol (EtOH). In humans, EtOH elicits sweet, bitter, and burning sensations. Here, we explore the relationship between variation in EtOH sensations and polymorphisms in genes encoding bitter taste receptors (TAS2Rs) and a polymodal nociceptor (TRPV1).

Methods: Caucasian participants (n = 93) were genotyped for 16 single nucleotide polymorphisms (SNPs) in *TRPV1*, 3 SNPs in *TAS2R38*, and 1 SNP in *TAS2R13*. Participants rated sampled EtOH on a generalized Labeled Magnitude Scale. Two stimuli were presented: a 16% EtOH whole-mouth sip-and-spit solution with a single time-point rating of overall intensity and a cotton swab saturated with 50% EtOH on the circumvallate papillae (CV) with ratings of multiple qualities over 3 minutes. Area-under-the-curve (AUC) was calculated for the time-intensity data.

Results: The EtOH whole-mouth solution had overall intensity ratings near "very strong." Burning/stinging had the highest mean AUC values, followed by bitterness and sweetness. Whole-mouth intensity ratings were significantly associated with burning/stinging and bitterness AUC values on the CV. Three TRPV1 SNPs (rs224547, rs4780521, rs161364) were associated with EtOH sensations on the CV, with 2 (rs224547 and rs4780521) exhibiting strong linkage disequilibrium. Additionally, the *TAS2R38* SNPs rs713598, rs1726866, and rs10246939 formed a haplotype, and were associated with bitterness on the CV. Last, overall intensity for whole-mouth EtOH associated with the *TAS2R13* SNP rs1015443.

Conclusions: These data suggest genetic variation in *TRPV1* and *TAS2Rs* influence sensations from sampled EtOH and may potentially influence how individuals initially respond to alcoholic beverages.

Key Words: Bitterness, Burn, Ethanol, Taste Phenotype, TRPV1.

TASTE STRONGLY INFLUENCES food intake (Glanz et al., 1998; IFIC 2011), including alcohol consumption (Barber and Grichting, 1987; Moore and Weiss, 1995). Ethanol (EtOH) activates olfactory, taste, and chemesthetic receptors, and each modality is carried centrally by different nerves; these inputs individually and jointly affect the percept evoked by EtOH. EtOH reportedly elicits sweet and bitter sensations in humans (Mattes and DiMeglio, 2001) and in mice (Blizard, 2007). Sour and salty sensations have also been reported, but with much lower intensities than bitter or sweet (Mattes and DiMeglio, 2001). EtOH also activates sweet taste fibers in nonhuman primates (Hellekant et al., 1997) and rodents (Lemon et al., 2004). Regarding alcohol behaviors, individual differences in bitterness and sweetness are predictors of alcohol liking and intake in young adults (Lanier et al., 2005).

Multiple studies have linked variation in *TAS2R* bitter receptor genes to alcohol intake. Duffy and colleagues reported *TAS2R38* haplotypes are associated with alcoholic intake, with AVI homozygotes (who perceive less bitterness from the bitter compound propylthiouracil [PROP]) consuming significantly more alcoholic beverages than heterozygotes or PAV homozygotes (Duffy et al., 2004a), a finding which was subsequently replicated in a separate cohort (Hayes et al., 2011). In a high-risk familial cohort (COGA), Wang and colleagues (2007) found the same haplotype associated with maximum number of drinks ever consumed within a 24-hour period in African-Americans. More recently, Dotson and colleagues (2012) reported associations between *TAS2R38* and *TAS2R13* polymorphisms and alcohol intake derived from the Alcohol Use Disorders Identification Test (AUDIT) in head and neck cancer patients.

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These differences in intake are presumably driven by differences in perceived intensity that lead to lower liking (Duffy, 2007; Duffy et al., 2009; Hayes et al., 2013b). However, this interpretation is complicated by previous reports where *TAS2R38* haplotypes fail to explain variation in sensations from EtOH (Duffy et al., 2004a) or blended whisky (Hayes et al., 2011). Mattes and DiMeglio (2001) observed variable EtOH thresholds and suprathreshold ratings of EtOH across individuals, but these differences did not associate with phenylthiocarbamide detection thresholds (another taste phenotype commonly associated with *TAS2R38* genotype; see Kim et al., 2003). This suggests EtOH may differentially activate bitter receptors beyond TAS2R38, consistent with other data (Dotson et al., 2012; Hinrichs et al., 2006). Of the handful of human *TAS2R*s previously reported to contain functional single nucleotide polymorphisms (SNPs), only 3 (*TAS2R13*, *TAS2R16*, and *TAS2R38*) have been implicated with regard to alcohol intake or dependence in prior literature (Dotson et al., 2012; Duffy et al., 2004a; Hinrichs et al., 2006). Due to an extremely low minor allele frequency, the relevant SNP in *TAS2R16* is largely irrelevant in European Americans, so we confined our analyses here to putatively functional variants in *TAS2R38* and *TAS2R13*.

In addition to bitter and sweet sensations, EtOH also causes irritation commonly described as burning or stinging (Green, 1987, 1988). Burning sensations in the mouth are due, in part, to activation of the transient receptor potential vanilloid receptor 1 (TRPV1). TRPV1 (formerly VR1) is activated by noxious heat, capsaicin (Caterina et al., 1999; Tominaga et al., 1998), and EtOH (Trevisani et al., 2002), even at relatively low concentrations (0.1 to 3% v/v). TRPV1 is a multimodal nociceptor activated by chemical and thermal stimuli, resulting in a substance P-dependent signal cascade that eventually culminates in sensations described as burning. In rodent-derived tissue culture, the release of substance P increases with increasing concentrations of EtOH (Trevisani et al., 2002). When the *trpv1* gene is knocked out in mice, knockouts have a higher preference for EtOH and consume more EtOH than wild-type mice (Blednov and Harris, 2009). Collectively, these data suggest the TRPV1 receptor likely plays a role in the perception and acceptability of EtOH.

The objectives of the present study were to determine whether polymorphisms in (i) *TRPV1* associate with the perception of EtOH, specifically EtOH burn, and whether polymorphisms in (ii) *TAS2R38*, and (iii) *TAS2R13* may explain differences in bitterness evoked by EtOH. Previously, EtOH intensity has been shown to associate with PROP tasting (Bartoshuk et al., 1993; Duffy et al., 2004a; Prescott and Swain-Campbell, 2000), but due to multiple sensations elicited from EtOH, we anticipated that measuring bitter and burning sensations separately would help elucidate the influence of both bitter taste receptors and heat/pain receptors on alcohol sensations, and potentially intake.

