A potential sex dimorphism in the relationship between bitter taste and alcohol consumption.

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Abstract

Background: Bitterness is an innate aversive taste important in detecting potentially toxic substances, including alcohol. However, bitter compounds exist in many foods and beverages, and can be desirable, such as in beer. TAS2R38 is a well-studied bitter taste receptor with common polymorphisms. Some have reported relationships between TAS2R38 genotypes, bitter taste phenotype and alcohol intake, however results have been mixed. These mixed results may be explained by the varying taste properties of different alcoholic beverages or a sex dimorphism in responses. ***Methods:*** Bitter taste phenotype was assessed using PROP taste test and TAS2R38-P49A genotype was assessed by RFLP-PCR. Alcohol intake was assessed by food frequency questionnaire and classified by beverage type (beer, wine, spirits or mixed drinks). The relationships between bitter taste phenotype and carriage of the P allele of the TAS2R38-A49P gene and alcohol intake were assessed adjusted for and stratified by sex, and the interaction between taste and sex was evaluated. ***Results:*** The relationship between alcohol intake and bitter taste phenotype varied by beverage type, with significant results for beer, spirits and mixed drinks, but not wine. When stratified, results varied by sex, and were only significant in males. Significant interactions were found for taster phenotype and sex (total alcohol intake and intake of beer and spirits). Results were similar for carriage of the TAS2R38-P49A P allele. **Conclusions:** Sex-specific interactions between bitter taste phenotype, TAS2R38 genotype and alcohol intake may explain variance in previous studies and may have implications for sex-specific disease risk and public health interventions.

Introduction

Bitter is an innate aversive taste important in protecting humans against the consumption of potentially toxic substances, including bacterial metabolites, products of food spoilage and many naturally occurring poisons [1, 2]. However, bitter compounds exist in many foods and beverages, and include bioactive compounds and nutrients, such as glucosinolates, isoflavones, and a range of other phytonutrients [3]. Bitterness may also be an acceptable or desirable property in some foods and beverages such as fermented foods, coffee, and beer [4-6].

Bitter tastants are detected by the TAS2R receptor family [7-9]. Significant genetic variance exists in TAS2R receptors, and this, along with varying taste bud density, largely explains the variance in bitter taste phenotypes [8, 9]. TAS2R38 detects compounds with a thiocyanate moiety, including two common test compounds used in taste research, phenylthiocarbamide (PTC) and 6-n-propyl-2-thiouracil (PROP) [7, 10-12]. The correlation between PTC/PROP tasting phenotype and sensitivity to a range of structurally unrelated bitter compounds is well demonstrated [13-18]. Therefore, threshold tasting of PTC/PROP is often used as a surrogate marker for bitter taste in general; non-tasters are insensitive to the bitterness of PTC or PROP and tasters are sensitive. The taster category can also be further divided into medium tasters and super tasters, depending on assessment methods used [19, 20].

Three common polymorphisms in the TAS2R38 gene (rs713598; rs1726866 and rs10246939), resulting in three amino acid substitutions (A49P, A262V, V296I), have been identified as being primarily responsible for the variation in PTC/PROP tasting status [7, 21]. These polymorphisms are in high linkage disequilibrium and despite the number of potential haplotypes, two common haplotypes Ala-Val-Ile (AVI) and Pro-Ala-Val (PAV) [7, 21] account for more than 90% of haplotypes found in the Caucasian population [7]. The ancestral PAV haplotype is associated with the PTC/PROP tasting phenotype and the mutant AVI haplotype is associated with the non-tasting phenotype [7, 22]. However, genotype and tasting phenotype do not correlate completely, with the common variants explaining only 45-80% of variance [7, 23]. Additional variation may be explained by other factors including fungiform papillae density, additional polymorphisms in TAS2R genes or genes for downstream signalling molecules [24], epigenetics or influences due to age [25], and sex [26]. Family studies suggest that tasting is the dominant trait and non-tasting the recessive [27, 28].

Several studies link bitter taster phenotype and TAS2R38 genotype to liking and intensity of ethanol [29-34], beer [35, 36], scotch [36], and red wines [37]. However, others have reported no relationship between bitter taste phenotype and ethanol taste and irritancy thresholds [38]. Genotypes and phenotypes associated with non-tasting have also been linked to increased risk of alcoholism in some studies [39-41], but not others [42-48]. Investigations on the influence of bitter taste phenotype and TAS2R38 genotype and habits of alcohol consumption have also yielded mixed results. Many studies have reported an association between

TAS2R38 genotype [30, 34, 36, 42, 49], bitter taster phenotype [16, 30, 35] and alcohol intake, however a number of others have reported no association [38, 48, 50-52] or varied relationships by alcohol type [36].

The inconsistency in these relationships may be due to several factors. Lack of assessment of different alcoholic beverage types independently in some studies may skew results due to the desirability of bitter elements in some alcoholic beverages, such as beer [35]. Additionally, while alcohol itself is bitter [32, 53] it is also reported to taste sweet [53]. Concentration of alcohol also varies by beverage type and in mixed drinks the bitter taste may be obscured. The hedonic response to alcohol may also be modified by the neuroactive influences of alcohol, which may be perceived as positive or negative [54]. Additionally, studies use varied measures of alcohol consumption, some treating it as a categorical [16, 38, 49, 52] and others as a continuous variable (mean [30, 35, 48, 50, 51], maximum drinks consumed [42] or frequency [34]). Differential exclusion or inclusion of non-drinkers may also account for some inconsistency in the published data, as avoidance of alcohol may occur for many reasons other than taste, including religious and cultural reasons [55-57]. The combined influence of these factors may vary by cohort and explain some of the variance in findings.

