Mapping Genotype to Phenotype: The genetics of taste.

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Student Outline

Learning Objectives

1. Distinguish fungiform papillae (with taste buds) from filiform papillae (lacking taste buds) on the anterior human tongue.
2. Learn about the differential response of supertasters, medium tasters, and nontasters to the bitter compound PROP (*(6-n-propylthiouracil).*
3. Understand how sensitivity to bitter taste may influence food choice and health.

Materials and Methods

Blue Food Coloring	Cotton-tipped Swab Applicators
Paper Cups, Small	PROP Test Paper (6-*n*-propylthiouracil)
Reinforcement Labels	Digital Camera

Procedure

1. Taste unsweetened vegetable juice and record taste reaction as *dislike*, *neutral*, or *like*. Rinse mouth with water.

2. Take one PROP taste paper and place on tongue. Identify reaction as *strongly bitter*, *bitter*, or *no taste* which is indicative of a super-taster, taster, or non-taster respectively. Record your taster status. Rinse mouth with water.

3. Dab some blue food color on a cotton swab. Using a mirror for guidance, swab the tip of the tongue with blue food color. Fungiform papillae which contain taste buds will not stain and appear pink against the background of filiform papillae which do not contain taste buds and stain blue (Figure 1). If the color is too dark, rub the tongue on the roof of the mouth. If too light, dab on a little more blue dye.

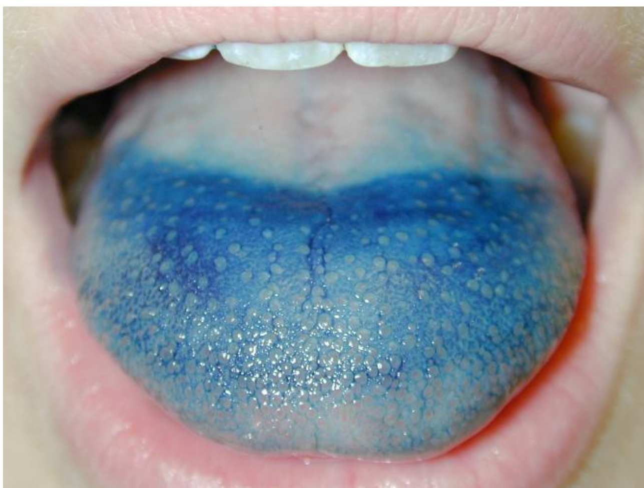


Figure 1. Anterior tongue stained with blue food coloring. Fungiform papillae appear pink against the background of blue filiform papillae.

4. Place a reinforcement label (Figure 2) on the tongue tip. Stick out your tongue to cover the lower lip. Gently close your mouth and use your teeth to hold the tongue in place. Shine a flashlight on the exposed tongue. Phototgraph the tongue. Count the number of pink fungiform papillae in the center hole of the reinforcement label. Have your lab partner verify the count. Record the number.

Figure 2. Adhesive reinforcement label placed on the tongue tip. Papillae are counted in the encircled area.

Example data table (data to be collated from the entire class).

Sl No.	Sex	PROP Taster status	Response to juice	No. of Papillae	Density
1	Male	Super Taster	Like		
2	Male	Taster	Dislike		
3	Male	Non-Taster	Indifferent		
4	Female	…	…		
…	…	…	…		
…	…	…	…		

5. Calculate the density of the fungiform papillae per cm2 in the area encircled. Recall that the area of a circle $= \pi r^2$. Calculate the density of the fungiform papillae per cm^2 in the area encircled.

6. How many super-tasters, tasters and non-tasters are there in your class? Is the distribution of super-tasters, tasters and non-tasters the same between the two sexes? Depict the results graphically (Bar Chart). How would you test this statistically?

7. Calculate the class mean for both the number and density of fungiform papillae for each PROP taster status: supertaster, medium taster, and nontaster. How does PROP taster status correspond to the mean number and density of fungiform papillae? Represent the

results in the form of a table as shown below. How would you test the differences statistically (Hint: Ananlysis of Variance or ANOVA).

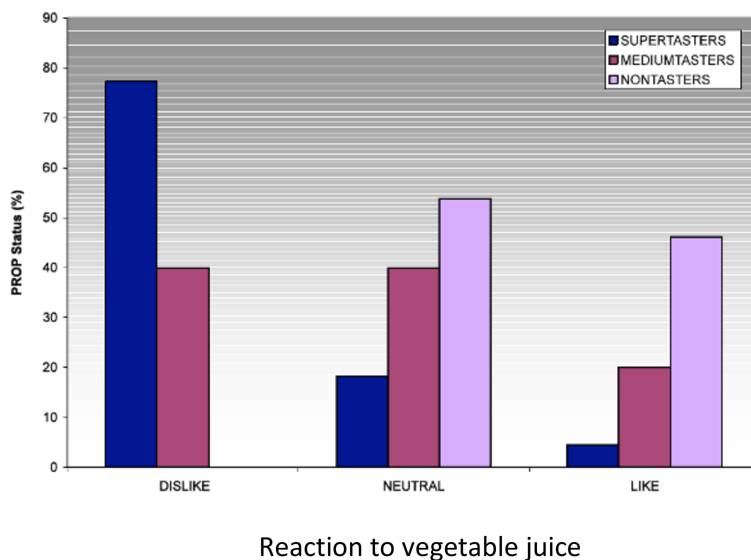
Example Result Table

Table 1. Mean number of papillae and mean density of papillae/cm ^{2} according to PROP taster status. (N_{total} = 50, +/- standard deviation, area of sample = 0.3165 cm^2 , *ANOVA Single Factor, p<0.001)

Fungiform Papillae	Supertasters (n=22)	Medium Tasters (n=15)	Nontasters (n=13)
Mean	20.68 +/- 8.78*	13.47 +/- 7.01*	10.69 +/- 4.21*
Mean Density ($/cm^2$)	65.38 +/- 27.74*	42.55 +/- 22.14*	33.78 +/- 13.30*

8. How does the taste reaction to vegetable juice correspond to PROP taster status? Represent the results using a bar diagram as shown below. Is the hypothesis, that a subject's taste response to vegetable juice will predict both PROP status and the relative density of fungiform papillae, supported? Explain.

Example Bar Chart



Reaction to vegetable juice

Background:
What makes us taste differently?
In 2003, Kim *et al.* located and sequenced the *TAS2R38* or *PTC* gene on chromosome 7 responsible for differently tasting Phenyl Thio Carbamide (PTC) a chemical very similar to PROP. This gene encodes for one of the estimated 25 bitter–taste receptor proteins present in taste buds. Three common SNPS (single nucleotide polymorphisms) based on three amino acid substitutions have been identified in the *TAS2R38* gene and account for five different haplotypes found in human populations. The two most common are PAV (proline-alaninevaline) identified as the major taster haplotype and AVI (alanine-valine-isoleucine) as the major
nontaster haplotype. Individuals with two copies of the AVI haplotype are largely nontasters whereas either one or two copies of the PAV haplotype were mostly tasters. PAV

homozygotes are more sensitive to PTC/PROP than PAV/AVI heterozygotes (Kim and Drayna 2004, Minella *et al.* 2005, Reed *et al*. 2006).

Miller and Reedy (1990) developed a method using methylene blue solution to stain the anterior tongue. Filiform papillae which do not contain taste buds stained a deep blue whereas tastebud containing fungiform papillae stained lightly and could be counted against the dark blue background of filiform papillae. They discovered that there were variations in both the number of fungiform papillae and the number of taste buds on the papillae among test subjects, and suggested that these differences might account for the observed variations in taste sensitivity among individuals. Several studies (Bartoshuk *et al*.1994, Delwiche *et al.* 2001) confirmed that the perceived bitterness of PROP tended to increase with the density of fungiform papillae.

Why should we bother?

People are suggested to eat 5-9 servings of fruits and vegetables daily to promote consumption of phytochemicals as a dietary strategy for disease prevention. For most people, taste is the main determinant in food selection and perceived bitterness in a food is often the primary reason for its rejection. Many phytochemicals, such as the flavonoid naringin in grapefruit juice and glucosinolates in cruciferous vegetables (broccoli, cabbage, kale, etc.) are bitter-tasting. Several studies reported that supertasters showed a tendency to avoid certain foods that they perceive as very bitter (Drewnowski *et al*.1997, Dinehart *et al*. 2006). The consequences of diet choice to health may be significant. A study of men over 65 who had been identified as supertasters had a significantly higher number of colon polyps, a finding which is associated with a higher risk of colon cancer (Milius, 2003). The supertasters reported that they avoided strong vegetable tastes. The diet of a supertaster appeared to be deficient in both protective phytochemicals and fiber which led to the higher formation of polyps, raising the risk of colon cancer.

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Linda Bartoshuk Honorary Symposium

Associations between oral sensation, dietary behaviors and risk of cardiovascular disease (CVD)

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Abstract

We hypothesize that variation in oral sensation influences chronic disease risk by impacting dietary behaviors. Bitterness of 6-*n*-propylthiouracil (PROP) and fungiform papilla (FP) number serve as genetic taste markers. Data support that nontasters (who taste PROP as least bitter or have lowest FP number) show dietary behaviors that increase CVD risk (e.g. higher alcohol intake, greater preference for and intake of high-fat and sweet foods) and have greater measured CVD risk (e.g. higher blood pressure, less favorable serum lipids). Taste genetics interacts with environmental factors (e.g. taste-related pathologies) to affect oral sensation, dietary behaviors and disease risk. The generalizability of oral sensory and CVD risk relationships has begun to be tested on diverse samples.
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Keywords: Taste genetics; Preference; Bitter; Sweet; Fat; Alcohol; Diet; Cardiovascular disease

Introduction

This paper summarizes a talk given to celebrate Linda Bartoshuk's election into the National Academy of Sciences. Bartoshuk has pioneered the characterization of genetic and environmental variation in oral sensation with advances in psychophysics. We apply this pioneering work to study variation in oral sensation, dietary behaviors (preference, intake) and CVD risks. Oral sensations from foods and beverages vary genetically and with conditions that affect nerve systems underlying these sensations. We hypothesize that variability in oral sensations explains differences in preference for foods/beverages, and, since we eat what we like and avoid what we do not, our dietary intake and ultimately CVD risk. Limiting fat, alcohol and salt intakes, increasing fruit/vegetable intakes, and maintaining healthy weight decreases dietary risk of CVD (AHA, 2000).

Fox discovered blindness to phenylthiocarbamide (PTC) bitterness (Fox, 1931). Family studies suggested this trait as homozygous recessive (Blakeslee, 1932; Snyder, 1931). PTC and PROP share a N–C=S group and produce bimodal thresholds to distinguish nontasters (elevated threshold) from tasters (low threshold). Bartoshuk, Duffy, and Miller (1994) applied modern psychophysical tools to scale PROP bitterness and separate tasters into medium tasters (PROP as bitter) and supertasters (PROP as exceptionally bitter). Supertasters taste salty, sweet, sour, and additional bitter compounds more intensely than do nontasters (Prutkin et al., 2000), in part because of greater numbers of fungiform papilla (FP) (structures housing taste buds on the anterior tongue) (Bartoshuk et al., 1994). Since taste buds hold pain and touch fibers (Whitehead, Beeman, & Kinsella, 1985), supertasters feel the most oral irritation (Prutkin et al., 2000) and touch sensations (reviewed below). The PTC/PROP gene (Kim et al., 2003) explains some of the variability in PROP bitterness. Supertasters carry one or both dominant PTC/PROP genes but must also have a high FP density. Additional genes controlling FP density should contribute to oral sensory effects on diet and health.

The study of taste genetics and dietary behaviors has been contentious. Some controversy stems from measuring oral thresholds, which may not reflect perception of concentrated stimuli and thus be irrelevant to dietary experiences. Other controversies involve invalid methods to compare sensory intensity and hedonic experiences

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across individuals (Bartoshuk, Duffy, Fast, Green, & Snyder, 2002) or limited control for variables that confound studies of oral sensation and diet (e.g. dietary restraint, intake studies with undergraduates). Some studies fail to show PROP effects on oral sensations and, not surprisingly, also fail to find PROP effects on intake. While oral sensation may play a large role in what we like to eat, humans have a vast array of cognitive and external influences to guide food choice. If PROP influences chronic disease through diet, the effect size should be the smallest as these diseases have multiple causes.

Our studies employ multiple phenotypical measures to characterize genetic and environmental (e.g. pathological, hormonal) variation in oral sensation. PROP bitterness and FP density each predict food preference (Duffy & Bartoshuk, 2000). FP density may provide a stable measure of genetic endowment least influenced by environment while taste markers reflect genetic and environmental influences. Individuals with elevated PROP bitterness and FP should have different oral sensations and dietary behaviors than those low on both measures or for whom the two measures are discordant (Duffy, Lucchina, & Bartoshuk, 2004). Based on Fischer, Griffin, England, and Garn (1961), use of PROP and other taste markers, including quinine (Duffy, Peterson, Dinehart, & Bartoshuk, 2003) and NaCl (Duffy, Peterson, & Bartoshuk, submitted), increases prediction of dietary behaviors.