MATERIALS AND METHODS

Overview

This study consisted of 4 sessions, each scheduled at least 1 week apart. All sessions (~1 hour each) were completed one-on-one in our laboratory with a member of the research team. During the first session, informed consent was obtained and participants were given a brief explanation of the study aims: to quantify the influence of specific genes on the sensations from capsaicin, piperine, and EtOH. Measures of alcohol use, misuse, or abuse were not collected, and no special emphasis was given to alcohol-related behaviors. EtOH was not tasted in the first session; the stimuli presented in session 1 have been reported elsewhere (Allen et al., 2013; Byrnes and Hayes, 2013), and are not described here for brevity. Upon completing session 1, participants were screened to determine whether they were eligible to participate in sessions 2, 3, and 4. Eligibility for sessions 2 to 4 was based on visibility of the individual's circumvallate papillae (CV) and the ability to tolerate stimulation with a wetted swab without gagging. Of participants who qualified, 130 individuals returned to complete all 4 sessions.

Participants

Participants, 18 to 45 years old, were recruited from the Pennsylvania State University campus and surrounding area. Those interested in participating completed an online survey to determine whether they met inclusion criteria. Qualifications include the following: not pregnant or breastfeeding, nonsmoker, no tongue, cheek, or lip piercings, no known smell or taste defect, no hyperactive thyroid, no history of chronic pain, and willingness to provide a salivary DNA sample. Of the participants who completed sessions 2 to 4 (total $n = 130$), the majority reported European ancestry ($n = 93$), with 18 reporting Asian ancestry, and 2 reporting African ancestry; 17 individuals declined to provide ancestry. Due to potential differences in allele frequencies across ancestry and the possibility of population stratification, all of the results here are restricted to individuals of European ancestry, resulting in a cohort of 58 women and 35 men with a mean age of 25 ($\pm$0.69 SEM) years.

Psychophysical Scaling of Test Stimuli

A generalized Labeled Magnitude Scale (gLMS) was used to collect psychophysical ratings for stimuli (Hayes et al., 2013a; Snyder et al., 2004). This scale ranges from 0 to 100 and asks participants to rate the intensity they experience relative to the "strongest imaginable sensation of any kind" (100). Adjective labels on the scale include: no sensation, barely detectable, weak, moderate, strong, and very strong, located at 0, 1.4, 6, 17, 35, and 51, respectively. This scale is believed to enhance the validity of comparisons across individuals, as compared to visual analog scales (Bartoshuk et al., 2003, 2004).

In sessions 2 to 4, participants were given instructions, identical to those provided during session 1, reorienting them to the scale. This included explanation of the top anchor, "strongest imaginable sensation of any kind," as well as reminding participants that they should click anywhere along the scale and to not let whether or not they like/dislike the sample to influence their intensity ratings. Before rating any sampled stimuli, participants completed a warm-up session where they rated 15 remembered sensations using a gLMS (e.g., Hayes et al., 2013a).

Test Stimuli and Protocol

Following orientation and warm-up, sessions 2 to 4 began by presenting 5 stimuli (sucrose, citric acid, NaCl, MSG/IMP, and quinine) on 4 quadrants of the tongue (right and left tip, right and left

CV) in a rotating fashion. Samples were presented in a blocked counterbalanced order, with all 5 stimuli being presented each day for a total of 20 samples (each of the 5 tastants in each of the 4 quadrants). After 10 applications, the participant took a break and performed a different task. All 5 tastants were presented before the same stimulus was presented again.

Participants completed a multiple attribute time intensity (MATI) task for a single irritant after the first 10 spatial stimuli described above. Each day consisted of a different irritant, with the irritant remaining constant throughout the session. The irritants presented in this study consisted of EtOH, piperine, and capsaicin; only EtOH results will be discussed here. A 50% v/v EtOH stimulus was presented to the posterior tongue by touching 2 saturated "buddy-taped" cotton swab applicators on either their left or right CV for 10 seconds. Intensity ratings were collected every 30 seconds for a total of 3 minutes. Intensity ratings for 6 qualities were collected (sweetness, bitterness, sourness, burning/stinging, umami/savory, and saltiness); the order of the attributes was fixed. Participants were asked to keep their tongue away from the roof of their mouth for the entire 3 minutes and to keep their lips closed to minimize evaporative cooling. Participants were not allowed to rinse for the 3-minute duration. Following the MATI task, there was a 4-minute break where participants were allowed to rinse with mouth temperature reverse osmosis (RO) water. Following the first MATI task, 10 additional spatial stimuli were applied, and then the second MATI task was completed for the CV on the opposite side. Next, Best Estimated Thresholds were collected using the 3 alternative forced choice method described in ASTM E-679; these data are not reported here, as detection thresholds do not predict ingestive behavior (Duffy et al., 2004b; Lucas et al., 2011). The final task within the session involved swishing a 15-mL sample of 16% v/v EtOH in the mouth for 5 seconds. Upon spitting out the sample, the participant rated the "overall intensity" on a gLMS.

The single time-point spatial data for the prototypical tastants serve as a negative control here; analysis of a superset of the present data (from Feeney and Hayes, 2014) indicate the means for the side tastes/sensations for each tastant were extremely low. For example, mean bitterness for sucrose, citric acid, and sodium chloride were 0.3, 3.0, 2.2, respectively. Similarly, mean burning/stinging were 0.35, 1.08, and 0.80. In contrast, means for the expected qualities of each (e.g., sourness for citric acid) were all 13 or higher (just below "moderate" on a gLMS). While the single point rating is slightly different than the MATI ratings for irritants in terms of participant demand, it suggests participants successfully distinguished between the various qualities in the rating task.

For a complete description of all of the phenotyping methods across sessions, please refer to the Supplemental material.