The influence of bitter taste phenotype or TAS2R38 genotype on alcohol consumption may also vary by sex. This may potentially relate to different cultural norms imposed upon genders, or may be due to different interactions with sex-specific biological pathways, such as hormones. Previous studies have used varied methods to account for potential sex differences in response. Some use sex matching or statistical correction for sex, others restrict analyses to single-sex cohorts or ignore the impact of sex distributions in the cohort. Therefore, it is possible that differing treatment of the sex variable explains some of the variance in the results summarised above.

It has been reported that females are more responsive and exhibit greater variance in sensitivity than males to the bitterness of PTC and PROP (reviewed in [4, 19, 26]). It has also been reported that males have higher taste thresholds for ethanol than females [38]. Additionally, in an African American cohort, the association between TAS2R38 genotype and the maximum number of drinks consumed was only significant in females [42]. Interestingly, in a study of undergraduate students, Driscoll *et al*. found a sex dimorphism; with male super-tasters reporting fewer problems with alcohol and a less significant family history of alcoholism, and female super-tasters reporting the opposite, with a greater incidence of family history of alcoholism and more current problems associated with alcohol use. However, Duffy *et al.* [58] reported no difference in PROP tasting or the association between tasting and alcoholic beverage consumption in males and females.

A sex specific response has been found in male and female taste preferences, with male non-tasters liking the taste of a test beer more than male super-tasters, a result that was not replicated in females,

however interaction between taste and sex was not assessed. In this study bitterness rating was inversely correlated to reported liking. Super-tasters also reported consuming less beer than non-tasters [35].

The potential sex-specific relationship between taste and alcohol consumption is not well elucidated in the context of total intake or for other defined types of alcoholic beverages. Therefore, in order to address this, we examined the relationships between bitter taster phenotype, TAS2R38-P49A variance, and alcohol consumption by beverage type in a convenience cohort sourced from patients undergoing routine colonoscopy.

Experimental

Subjects

This cohort is part of a larger study (n=263) investigating the gene-nutrient interactions and risk for colorectal cancer in patients undergoing routine colonoscopy at Gosford Hospital, NSW, Australia, and is used as a convenience sample here. Ethics committee approval was obtained from the University of Newcastle Human Research Ethics Committee (approval number H-429-0407) for collection of all samples and data used in this study. Study participants were fully informed regarding the purposes of the study and consent was obtained. Participants were excluded if blood was not collected for DNA analysis, or if extreme values in their food frequency questionnaire indicated possible errors in completion, or if they did not report consuming alcohol. Following these exclusions 180 participants remained for inclusion in this study (51% female; aged 18-88 years, mean 61.6 years).

Estimated habitual alcohol intake and smoking history

Estimated habits of daily intake of alcohol and total energy were assessed by interviewer administered food frequency questionnaire, analysed using FoodworksTM 2.10.146 (Xyris Software, Brisbane, QLD, Australia) [59]. Alcohol consumption was converted to standard drinks, using the Australian definition of one standard drink containing 10ml of alcohol [60]. For stratified analyses, alcoholic beverages were assigned to the categories beer, wine, mixed drinks and spirits. Cigarette smoking history of participants was coded as current smokers, ex-smokers, or never smokers.

Bitter taste phenotyping and TAS2R38-P49A genotyping

Bitter taste phenotype was assessed using taste tests in which participants rate PROP solutions of varying concentrations on a continuous scale, as previously described [61]. The overall average index of these ratings was used to assign participants into bitter taster phenotype categories of “taster” and “non-taster” [61]. Genomic DNA was extracted from whole blood by standard procedures using the QIAamp DNA Blood Mini Kit and the P49A variant of the TAS2R38 gene (rs713598) was assessed using RFLP-PCR as previously described [61]. Water blanks were used as negative controls, at all stages, and samples

of known genotype were used as positive controls to confirm success of reactions. Genotypes were grouped by carriage (AP and PP genotypes) or absence (AA genotype) of the dominant allele [28, 62].

Statistics

Alcohol intake (standard drinks per day) variables were transformed ($\log_{10}(x+1)$) to normalise distributions. Results are presented as back transformed means and 95% confidence intervals. All analyses were adjusted for age and cigarette smoking history as both have been reported to influence the relationship between TAS2R38 genotype and bitter taste phenotype [25, 63]. Analyses were either adjusted for or stratified by sex, as appropriate. Multifactorial modelling was conducted using least-squares regression with interaction terms included ($p_{interaction}$). Pairwise comparison of least-squares means made by t-tests or χ^2 tests with likelihood ratios. Outcomes were considered to be statistically significant at $p \leq 0.05$.