Two sources of data fuel our investigation of oral sensation, diet and CVD risk. One is a laboratory study primarily of Caucasian adults (range 20–39 years) who were healthy, denied smoking, or having high dietary restraint, which causes underreporting of intake (Bathalon et al., 2000). Males and females were recruited for variability in PROP bitterness, but without significant history of taste-related pathology to confound taste genetic classification (Bartoshuk, Duffy, Reed, & Williams, 1995). Over three visits we assessed oral sensations, food/beverage preference and intake, body composition, PROP tasting and FP number, and collected blood samples for serum lipids and gene analyses. A PROP threshold and FP counting with videomicroscopy and blue food coloring (Miller & Reedy, 1990) were completed during the first session. PROP was scaled on the general Labeled Magnitude Scale (gLMS) (Bartoshuk et al., 2002) during the last session. Intake was assessed with a frequency questionnaire (Block, 1997) and five, 24-h food records. Measures of taste genetics neither associated significantly with total energy intake nor that expressed to body weight.

The second source was an oral sensory screening within a health risk appraisal of primarily middle-aged Caucasian males (range 25–60 years). The employees completed health/nutrition questionnaires, were measured for CVD risk (adiposity, blood pressure, serum lipids), and screened for taste genetic markers (visual FP counting and bitterness of PROP paper (Bartoshuk et al., 1995) on the gLMS).

Alcohol preference and intake

Alcohol in ethanol solutions (Bartoshuk et al., 1993; Duffy et al., submitted; Prescott & Swain-Campbell, 2000), some beers (Intranuovo & Powers, 1998) and wines (Pickering, Simunkova, & DiBattista, 2004) is more bitter and irritating to those who taste PROP as more bitter than those who do not. Is this a noxious enough experience for PROP supertasters to act as a sensory hindrance to over-consumption of alcoholic beverages? Nontasters are reported to be high beer consumers (Guinard et al., 1996) especially during the first year of drinking (Intranuovo & Powers, 1998). Use of thresholds may explain some of the inconsistency for PROP effects on risk of alcoholism: studies support (Pelchat & Danowski, 1992) or fail to support (Kranzler, Moore, & Hesselbrock, 1996; Kranzler, Skipsey, & Modesto-Lowe, 1998) more nontasters among the offspring of alcoholics than non-alcoholics. One study showed that college students having individual or family problems with alcoholism were more likely to report PROP as strongly bitter (DiCarlo & Powers, 1998).

From our screening and laboratory studies, those who tasted PROP as least bitter reported consuming alcoholic beverages most frequently, effects separate of sex and age. The screening study found that lowest PROP bitterness also associated with highest blood pressure, independently of age and sex (Hutchins, Pescatello, Allen, & Duffy, 2002a,b). The PROP-blood pressure association could be partially mediated through alcohol intake as over-consumption increases risk of hypertension (Flack et al., 2003). Our laboratory study predicted alcohol intakes from markers of taste genetics and those linked with environmental insult. Individuals who reported PROP and NaCl as most intense had the highest alcohol intakes (Duffy et al., submitted). The latter finding was similar to a study showing that individuals with a family history of alcoholism tasted NaCl as most intense (Sandstrom, Rajan, Feinn, & Kranzler, 2003). Elevated NaCl intensity may be more reflective of environmental rather than genetic influences on taste. Our study showed that those with depressed chorda tympani taste relative to intensified whole mouth NaCl, a spatial taste pattern that could result from taste-related pathology, drank the most alcohol.

Sweet preference and intake

A number of sweeteners are more intense to those who taste PROP as more bitter (Prutkin et al., 2000). Less liking for sweetness has been reported in PROP tasters (Looy & Weingarten, 1992) and supertasters (Duffy & Bartoshuk, 2000), effects seen primarily in females. These studies fueled the question: could PROP effects on sweet sensation/hedonics influence intake of sugars and ultimately increase risk of dyslipidemia associated with high-sugar diets (Parks & Hellerstein, 2000)?

Based on the logic of Fischer et al. (1961), we found effects of PROP bitterness *and* quinine bitterness on sweet preference and intake (Duffy et al., 2003). Those who tasted PROP as less bitter or QHCl as more bitter reported a higher sweet preference and greater intake of added sugars (frequency of consuming sweet foods or energy from added sugars). Individuals discordant in PROP versus QHCl bitterness (e.g. low PROP but high QHCl bitterness) also varied in sweet preference and intake. Interactions between genetics and environment may contribute to this discordance. Using both markers increased the ability to predict sweet behaviors. As with the alcohol findings, there appears to be environmentally and genetically mediated effects on oral sensation and sweet intake. Genetic and environmental effects on oral sensations may also offer explanation for PROP/sex interactions on sweet preference.

Fat preference and intake

PROP bitterness and/or FP number shows positive associations with creamy/oiliness of high-fat milks and foods (Duffy et al., 2004), salad dressings (Tepper & Nurse, 1997) and corn oil (Prutkin, Fast, Lucchina, & Bartoshuk, 1999), viscous sensations from guar gum (Prutkin et al., 1999) and tongue tactile sensitivity (Essick, Chopra, Guest, & McGlone, 2003).

For preference, Tepper and Nurse (1998) reported that nontasters liked sampled salad dressings more than medium or supertasters. Our laboratory study found PROP and sex effects on preference for sampled high-fat foods (Duffy et al., 2004). Lower PROP bitterness was associated with greater fat preference in females; males showed opposite responses. Similar sex/PROP interactions have been seen from questionnaire preference ratings (Duffy & Bartoshuk, 2000; Duffy et al., 2004) and in preschool children (Keller, Steinman, Nurse, & Tepper, 2002). Applying multiple regression analyses to our laboratory data revealed that PROP, sex and creaminess were significant predictors of preference for sampled high-fat foods. Men and those reporting the foods as more creamy as well as tasting PROP as least bitter preferred these foods most. Nonetheless, females who tasted PROP above strong reported that greater creaminess was less pleasant.

FP number may predominate contributions of taste genetics to predicting fat sensations and dietary behaviors and offer a sensory explanation for PROP/sex interactions on fat behaviors. Females in the laboratory study were skewed toward highest FP number, a finding seen previously (Prutkin et al., 2000). Women who taste PROP as very bitter and have high FP may experience a much different array of oral sensations from high-fat foods than men who taste PROP as very bitter, but do not possess as many FP. Number of FP was a better predictor of fat preference *and* intake than was PROP in our screening study (Duffy, Hutchins, Allen, & Pescatello, 2002; Hutchins et al., 2002a,b). From the laboratory study, PROP bitterness and FP number were negatively correlated with fat intake across 40 high-fat foods, which approached significance in females. However, the number of negative associations with FP number (29 of 40) exceeded that for PROP (25 of 40). In multiple regression analyses, men and those with fewer FP consumed these foods significantly more frequently. Similar analyses showed significant sex effects but the PROP effects only neared significance ($p = 0.06$).

Preliminary evidence also shows environmental influences on fat sensations and dietary behaviors including aging (Chapo, Bartoshuk, Ilich, & Duffy, 2002) and exposure to taste-related pathology (Snyder, Duffy, Chapo, Cobbett, & Bartoshuk, 2003). Interplay between genetics and environment may also explain some of the PROP/sex interactions on fat behaviors.

Adiposity and serum lipids

Fischer, Griffen, and Rockey (1966) concluded that nontasters of quinine and PROP were short, soft, round, and fat while those with low thresholds to these compounds, tall and lean. Recent data support these conclusions. From Lucchina's (1995) doctoral work, elderly females who tasted PROP as more bitter had lower measured adiposity and more favorable serum lipids (Duffy et al., 2004). Two screening studies find that greater PROP bitterness was associated with lower fat preference and lower body mass indices in non-obese individuals, one self-reported height/weight (Duffy, Fast, Cohen, Chodos, & Bartoshuk, 1999) and the other measured (Hutchins et al., 2002a,b). PROP effects on adiposity in the obese are probably overshadowed by multiple causes of obesity, including socio-cultural factors (Tepper & Ullrich, 2001). Environmental influences on oral sensation and preference also may contribute. Our screening study shows greater risk of dyslipidemia in those tasting PROP as least bitter and/or having least FP (Duffy et al., 2004; Hutchins et al., 2002a,b).

Summary thoughts

The application of Bartoshuk's work on characterization of oral sensation has proven fruitful for investigations on oral sensation, diet and diet-related diseases. Application of psychophysical and genetic markers of oral sensation holds promise to delineate interactions between genetic and environmental determinants of diet and health.

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Bitter Receptor Gene (*TAS2R38*), 6-*n*-Propylthiouracil (PROP) Bitterness and Alcohol Intake

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Abstract

Background—Phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP), chemically related compounds, are probes for genetic variation in bitter taste, although PROP is safer with less sulfurous odor. Threshold for PROP distinguishes nontasters (increased threshold) from tasters (lower threshold); perceived intensity subdivides tasters into medium tasters (PROP is bitter) and supertasters (PROP is very bitter). Compared with supertasters, nontasters have fewer taste papillae on the anterior tongue (fungiform papillae) and experience less negative (e.g., bitterness) and more positive (eg, sweetness) sensations from alcohol. We determined whether the *TAS2R38* gene at 7q36 predicted PROP bitterness, alcohol sensation and use.

Methods—Healthy adults (53 women, 31 men; mean age 36 years)—primarily light and moderate drinkers—reported the bitterness of five PROP concentrations (0.032–3.2 mM) and intensity of 50% ethanol on the general Labeled Magnitude Scale. PROP threshold and density of fungiform papillae were also measured. Subjects had common *TAS2R38* gene haplotypes [alanine-valine-isoleucine (AVI) and proline-alanine-valine (PAV)].

Results—PROP bitterness varied significantly across genotypes with repeated measures ANOVA: 26 AVI/AVI homozygotes tasted less bitterness than either 37 PAV/AVI heterozygotes or 21 PAV/PAV homozygotes. The PAV/PAV group exceeded the PAV/AVI group for bitterness only for the top PROP concentrations. The elevated bitterness was musch less than if we defined the groups using psychophysical criteria. With multiple regression analyses, greater bitterness from 3.2 mM PROP was a significant predictor of greater ethanol intensity and less alcohol intake—effects separate from age and sex. Genotype was a significant predictor of alcohol intake, but not ethanol intensity. With ANOVA, AVI/AVI homozygotes reported higher alcohol use than either PAV/AVI heterozygotes or PAV/PAV homozygotes. When age effects were minimized, PROP bitterness explained more variance in alcohol intake than did the *TAS2R38* genotype.

Conclusions—These results support taste genetic effects on alcohol intake. PROP bitterness serves as a marker of these effects.

Keywords

Taste; Alcohol Consumption; Genetics; PTC/PROP Bitterness; *TAS2R38*

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STRONG SUPPORT EXISTS for a familial component in the etiology of alcoholism and alcohol use [see Dick and Foroud (2003) for review]. Twin studies show that the heritability of alcoholism ranges from 50 to 60% (Heath et al., 1997) and that genetic influences can explain a 5-fold difference in alcohol use among adolescents in alcohol-predisposing environments (Dick et al., 2001). Genetic risk for alcoholism is complex; several different genes undoubtedly exert effects on the rewarding influence of drinking alcohol, on the metabolic tolerance of alcohol overconsumption, on brain systems that respond to reward, and on response to alcohol withdrawal (Crabbe, 2002). Specific gene mechanisms have been linked to, for example, the metabolism of alcohol via alcohol dehydrogenase (Mulligan et al., 2003; Osier et al., 1999) and aldehyde dehydrogenase (Oota et al., 2004), as well as dependence via γ-aminobutyric acid receptors (Song et al., 2003). The purpose of this study was to examine the association between genetic variation in taste and alcohol use in a group of reportedly healthy young adults.

Genetic variation in taste influences the sensations from alcoholic beverages and could be one of the genetic factors that interacts with environmental factors to determine the risk of alcohol overconsumption, as suggested by models of gene-environment interaction (Heath and Nelson, 2002). The ability to taste the bitterness of phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP), which share an N—C=S group, is a well documented phenotypic polymorphism. The distribution of thresholds for PTC or PROP tasting is bimodal: "nontasters" have increased thresholds (low sensitivity), and "tasters" have lower thresholds (higher sensitivity). Family studies have generally supported the model that tasting was a dominant trait and nontasting a recessive trait (Blakeslee, 1932; Snyder, 1931).

An important gene contributing to PTC perception has been identified (Kim et al., 2003). The gene (*TAS2R38*), located on chromosome 7q36, is a member of the bitter taste receptor family. There are two common molecular forms [proline-alanine-valine (PAV) and alanine-valine-isoleucine (AVI)] of this receptor defined by three nucleotide polymorphisms that result in three amino acid substitutions: Pro49Ala, Ala262Val, and Val296Ile. The ancestral human haplotype at these three amino acids—determined by sequencing DNA from several other ape species, an old world monkey, and a new world monkey—is PAV (Kim et al., 2003; Wooding et al., 2004). This molecular form is common in humans and is associated with tasting; the other common form, the triply derived molecular form, AVI, is associated with nontasting. Three other haplotypes have been observed: AAV, AAI, and PVI. The original report (Kim et al., 2003) studied 200 Europeans and 118 individuals from other regions.

Historically, researchers have used detection thresholds to classify individuals as nontasters or tasters of these bitter compounds (e.g., Fox, 1931; Harris and Kalmus, 1949). Fischer and Griffin (1964) replaced PTC with its chemical relative PROP, which lacks the sulfurous odor of PTC and may be less toxic (Barnicot et al., 1951; Lawless, 1980). Insensitivity to PTC or PROP is estimated at 30% in European populations, although the percentages vary with sex and among ethnic groups globally (Bartoshuk et al., 1994; Guo and Reed, 2001).