Genetic Analysis

DNA was collected using Oragene salivary collection kits per manufacturer instructions (Genotek Inc, Ontario, Canada). To maximize coverage of *TRPV1* (chr 17) variation, a tag SNP approach was used with tag SNPs identified in HapMap using the CEU reference population: rs4790521, rs4790522, rs224547, rs4790151, rs161364, rs8065080, rs150908, rs224534, rs222747, rs150846, rs161386, rs222749, rs7217945, rs161381, rs17707155, and rs222741. Additionally, bitter taste receptors SNPs in *TAS2R13* (chr 12; rs1015443) and *TAS2R38* (chr 7; rs713598, rs1726866, and rs10246939) were chosen based on previous literature. Genotypes were determined using Sequenom MassARRAY technology (Sequenom, San Diego, CA). Primers were purchased from Integrated DNA Technologies (Coralville, IA). Genotypes were automatically assigned via MassARRAY software (Sequenom). Two technicians independently inspected all genotypes and 15% of samples were rerun to ensure reliability.

Statistical Analysis

Data were analyzed using SAS 9.2 (Cary, NC). For MATI data, area-under-the-curve (AUC) was calculated as a summary measure.

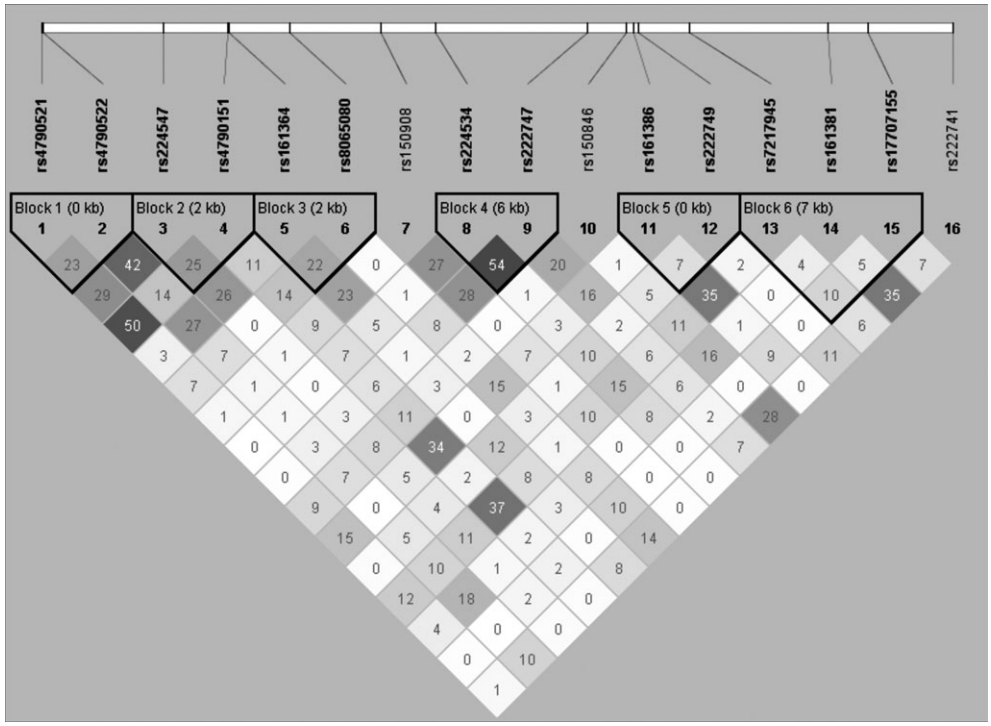

Fig. 1. Linkage disequilibrium plot (r^2) for 16 TRPV1 SNPs from 93 participants of European ancestry. Darker gray indicates higher r^2 values.

Table 1. List of TRPV1 SNPs Included in the Analysis

								Reported *p*-values	
									AUC ratings
Receptor	Chr.	SNP ID	Call rate, %	HWE *p*-value	Maj/min allele	MAF	SNP location	EtOH WM	Burn	Bitter
TRPV1	17	rs4790521	97.9	0.71	T/C	0.37	3′ UTR	0.35	0.19	0.0033
TRPV1	17	rs4790522	98.9	0.95	C/A	0.42	3′ UTR	0.12	0.79	0.50
TRPV1	17	rs224547	98.9	0.57	A/G	0.43	Intronic	0.07	0.054	0.0044
TRPV1	17	rs4790151	98.9	1.0	G/A	0.30	Intronic	0.16	0.84	0.35
TRPV1	17	rs161364	98.9	0.39	C/T	0.30	Intronic	0.63	0.0021	0.30
TRPV1	17	rs8065080	96.8	0.96	T/C	0.37	Ile585Val	0.17	0.70	0.43
TRPV1	17	rs150908	95.7	0.07	G/A	0.35	Intronic	0.80	0.53	0.33
TRPV1	17	rs224534	98.9	0.56	G/A	0.37	Thr469Ile	0.17	0.94	0.49
TRPV1	17	rs222747	96.8	0.56	C/G	0.28	Met315Ile	0.48	0.78	0.74
TRPV1	17	rs150846	96.8	1.0	C/T	0.39	Intronic	0.22	0.86	0.55
TRPV1	17	rs161386	98.9	0.59	C/T	0.38	Intronic	0.88	0.62	0.23
TRPV1	17	rs7217945	98.9	0.28	G/A	0.28	Intronic	0.83	0.06	0.27
TRPV1	17	rs17707155	98.9	0.58	C/T	0.26	Intronic	0.78	0.91	0.39
TAS2R13	12	rs1015443	98.5	0.28	C/T	0.35	Ser259Asn	0.04	0.98	0.36
TAS2R38	7	rs713598	98.4	0.37	G/C	0.49	Ala49Pro	0.32	0.87	0.0048
TAS2R38	7	rs1726866	97.7	0.53	C/T	0.50	Val262Ala	0.86	0.57	0.21
TAS2R38	7	rs10246939	98.5	0.73	C/T	0.48	Ile296Val	0.87	0.68	0.055

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; EtOH WM, ethanol whole-mouth sample 16% v/v; AUC, area-under-the-curve. *p*-Values are Generated via ANOVA. The Reported *p*-Values are Unadjusted.

To test AUC values for individual SNPs, analysis of variance (ANOVA) was performed via *proc mixed,* and post hoc comparisons were made via the Tukey-Kramer method. For SNPs that showed significant associations with AUC ratings, repeated measures ANOVAs were conducted for bitter and burning/stinging ratings using time as a repeated factor via *proc mixed*. If the SNP-by-time interaction was significant, means for the 2 most extreme groups at each time-point were compared using unadjusted *t*-tests via the LSMEANS option.