Results

Cohort distributions

The distribution of phenotype did not vary significantly by sex ($\chi^2=0.3$, $p=0.6$), with 76.1% of females and 79.5% of males categorised as tasters. Additionally, the distribution of genotypes did not vary significantly by sex ($\chi^2=1.8$, $p=0.2$), with 59.9% of females and 69.3% of males possessing at least one P allele. Genotype frequency did not deviate from Hardy-Weinberg equilibrium expectations. Genotype was closely, but not completely linked to phenotype in the complete cohort ($\chi^2=59.11$, $p<0.0001$; Figure 1 A), and by sex (females $\chi^2=35.11$, $p<0.0001$; males $\chi^2=23.13$, $p<0.0001$; Figures 1B & C, respectively).

The mean age of the males in this cohort was significantly older than the females (60.4±1.0 years vs. 64.4±1.2 years, $p=0.009$). Therefore, all analyses were adjusted for age. Males also reported drinking significantly more alcohol, relative to females in this cohort with males consuming an average of 2.6 standard drinks per day (95% CI 2.0-3.2) compared to 1.0 standard drinks per day (95% CI 0.7-1.2) in the female portion of the cohort ($p=0.0001$; Figure 2A).

In this cohort, males reported consuming more beer than females. Males reported consuming an average of 1.6 standard drinks (95% CI 1.2-1.9) of beer per day, whilst females reported consuming just 0.04 standard drinks per day (95% CI -0.3-0.4; $p<0.0001$; Figure 2B). There were no differences between the sexes in the number of standard drinks consumed from spirits, mixed drinks or wine (Figure 2C-E).

Bitter taste phenotype predicts total alcohol intake

In the complete cohort, bitter taste phenotype was a significant predictor of the total number of standard drinks reportedly consumed per day (p=0.01; Table 1). Tasters reported consuming, on average, 0.66 fewer standard drinks per day, compared to non-tasters (Table 1).

The relationship between bitter taste phenotype and alcohol intake varies by alcoholic beverage type

When analyses were stratified by alcohol type, bitter taste phenotype predicted the average consumption of standard drinks contained in beer (p=0.006), mixed drinks (p=0.002) and spirits (p=0.02), but not wine (p=0.7; Table 1). The largest difference was seen in beer consumption, with tasters reporting their consumption, on average, to be 0.38 fewer standard drinks per day compared to non-tasters. For both mixed drinks and spirits, although the results were statistically significant, the difference in consumption between tasters and non-tasters was small, approximately 0.15 standard drinks per day and 0.08 standard drinks per day, respectively (Table 1).

The relationship between bitter taste phenotype and alcohol intake (total and by beverage type) varies by sex

Bitter taster phenotype was a significant predictor of total alcohol consumption in males (p=0.00; Table 2), with tasters consuming an average of 1.87 fewer standard drinks per day, compared to non-tasters. However, bitter taste phenotype did not predict the number of standard drinks consumed in females (p=0.7; Table 2). A significant interaction was found between bitter taster phenotype and sex in the prediction of alcohol intake ($p_{interaction}$=0.02; Table 2).

In the male portion of the cohort, bitter taster phenotype was a significant predictor of intake of standard drinks consumed as beer (p=0.004), mixed drinks (p=0.01) and spirits (p=0.02), but not wine (p=0.8; Table 2). However, in the female portion of the cohort, bitter taster phenotype did not significantly predict the intake of alcohol, regardless of beverage type (Table 2). In the males, the difference between phenotypes was greatest for beer, with tasters reporting consuming an average of 1.24 standard drinks contained in beer per day, relative to the non-taster males (Table 2). The effect on mixed drinks and spirits, although significant was smaller, with tasters reporting a reduction of 0.2 standard drinks per day from mixed drinks, and 0.16 standard drinks per day from spirits (Table 2). No difference between taster phenotypes was found for wine in the male portion of the cohort (Table 2).

A significant interaction was found between bitter taster phenotype and sex in the prediction of the consumption of standard drinks consumed in beer ($p_{interaction}$=0.002) and spirits ($p_{interaction}$=0.05; Table 2). However, no significant interaction was found between bitter taster phenotype and sex in the prediction of wine or mixed drinks.

TAS2R38-A49P genotype predicts total alcohol intake

As with phenotype, TAS238-P49A genotype was also a significant predictor of the number of standard drinks reportedly consumed per day (p=0.04). Those possessing a P allele, commonly associated with the taster phenotype, reported consuming an average of 0.43 fewer standard drinks per day than those without a P allele (Table 3).

The relationships between TAS2R38-A49P genotype and alcohol intake varies by alcoholic beverage type, but differ from phenotype

Unlike phenotype, TAS2R38-A49P genotype did not predict consumption of alcohol contained in beer (p=0.2; Table 3). However, genotype did predict the consumption of alcohol in mixed drinks (p=0.004) and spirits (p=0.02; Table 3). Those with the P allele consumed 0.08 fewer standard drinks per day from spirits, and 0.12 fewer standard drinks per day from mixed drinks, compared those without the P allele (Table 3). As with phenotype, genotype did not predict the consumption of alcohol in wine (p=0.9; Table 3).

The relationship between TAS2R38-A49P genotype and alcohol intake (total and by beverage type) varies by sex

Similar to phenotype, TAS2R38-A49P genotype did predict the total number of standard drinks consumed by males (p=0.02), but not females (p=0.9; Table 4), with a significant interaction found between TAS2R38-A49P genotype and sex in the prediction of alcohol intake ($p_{interaction}$=0.04; Table 4).