The taster group shows significant variability in the perceived bitterness of PROP. Although threshold measures may be used to separate individuals with low thresholds (tasters) from individuals with increased thresholds (nontasters), subsequent work by Bartoshuk et al. (1994) identified two distinct populations within the taster group. By comparing the perceived intensity of concentrated PROP, the taster group is subdivided into those who taste concentrated PROP (3.2 mM) as "strongly" bitter (medium tasters) and those who taste PROP as greater than "very strongly" bitter (supertasters) (Bartoshuk et al., 1994). Supertasters cannot be identified via thresholds, because the distributions between those who are sensitive and extremely sensitive to PROP overlap (Reed et al., 1995). Supertasters differ from medium tasters and nontasters in the number of taste papillae on the anterior tongue (fungiform papilla); PROP supertasters have, on average, the most fungiform papillae and taste buds as assessed

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with videomicroscopy (Bartoshuk et al., 1994). A positive relationship between PROP bitterness and fungiform papillae number is also observed by using lower magnification for papillae counting (Delwiche et al., 2001; Tepper and Nurse, 1997). Supertasting may result from an anatomical difference related to the density of fungiform papillae on the tongue, as well as an allelic variation of *TAS2R38* that results in the presence or absence of a functional receptor, as proposed by Bartoshuk et al. (2001) and as supported by data shown in this article. The genetic control of fungiform papilla density is unknown.

The perceived bitterness of PROP is correlated with unpleasant and pleasant sensations from alcohol. Those who taste PROP as more bitter also report ethanol (Bartoshuk et al., 1993; Duffy et al., 2004; Prescott and Swain-Campbell, 2000), some types of beer (Intranuovo and Powers, 1998), scotch (Lanier et al., 2004), and red wines (Pickering et al., 2004) as more bitter or irritating. Nontasters not only perceive scotch as less bitter but also as more sweet than do supertasters (Lanier et al., 2005). The density of fungiform papillae can explain some of the oral sensory differences associated with PROP tasting, as first suggested by Miller and Reedy (1990). The taste buds are surrounded by fibers of the trigeminal nerve (cranial nerve V), which are believed to mediate oral burn (Finger et al., 1994; Whitehead et al., 1985; Whitehead and Kachele, 1994). It is interesting to note that sucrose and ethanol stimulate similar central brain centers in rats (Lemon et al., 2004) and that ethanol stimulates taste nerve fibers responsive to sucrose in primates (Hellekant et al., 1997).

Supertasters may have an inherent sensory aversion to consuming alcoholic beverages with high levels of ethanol and a pronounced alcohol flavor. Young adults who taste PROP as more bitter have been found to consume less beer (Guinard et al., 1996), including during their first year of drinking (Intranuovo and Powers, 1998). In young adults who were not college undergraduates, we found that PROP supertasters reported consuming alcoholic beverages less frequently than did nontasters (Duffy et al., 2004), a finding that was also seen in preliminary data in adults (primarily men) recruited through an industrial worksite wellness program (Hutchins et al., 2002). Not all studies, however, find associations between PROP bitterness and alcohol intake (e.g., Mattes and DiMeglio, 2001).

The literature is inconsistent with respect to a relationship between PROP tasting and risk of alcoholism. In studies with alcoholics compared with controls, some report an excess of nontasters among alcoholics (DiCarlo and Powers, 1998; Peeples, 1962; Spiegel, 1972), whereas other studies do not (Reid et al., 1968; Smith, 1972; Swinson, 1973). In studies examining family history of alcoholism, Pelchat and Danowski (1992) found significantly more PROP nontasters among children of alcoholics than among children of nonalcoholics, whether or not the children themselves were alcoholic. Kranzler and colleagues, however, were unable to show a significant relationship between PROP threshold and parental history of alcohol dependence in nonalcoholic young adults (Kranzler et al., 1998) or in those with alcohol dependency (Kranzler et al., 1996). One study found comorbidity between depression and alcoholism in college students who reported PROP as very bitter (DiCarlo and Powers, 1998).

Some of the inconsistencies in PROP effects on alcohol consumption behaviors could relate to the measurement of PROP tasting. A number of studies relating alcohol-ingestive behaviors to PROP have relied on a threshold procedure (Kranzler et al., 1996, 1998; Peeples, 1962; Pelchat and Danowski, 1992; Spiegel, 1972), which, because it cannot identify supertasters (Bartoshuk et al., 1994), has the potential to fail to find PROP effects. In fact, we reported positive and significant associations between the frequency of consuming alcoholic beverages and PROP bitterness, but not PROP threshold (Duffy et al., 2004). Distinguishing PROP supertasters from medium tasters and nontasters requires valid scaling methods, as reviewed previously (Bartoshuk et al., 2002b, 2004a,Bartoshuk et al., b).

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Discovery of allelic variation in *TAS2R38*, the gene for the PTC receptor, presented the opportunity for examining its ability to predict the oral sensation from an ethanol probe and the frequency of consuming alcoholic beverages in a sample of healthy adults who were recruited into a study of taste genetics and dietary behaviors and who reported consuming alcoholic beverages. Analysis of these data showed that genotype predicts PROP bitterness and, because of its association with PROP bitterness, predicts alcohol intake. However, genotype fully accounts neither for supertasting nor for some of the oral sensations from alcohol.

METHODS

Subjects and Procedure

A convenience sample of reportedly healthy adults was recruited into an observational study to test the relationship between genetic variation in taste and dietary behaviors. A telephone screening and the first visit served to recruit healthy adults who did not smoke tobacco or have a high level of dietary restraint, as described previously (Duffy and Bartoshuk, 2000; Duffy et al., 2004). Of the potential 94 subjects, 4 with uncommon haplotypes and 6 non–alcohol drinkers were removed from the dataset. Eighty-four subjects (53 women and 31 men) with an age (mean ± SD) of 36 ± 13 years (range, 21–59 years) participated in 2 or 3 visits to the taste laboratory to collect the data reported in this article, with a separate visit to draw blood samples for genotyping. The only exception was that the PROP threshold was available for only 48 of the 84 subjects.

The sample was primarily of European ancestry, according to their responses to the following categories: 72 Caucasians, 4 Asians, 1 African American, 6 Hispanics, and 1 Asian Indian. The University of Connecticut and Yale University Institutional Review Boards approved all study procedures. Subjects gave written consent and were paid for their participation.

Subjects rated the intensity of oral stimuli on a computer that displayed the general Labeled Magnitude Scale (gLMS) (Bartoshuk et al., 2002a,b), an adjective-labeled ratio scale labeled as 0 for no sensation and 100 (at the top) for the “strongest imaginable sensation of any kind.” Intermediate-intensity descriptors were located at “barely detectable” (1.4), “weak” (6), “moderate” (17), “strong” (35), and “very strong” (53). Subjects were instructed to consider the top of the scale across all sensory domains. The ratings, thus, were not confined to the context of oral sensation, because previous research has shown that the intensity descriptors denote different absolute perceived intensities to groups who vary in their experiences with oral sensations (Bartoshuk et al., 2002b). To restrict the ratings just to an oral sensory domain would violate the assumption that the adjectives applied to oral sensation denote the same perceived intensities to all. Subjects pointed and clicked with the computer mouse to the location on the gLMS that represented the intensity of the sensation. A BASIC program (Microsoft BASIC, version 2.43; Microsoft, Redmond, WA) converted the response into a whole number visible to the subject. The experimenter was present to provide the samples and to record the intensity ratings.

Alcohol Sensory Intensity

During two visits, subjects rated the intensity of the 50% ethanol probe applied to the left tip of the tongue with a cotton-tipped applicator. The probe was selected as a measure of alcohol irritation and was prepared from dehydrated 200-proof ethyl alcohol diluted to 50% (v/v) with deionized water. Subjects extended their tongues, and the alcohol was swabbed onto the left anterior tongue. They were asked to keep their tongues extended and wait until the burning sensation had reached the strongest point before making their ratings. The mean of the two intensity ratings was used in the analysis.

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PROP Tasting

Threshold—A PROP threshold test was available on the first 48 of 84 subjects who participated in the study. In these subjects, testing was completed on the first day. The threshold was related to the PTC genotype for comparison with the data reported by Kim et al. (2003). A modified up-down procedure (Bartoshuk, 1978; McBurney and Collings, 1984) was used with room temperature solutions ranging in quarter-log steps from 0.001 to 3.2 mM reagent-grade PROP dissolved in deionized water (Picotech System; Hydro, Garfield, NJ; 18 MΩ/ml). Subjects tasted two 10-ml samples served at room temperature (one was water, and one was a given concentration of PROP). Each tasting was preceded with a water rinse. Subjects were instructed to choose the sample with the stronger taste. With one correct choice, the same PROP concentration was presented again. After two correct choices, the next lower concentration was presented (a reversal). With one incorrect choice, the next highest concentration was presented (a reversal). The threshold was considered to be the geometric mean of the second through seventh reversals. A taster threshold was ≤0.1 mM, and a nontaster threshold was ≥0.2 mM PROP.

Perceived Intensity—On the final day of testing, at the end of the session, all subjects rated the intensity of PROP with a protocol that included intensity ratings of NaCl and 1000-Hz tones (Bartoshuk et al., 1994). Taste stimuli were presented in half-log steps: five NaCl solutions (from 0.01 to 1 M) and five PROP solutions (from 0.032 to 3.2 mM). Tones were presented in 12-dB steps (from 50 to 98 dB). Blocks of stimuli were presented in the following order: tones, NaCl, tones, NaCl, tones, PROP, tones, PROP, tones. The stimuli were randomized within each block. The PROP ratings were analyzed as raw gLMS ratings. NaCl and tones have been used as sensory standards to normalize the oral sensory data, but because raw and normalized data produce similar results (Duffy et al., 2004), this study used raw gLMS ratings.

Fungiform Papilla Number

By following a procedure similar to the method of Miller and Reedy (1990), the number of fungiform papillae on the right and left anterior tongue tip was determined with videomicroscopy. This procedure took place on the last day of testing. The subject's tongue was painted with blue food coloring to contrast between stained filiform and unstained fungiform papillae. Subjects held their tongue tip between two plastic slides attached to each other with screws. With a Zeiss (Jena, Germany) operating microscope, magnification (× 15) allowed fungiform papillae to be easily distinguished from filiform papillae, which contain no taste buds. The images were recorded for 3 to 5 min to allow subsequent counting of the fungiform papillae in a 6-mm-diameter circle on right and left tongue tips on a high-resolution monitor. The average of counts from the two sides was used to compare with the alcohol sensory and intake measures.

Alcohol Intake

Yearly intake of beer, wine or wine coolers, and liquor or mixed drinks was assessed by using the Block Food Survey (Berkley Nutrition Services, 2000; Block et al., 1986), version 98.1. A registered dietitian interviewed each subject on the first or second day of testing by using this survey, and subjects reported how often they consumed each beverage (categories range from "never" to "every day") and the amount consumed per time interval (e.g., glass, bottle, and drink and the size of the serving). Categories were coded to a number of drinks per year as follows: "few times per year" as 4 drinks, "once per month" as 12 drinks, "2–3 times per month" as 30 drinks, "once per week" as 52 drinks, "2 times per week" as 104 drinks, "3–4 times per week" as 182 drinks, "5–6 times per week" as 286 drinks, and "every day" as 365 drinks. Subjects reported consuming alcoholic beverages at least a "few times per year." For each time,

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the number of servings of alcoholic beverage consumed was recorded. The total alcohol intake was the sum of the yearly intakes of beer, wine, and liquor.

TAS2R38 Gene Analyses

A trained phlebotomist drew blood samples from subjects in a visit that involved only drawing the blood samples. DNA was extracted from whole-blood samples that had been stored at subzero (−60°C) freezer temperatures with standard methods that generally followed the manufacturer's instructions (Gentra, Minneapolis, MN), with occasional modification required for old, lysed samples. Purified DNA samples were stored at 4°C in Tris 10 mm; EDTA 1 mm (TE) until analyzed. Samples were analyzed by using the 5′-exonuclease reaction (TaqMan) with assays provided by Applied Biosystems (assay numbers C_9506826, C_9506827, and C_8876467; Foster City, CA) in 384-well format and read on an ABI Prism 9700 (Applied Biosystems, Foster City, CA). Samples that failed to give a clean genotype were repeated once.

Statistical Analyses

Data were analyzed with Statistica (Macintosh version 4.1, StatSoft, Tulsa, OK). The criterion for significance was $p \leq 0.05$. Standard multiple regression analyses were used to predict the alcohol sensory and intake data from the taste phenotype, *TAS2R38* genotype, sex, and age. Two levels of prediction were completed. The first used only the phenotype (PROP intensity and fungiform papillae number), age, and sex to predict alcohol sensation and intake. The second used the genotype, age, sex, and fungiform papillae number to predict the alcohol data. PROP intensity was not included because of the covariance with the genotype. Skewed variables were transformed to improve the normality of the distribution for this statistical procedure (Tabachnick and Fidell, 2001). Univariate and multivariate outliers were removed by the standardized residual (≥ 2.5) and the Mahalanobis distance criteria (critical χ^2 table with $p < 0.001$; degrees of freedom are the number of independent variables) (Tabachnick and Fidell, 2001). The "Results" section presents the multiple regression coefficient (r) and semipartial correlations (sr) of significant contributors to the multiple r.

The degree of difference between genotypes was examined with ANOVA by using planned comparisons with t tests and the error term generated by the ANOVA (Keppel, 1991), as well as the χ^2 statistic. Kendall's τ statistic, which accounted for ties, was used to test the level of association between ranking individuals by genotype (AVI/AVI, PAV/AVI, and PAV/PAV) and phenotype according to the bitterness of 3.2 mM PROP (nontasters, medium tasters, and supertasters).