Haploview (Barrett et al., 2005) was used to examine the extent of linkage disequilibrium (LD) between each SNP. Haplotype blocks were defined according to Solid Spine of LD criteria (Barrett et al., 2005). LD plots show rounded R-squared values in individual squares.

RESULTS

On the posterior tongue (i.e., on the CV), burning/stinging was the predominant quality for the swab saturated with 50% v/v EtOH, followed by bitterness and sweetness. Means were taken from the ratings for the left and right CV, and the AUC across time was calculated for each participant. Burning/stinging AUCs and bitter AUCs were positively correlated (R^2 = 11%; p = 0.0013). Sweetness and burning/stinging AUCs were significantly correlated (R^2 = 6.0%; p = 0.0183); however sweetness AUCs were not significantly correlated with bitter AUC (p = 0.2). For the whole-mouth sip-and-spit procedure using 16% v/v EtOH in water, overall intensity means fell near "very strong" on the gLMS (47.0 ± 2.1 SEM). Whole-mouth ratings of "overall intensity" for 16% EtOH were also associated with both bitterness and burning/stinging AUCs from 50% EtOH swabs on the CV (R^2 = 4.8%; p = 0.034 and R^2 = 4.5%; p = 0.042, respectively).

Figure 1 shows the LD for the TRPV1 SNPs. R^2 values were reported and haplotypes were generated using Solid Spine of LD criteria (Barrett et al., 2005). Overall, 6 haplotypes were generated showing strong LD between neighboring SNPs, with the exception of rs150908, rs150846, and rs222741, all of which are located within the intronic region. Of the 16 *TRPV1* SNPs included in the genetic analysis (shown in Fig. 1), 3 SNPs exhibited a minor allele frequency (MAF) below 0.25 in our cohort: rs222741, rs161381, and rs222749. As these genotypes had too few participants to perform meaningful statistical analysis due to low sample number in the minor allele group, they were excluded from further analysis. The SNPs used in the present analysis are detailed in Table 1.

Exploratory Genotype–Phenotype Associations Based on AUC Scores Over Time

An intronic *TRPV1* SNP rs224547 (chr. 17) was associated with the summary AUC scores for both bitterness and burning/stinging from 50% v/v EtOH applied to the CV. Burning/stinging AUC scores were associated with rs224547 genotype, $F(89, 2)$ = 3.02, p = 0.0539. The AA homozygotes (n = 32) had the greatest mean AUC with 936.68 (±132.70 SEM) compared with the AG heterozygotes (n = 40) which had the smallest mean area of 505.03 (±118.69), with the GG homozygotes (n = 20) having a mean area of 772.69 (±167.86). Bitterness AUC scores were also significant for rs224547, $F(89, 2)$ = 5.76, p = 0.0044, and in the same direction as burning/stinging values, with the AA homozygotes having the highest mean area. The AA homozygotes reported the greatest mean area, 687.54(±108.92), with the

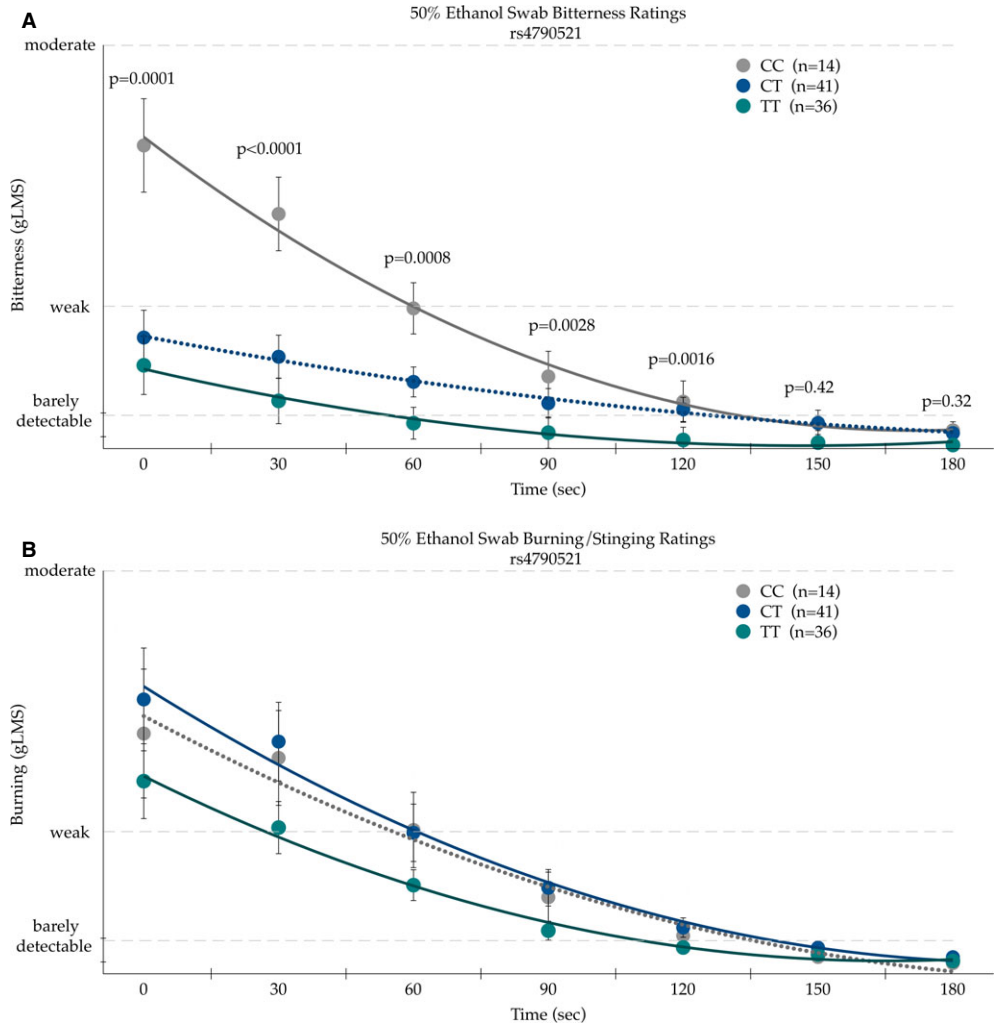

Fig. 2. Bitterness (**A**) and burning/stinging (**B**) from 50% ethanol applied to the posterior tongue differs by the TRPV1 SNP rs4790521 SNP in repeated measures ANOVA (see text for details). Points are arithmetic means with bars showing the standard error of the mean. *p*-Values indicate unadjusted *t*-tests at each time point comparing the 2 groups of homozygotes to decompose the significant time by SNP interaction.

heterozygotes having a mean of 256.22(±97.42). The GG homozygotes had the smallest area for bitterness: 186.75 (±137.77).