Reflecting the previous results for phenotype, TAS2R38-A49P genotype also predicted intake of alcohol from beer (p=0.05), mixed drinks (p=0.02) and spirits (p=0.02; Table 4). Genotype did not predict intake for any alcoholic beverage type in the female portion of the cohort (Table 4.)

A significant interaction was found between TAS2R38-A49P genotype and sex in the prediction of the consumption of standard drinks contained in beer ($p_{interaction}$=0.05; Table 4). However, no significant interaction was found between genotype and sex in the prediction of the number of standard drinks consumed in mixed drinks, spirits or wine.

In multifactorial models genotype and phenotype are both predictors of alcohol intake, with sex an independently significant predictor in both models

When bitter taste phenotype, age and sex were modelled together, the model explained 17% of the estimated alcohol consumption (p<0.0001), with both sex and phenotype being identified as independent significant variables (p<0.0001 and p=0.001, respectively). When an additional adjustment

was applied for total estimated energy intake, the model explained 24% of alcohol consumption ($p<0.0001$), and both sex and phenotype remained significant independent predictors.

When bitter taste genotype, age and sex were modelled together, the model explained 15% of the estimated alcohol consumption ($p<0.0001$), with both sex and genotype being identified as independent significant variables ($p<0.0001$ and $p=0.004$, respectively). When an additional adjustment was applied for total estimated energy intake, the model explained 21% of alcohol consumption ($p<0.0001$), and both sex and genotype remained significant independent predictors.

As this was a convenience sample obtained from patients undergoing routine colonoscopy, additional adjustment for diagnosis following colonoscopy (presence or absence of adenomatous colon polyps) was applied to the above analyses. This adjustment did not significantly alter the results.

The relative contribution of alcohol to energy intake varies by genotype and phenotype in males only

In the complete cohort, bitter taste phenotype was a significant predictor of the proportion of average energy intake that could be attributed to alcohol, with non-tasters consuming a significantly higher percentage of total kilojoules from alcohol ($p=0.003$, Table 5). Sex was also a significant independent predictor of the proportion of average energy intake that could be attributed to alcohol, with women consuming less kilojoules from alcohol than men ($p<0.0001$). There was a significant interaction between sex and phenotype in predicting the proportion of kilojoules consumed from alcohol ($p_{interaction}=0.006$). Similar results were seen when analysis was repeated by genotype (Table 5). When stratified by sex, phenotype and genotype predicted proportion of energy consumed as alcohol in males only (Table 5).

Discussion

The data presented here demonstrate that the relationships between TAS2R38-P49A genotype and alcohol intake vary both by sex and by type of alcohol consumed. Similarly, the relationships between bitter taster phenotype and alcohol intake vary both by sex and by type of alcohol consumed.

While other studies have previously investigated the relationship between taste and alcohol consumption, this study adds to this body of knowledge by comprehensively assessing a range of alcohol types, both adjusted for and stratified by sex. Further, we demonstrate a potential interaction between sex and genotype, and sex and phenotype, in predicting alcohol intake, particularly for total alcohol intake and consumption of beer. These interactions, not previously considered, may explain some of the inconsistent results between other studies, where some found associations between alcohol and bitter taste genotype and phenotype [29-37, 39-42, 49], bitter taster phenotype [16, 30, 35], and others did not [38, 42-48, 50-52].

It appears that bitter taste phenotype and TAS2R38-P49A genotype both predict alcohol intake (total, beer, spirits and mixed drinks) in males, but not females. Significant interactions were found between sex and phenotype for total alcohol, beer and spirits consumption, and between sex and genotype for total alcohol and beer consumption.

However, in this study, men did report consuming significantly more total alcoholic beverages and beer than females. Therefore, the results presented here may not represent a true sex-specific response and may reflect bitter taste being a greater predictor of intake in those who consume more alcohol. This could also potentially explain inconsistencies in previous studies. Additional studies, of larger sample size, are needed to allow stratification by level of alcohol consumption to assess the influence of sex on alcohol consumption within these strata. Nevertheless, when data was analysed as a proportion of the energy consumption that is attributable to alcohol intake, in order to account for differing dietary patterns and requirements between the sexes, the sex-specific patterns remained.

In interpreting these results it is important to acknowledge that taste is not the only reason people drink. Additional explanatory factors include different societal pressures on males and females regarding alcohol consumption. Females may be more influenced by other factors such as health consequences and behavioural norms over taste decisions. Reporting bias also needs to be considered. Food frequency questionnaires reflect reported normal habits of consumption, however, under-reporting is a common problem with this method of data collection, and this may be more prevalent in females. Socio-economic factors may also influence these outcomes. Furthermore, sex-specific results may be due to hormonal interactions not assessed here. Hormonal variation within or between sex groups may be an explanatory or confounding factor and the data presented here justifies further exploration of these potential interactions."

Food frequency questionnaires also capture average consumption over time, which is translated into average drinks per day. However, they do not describe the temporal dispersion of consumption, therefore, the differences between consumption patterns between males and females are not captured. Binge drinking is more common in males than females [64], and this may account for a portion of the higher alcohol consumption in males seen here. Cultural norms leading to different learned responses to bitter tastants in alcohol may also contribute. However, the taste specific differences found here in males, who consumed more alcohol than females, suggests that higher consumption does not mask the influence of taste.