RESULTS

Relationship Between Genotype and Taste Phenotype

Individuals had only three patterns on genotyping for the three polymorphic sites: only P, A, and V present; only A, V, and I present; and both alleles present at all three sites. Given extensive population data (Bamshad et al., 2004; Kidd et al., 2004), these correspond to PAV homozygotes, AVI homozygotes, and PAV/AVI heterozygotes, respectively, with probabilities greater than 99%. The four individuals with other results were excluded from subsequent analyses, as reported previously.

Figure 1 shows the distribution of taste thresholds across genotypes. The PROP threshold was significantly different across genotypes [$F(2,46) = 89.783$; $p < 0.0001$] such that the mean threshold for the AVI homozygotes (0.579 ± 0.10 mM; mean ± SEM) was greater than for PAV/AVI heterozygotes (0.038 ± 0.001 mM), which it turn was greater than for PAV homozygotes (0.011 ± 0.003 mM). There was strong concordance between nontasters defined

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by PROP threshold ($n = 17$) and by genotype (AVI/AVI; $n = 18$). One individual had an AVI/AVI genotype but a taster threshold (0.0468 mM PROP).

Figure 2 shows the PROP bitterness functions for individuals with the three common genotypes. The genotype and phenotype groups were similar for age across groups but had some variability in numbers of men and women (Table 1). The genotype × PROP intensity ANOVA showed significant main effects of genotype [$F(2,81) = 28.594$; $p < 0.0001$] and concentration [$F(4,324) = 215.01$; $p < 0.0001$] and a significant genotype × concentration interaction [$F(8,324) = 14.15$; $p < 0.0001$]. By pairwise comparisons, the three functions differ significantly for the three highest concentrations of PROP (PAV/PAV homozygotes > PAV/AVI heterozygotes > AVI/AVI homozygotes, $p \leq 0.001$). However, the function for the PAV/PAV homozygotes was only slightly above that for the PAV/AVI heterozygotes. Figure 2 also shows the same subjects classified by psychophysical criteria: the 25% with the lowest ratings for 3.2 mM PROP (near saturation) were classified as nontasters, the 25% with the highest ratings were classified as supertasters, and the remaining 50% were classified as medium tasters. There was a significant correspondence between the genotype and phenotype rankings according to a Kendall's τ of 0.46 ($p < 0.01$; Table 2).

In a genotype × sex ANOVA, the average number of fungiform papillae did not vary significantly across genotypes. The average number was higher in women (26.48 ± 0.96; mean ± SEM) than in men (22.60 ± 1.05; $t = 2.688$; $p < 0.01$), and PAV/PAV homozygous women tended to be distributed toward more fungiform papilla than did AVI/AVI homozygous women. In χ^2 analyses, PAV/PAV women tended to be in the category of ≥ 25 papillae in the 6-mm area, whereas AVI/AVI women tended to be in the category of fewer than 25 papillae ($\chi^2 = 3.012$; $p = 0.08$).

Via multiple regression analyses, genotype and fungiform papillae number were significant contributors to predicting PROP bitterness. The model to predict 3.2 mM PROP bitterness contained genotype, fungiform papillae number, sex, and age, yet only genotype and fungiform papillae number were significant predictors ($r = 0.64$; $p < 0.0001$; $R^2 = 0.41$). Although genotype predicted most of the variance in PROP bitterness ($sr = 0.55$; $p < 0.00001$), the fungiform papillae number explained an additional 5% ($sr = 0.21$; $p < 0.05$).

Predicting Alcohol Sensation

The intensity of the ethanol probe averaged nearly "strong" (33.98 ± 1.91; mean ± SEM) and ranged from "weak" to above "very strong." Phenotypical measures of taste accounted for significant variance in intensity ratings of the ethanol probe. The regression model contained the phenotypical measures (PROP bitterness and fungiform papillae), age, and sex, yet only PROP bitterness and age were significant contributors ($r = -0.46$; $p < 0.001$; $R^2 = 0.22$). The intensity of the ethanol probe was greater in the older subjects ($sr = 0.38$; $p < 0.001$). PROP bitterness explained an additional 7% of the variance in intensity of the ethanol probe; those who tasted PROP as more bitter also reported that the probe was more intense ($sr = 0.26$; $p < 0.01$).

The genotype was not an adequate substitute for PROP bitterness in predicting the intensity of the ethanol probe. By replacing PROP bitterness with genotype in the regression model with fungiform papillae, age, and sex, only age and fungiform papillae were significant contributors ($r = 0.47$; $p < 0.001$; $R^2 = 0.22$). The fungiform papillae number explained an additional 5% of the variance in intensity of the ethanol probe; those with the most papillae reported that the probe was the most intense ($sr = 0.23$; $p < 0.05$).

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Predicting Alcohol Intake From Taste Phenotype and Genotype

Subjects reported consuming an average of 199 ± 22.35 (mean ± SEM) drinks per year (ranging from a "few times per year" to 3 drinks per day), which translates to approximately 3 to 4 drinks per week. Presented according to categories of alcohol drinking from the National Longitudinal Alcohol Epidemiologic Survey (Stinson et al., 1998), there were 40 "light drinkers" (a couple of drinks per year but fewer than 3 drinks per week), 42 "moderate drinkers" (3 to fewer than 14 drinks per week), and 2 "heavy drinkers" (2 or more drinks per day). The average yearly intake of alcoholic beverages was significantly greater in men (261.74 ± 45.00; mean ± SEM) than in women (162.19 ± 22.85; $t = 2.188$; $p < 0.05$).

By psychophysical groups according to 3.2 mM PROP bitterness, nontasters consumed 288.81 ± 64.65 drinks per year, medium tasters 188.05 ± 28.10, and supertasters 134.43 ± 21.59. In multiple regression analyses, those who tasted PROP as more bitter reported consuming less alcohol, an effect that was separate from age and sex effects on alcohol intake. In the model with phenotypical measures, sex, and age, only age and PROP bitterness were significant predictors of alcohol intake ($r = 0.38$; $p < 0.01$; $R^2 = 0.15$). Although those who were older ($sr = 0.27$; $p = 0.01$) reported less frequent intake of alcoholic beverages, PROP bitterness predicted an additional 4.4% of the variance in intake of alcoholic beverages ($sr = -0.21$; $p = 0.05$; Fig. 3).

Genotype served as an adequate substitute for PROP bitterness in predicting alcohol intake. By replacing perceived PROP bitterness with genotype in the regression model with age and sex, only age and genotype were significant predictors ($r = 0.38$; $p < 0.01$; $R^2 = 0.14$). The *TAS2R38* genotype explained an additional 5% of the variance in alcohol intake beyond that explained by age ($sr = 0.21$; $p = 0.05$). Alcohol intake by genotype is shown in Fig. 4. ANOVA across the entire sample showed significant effects of genotype [$F(2,81) = 3.60$; $p < 0.05$]. By pairwise comparisons, the AVI/AVI homozygotes consumed significantly more alcoholic beverages (285.16 ± 55.82 drinks per year; mean ± SEM) than either the PAV/AVI heterozygotes (180.49 ± 29.32; $p < 0.05$) or the PAV/PAV homozygotes (132.90 ± 21.98; $p = 0.01$). Because two individuals in the AVI/AVI group seemed to be outliers (Fig. 4), the difference in alcohol intake between AVI/AVI homozygotes and PAV/AVI heterozygotes was tested with the χ^2 statistic. Those who were PAV/AVI were significantly more likely than those who were AVI/AVI to consume 250 or less drinks per year (31 vs. 13, respectively) and were significantly less likely to consume more than 250 drinks per year (6 vs. 13, respectively; $\chi^2 = 6.748$; $df = 1$; $p < 0.01$).

Putting perceived PROP bitterness and genotype in the regression model showed that PROP bitterness exceeded the ability of genotype to predict alcohol intake. For this analysis, the effect of age on alcohol intake was minimized by only examining individuals who were ≤ 40 years old (23 women and 26 men). In multiple regression analyses with genotype, PROP bitterness, and sex in the model, only PROP bitterness contributed significantly to the prediction of alcohol intake ($r = 0.46$; $p = 0.01$; $R^2 = 0.21$).

DISCUSSION

The *TAS2R38* genotype for common haplotypes explained significant variance in the intensity of bitterness from PROP and intake of alcoholic beverages in the sample of reportedly healthy adults who were classified as primarily light and moderate drinkers. Individuals who were AVI/AVI homozygotes tasted PROP as least bitter and reported significantly greater intake of alcoholic beverages than either AVI/PAV heterozygotes or PAV/PAV homozygotes. The genotype neither explained full variability in PROP bitterness nor was a probe of alcohol sensation. Using fungiform papillae number and genotype increased the ability to predict the bitterness of PROP and the intensity from the alcohol probe.

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The sample was recruited for diversity in PROP bitterness. Dividing individuals by tertiles of PROP bitterness produced a group of nontasters ($n = 21$) to whom PROP was less than "moderately" bitter, a group of medium tasters ($n = 42$) to whom PROP tasted "strongly" bitter, and a group of supertasters ($n = 21$) to whom PROP tasted "very strongly" bitter. A similar distribution of common genotypes of the *TAS2R38* gene was observed: 26 AVI/AVI homozygotes (31%), 37 PAV/AVI heterozygotes (44%), and 21 PAV/PAV homozygotes (25%). However, the PROP bitterness functions by genotype were not as distinct as those divided by phenotype (Fig. 2). The PAV/AVI heterozygotes had steeper bitterness functions than the medium tasters, and the PAV/PAV homozygote functions were less steep than the supertasters'. With multiple regression analyses, the number of fungiform papillae and genotype were separate predictors of PROP bitterness. Supertasting thus seems to result from both homozygosity at *TAS2R38* and at least one other genetic determinant, such as that determining the density of fungiform papillae. PROP bitterness also differs between men and women (Bartoshuk et al., 1994;Prutkin et al., 2000) and with exposure to taste-related pathology (Bartoshuk et al., 1995). Thus, PROP bitterness responses likely result from a functional receptor that responds specifically to PROP, the fungiform papilla density, and a number of factors not directly related to genetic endowment (e.g., taste-related pathology). In addition, a formal possibility is regulatory variation of *TAS2R38* such that more receptors are produced to increase the taste responsiveness. To date, no regulatory variation has been identified because the regions that regulate expression of the gene are not fully defined.

This study seems to be the first to report on the association between *TAS2R38* gene and fungiform papilla density. The genotype did not account for differences in number of fungiform papillae across the entire sample, although there was a trend for PAV/PAV women to have a higher density of fungiform papillae than AVI/AVI women. Ample evidence exists for an association between PROP bitterness and fungiform papillae number; individuals who taste PROP as more bitter have, on average, more fungiform papillae numbers those for whom PROP is less bitter, as assessed with videomicroscopy (Bartoshuk et al., 1994; Miller and Reedy, 1990) and by counting with handheld magnification (Delwiche et al., 2001; Tepper and Nurse, 1998). The present dataset may have been too small to detect an association between *TAS2R38* genotype and fungiform papillae. However, it is unknown whether the *TAS2R38* gene should be associated with fungiform papilla density. Because the *TAS2R38* gene codes for a receptor that responds to compounds with the $N-C=S$, it is uncertain why fungiform papilla density would associate with the presence or absence of the ability to taste PTC/PROP. The lack of knowledge on the location of genes that mediate fungiform density also makes specific genetic linkages uncertain.

Intensity from the alcohol probe was predicted by either PROP bitterness or the number of fungiform papillae, but not by genotype. This probe likely stimulated burning but also could have stimulated taste sensations of bitterness (Bartoshuk et al., 1993; Pickering et al., 2004; Prescott and Swain-Campbell, 2000) or sweetness, as shown in rats (Lemon et al., 2004) and in primates (Hellekant et al., 1997). The lack of association between alcohol intensity and genotype suggests that the *TAS2R38* gene codes for the receptor that responds to PTC and chemically related compounds, but not for perceived irritation. Fungiform papillae are innervated by taste and oral somatosensory nerves—this indicates that responses to alcohol sensation on the tongue tip are related to PROP bitterness and the number of fungiform papillae (Duffy et al., 2004).

The relationship between genotype and alcohol intake is probably mediated through the bitterness of PROP. PROP bitterness is associated with oral sensations from alcohol, including bitterness, irritation/astringency (Bartoshuk et al., 1993; Pickering et al., 2004; Prescott and Swain-Campbell, 2000), and sweetness (Lanier et al., 2005). Individuals who taste PROP as

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most bitter may experience more negative oral sensations from alcoholic beverages (e.g., bitterness and irritation) and fewer positive sensations (e.g., sweetness) as a sensory hindrance for overconsumption, as suggested by data from our laboratory (Duffy et al., 2004) and others (Guinard et al., 1996; Intranuovo and Powers, 1998). Thus, the relationship between genotype and alcohol intake serves to verify the results of PROP relations with alcohol intake.