A second *TRPV1* SNP, rs4790521, was also a significant predictor of bitterness AUC ratings of 50% v/v EtOH on the CV, $F(88, 2) = 6.09$, $p = 0.0033$. This finding is not surprising as rs4790521 is in strong LD with rs224547, as shown in Fig. 1. The CC homozygotes ($n = 14$) had the highest mean area for bitterness: 860.09(±164.70). The CT homozygotes ($n = 41$) had a mean area of 419.45 (±96.25), with the TT homozygotes ($n = 36$) with the lowest mean area of 185.73 (±102.71).

A third *TRPV1* SNP, rs161364, also associated with the AUC burning/stinging ratings for 50% v/v EtOH on the CV, $F(89, 2) = 6.61$, $p = 0.0021$. The TT homozygotes (n = 7) had a mean area of 1,528.93(±273.59), which was significantly greater ($p = 0.001$) than the CT heterozygotes ($n = 37$), which had a mean area of 476.55(±119.00). The CC ($n = 48$) homozygotes had a mean area of 746.95 (±104.48), which was significantly lower than the TT homozygotes ($p = 0.03$); the CC homozygotes did not differ from the CT heterozygotes (746.95 vs. 476.55; $p = 0.15$).

TRPV1 SNPs Associate with the Perception of EtOH

Two SNPs that were significant for the summary AUC estimate across time for the 50% v/v EtOH swab (rs224547 and rs4790521) were analyzed further to explore effects across time; bitterness and burning/stinging at each timepoint (0, 30, 60, 90, 120, 150 and 180 seconds) were tested via repeated measures ANOVA. The third significant *TRPV1* SNP, rs161364, was not analyzed further across time due to low frequency of the TT homozygotes ($n = 7$).

In repeated measures ANOVA on the bitterness ratings, the time by SNP interaction was significant for the *TRPV1* rs4790521 SNP, $F(12, 528) = 3.51$, $p < 0.001$, as shown in Fig. 2*A*. In the first 2 minutes after application (i.e., at 0, 30, 60, 90, and 120 seconds), bitterness ratings were significantly

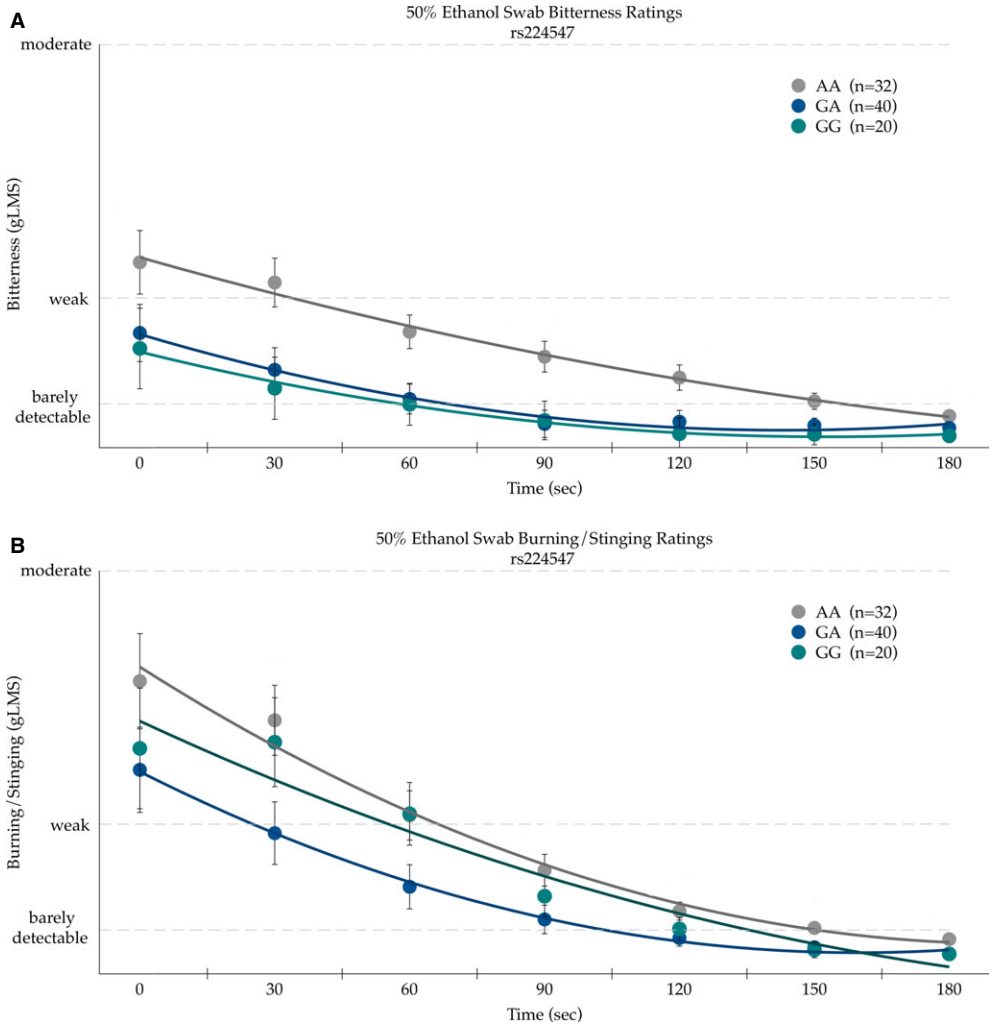

Fig. 3. Bitterness (**A**) and burning/stinging (**B**) from 50% ethanol on the posterior tongue differ by the rs224547 SNP in TRPV1 in repeated measures ANOVA (see text for details). Points are arithmetic means with bars showing the standard error of the mean.

different across rs4790521 genotype, with the TT homozygotes giving significantly higher ratings than the CC homozygotes. However, as bitterness decayed after 120 seconds, genotype no longer associated with bitterness, presumably due to floor effects. In repeated measures ANOVA on the burning/stinging ratings, we observed significant main effects for SNP, $F(2, 88) = 5.36$, $p = 0.0064$, and time, $F(6, 528) = 25.71$, $p < 0.0001$; the time by SNP interaction for the rs4790521 SNP was not significant, $F(12, 528) = 0.53$, $p = 0.89$. Nonetheless, the pattern was similar to the bitter results as the TT homozygotes tended to report the lowest sensations.