The strongest beverage type specific results were seen for beer, whilst wine consumption was not related to taste genotype or phenotype in any analysis. This may reflect the different taste profiles of different alcohol types, with bitterness perceived as a desirable feature in beer [4-6]. It is important to note that the level of bitter compounds can vary between type and brand of beer, and therefore the different beers

consumed may influence results. The food frequency questionnaires also assess intake and not "liking" or hedonic rating, and this should be explored further in future studies. However, in a taste test using two different beers with different perceived bitterness, Intranuovo et al, found a correlation between the subjects rating of bitterness and their level of liking for both beers [35]. Sample size may be considered a limitation of this study, however, it is similarly sized to many preceding studies. It is unlikely that the sex-specific responses are an artefact of the post-stratification sample size, as there were more females in the study sample here. Lack of information of socio-economic status and family history of alcoholism and biometric measurements, such as BMI, are also notable limitations and these should be collected in future studies of this nature. However, these limitations should not detract from the potential importance of understanding the sex-specificity of the influence of taste on habits such as alcohol consumption.

These sex-specific findings may have public health implications in terms of disease risks that vary by sex, particularly diseases where alcohol consumption is a risk factor. They may also inform sex-specific interventions and recommendations in regards to alcohol intake. These findings extend our understanding of the variances that exist in bitter taste profiles and how they may modulate dietary intake. By modulating intake, taste profiles have the potential to modify disease risk. The sex-specific findings presented here demonstrate the ongoing need to consider sex dimorphism in the analyses of taste and dietary studies.

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Figure Legends

Figure 1: *Relationship between TAS2R38 genotype (presence vs. absence of P variant) and bitter taste phenotype (taster vs non-taster) in A) the complete cohort and by sex, B) females, C) males.*

Figure 2: *Comparison of alcohol consumption by sex A) total standard drinks B) beer C) spirits D) mixed drinks E) wine.*

Tables

Table 1: *Alcohol intake by bitter taster phenotype.*

	Tasters	**Non-Tasters**	
	Mean (95% CI)		***p-value***
Total	2.02 (1.81-2.24)	2.68 (2.20-3.26)	*0.01*
Beer	1.34 (1.23-1.46)	1.72 (1.47-2.01)	*0.006*
Spirits	1.05 (1.02-1.08)	1.13 (1.07-1.20)	*0.02*
Mixed drinks	1.07 (1.03-1.11)	1.22 (1.14-1.31)	*0.002*
Wine	1.39 (1.28-1.50)	1.35 (1.16-1.57)	0.7

Table 2: *Alcohol intake by bitter taster phenotype and sex.*

	Males			**Females**			
	Tasters	**Non-Tasters**		**Tasters**	**Non-Tasters**		
	Mean (95% CI)		***p-value***	***Mean (95% CI)***		***p-value***	$p_{interaction}$
Total	2.48 (2.08-2.95)	4.35 (3.14-6.03)	*0.003*	1.65 (1.45-1.87)	1.73 (1.37-2.19)	0.7	*0.02*
Beer	1.71 (1.44-2.03)	2.95 (2.13-4.07)	*0.004*	1.04 (1.00-1.07)	1.03 (0.97-1.09)	0.8	*0.002*
Spirits	1.06 (1.00-1.12)	1.22 (1.10-1.35)	*0.02*	1.05 (1.02-1.08)	1.07 (1.02-1.13)	0.4	*0.05*
Mixed drinks	1.10 (1.03-1.17)	1.30 (1.15-1.47)	*0.01*	1.04 (0.99-1.10)	1.15 (1.05-1.26)	0.06	0.3
Wine	1.38 (1.22-1.57)	1.42 (1.21-1.58)	0.8	1.42 (1.27-1.58)	1.32 (1.09-1.61)	0.6	0.5

Table 3: *Alcohol intake by TAS2R38-A49P genotype.*

	Presence of P variant	**Absence of P variant**	
	Mean (95% CI)		***p-value***
Total	2.01 (1.79-2.26)	2.44 (2.08-2.86)	*0.04*
Beer	1.37 (1.25-1.51)	1.51 (1.33-1.72)	0.2
Spirits	1.04 (1.01-1.08)	1.12 (1.07-1.16)	*0.02*
Mixed drinks	1.06 (1.02-1.11)	1.18 (1.11-1.25)	*0.004*
Wine	1.38 (1.26-1.50)	1.39 (1.23-1.56)	0.9

Table 4: *Alcohol intake by TAS2R38-A49P genotype and sex.*

	Males			**Females**			
	Presence of P variant	**Absence of P variant**	**p-value**	**Presence of P variant**	**Absence of P variant**	**p-value**	$p_{interaction}$
	Mean (95% CI)			***Mean (95% CI)***			
Total	2.47 (2.05-2.98)	3.70 (2.81-4.88)	*0.02*	1.66 (1.43-1.93)	1.68 (1.41-2.01)	0.9	*0.04*
Beer	1.77 (1.47-2.04)	2.41 (1.95-3.06)	*0.05*	1.05 (1.01-1.09)	1.02 (0.97-1.06)	0.3	*0.05*
Spirits	1.05 (0.99-1.11)	1.18 (1.09-1.29)	*0.02*	1.04 (1.01-1.08)	1.06 (1.02-1.11)	0.5	0.06
Mixed drinks	1.09 (1.02-1.17)	1.25 (1.13-1.39)	*0.02*	1.03 (0.97-1.09)	1.12 (1.05-1.20)	0.06	0.4
Wine	1.37 (1.20-1.57)	1.44 (1.18-1.75)	0.7	1.41 (1.25-1.60)	1.37 (1.18-1.59)	0.7	0.6