Associations between the *TAS2R38* gene and alcohol drinking add to the evidence of genetic influences on alcohol use and suggest a new region that might be related to oral sensory motivations to drink alcohol. Genome-wide scans link maximum alcohol consumption with gene regions on chromosome 9 (9q21.11) (Bergen et al., 2003). Alcohol dependence defined by clinical criteria and family history has been linked through a genome-wide scan by the Collaborative Study on the Genetics of Alcoholism to regions on chromosomes 1 and 7, with some evidence for regions on chromosomes 2 and 3 (Foroud et al., 2000). The Collaborative Study on the Genetics of Alcoholism data confirm earlier findings of Reich et al. (1998) with linkages on chromosomes 1, 2, and 7. The *TAS2R38* gene location on chromosome 7 (7q36) is unlinked to the loci for alcohol dependence (7p12.3). Regions on chromosome 7 identified with alcohol dependence are near the markers D7S1793 (Reich et al., 1998), D7S821, D7S1830, and D7S1797 (Foroud et al., 2000). Nonetheless, as discussed by Foroud et al. (2000), multiple loci on chromosome 7 may influence susceptibility to alcoholism. Thus, genetic control of alcohol behaviors, involving bingeing (i.e., maximal drinking) and alcohol dependence, may not be linked to those that involve alcohol preference.

Alcohol sensations and intake showed interesting age relationships within this sample. The intensity of the alcohol probe showed age-related increases. Interactions between taste and trigeminal innervation on the tongue tip may offer one explanation for this age-related increase in alcohol intensity. The probability of exposure to conditions that can damage taste on the anterior tongue increases with aging. The chorda tympani branch of cranial nerve VII mediates taste on the anterior tongue. Taste from the chorda tympani nerve is depressed with a history of diseases such otitis media and head trauma (Bartoshuk et al., 1995). Decreased taste on the anterior tongue can remove the usual inhibition that taste has on trigeminal sensations from the anterior tongue, as seen in experimental evidence with temporary anesthesia of the chorda tympani nerve (Tie et al., 1999) and oral pain phantoms arising from the anterior tongue (Grushka and Bartoshuk, 2000). Thus, the increased intensity from the alcohol probe in the older subjects could have resulted from changes in the interactions between taste and trigeminal sensations. Our sample showed age-related decreases in consumption of alcoholic beverages, a finding that parallels that seen in national statistics from the Behavioral Risk Factor Surveillance System for 1994 to 1997 and from the National Health Interview Survey (Kamimoto et al., 1999). Thus, increased intensity from alcohol, such as increased burn or astringency, could also serve as a sensory hindrance for overconsuming alcoholic beverages with aging.

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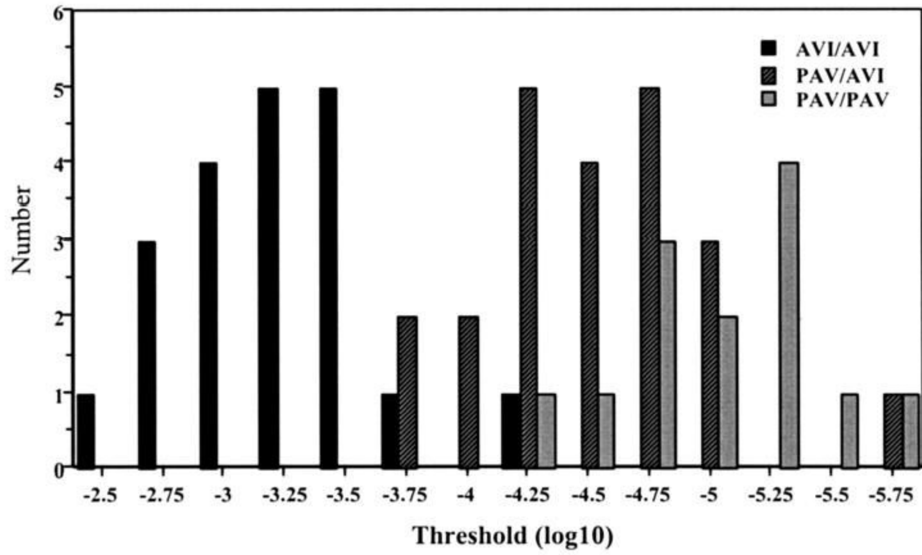

Fig. 1.
Distribution of common genotypes for the *TAS2R38* gene by PROP threshold, with number of subjects (*y* axis) and PROP molar concentration ($\log_{10}$ transformed).

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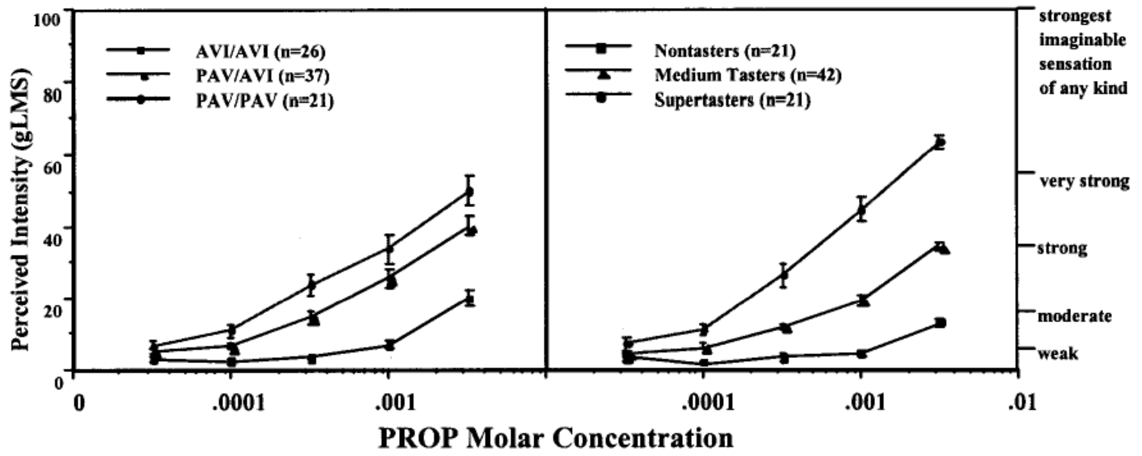

Fig. 2.

PROP bitterness functions (perceived intensity on the general Labeled Magnitude Scale by molar concentration) in genotype groups (left) and psychophysical groups defined from the bitterness of 3.2 mM PROP (right).

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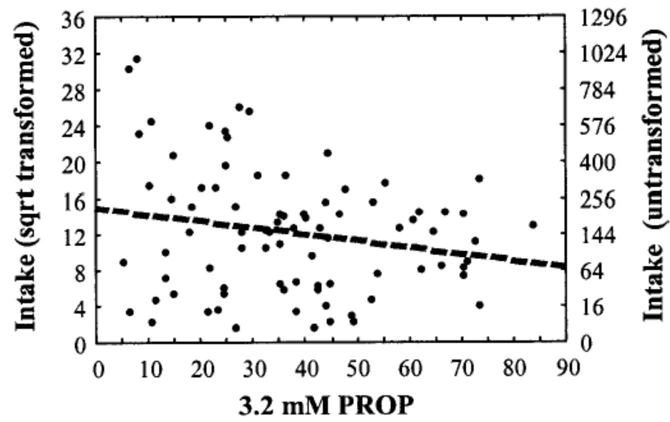

Fig. 3.

Yearly intake of alcoholic beverages by the bitterness of 3.2 M PROP rated on the general Labeled Magnitude Scale. The intake data were square root–transformed because of the positive skew. The *x* axis on the left is labeled as the transformed value and on the right as the untransformed value.

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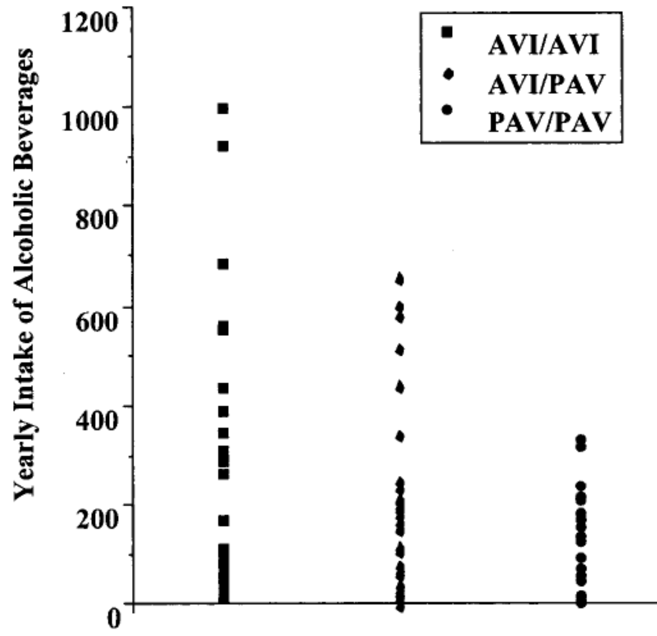

Fig. 4.
Yearly intake of alcoholic beverages across the entire sample by *TAS2R38* genotype groups.

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Table 1

Age and Sex Breakdown for Genotype and Phenotype Groups[1]

Variable	Sex (F/M)	Age, years (mean ± SEM)
AVI/AVI	15/11	34.19 ± 2.47
PAV/AVI	21/16	37.41 ± 2.02
PAV/PAV	17/4	35.81 ± 2.87
Nontasters	13/8	37.43 ± 3.10
Medium tasters	27/15	35.64 ± 1.99
Supertasters	13/8	35.33 ± 2.34

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Table 2

Individuals Classified by Psychophysical Criterion (Columns[a]) by Genotype (Rows[b])

Variable	Nontasters	Medium tasters	Supertasters	*n*
AVI/AVI	15	9	2	26
PAV/AVI	5	23	9	37
PAV/PAV	1	10	10	21
n	21	42	21	

[a]Based on the bitterness of 3.2 mM PROP (nontasters, ≤moderately bitter; supertasters, ≥very strong; and medium tasters in between).

[b]*TAS2R38* gene (Kim et al., 2003).

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Associations between taste genetics, oral sensation and alcohol intake

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Abstract

Alcohol produces a range of oral sensations, some of which have been shown to vary with the perceived bitterness of 6-*n*-propylthiouracil (PROP), one marker for genetic variation in taste. Some studies report that offspring of alcoholics are most likely to be PROP nontasters [*Physiol. Behav.* 51 (1992) 1261; *Physiol. Behav.* 64 (1998) 147], yet others report the offspring as more responsive to sodium chloride (NaCl) and citric acid, which appears to contradict the taste genetic hypothesis. We predicted alcohol sensation and intake from measures of taste genetics (PROP bitterness and number of fungiform papilla), NaCl and citric acid intensity, and spatial taste pattern in 40 females and 43 males. Subjects used the general Labeled Magnitude Scale (gLMS) [*Chem. Senses* 18 (1993) 683; *J. Food Qual. Pref.* 14 (2002) 125] as an intensity and hedonic scale. Those who tasted PROP as most bitter or had highest numbers of fungiform papilla reported greatest oral burn from an alcohol probe; those who tasted least PROP bitterness consumed alcoholic beverages most frequently. Although higher NaCl and citric acid ratings associated with more frequent consumption of alcoholic beverages, the findings could be explained by lower intensity of tastants on the tongue tip (chorda tympani nerve) relative to whole mouth perception. In multiple regression analyses, PROP bitterness and the spatial pattern of taste perception were independent contributors to the prediction of alcohol intake. In summary, the results support that variation in oral sensation associates with alcohol intake. Those who taste PROP as least bitter and have low chorda tympani relative to whole mouth taste intensity appear to have fewest oral sensory hindrances to the consumption of alcoholic beverages.
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Keywords: Taste; Genetics; 6-*n*-Propylthiouracil; Alcohol; Intake; Preference; Bitter taste; Fungiform papilla

1. Introduction

Numerous studies support a familial component in the etiology of alcoholism (see Ref. [1] for review). A study of more than 3500 male twins in the United States [2] suggests both direct and indirect mechanisms in the heritability of alcoholism. Although direct mechanisms could include specific gene loci that control alcohol metabolism (e.g., alcohol dehydrogenase [3]), they are more likely to involve multiple chromosomes [4]. Indirect mechanisms include comorbid conditions, such as affective and conduct disorders [5] as well as personality disorders [6]. One direct mechanism could involve genetic variation in taste and oral sensation. This paper explores associations between genetic variation in taste, oral responses to an alcohol probe and consumption of alcoholic beverages.

Bitterness of phenylthiocarbamide (PTC) or the chemically related compound, 6-*n*-propylthiouracil (PROP), provides a phenotypic marker for genetic variation in taste and oral sensation. Historically, researchers have used detection thresholds to classify individuals as nontasters or tasters of these bitter compounds (e.g., Refs. [7,8]). Family studies have shown that individuals who are nontasters have two recessive alleles, while tasters may carry one or both dominant alleles [9,10]. Insensitivity to PTC or PROP is estimated at 30% of the Caucasian population; the percentages vary with sex and race [11].

Scaling the intensity of PROP bitterness allows separation of tasters into “medium tasters” (those who taste PROP as bitter) and “supertasters” (those who taste PROP as exceptionally bitter) [12]. Supertasters cannot be identified via thresholds [13] and thus, effects due to supertasters cannot be revealed in studies classifying subjects by PROP threshold only. Responses to PTC/PROP associate with allelic variation on chromosome 5 [14] and 7 [14,15], regions that contain genes for putative bitter receptors (e.g., Refs. [16,17]). Single nucleotide polymorphisms in putative bitter receptors TAS2R3, TAS2R4 and TAS2R5 do not explain variation in PROP bitterness [18]. Supertasting

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may result from increased density of fungiform papilla as well as allelic variation that results in the presence or absence of a specific PROP receptor as proposed [19] and supported by preliminary data [20].