The second significant SNP in the AUC analysis for bitterness and burn, rs224547, was subsequently analyzed across time. In repeated measures ANOVA for bitterness, there was a main effect of SNP, $F(2, 89) = 21.40$, $p < 0.0001$, and time, $F(6, 534) = 13.33$, $p < 0.0001$, but the influence of the rs224547 SNP did not differ over time, $F(12, 534) = 0.13$, $p = 0.99$. As shown in Fig. 3*A*, the AA homozygotes consistently reported more bitterness than the GG homozygotes. In repeated measures ANOVA on the burning/stinging ratings (Fig. 3*B*), there was a main effect of the rs224547 SNP, $F(2, 89) = 9.10$, $p = 0.0003$, and time, $F(6, 534) = 31.14$, $p < 0.0001$, but the influence of this SNP did not differ over time, as the interaction was not significant, $F(12, 534) = 0.83$, $p = 0.62$.

In contrast to the time course data on the posterior tongue, none of the *TRPV1* SNPs tested explained differences in "overall intensity" ratings of a whole-mouth sip-and-spit solution of 16% v/v EtOH.

TAS2R SNPs Associate with the Perception of EtOH

Three SNPs in *TAS2R38* (chr. 7, rs713598, rs1726866, and rs10246939, resulting in A49P, V262A, and I296V, respectively) were explored for their association with EtOH bitterness. There was a significant association with A49P (rs713598) with AUC bitterness values for the 50% EtOH swab on the CV, $F(2, 89) = 3.13$, $p = 0.048$. The Pro49Pro homozygotes ($n = 18$) reported the most bitterness

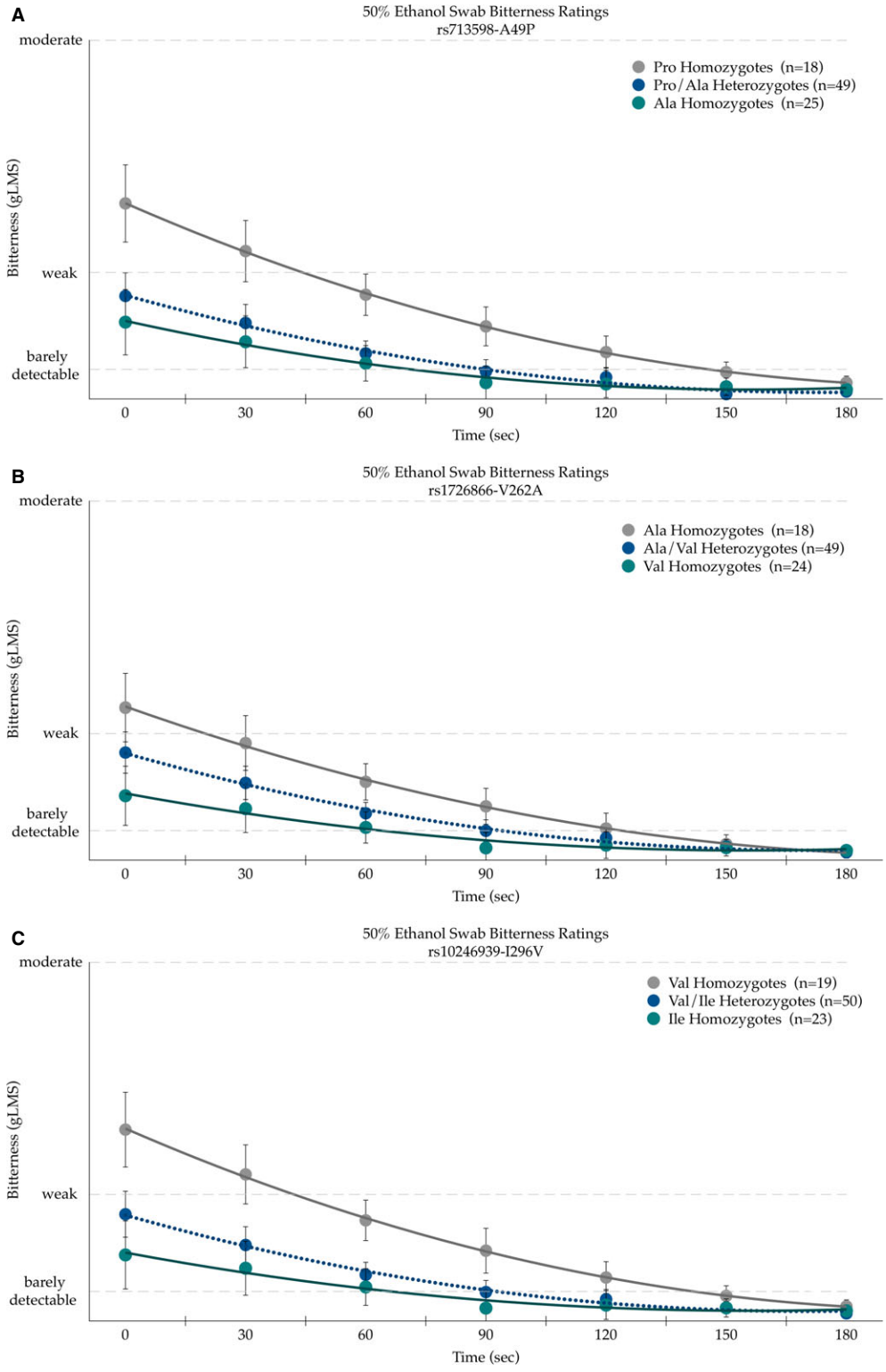

Fig. 4. Bitterness from 50% ethanol applied to the posterior tongue differs by the TAS2R38 SNPs rs713598 (**A**), rs1726866 (**B**), and rs10246939 (**C**) in repeated measures ANOVAs (see text for details). Points are arithmetic means with bars showing the standard error of the mean.