Table 5: *Percentage contribution of alcohol to overall energy intake, by bitter taster phenotype and TAS2R38 genotype.*

	Phenotype			**Genotype**		
	Tasters	***Non-tasters***	***p-value***	***Presence of P variant***	***Absence of P variant***	***p-value***
Complete cohort	4.0% (3.1-4.9%)	7.0% (5.3-8.85)	*0.003*	4.0% (3.0-5.1%)	5.9% (4.5-7.3%)	*0.04*
Females	2.5% (1.7-3.4%)	3.0% (1.5-4.5%)	0.6	2.5% (1.5-3.4%)	2.9% (1.8-4.1%)	0.5
Males	5.5% (3.9-7.1%)	11.5% (8.3-14.7%)	*0.001*	5.7% (3.9-7.5%)	9.1% (6.4-11.8%)	*0.03*
$p_{interaction}$ (sex)		*0.006*			*0.05*	

References

1. W. Meyerhof, M. Behrens, A. Brockhoff, B. Bufe and C. Kuhn, Human bitter taste perception, *Chem Senses*, 2005, **30 Suppl 1**, i14-15.
2. W. Meyerhof, Elucidation of mammalian bitter taste, *Rev Physiol Biochem Pharmacol*, 2005, **154**, 37-72.
3. A. Drewnowski and C. Gomez-Carneros, Bitter taste, phytonutrients, and the consumer: a review., *Am J Clin Nutr.*, 2000, **72**, 1424-1435.
4. A. Drewnowski, The science and complexity of bitter taste, *Nutr Rev*, 2001, **59**, 163-169.
5. V. Duffy and L. Bartoshuk, Food acceptance and genetic variation in taste., *J Am Diet Assoc*, 2000, **1006647-55**.
6. K. Maehashi and L. Huang, Bitter peptides and bitter taste receptors, *Cell Mol Life Sci*, 2009, **66**, 1661-1671.
7. U. K. Kim, E. Jorgenson, H. Coon, M. Leppert, N. Risch and D. Drayna, Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide, *Science*, 2003, **299**, 1221-1225.
8. E. Adler, M. Hoon, K. Mueller, J. Chandrashekar, N. Ryba and C. Zuker, A novel family of mammalian taste receptors., *Cell Biochem Biophys*, 2000, **100**, 693-702.
9. J. Chandrashekar, K. Mueller, M. Hoon, E. Adler, L. Feng, W. Guo, C. Zuker and N. Ryba, T2Rs function as bitter taste receptors., *Cell*, 2000, **100**, 703-711.
10. E. E. Harris and D. Meyer, The molecular signature of selection underlying human adaptations, *Am J Phys Anthropol*, 2006, **Suppl 43**, 89-130.
11. U. K. Kim, P. A. Breslin, D. Reed and D. Drayna, Genetics of human taste perception, *J Dent Res*, 2004, **83**, 448-453.
12. U. K. Kim and D. Drayna, Genetics of individual differences in bitter taste perception: lessons from the PTC gene, *Clin Genet*, 2005, **67**, 275-280.
13. B. J. Tepper, E. A. White, Y. Koelliker, C. Lanzara, P. d'Adamo and P. Gasparini, Genetic variation in taste sensitivity to 6-n-propylthiouracil and its relationship to taste perception and food selection, *Ann N Y Acad Sci*, 2009, **1170**, 126-139.
14. J. Hansen, D. Reed, M. Wright, N. Martin and P. Breslin, Heritability and genetic covariation of sensitivity to PROP, SOA, quinine HCl, and caffeine., *Chem Senses.*, 2006, **31**, 403-413.
15. A. Drewnowski, S. Henderson and A. Shore, Taste responses to naringin, a flavonoid, and the acceptance of grapefruit juice are related to genetic sensitivity to 6-n-propylthiouracil., *Am J Clin Nutr.* , 1997, **66**, 391-397.
16. J. Guinard, D. Hong, C. Zoumas-Morse, C. Budwig and G. Russell, Chemoreception and perception of the bitterness of isohumulones., *Physiol Behav.*, 1994, **56**, 1257-1263.
17. D. Mela, Gustatory perception of isohumulones: influence of sex and thiourea taster status, *Chem. Senses*, 1990, **15**, 485-490.
18. J. Delwiche, Z. Buletic and P. Breslin, Relationship of papillae number to bitter intensity of quinine and PROP within and between individuals., *Physiol Behav.* , 2001, **74**, 329-337.
19. L. M. Bartoshuk and G. K. Beauchamp, Chemical senses, *Annu Rev Psychol*, 1994, **45**, 419-449.
20. D. Reed, L. Bartoshuk, V. Duffy, S. Marino and R. Price, Propylthiouracil tasting: determination of underlying threshold distributions using maximum likelihood., *Chem Senses.* , 1995, **20**, 529-533.
21. B. Bufe, P. A. Breslin, C. Kuhn, D. R. Reed, C. D. Tharp, J. P. Slack, U. K. Kim, D. Drayna and W. Meyerhof, The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception, *Curr Biol*, 2005, **15**, 322-327.