PROP bitterness influences oral sensations from alcohol, a relationship that appears to be mediated through fungiform papilla. Greater PROP bitterness associates with more bitterness from ethanol [21] and some types of beer [22], more bitterness, astringency and acidity from red wines [23], and greater irritation from ethanol [21,24]. A PROP bitterness and fungiform papilla relationship was first shown by Miller and Reedy [25]; PROP supertasters have, on average, the greatest number of fungiform papillae and taste buds as assessed with videomicroscopy [11]. A positive relationship between PROP bitterness and fungiform papillae number is also observed using lower magnification for papillae counting [26,27]. Fungiform papillae hold taste buds that are innervated for taste by the chorda tympani branch (CTN) of the facial nerve (cranial nerve VII). These taste buds are surrounded by fibers of the trigeminal nerve (cranial nerve V), which are believed to mediate oral burn [28–30].

Oral sensory differences in alcohol sensation with PROP tasting may explain some of the variability in alcohol preference and drinking behaviors [31]. Nontasters of PROP may experience the least bitterness/oral burn from alcohol and thus have greater preference for and consumption of alcoholic beverages. By scaling PROP bitterness, Intranuovo and Powers [22] found that those who tasted PROP as least bitter consumed significantly more beers in their first year of drinking. Guinard et al. [32] also reported that high users of beer (greater than 3.6 L per week) were more likely to be PTC/PROP nontasters than were low users (less than 720 ml per week). However, the method for PROP and PTC was described as a screening procedure without clear indication of how nontasters were defined. Mattes and DiMeglio [33] did not find differences in intake of alcoholic beverages between PTC tasters and nontasters. In this study, subjects tasted filter papers without PTC and those saturated with PTC. Nontasters were those who reported both papers as tasteless; tasters were those who rated the PTC-saturated paper as bitter. Differences in psychophysical methodologies used to define PROP/PTC may explain some differences across these studies (see discussion below).

There is inconsistent support for PROP as a genetic marker for risk of alcoholism. In studies with alcoholics compared with controls, some report an excess of nontasters among the alcoholics [34–36] while other studies do not [37–39]. DiCarlo and Powers [36] also found a higher proportion of PROP supertasters in college students who reported both problems with alcoholism and depression in themselves and their parents than in nontasters. In studies examining family history of alcoholism, Pelchat and Danowski [31] found significantly more PROP nontasters among children of alcoholics than among children of nonalcoholics, whether or not the children themselves were alcoholic. Kranzler et al. [40] did not find a significant relationship between PROP threshold and parental history of alcohol dependence in nonalcoholic young adults or in those with alcohol dependency [41].

Some of the inconsistencies in PROP effects on alcohol consumption behaviors could relate to the measurement of PROP tasting. Some of the studies that fail to find a PROP–alcohol association have methodological problems as reviewed by Pelchat and Danowski [31], including inappropriate matches between alcoholics and controls [39] and procedures that may falsely classify nontasters through a "yes/no" response to a PTC-impregnated paper [37] or a single PTC solution [38]. Studies on alcohol ingestive behaviors that use a threshold procedure [31,34,35,40,41] will fail to reveal PROP effects if the behavioral differences are most apparent across those who vary most in PROP tasting (i.e., nontasters and supertasters). DiCarlo and Powers [36] used the bitterness of the PROP-impregnated paper [42] to examine PROP effects on alcohol ingestive behaviors. Subjects were defined as nontasters, medium tasters and supertasters based on their ratings of bitterness of PROP using a nine-point category scale. Methodological advances show that these category scales may not accurately classify supertasting [13,43]. Characterization of supertasters and related sensory behaviors requires scaling methods that permit valid comparisons across subjects. The methodological difficulties in identifying supertasting has been reviewed previously [13,43] and will be reviewed here briefly.

Adjective-labeled, self-rating scales (e.g., Likert, category and visual analogue) are commonly used in taste studies. They are valid for within-subject comparisons; however, they are invalid for across-subject/group comparisons unless the adjectives denote the same perceived intensity, on average, to all groups of interest. However, intensity adjectives denote different absolute perceived intensities within subjects, depending on the domain to which they are applied. For example, a "strong" oral burn from a chili pepper reflects a greater perceived intensity than a "strong" rose odor. Intensity adjectives also denote different absolute perceived intensities across subjects depending on the subject's experience with the domain of interest. For taste, supertasters experience greater perceived intensities than do nontasters (see Refs. [13,43,44] for reviews); thus, a "strong" bitter to a supertaster is more intense than a "strong" bitter to a nontaster. Using adjective-labeled scales to make across-group comparisons when the groups, on average, use the adjectives to refer to different perceived oral sensory intensities obviously invalidates the comparisons [45]. Most of the time, the invalid comparison will simply underrepresent the actual effect size (e.g., Ref. [46]). However, in some cases, the invalid comparison will produce apparent differences that are actually in the wrong direction (see Ref. [45] for a review). For example, suppose that the adjective "strong" reflects a

perceived intensity that is twice as great to supertasters as it is to nontasters. Suppose an alcoholic beverage were 10% more intense to supertasters. Treating "strong" as if it reflected the same perceived intensity to both groups effectively reduces all of the supertaster ratings by half. Thus, a beverage that is 10% more intense would be reduced so far that the reduced rating for supertasters would fall below that for nontasters. We call this a reversal artifact.

Environmental factors, which impact oral sensation, affect the study of taste genetic influences on alcohol ingestive behaviors [42]. Depressed taste from the cranial nerves can alter oral sensations by changing the interactions among taste nerves [47], between taste nerves and trigeminal nerves [48] and possibly between taste and retronasal olfaction [49]. For example, an individual with depressed CTN taste relative to density of fungiform papillae or PROP taster status may have altered taste and somatosensory sensations that appear as phantom taste or pain sensations [50] or intensified taste and somatosensory sensations in response to oral stimuli [48,51]. Otherwise healthy adults can show depressed CTN taste relative to whole mouth sensations because of common illnesses, such as otitis media, middle-ear infection [42]. The logic of these findings is that damage to the CTN releases the usual inhibition from other nerves to intensify oral sensations. In relation to taste and alcohol, some studies have reported that individuals with a paternal history of alcoholism rated greatest intensity to concentrated sodium chloride (NaCl) and citric acid [52,53]. If these individuals were more likely nontasters, following the taste genetic hypothesis, those with the paternal history should have lowest intensity ratings of NaCl and citric acid (e.g., Refs. [44,54,55]). The question remains if these opposing findings result from interactions between genetic taste and environmental influences, which affect oral sensations and alcohol ingestive behaviors. Intensification of NaCl intensity has been seen in aged versus young women and the intensification is thought to result from increased trigeminal sensations as the result of taste damage [51].

The primary goal of the present study was to examine relationships between markers of taste genetics (perceived bitterness of PROP, PROP threshold and fungiform papilla number) and sensory responses to ethyl alcohol as well as reported intake of alcoholic beverages in adults. Existing data afforded analysis of relationships between the alcohol variables, NaCl and citric acid intensity, and a measure of CTN taste functioning. Multiple regression analyses were used to determine the ability of taste genetic and other taste markers to predict alcohol variables.

For intensity and hedonic ratings, subjects used the general Labeled Magnitude Scale (gLMS) [43,45], which is a generalization of the adjective-labeled, ratio scale devised by Green et al. [56,57]. The important change concerns the label at the top of the scale: "strongest imaginable sensation of any kind." The idea behind the choice of this label was to "stretch" the adjective-labeled scale to its maximum. To the extent that this maximal experience is equivalent across subjects, the gLMS will act as a universal sensory ruler. Even if this is not the case, this maximal experience is unlikely to be associated with taste. This means that the gLMS should produce valid comparisons, on average, across nontasters, medium tasters and supertasters of PROP. Previous research has shown that PROP taste functions for nontasters, medium tasters and supertasters produced by the gLMS are equivalent to those obtained by magnitude matching [13,58].

2. Methodology

2.1. Subjects and procedure

Subjects participated in an observational study designed to examine the relationship between genetic variation in taste and food/beverage sensations, dietary behaviors and nutritional status in adults. The goal of subject recruitment was to obtain diversity in genetic variation in taste in males and females and to minimize confounding factors that would affect the ability to examine taste genetic influences on dietary behaviors.

A telephone screening and the first visit served to recruit healthy adults who did not smoke tobacco or have a high level of dietary restraint. Because dietary restraint may influence accuracy of reporting dietary intake [59], potential subjects with high dietary restraint were identified by telephone with the concern for dieting subscale of the Restrained Eating Scale [60,61]. During the first visit, subjects completed the Three-Factor Eating Questionnaire [62]. Those who scored ≤ 12 on the "cognitive restraint of eating" [63] from this instrument were invited to participate in the complete study.

All subjects who met the screening criteria described above were accepted into the study. However, as subject recruitment continued, there was a need to oversample for nontasters and supertasters; this sampling occurred in the first visit. The PROP threshold procedure (described below) was a screen for nontasters; nontasters have a threshold of >0.2 mM PROP. The perceived bitterness of 0.32 mM PROP served as a screen for supertasting. This concentration was selected to be strong enough to allow relatively good separation of medium tasters from supertasters based on pilot data and previous studies (e.g., Ref. [11]). Higher PROP concentrations were avoided to minimize a context effect in later sessions (e.g., see Ref. [64]). Eight subjects who were suspected to be medium tasters were not invited to continue through the second and third visits.

Eighty-three adults (40 females, 43 males) participated in the present study. The subjects were primarily Caucasian (62 Caucasians, 11 Asians, 1 African American, 5 Hispanic and 4 Asian Indian) with a mean age of 26 ± 4 S.D. (range

21–39 years). Study subjects completed three visits that were approximately 1 week apart. The majority of the sample (60 of 80) had a normal body mass index [BMI; weight (kg)/height $(m)^2$ from 17 to 25]; 20 were overweight (BMI 25 to 30) and 3 were obese (BMI >30). There was no significant association between PROP bitterness and BMI in this sample. The University of Connecticut and Yale University Institutional Review Boards approved all study procedures. Subjects gave written consent and were paid for their participation.

Subjects used the gLMS to rate the intensity of oral stimuli and tones as well as the degree of liking/disliking of the alcohol probe. Subjects were instructed to consider the top of the scale across all sensory domains. For sensory intensity, the distances are treated as 0 for no sensation and as 100 for "strongest imaginable sensation of any kind." For hedonic ratings, subjects were instructed to consider the intensity of affective rather than sensory experiences. For pleasant experiences, the top of the scale was the "strongest imaginable pleasant experience of any kind" (i.e., + 100); for unpleasant experiences, the top of the scale was the "strongest imaginable unpleasant experience of any kind" (i.e., − 100).

Subjects made their ratings on a computer that displayed the gLMS and, through a basic program (Microsoft Basic, Version 2.43), converted the response into a whole number distance score visible to the subject. The experimenter was present to provide the samples and to assist the subjects in using the computer to make their ratings. Subjects pointed and clicked with the computer mouse to the location on the gLMS that represented the intensity of the sensation. The next screen showed the distance in whole numbers, which was recorded by the experimenter. The computer program then asked the subjects if they were ready for another sample; clicking "yes" provided a new gLMS to make the next rating. For hedonic ratings, subjects were instructed to first tell the researcher if they liked or disliked the alcohol stimulus. If they neither liked nor disliked the stimulus, a zero was registered.

2.2. Sensory responses to alcohol

During each of the three visits, subjects rated the intensity of tones as well as the intensity and the degree of liking/disliking of the 50% ethanol probe applied to the left tip of the tongue with a cotton-tipped applicator. The probe was selected as a measure of alcohol irritation and was prepared from dehydrated 200 proof ethyl alcohol diluted to 50% (volume/volume) with deionized water. Subjects extended their tongue and the alcohol was swabbed onto the left anterior tongue. They were asked to keep their tongue extended and wait until the burning sensation had reached the strongest point before making their ratings. Mean intensity and hedonic ratings were calculated for the three visits and associated with the taste genetic, NaCl and citric acid, and spatial taste measures.

2.3. NaCl and citric acid intensity and CTN taste functioning

These measures came from the spatial taste test during the first visit. The test measures taste functioning on areas innervated by chorda tympani and glossopharyngeal nerves as well as whole mouth perception. The procedures were those reported previously [65,66] except that subjects used the gLMS to rate the intensity of 1.0 M NaCl, 1.0 M sucrose, 32 mM citric acid and 1.0 mM quinine hydrochloride (QHCl). Stimuli were unilaterally painted with sterile cotton-tipped applicators onto fungiform papillae on the anterior tongue, the foliate papillae, the circumvallate papillae and the palate (quality and area presented in the order listed). Each taste stimulus was given in pairs, right and left side at each location (the initial side at each site varied). Whole mouth intensity was obtained following localized testing of all qualities. The experimenter asked subjects to fill their mouths sequentially with each tastant, swish, spit and then swallow the residual to stimulate the vagus nerve. Subjects rinsed with water before each presentation.

A measure of CTN taste functioning was calculated as the ratio of average intensities for CTN to whole mouth stimulation for all qualities. The ratio is thus a measure of CTN taste relative to oral sensory contributions from the glossopharyngeal, vagal and trigeminal nerves. Using the ratio versus absolute CTN allowed control for genetic taste effects on CTN ratings. That is, equal ratios would be seen in a PROP supertaster who reported higher CTN taste intensities relative to higher whole mouth intensities compared with a PROP nontaster who reported lower CTN taste intensities relative to lower whole mouth intensities. However, a lower ratio indicated lower CTN intensity, which may release the usual inhibition to taste from the glossopharyngeal and vagus nerves to produce higher whole mouth intensity. Intensities of NaCl and citric acid from the whole mouth stimulation and the CTN/whole mouth ratio were used to predict alcohol intensity and intake.