(717.9 ± 149.2); heterozygotes (n = 49) and the Ala49Ala homozygotes (n = 24) had similar mean areas (240.5 ± 90.4 and 255.2 ± 126.6, respectively). The second SNP Val262Ala (rs1726866) was not associated (p = 0.21) with bitterness AUC, whereas the third SNP Ile296Val (rs10246939) was associated with bitterness AUC, $F(2, 89) = 3.00$, $p = 0.0549$. The Val296Val homozygotes (n = 19) had the greatest bitterness AUC (696.1 ± 145.4),

followed by the heterozygotes (n = 50) (345.7 ± 89.6), while the Ile296Ile homozygotes (n = 23) reported the least bitterness (238.04 ± 132.2). Accordingly, these 3 SNPs were chosen for further analysis to explore associations between genotype and mean bitterness on the CV over time (Fig. 4).

Repeated measures ANOVA indicated the main effects of Ala49Pro genotype, $F(2, 89) = 13.40$, $p < 0.0001$, and time, $F(6, 534) = 14.84$, $p < 0.0001$, were associated with bitterness, although the SNP effect did not vary across time as the time by SNP interaction was not significant, $F(12, 534) = 1.0$, $p = 0.44$. As shown in Fig. 4*A*, the Pro49 homozygotes experienced greater bitterness than Ala49 homozygotes. A similar pattern was observed for Val262Ala (Fig. 4*B*), with significant main effects of genotype, $F(2, 88) = 6.50$, $p = 0.0023$, and time, $F(5, 528) = 14.12$, $p < 0.0001$, for bitterness. The time by genotype interaction was not significant for Val262Ala, $F(12, 528) = 0.76$, $p = 0.69$, indicating the effect of genotype did not change over time. As shown in Fig. 4*B*, the Ala262 homozygotes reported more bitterness. The Ile296Val SNP in *TAS2R38* showed a similar pattern as the Ala49Pro and Val262Ala SNPs; the main effects of genotype, $F(2, 89) = 12.96$, $p < 0.0001$, and time, $F(6, 534) = 14.07$, $p < 0.0001$, were significant for bitterness, and the effect of genotype did not differ over time, $F(12, 534) = 1.13$, $p = 0.34$. As shown in Fig. 4*C*, the Val296 homozygotes reported more bitterness than the Ile296 homozygotes. In summary, the consistency across these 3 SNPs is to be expected due to high LD (Kim et al., 2003). However, due to the novel association with EtOH sensations described here, we report each separately as prior site-directed mutagenesis studies that indicate the relative importance of each site for PROP may not generalize to EtOH.

In contrast to the time course data, these same 3 SNPs (rs713598, rs1726866, and rs10246939, resulting in A49P, V262A, and I296V) were not associated with the "overall intensity" ratings of whole-mouth 16% EtOH ($p = 0.32$; $p = 0.86$; and $p = 0.87$, respectively).

Finally, Asn259Ser (rs1015443) in TAS2R13 **(chr. 12)**, a SNP gene previously implicated with regard to alcohol intake (Dotson et al., 2012), was significantly associated with "overall intensity" ratings of whole-mouth 16% EtOH, $F(2, 89) = 3.31$, $p = 0.041$ (Fig 5). The Asn homozygotes (n = 12) reported having the highest mean ratings 52.7 (±5.8), with the heterozygotes (n = 49) rating 50.3 (±2.9) and the Ser homozygotes (n = 31) reporting the least intensity 39.5 (±3.6). Although AUC ratings for bitterness and burning/stinging were not significant (Table 1), we performed a repeated measures ANOVA for the MATI data due to significant associations between Asn259Ser and whole-mouth intensity ratings. There were significant main effect of genotype, $F(2, 89) = 4.05$, $p = 0.021$, and time, $F(6, 534) = 11.16$, $p < 0.0001$, for bitterness; however, the interaction of time and SNP was not significant, $F(12, 534) = 0.4$, $p = 0.96$ (not shown).

DISCUSSION

In our cohort, burning/stinging was reported to be the predominate sensation for a 50% EtOH solution applied to the CV, followed by bitterness and sweetness. Summary measures of burning/stinging across time (AUC values) were significantly correlated with summary measures of bitterness across time. Additionally "overall intensity" ratings of a whole-mouth 16% EtOH solution at a single time-point were significantly associated with summary measures of burning/stinging and bitterness over time. Collectively, this suggests those who experience more burn from EtOH also experience more bitterness.

As EtOH activates *TRPV1* in vitro (Trevisani et al., 2002), there is reason to believe that polymorphisms in *TRPV1* might alter the perceived burn from EtOH if the SNPs are functional. The TRP box, a 6-mer region located near the channel gate (C-terminus domain) found in all TRP channels, has shown to be key in TRPV1 function (García-Sanz et al., 2007; Gaudet, 2007; Valente et al., 2008). This same finding has been shown for TRPM8 for activation from menthol (Bandell et al., 2006). Mutations within the TRP box in TRPV1 eliminate response to 1uM capsaicin in vitro (Valente et al., 2008). Valente and colleagues (2008) hypothesize reduced activation is due to maintaining the gate in a closed state. *TRPV1* SNPs rs4790521 and rs224547 exhibit strong LD and surround the TRP box. These 2 SNPs may be in LD with polymorphisms within the TRP box.

Differences in the burning/stinging of EtOH associated with the *TRPV1* SNP rs224547, with AA homozygotes experiencing greater burning/stinging compared to GG homozygotes. However, we did not expect this SNP to associate with bitterness, as was also observed here. Moreover, the *TRPV1* SNP rs4790521 was also found to be associated with bitter AUC ratings, with CC homozygotes reporting significantly more bitterness within the first minute of the CV being

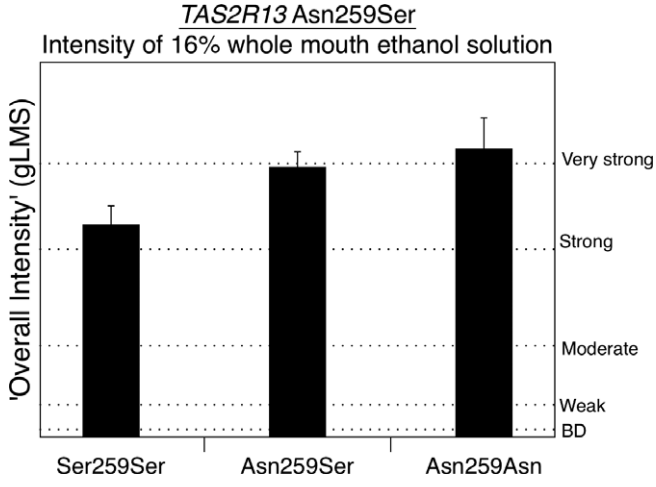

Fig. 5. Ratings of "overall intensity" from a 16% whole mouth sip and spit ethanol solution differs by the rs1015443 SNP in TAS2R13 in one-way ANOVA. Bars are arithmetic means ± standard error of the mean.