22. S. Wooding, U. K. Kim, M. J. Bamshad, J. Larsen, L. B. Jorde and D. Drayna, Natural selection and molecular evolution in PTC, a bitter-taste receptor gene, *Am J Hum Genet*, 2004, **74**, 637-646.
23. U. Kim, S. Wooding, D. Ricci, L. B. Jorde and D. Drayna, Worldwide haplotype diversity and coding sequence variation at human bitter taste receptor loci, *Hum Mutat*, 2005, **26**, 199-204.
24. D. Drayna, H. Coon, U. Kim, T. Elsner, K. Cromer, B. Otterud, L. Baird, A. Peiffer, M. Leppert and U. G. R. Project., Genetic analysis of a complex trait in the Utah Genetic Reference Project: a major locus for PTC taste ability on chromosome 7q and a secondary locus on chromosome 16p., *Hum Genet.* , 2003, **112**, 576-572.
25. J. Mennella, M. Pepino, F. Duke and D. Reed, Age modifies the genotype-phenotype relationship for the bitter receptor TAS2R38., *BMC Genet.*, 2010, **11**, doi: 10.1186/1471-2156-1111-1160.
26. S. W. Guo and D. R. Reed, The genetics of phenylthiocarbamide perception, *Ann Hum Biol*, 2001, **28**, 111-142.
27. A. F. Blakeslee, Genetics of Sensory Thresholds: Taste for Phenyl Thio Carbamide, *Proceedings of the National Academy of Sciences of the United States of America*, 1932, **18**, 120-130.
28. L. H. Snyder, INHERITED TASTE DEFICIENCY, *Science*, 1931, **74**, 151-152.
29. L. M. Bartoshuk, Genetic and pathological taste variation: what can we learn from animal models and human disease?, *Ciba Found Symp*, 1993, **179**, 251-262; discussion 262-257.
30. V. Duffy, A. Davidson, J. Kidd, K. Kidd, W. Speed, A. Pakstis, D. Reed, D. Snyder and L. Bartoshuk, Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake., *Alcohol Clin Exp Res.*, 2004, **28**, 1629-1637.
31. J. Prescott and N. Swain-Campbell, Responses to repeated oral irritation by capsaicin, cinnamaldehyde and ethanol in PROP tasters and non-tasters., *Chem Senses.*, 2000, **25**, 239-246.
32. A. A. Nolden, J. E. McGeary and J. E. Hayes, Differential bitterness in capsaicin, piperine, and ethanol associates with polymorphisms in multiple bitter taste receptor genes, *Physiol Behav*, 2016, **156**, 117-127.
33. A. L. Allen, J. E. McGeary and J. E. Hayes, Polymorphisms in TRPV1 and TAS2Rs associate with sensations from sampled ethanol, *Alcohol Clin Exp Res*, 2014, **38**, 2550-2560.
34. J. E. Hayes, M. R. Wallace, V. S. Knopik, D. M. Herbstman, L. M. Bartoshuk and V. B. Duffy, Allelic variation in TAS2R bitter receptor genes associates with variation in sensations from and ingestive behaviors toward common bitter beverages in adults, *Chem Senses*, 2011, **36**, 311-319.
35. L. R. Intranuovo and A. S. Powers, The perceived bitterness of beer and 6-n-propylthiouracil (PROP) taste sensitivity, *Ann N Y Acad Sci*, 1998, **855**, 813-815.
36. S. Lanier, J. Hayes and V. Duffy, Sweet and bitter tastes of alcoholic beverages mediate alcohol intake in of-age undergraduates., *Physiol Behav.* , 2005, **83**, 821-831.
37. G. J. Pickering, K. Simunkova and D. DiBattista, Intensity of taste and astringency sensations elicited by red wines is associated with sensitivity to PROP (6-n-propylthiouracil), *Food Quality and Preference*, 2004, **15**, 147-154.
38. R. D. Mattes and D. DiMeglio, Ethanol perception and ingestion, *Physiol Behav*, 2001, **72**, 217-229.
39. K. A. Driscoll, M. Perez, K. C. Cukrowicz, M. Butler and T. E. Joiner, Jr., Associations of phenylthiocarbamide tasting to alcohol problems and family history of alcoholism differ by gender, *Psychiatry research*, 2006, **143**, 21-27.
40. S. T. DiCarlo and A. S. Powers, Propylthiouracil tasting as a possible genetic association marker for two types of alcoholism, *Physiol Behav*, 1998, **64**, 147-152.