2.4. Measurement of PROP tasting

The ability to taste PROP was assessed by threshold and scaling methods. Both measures were compared with the sensory and hedonic responses to an alcohol probe and the frequency of alcohol intake.

2.4.1. Threshold

A PROP threshold test was determined on the first day of testing using a modified up–down procedure [67,68] with room temperature solutions ranging in quarter-log steps from 0.001 to 3.2 mM reagent grade PROP dissolved in deionized water (Hydro Picotech System, 18 MΩ/cc). Subjects tasted two samples (10 ml each, room temperature); one was water and the other was a given concentration of PROP. Each tasting was preceded with a water rinse. Subjects were instructed to choose the sample with the stronger taste. After

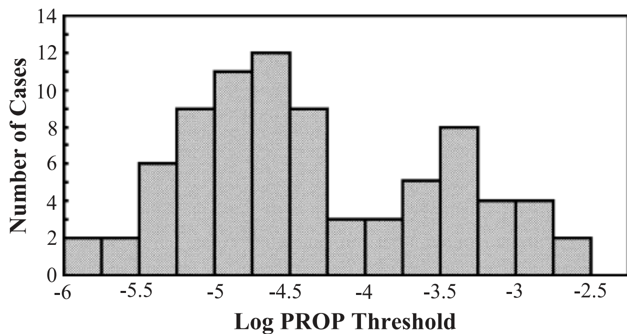

Fig. 1. PROP threshold distribution.

a correct choice, the same concentration was presented again. After two correct choices, the next lower concentration was presented (a reversal). After an incorrect choice, the next highest concentration was presented (a reversal). The first reversal was discarded and the threshold was considered to be the geometric mean of the next six reversals.

2.4.2. PROP scaling

Perceived bitterness of PROP was measured with the gLMS on the final day of testing, last within the session, using a protocol that included intensity ratings of sodium chloride (NaCl) and 1000-Hz tones [11]. Taste stimuli were presented in half-log steps: five NaCl solutions (from 0.01 to 1 M) and five PROP solutions (from 0.032 to 3.2 mM). Tones were presented in 12-dB steps (from 50 to 98 dB). Blocks of stimuli were presented in the following order: tones, NaCl, tones, NaCl, tones, PROP, tones, PROP, tones. The stimuli were randomized within each block. The PROP ratings were analyzed as raw gLMS ratings as well as normalized to tone ratings that preceded the tasting of PROP. For normalization, a factor was calculated for each subject from the geometric mean of 86- and 98-dB tones divided into the arithmetic mean of all geometric means. Each subject's raw data was then multiplied by that subject's normalization factor to provide comparable data for all subjects [69]. The NaCl data from the PROP scaling are part of an ongoing evaluation of standards in PROP studies and were not used in the analyses in the present study.

2.5. Fungiform papilla number

The number of fungiform papillae at the tongue tip was determined with videomicroscopy similar to the method of Miller and Reedy [25]. For this procedure, the subject's tongue was painted with blue food coloring to contrast between stained filiform and unstained fungiform papillae. Subjects reclined and steadied their stained tongues between two plastic slides attached with screws. Magnification (× 15) easily distinguished fungiform from filiform papillae, which contain no taste buds. The images were recorded for 3 to 5 min to allow subsequent counting of the fungiform papillae in a 6-mm-diameter circle on the right and left tongue tips. For counting, images were viewed on a high-resolution television and a circle template was placed on the image so that the edge touched the midline of the tongue as well as the tongue tip. The average of counts from the two sides was used to compare with the alcohol sensory and intake measures.

2.6. Alcohol intake

The Block Food Questionnaire [70,71] version 98.1 was used to evaluate yearly intake of beer, wine/wine coolers and liquor/mixed drinks. In an interview during the second visit, subjects reported how often they consumed each beverage

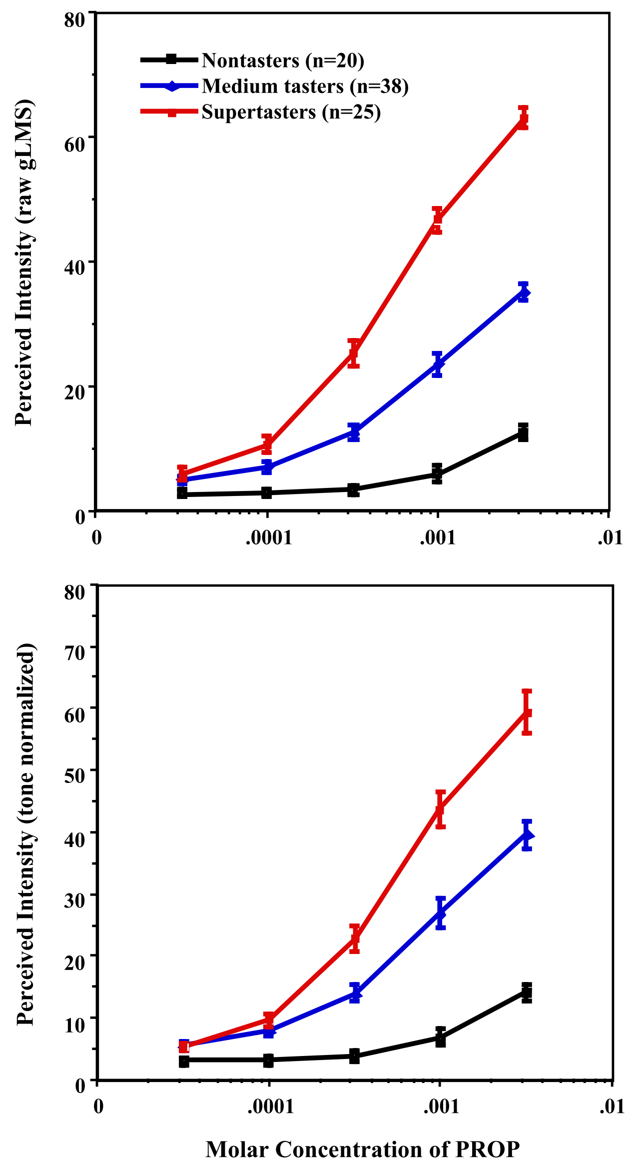

Fig. 2. Perceived bitterness (±SEM) PROP plotted against PROP concentration for nontasters, medium tasters, and supertasters using ratings from the gLMS (top graph) and those normalized to the intensity of 1000 Hz tones at 86 and 98 dB (bottom graph). Subjects were divided by bitterness of 3.2 mM PROP into 20 subjects who tasted PROP as less than "moderate," 38 who tasted PROP between "moderate and "very strong," and 22 who tasted PROP as "very strong" or greater.

(categories range from "every day" to "never") and the amount consumed per time. A yearly intake of each alcoholic beverage was calculated from reported frequency of intake multiplied by amount consumed each time. Total alcohol intake per year was the sum of beer, wine and liquor consumption.

2.7. Analysis

Data were analyzed using STATISTICA (Macintosh version 4.1, StatSoft, Tulsa, OK). Criterion for significance was $P \leq .05$. Simple regression was used to predict the alcohol data from the taste genetic, NaCl and citric acid intensity and measure of CTN taste functioning. These independent variables and sex were entered into standard multiple regression to predict alcohol intensity and intake. The Results section presents the multiple regression coefficient (r) and semi-partial correlations (sr) of significant contributors to the multiple r. Skewed variables were transformed to improve the normality of the distribution for this statistical procedure [72]. Univariate and multivariate outliers were removed by the standardized residual (≥ 2.5) and the Mahalanobis distance criteria (critical chi-square table with $P < .001$ and the degrees of freedom as the number of independent variables) [72].

3. Results

The sample had diversity in PROP tasting and fungiform papilla number. PROP threshold scores ranged from 0.0015 to 2.18 mM and had the usual bimodal distribution (Fig. 1). Fig. 2 shows PROP functions for subjects divided by bitterness of 3.2 mM PROP into 20 subjects who tasted PROP as less than moderate (≤ 22 on the gLMS), 38 who tasted PROP between moderate and very strong (>22 to 53 on the gLMS), and 25 who tasted PROP as very strong or greater (>53 on the gLMS); the normalized ratings produced similar functions. These subject groups are designated *nontasters*, *medium tasters*, and *supertasters*, respectively for the purpose of this manuscript. There was no significant average or distribution difference in 3.2 mM PROP bitterness ratings between females and males. The fungiform papilla number averaged from 11.75 to 42.50 papilla per 6-mm area and PROP bitterness showed significant correlation with fungiform papillae density in raw and tone normalized PROP bitterness ratings (Fig. 3). Women were more likely to have fungiform papilla numbers that exceeded 25 papilla in the circular template than were men ($\chi^2 = 4.966$, $P < .05$). Because raw and normalized PROP ratings produced similar functions and equivalent associations

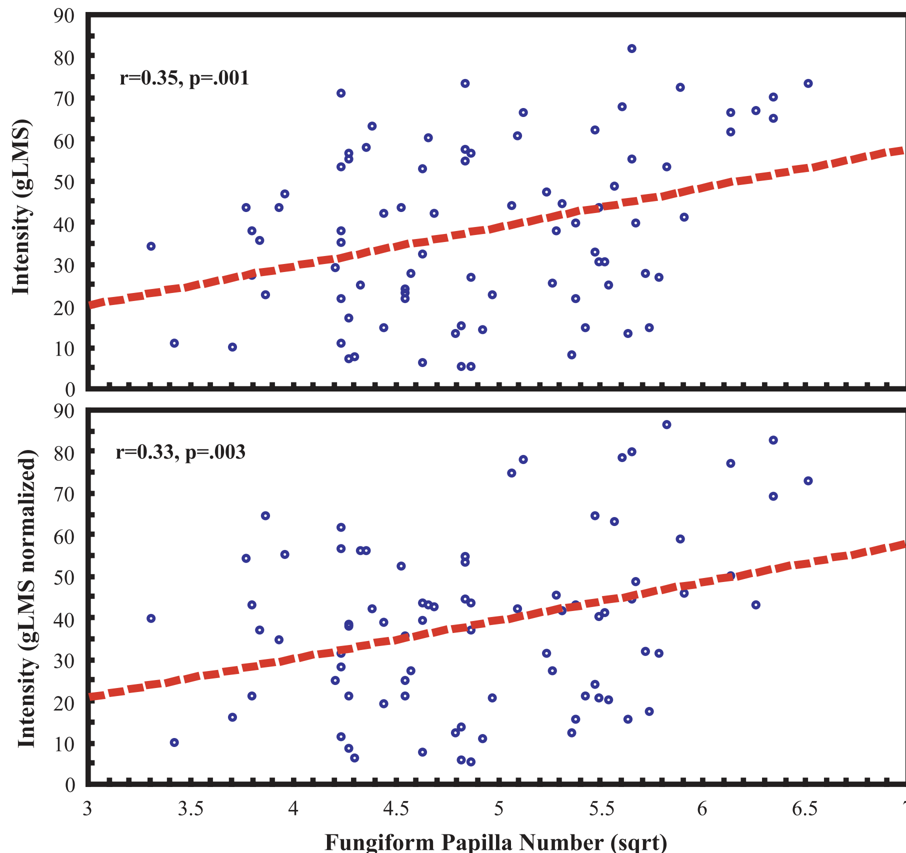

Fig. 3. Scatterplots of 3.2 mM PROP bitterness on the general Labeled Magnitude Scale (top) and that normalized to the intensity of 1000 Hz tones at 86 and 98 dB (bottom) by the number of fungiform papilla (square root transformed).

Table 1
Correlation matrix: measures of taste genetics, NaCl and citric acid taste, spatial taste pattern, alcohol intensity/hedonics and intake

	PROP threshold	PROP bitterness	Fungiform papilla number	Whole mouth NaCl intensity	Whole mouth citric acid intensity	CTN/swallow intensity	Alcohol intensity	Alcohol hedonics	Alcohol intake
PROP threshold	1.00	− .72‡	− .17	.08	.02	− .13	.09	.15	.14
PROP bitterness		1.00	.37‡	.31 *	.39‡	.06	.30†	− .27 *	− .29†
Fungiform papilla number			1.00	.18	.18	− .07	.31†	− .26 *	.09
NaCl intensity				1.00	.54‡	− .24 *	.47‡	.09	.27†
Citric acid intensity					1.00	− .15	.36‡	.04	.23 *
CTN taste/ swallow intensity (all qualities)						1.00	.28 *	− .19	− .24 *
Alcohol intensity							1.00	− .45‡	− .20
Alcohol hedonics								1.00	.19
Alcohol intake									1.00

* $P < .05$.
† $P = .01$.
‡ $P = .005$.

with fungiform papilla number, raw ratings are used for testing associations with alcohol and oral sensations.

Greater PROP bitterness associated with greater intensity from whole mouth NaCl and citric acid; fungiform papilla number only showed a modest association with the intensity of these tastes (Table 1).