exposed to 50% EtOH. While unexpected, previous reports suggest that bitter and burn sensations are perceptually similar, even though they are thought to be transduced through separate pathways. Lim and Green (2007) reported bitterness from quinine was more similar to the burn from capsaicin than the other prototypical tastes (sour, salty and sweet), suggesting that these 2 sensations are similar, serving as part of a "chemofensor complex" (Green, 2012). Other evidence shows the prototypical burning stimulus capsaicin evokes bitterness in some individuals (Green and Hayes, 2004; Green and Schullery, 2003; Lawless and Stevens, 1984). Additionally, nonnutritive sweeteners activate TRPV1 in vitro (Riera, 2007; Riera et al., 2008) and are often described as bitter by humans (e.g., Allen et al., 2013). Collectively, these data might suggest a simple labeled line receptor-percept hypothesis may be overly reductionist, as burn and bitterness may not be as independent as previously believed. However, this explanation is complicated by the weak phenotypic association observed between burning and stinging, and bitterness in our phenotypic data. Alternatively, we cannot rule out that the genotype–phenotype associations observed here could potentially be an artifact caused by the fixed order of the scales (Bennett et al., 2012; Green et al., 2010), semantic confusion between attributes (Bennett et al., 2012), affective dumping, or some combination thereof. That is, participants always rated sweetness, followed by bitterness and sourness before burning/stinging, which may bias participants toward dumping aversive sensations into the first aversive attribute available to them (bitterness vs. burning). Additional work is needed to clarify the relationship between TRPV1 and aversive sensations. Nonetheless, present data suggest that sensations from sampled EtOH vary as a function of genetics, consistent with the idea that variation in chemosensory genes can influence ingestive behavior (Hayes et al., 2013b).

These findings suggest that individuals with AA genotype for SNP rs224547 and/or CC genotype for rs4790521 may potentially associate with reduced alcoholic consumption if they perceive greater bitterness and/or burn from alcohol, which would be expected to deter consumption of alcoholic beverages, at least initially before dependence and reward-related associations develop. Present results should be considered provisional until replicated, and additional work testing whether these SNPs associate with alcohol use is warranted.

The *TRPV1* SNP rs161364 was significantly associated with burning AUC ratings. The C allele carriers (CC and CT) did not differ from each other, but the TT homozygotes were different from both groups of C allele carries. This suggests the C allele may be associated with decreased function. However, due to the low frequency of TT individuals, we caution that this finding needs to be replicated to determine the possible functionality of this SNP.

Variations in bitter taste receptor genes have been shown to explain reported bitterness from a wide range of compounds and foods (reviewed by Hayes et al., 2013b). Here, we report the perceived bitterness from 50% EtOH on the posterior tongue was significantly associated with the *TAS2R38* SNPs rs713598 (A49P), rs172866 (Val262A) and rs10246939 (I296V). This is not surprising, as bitter sensations have been reported previously from EtOH (e.g., Mattes and DiMeglio, 2001; Scinska et al., 2000) and alcoholic beverages (e.g., Lanier et al., 2005). Previous work exploring relationships between taste perception and PROP phenotype reported that individuals who perceived PROP as being more bitter also reported greater irritation for EtOH solutions than individuals who reported no bitterness from PROP (Bartoshuk et al., 1993). Since 2003, it has been widely accepted that the perception of PROP is largely explained by 3 SNPs in *TAS2R38* (Bufe et al., 2005; Duffy et al., 2004a; Hayes et al., 2008; Meyerhof et al., 2010) as they exhibit strong LD (Kim et al., 2003). Polymorphism in *TAS2R38* are associated with food intake via differential sensations (see Duffy et al., 2010; Feeney, 2011) and polymorphisms in other TAS2Rs have also associated with differences in the sensations from foods and beverages. For example *TAS2R19* variation associates with the bitterness of quinine, while SNPs in *TAS2R9* and *TAS2R31* associate with the bitterness from acesulfame K. Accordingly, it should not be surprising that the bitterness of EtOH associates with variation in *TAS2R* genes that encode bitter taste receptors.

Previously, SNPs in another bitter receptor gene, *TAS2R13,* were found to associate with alcohol intake frequency in a cohort of head and neck cancer patients. Dotson and colleagues (Dotson et al., 2012) reported that rs1015443 CC homozyogtes reported greater frequency of drinking days, drinks per drinking day, and heavy episodic drinking. Here, we provide evidence that the same SNP associates with the overall intensity of whole-mouth EtOH, with the CC homozygotes reporting lower intensities than heterozygotes or TT homozygotes.

LIMITATIONS AND CONCLUSIONS

Previous work associates SNPs in *TAS2R13* and *TAS2R38* with alcohol intake, and it was assumed differential intake results from differences in sensation. Here, we provide the first evidence EtOH sensations differ with these TAS2R polymorphisms. However, one must also keep in mind the differences between EtOH and alcoholic beverages, as our participants did not sample alcoholic beverages, only EtOH diluted with water. Spirits, beer and wine, contain sensory active compounds beyond just EtOH, and these compounds may suppress bitterness (via perceptual masking) or add additional bitterness (e.g., hops). For the whole-mouth 16% EtOH solution used here, only "overall intensity" ratings were collected, so we cannot distinguish between separate percepts like burning and bitterness when regions beyond the circumvallate, like the palate or anterior tongue, are stimulated. Also, this stimulus was delivered via a sip-and-spit method, so involvement of the pharynx was minimal.

Genetic association studies are by definition quasi-experimental (since one cannot randomly assign to genotype); therefore, associations may reflect the impact of unmeasured third variables (genetic or otherwise). While limiting the study to Caucasians reduces the threat of population stratification, it does not completely control for this possibility. The novel results reported here should be considered provisional until such a time as they may be replicated in a larger sample. Nonetheless, present data suggest a relationship between sensations from EtOH and genetic variation in *TAS2Rs* and possibly *TRPV1*.

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CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.