41. M. L. Pelchat and S. Danowski, A possible genetic association between PROP-tasting and alcoholism, *Physiol Behav*, 1992, **51**, 1261-1266.
42. J. C. Wang, A. L. Hinrichs, S. Bertelsen, H. Stock, J. P. Budde, D. M. Dick, K. K. Bucholz, J. Rice, N. Saccone, H. J. Edenberg, V. Hesselbrock, S. Kuperman, M. A. Schuckit, L. J. Bierut and A. M. Goate, Functional variants in TAS2R38 and TAS2R16 influence alcohol consumption in high-risk families of African-American origin, *Alcohol Clin Exp Res*, 2007, **31**, 209-215.
43. H. R. Kranzler, P. J. Moore and V. M. Hesselbrock, No association of PROP taster status and paternal history of alcohol dependence, *Alcohol Clin Exp Res*, 1996, **20**, 1496-1500.
44. H. R. Kranzler, K. Skipsey and V. Modesto-Lowe, PROP taster status and parental history of alcohol dependence, *Drug and alcohol dependence*, 1998, **52**, 109-113.
45. N. C. Reid, P. W. Brunt, W. B. Bias, W. C. Maddrey, B. A. Alonso and F. L. Iber, Genetic characteristics and cirrhosis: a controlled study of 200 patients, *British medical journal*, 1968, **2**, 463-465.
46. S. E. Smith, Taste thresholds in drug addicts and alcoholics, *The British journal of addiction to alcohol and other drugs*, 1972, **67**, 317-321.
47. R. P. Swinson, Phenylthiocarbamide taste sensitivity in alcoholism, *The British journal of addiction to alcohol and other drugs*, 1973, **68**, 33-36.
48. N. Gorovic, S. Afzal, A. Tjonneland, K. Overvad, U. Vogel, C. Albrechtsen and H. E. Poulsen, Genetic variation in the hTAS2R38 taste receptor and brassica vegetable intake, *Scand J Clin Lab Invest*, 2011, **71**, 274-279.
49. O. Ramos-Lopez, S. Roman, E. Martinez-Lopez, K. Gonzalez-Aldaco, C. Ojeda-Granados, M. Sepulveda-Villegas and A. Panduro, Association of a novel TAS2R38 haplotype with alcohol intake among Mexican-Mestizo population, *Annals of hepatology*, 2015, **14**, 729-734.
50. J. H. Choi, J. Lee, I. J. Choi, Y. W. Kim, K. W. Ryu and J. Kim, Genetic Variation in the TAS2R38 Bitter Taste Receptor and Gastric Cancer Risk in Koreans, *Scientific reports*, 2016, **6**, 26904.
51. M. Keller, X. Liu, T. Wohland, K. Rohde, M. T. Gast, M. Stumvoll, P. Kovacs, A. Tonjes and Y. Bottcher, TAS2R38 and its influence on smoking behavior and glucose homeostasis in the German Sorbs, *PLoS One*, 2013, **8**, e80512.
52. N. Timpson, M. Christensen, D. Lawlor, T. Gaunt, I. Day, S. Ebrahim and G. Davey Smith, TAS2R38 (phenylthiocarbamide) haplotypes, coronary heart disease traits, and eating behavior in the British Women's Heart and Health Study., *Am J Clin Nutr.*, 2005, **81**, 1005-1011.
53. A. Scinska, E. Koros, B. Habrat, A. Kukwa, W. Kostowski and P. Bienkowski, Bitter and sweet components of ethanol taste in humans, *Drug and alcohol dependence*, 2000, **60**, 199-206.
54. R. Mattes, Influences on acceptance of bitter foods and beverages., *Physiol Behav.*, 1994, **56**, 1229-1236.
55. A. M. J. Anacker and A. E. Ryabinin, Biological Contribution to Social Influences on Alcohol Drinking: Evidence from Animal Models, *International Journal of Environmental Research and Public Health*, 2010, **7**, 473-493.
56. M. J. Smith, A. Abbey and R. O. Scott, Reasons for Drinking Alcohol: Their Relationship to Psychosocial Variables and Alcohol Consumption, *International Journal of the Addictions*, 1993, **28**, 881-908.
57. H. W. Perkins, Religious Traditions, Parents, and Peers as Determinants of Alcohol and Drug Use among College Students, *Review of Religious Research*, 1985, **27**, 15-31.
58. V. B. Duffy, J. M. Peterson and L. M. Bartoshuk, Associations between taste genetics, oral sensation and alcohol intake, *Physiol Behav*, 2004, **82**, 435-445.
59. M. Lucock, Z. Yates, C. Martin, J. H. Choi, E. Beckett, L. Boyd, K. LeGras, X. Ng, V. Skinner, R. Wai, J. Kho, P. Roach and M. Veysey, Methylation diet and methyl group genetics in risk for adenomatous polyp occurrence, *BBA clinical*, 2015, **3**, 107-112.
60. D. o. H. Australian Government, *Journal*.

61. M. Lucock, X. Ng, L. Boyd, V. Skinner, R. Wai, S. Tang, C. Naylor, Z. Yates, J. H. Choi, P. Roach and M. Veysey, TAS2R38 bitter taste genetics, dietary vitamin C, and both natural and synthetic dietary folic acid predict folate status, a key micronutrient in the pathoaetiology of adenomatous polyps, *Food Funct*, 2011, **2**, 457-465.
62. A. F. Blakeslee, Genetics of Sensory Thresholds: Taste for Phenyl Thio Carbamide, *Proc Natl Acad Sci U S A*, 1932, **18**, 120-130.
63. N. Jacob, J.-L. Golmard and I. Berlin, Differential Perception of Caffeine Bitter Taste Depending on Smoking Status, *Chemosensory Perception*, 2014, **7**, 47-55.
64. CDC, Vital signs: binge drinking prevalence, frequency, and intensity among adults—U.S., 2010., *MMWR Morb Mortal Wkly Rep*, 2012, **61**, 14-19.