The mean intensity rating of ethyl alcohol was 30.5 ±1.6 S.E.M. (between moderate and strong) and mean liking/disliking rating was − 10.42 ± 2.51 S.E.M. (between weakly and moderately dislike). Females were skewed toward higher intensities and more disliking (Fig. 4). Greater intensity from the alcohol probe was reported in those who rated the taste markers (PROP, NaCl and citric acid) as more intense, had greater CTN to whole mouth ratios and higher numbers of fungiform papilla (Table 1). Through multiple regression, significant variance in alcohol intensity ratings was explained by taste genetic measures, NaCl and citric acid intensities, sex and CTN to whole mouth ratio (r=.65, P < .000005). More intense sensations from the alcohol probe were reported by those who found PROP (sr=.21, P < .05) and NaCl (sr=.33, P < .001) as more intense and had greater CTN to whole mouth ratios (sr=.27, P < .01).

Sixty-eight of 83 subjects reported consuming alcoholic beverages more often than once per month. The reported yearly consumption of alcoholic beverages did not differ significantly between males and females, either through testing mean or distribution differences (male average = 235.69 ± 39.49 S.E.M.; female average = 170.65 ± 28.87). The reported intake of alcoholic beverages correlated significantly with bitterness of PROP (Fig. 5), especially when those who “never” report drinking alcohol were removed from the analyses (r=.36, P=.002), but not with fungiform papilla number. Average yearly intake of alcoholic beverages for nontasters (300.75 ± 66.82 S.E.M.) was greater than that for medium (177.49 ± 32.62) or supertasters (118.17 ± 20.29). There was a consistent negative relationship between PROP bitterness and intake across the beer, wine and liquor. In multiple regression, PROP effects were separate from those of sex on alcohol intake.

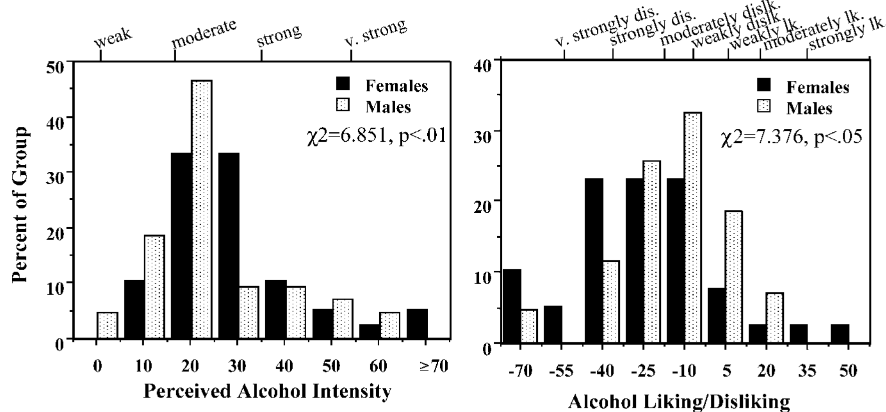

Fig. 4. Distributions of perceived alcohol intensity (left) and alcohol hedonics (right) for females and males. Frequencies are expressed as percentage of each sex. The distributions were tested with the chi square analyses; the categories were > or <30 on the gLMS for intensity ratings and <−23, ≥−23 and <−9, and ≥−9 on the gLMS for hedonic ratings.

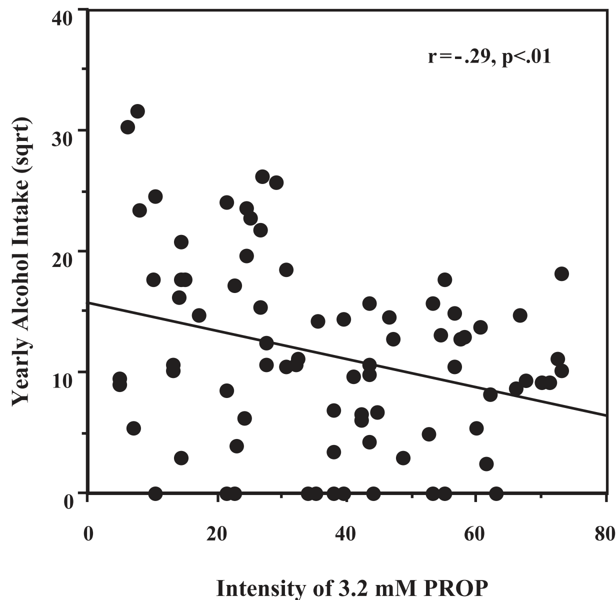

Fig. 5. Frequency of intake of alcoholic beverages over 1-year (square root transformed) by the bitterness of 3.2 M PROP rated on the general Labeled Magnitude Scale.

Multiple regression with sex, fungiform papilla number, PROP bitterness, NaCl and citric acid intensities and CTN/whole mouth ratio explained significant variance in intake of alcoholic beverages (r=.48, P < .002). Only PROP bitterness and CTN/whole mouth ratio were significant predictors: those who reported lower PROP bitterness (sr = − .26, P=.01) and lower CTN/whole mouth ratios (sr = − .24, P=.01) reported more frequent consumption of alcoholic beverages.

4. Discussion

This study of healthy adults showed significant associations between oral sensation and intake of alcoholic beverages. Those who tasted the least bitterness from concentrated PROP or had lowest numbers of fungiform papilla, as markers for genetic nontasters, reported less burn and disliking of a 50% alcohol probe painted on the tongue tip as well as more frequent consumption of alcoholic beverages. The spatial pattern of oral sensation also explained variation in burn from the alcohol probe and consumption of alcoholic beverages. Individuals who showed the most potential for chorda tympani nerve damage (low CTN to whole mouth ratio) reported the least intensity from the alcohol probe and the most frequent intake of alcohol beverages. Multiple regression analyses showed separate influences of taste genetics and spatial patterns of oral sensation on alcohol intake.

These findings support genetic taste influences on oral sensations from alcohol, which may influence the liking/disliking and ultimately consumption of alcoholic beverages. The present study and others suggest that genetic nontasters have less deterrence for consuming alcoholic beverages because they experience less negative oral sensations. Negative responses to alcohol sensations have been shown to deter the initiation of drinking in adolescents [73] and positive oral sensations from alcohol are reported as a reason for drinking alcohol in adults [22]. One study reported that over 80% of alcoholics liked the taste of alcoholic beverages [41]. Genetic variation in taste may have less affect on consumption patterns of beverages where bitter and irritation sensations are minimized or in social and physical environments that support drinking alcoholic beverages.

Taste genetic influences on alcohol sensation are consistent with previous studies with PROP bitterness related to alcohol sensations most frequently reported. Through magnitude matching and the standardization of oral sensations to the intensity of sodium chloride, Bartoshuk et al. [21] found that PROP medium and supertasters report greater bitterness and irritation from 30% to 50% ethanol applied to the tongue tip than do nontasters. Using the Labeled Magnitude Scale [56,57] for measuring intensity of oral sensations, Itranuovo and Powers [22] extended these findings to sampled beer; PROP supertasters tasted the most bitterness in bitter ale (Pilsner Urquell). Note that the differences in the perceived intensities of alcohol sensations across taster groups are sufficiently large to produce significant differences even with these earlier scaling methods. Recent data show that the gLMS produces a more accurate assessment of PROP effects on oral sensations [43,45]. Pickering et al. [23] used the gLMS and showed that individuals who tasted 3.2 mM PROP as greater than very strong also reported significantly more bitterness, astringency and acidity in red wines; advances in psychophysical techniques [13,43,45] may have revealed these associations where previous attempts did not [74].

Liking/disliking of alcohol associates with alcohol sensations and measures of taste genetics according to findings from this study and others. The more irritating the alcohol probe (present study) as well as more bitter a beer [22], the less it was liked. Greater PROP bitterness and number of fungiform papilla associated with more dislike of the alcohol probe; this is consistent with previous studies associating PROP bitterness with level of liking from sampled beer [22].

Associations between suprathreshold measures of PROP bitterness and alcohol intake are consistent with previous studies. Intranuovo and Powers [22] found that PROP nontasters had the highest intake of alcoholic beverages when they first started drinking; their findings did not extend to current alcohol consumption. The present study did find PROP effects on intake of alcohol during the year preceding the study; history of alcohol use and initiation of alcohol consumption was not determined. The sample size of the present study did not allow examination of relationships between PROP tasting and intake of specific alcoholic beverages. It may be that PROP effects would be less on alcoholic beverages that have less bitterness or irritation. The present study found that PROP bitterness was a better marker for alcohol intake than number of fungiform papilla;

that PROP shows significant and stronger correlation with alcohol intake than fungiform papillae number has also been found in preliminary data on middle-aged adults [75]. PROP bitterness may be a marker of all oral sensations from alcohol (e.g., taste, oral somatosensation and retronasal olfaction) whereas fungiform papilla number is more salient to the oral somatosensory properties.

The present study failed to find a significant relationship between PROP threshold and alcohol sensation, hedonics or intake. Although threshold showed a strong negative correlation with bitterness of 3.2 mM PROP (i.e., high threshold and low PROP bitterness), thresholds cannot consistently identify supertasters [11]. Factors not directly related to alcoholism (e.g., viral infection of the respiratory system and head trauma) damage taste, particularly bitterness [42], making an individual appear to be genetically less sensitive to PROP. Individuals who have depressed bitter taste perception on the anterior tongue show heightened response to burn from oral irritants [48] as well as phantom pain sensations [50]. It is not surprising that inconsistent findings exist on PROP tasting related to alcohol ingestive behaviors in studies that employ threshold as the measure of PROP tasting. A consistent relationship between history of alcoholism and PROP tasting may be more apparent with psychophysical techniques that clearly separate PROP nontasters, medium tasters and supertasters [13,23,43,45]; PROP supertasters may experience the most negative sensory cues from alcohol.

Females were skewed toward higher alcohol burn and more aversive ratings of this sensation. This sex difference cannot be explained by differences in PROP tasting across males and females; although previous studies show a sex difference in PROP tasting (see Ref. [11] for a review), females and males in the present study did not differ in PROP tasting. Women in the present study were skewed toward higher density of fungiform papillae; this has been reported previously [44]. The density of fungiform papilla and interactions between taste and trigeminal nerves influence the burn from alcohol. Because fungiform papillae are innervated by both taste and trigeminal fibers, individuals with highest density would likely experience greater burn from the alcohol probe.

Oral sensory responses to the 50% alcohol probe, intensity or hedonic ratings, did not correlate significantly with alcohol intake. This was expected as the probe provided primarily a measure of alcohol irritation that may not generalize to the full array of sensory and learned experiences associated with alcoholic beverages. Additionally, the probe was limited to the left tongue tip and drinking stimulates the entire mouth. Those who taste PROP as more bitter get more intensity and greater disliking from the probe of alcohol irritation, which might explain why these individuals consume alcohol less frequently. Intranuovo and Powers [22] did find that subjects reported the main reason for drinking beer was because they "liked the taste." Future investigations would benefit from testing the association between PROP intensity, oral sensations from alcoholic beverages and alcohol intake.

The NaCl and citric acid effects on alcohol intake appear to correspond with findings of Sandstrom et al. [53]; individuals who consumed alcoholic beverages most frequently (present study) or had a positive paternal history of alcoholism [53] perceived concentrated NaCl and citric acid as most intense. A direct comparison is difficult however because the studies employed different alcohol outcomes and the latter used a scaling methodology that limits ability to make valid across group comparisons. The present study revealed that the spatial pattern of taste (lower taste on the anterior tongue relative to whole mouth) explained some of the contribution of NaCl and citric acid to predict less burn from the alcohol probe and to predict more frequent intake of alcoholic beverages. Thus, it may be lower CTN taste functioning that leads to intensified whole mouth sensations from NaCl and citric acid. The concentrations of NaCl and citric acid in the Sandstrom et al. study would act as trigeminal stimuli: NaCl: 0.31, 0.62, 0.92, 1.23 and 1.54 M; and citric acid: 0.10, 0.19, 0.29, 0.38 and 0.48 M (correction of concentrations published in error, personnel communication from H. Kranzler—August 2003). Reductions of CTN taste intensify oral trigeminal sensations as shown by experimental [48] and clinical [50] evidence. It is unknown if the lower CTN taste is an antecedent or a consequence of the alcohol intake. As an antecedent, environmental insults (e.g., viral and trauma induced) could reduce oral sensations on the tongue tip and limit this barrier to consuming bitter and irritating alcoholic beverages. There may also be physiologic connections between salt sensations/hedonics and alcohol ingestive behaviors as suggested by preliminary evidence [53]. Low CTN taste could also be a consequence of disease and pathologies associated with high consumption of alcohol intake [76,77]. This deserves further evaluation.

The clinical significance of the intake data must be evaluated. While a greater percentage of the nontasters reported consuming alcohol daily than did supertasters, consumption of one to two alcoholic beverages per day can be part of a healthy diet as outlined in the 2000 edition of the Dietary Guidelines for Healthy Americans [78]. The subjects in the present study were recruited for a range of PROP tasting in both females and males and to control variables that could confound the ability to examine the influence of PROP tasting on dietary behaviors. The sample was diverse in PROP tasting; the bimodal distribution of PROP thresholds demonstrated both nontasters and tasters and the PROP functions suggested medium and supertasters (Fig. 2). Alcohol use in the sample approximated national statistics; according to the 1999 National Household Survey on Drug Abuse [79] data, approximately 60% of individuals aged 21–39 consumed alcohol in the month preceding the survey and that the rates of alcohol consumption were up to 70% in college students in New England. This compares to the present sample in which 65 of 80 subjects report consuming

alcoholic beverages greater than once per month. Inclusion of subjects with low levels of dietary restraint may have improved the accuracy of self-reported alcohol consumption as high dietary restraint has been shown to decrease the accuracy of dietary assessment [59].

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