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Variation in the Gene *TAS2R13* is Associated with Differences in Alcohol Consumption in Patients with Head and Neck Cancer

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Abstract

Variation in responsiveness to bitter-tasting compounds has been associated with differences in alcohol consumption. One strong genetic determinant of variation in bitter taste sensitivity is alleles of the *TAS2R* gene family, which encode chemosensory receptors sensitive to a diverse array of natural and synthetic compounds. Members of the *TAS2R* family, when expressed in the gustatory system, function as bitter taste receptors. To better understand the relationship between *TAS2R* function and alcohol consumption, we asked if *TAS2R* variants are associated with measures of alcohol consumption in a head and neck cancer patient cohort. Factors associated with increased alcohol intake are of strong interest to those concerned with decreasing the incidence of cancers of oral and pharyngeal structures. We found a single nucleotide polymorphism (SNP) located within the *TAS2R13* gene (rs1015443 [C1040T, Ser259Asn]), which showed a significant association with measures of alcohol consumption assessed via the Alcohol Use Disorders Identification Test (AUDIT). Analyses with other SNPs in close proximity to rs1015443 suggest that this locus is principally responsible for the association. Thus, our results provide additional support to the emerging hypothesis that genetic variation in bitter taste receptors can impact upon alcohol consumption.

Key words: alcohol consumption, alcohol use disorders identification test (AUDIT), bitter taste, behavioral genetics, taste receptor, *TAS2R*

Introduction

Cancers of the oral and pharyngeal structures have a high incidence worldwide and a grim prognosis (Zygogianni et al. 2011). Lifestyle risk factors play a major role in the development of these carcinomas. Alcohol intake, particularly in combination with smoking, is a significant lifestyle risk factor associated with the incidence of these cancers (Hayes RB et al. 1999; Anantharaman et al. 2011; Groome et al. 2011; Lambert et al. 2011; Zygogianni et al. 2011). Thus, factors associated with increased alcohol intake are of strong interest to those concerned with decreasing the incidence of these malignancies.

One of the many factors thought to influence alcohol intake is its perceived taste. Researchers have attempted to perceptually categorize the taste evoked by ethanol. Results from such experiments suggest that ethanol evokes both sweet and bitter percepts, depending on concentration (Di Lorenzo et al. 1986; Lawrence and Kiefer 1987; Kiefer and Lawrence 1988; Kiefer and Mahadevan 1993; Lanier et al. 2005; Blizard 2007). Sensitivity to the sweet component may contribute substantially to preference for alcohol, while sensitivity to the bitter component may negatively influence alcohol consumption. Indeed, research suggests

that individuals who possess enhanced perception of bitter taste, as defined by sensitivity to the bitter tasting chemical 6-*n*-propylthiouracil (PROP), consume alcoholic beverages less frequently than individuals who are less sensitive to the compound (Intranuovo and Powers 1998; Duffy et al. 2004; Basson et al. 2005; Lanier et al. 2005).

In addition to influencing alcohol intake, a large body of research indicates that PROP-defined bitter taste sensitivity also affects the intake of certain bitter-tasting foods, including specific fruits and vegetables (Fischer et al. 1961; Glanville and Kaplan 1965; Jerzsa-Latta et al. 1990; Drewnowski et al. 1997, 1998, 1999, 2000; Dinehart et al. 2006; Tepper et al. 2009; Duffy et al. 2010; Feeney et al. 2011). Members of the T2R family of GPCRs have been shown to function as taste receptors for chemicals that humans and mammals perceive as bitter tasting (Chandrashekar et al. 2000; Mueller et al. 2005; Meyerhof et al. 2010). T2R receptors are encoded by members of the *TAS2R* gene family. The principal genetic determinants of phenotypic variation in PROP taste sensitivity are alleles of the gene *TAS2R38* (Kim et al. 2003; Bufe et al. 2005). Indeed, variation in *TAS2R38* is also associated with vegetable intake (Sacerdote et al. 2007; Duffy et al. 2010). Moreover, polymorphisms in or near *TAS2R38*, as well as the gene *TAS2R16*, have been associated with measures of alcohol consumption (Duffy et al. 2004; Hinrichs et al. 2006; Wang et al. 2007; Hayes JE et al. 2011). There are 25 functional *TAS2R* genes in the human genome (Kim et al. 2005; Meyerhof 2005). However, the influence of genetic variation in bitter taste receptor genes on alcohol consumption has been limited to the assessment of just a few genes (i.e., *TAS2R38* and *TAS2R16*). Since many *TAS2R* receptors are broadly tuned (Meyerhof et al. 2010), it is entirely possible that other *TAS2R*s may influence alcohol intake.

Because of the observed role that bitter taste plays in alcohol intake, we hypothesized that allelic variation in individual *TAS2R*s could significantly impact upon alcohol consumption. Thus, we initiated a candidate gene study to identify specific sequence variants in *TAS2R* genes that are associated with measures of alcohol intake, using a cohort of head and neck cancer patients. These patients have relatively high premorbid levels of alcohol intake and may possess genetic factors that predispose them to increased levels of alcohol consumption, making head and neck patients an ideal group. This is one of the first studies attempting to link a known risk factor (alcohol intake) to allelic variations in bitter taste receptors in a head and neck cancer clinical population.

Materials and methods

Head and neck cancer patients

This study was conducted according to the principles of the 1975 Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the University of Florida Institutional Review Board. A convenience sample of 173 (126 men and 47 women) head and neck cancer patients was recruited from clinics at the University of Florida. Sixty-five percent were newly diagnosed head and neck cancer patients. The characteristics of this group have been detailed elsewhere (Logan et al. 2010, also see Table 1).

DNA extraction and genetic analysis

DNA was extracted from leukocytes from whole blood following manufacturer's instructions (PureGene system, Qiagen), with occasional modification for hemolyzed samples. Purified DNA samples were stored at 4°C in 1xTE (Tris 10 mM and EDTA 1 mM, pH 8.0) until analyzed. When necessary, DNA was genome-amplified using the Illustra GenomiPhi V2 D amplification kit from GE Lifesciences. Genotypes were determined using vendor-supplied TaqMan SNP assays from Applied Biosystems with the plates read on an ABI Prism 7900 HT (Applied Biosystems/Life Technologies).

We identified candidate SNPs in coding and regulatory regions from the Entrez SNP database and from the literature (e.g. Kim et al. 2005). In total, 23 *TAS2R*-associated SNPs were genotyped (Table 2). SNP frequencies in our sample were similar to values seen in reference data sets. Three additional haplotype-tagging SNPs ($r^2 \geq 0.8$), identified from the HapMap (International Hapmap Consortium 2005), were genotyped for the purpose of defining linkage disequilibrium (LD; bottom of Table 2).

Measures

Measures were drawn from the 10-item Alcohol Use Disorders Identification Test (AUDIT; Saunders et al. 1993). The AUDIT was developed by the World Health Organization as a simple method of screening for excessive

Table 1 Characteristics of cancer patients

	N = 173
Sex	
Men	126
Women	47
Race	
White	94% (*N* = 162)
Other	6% (*N* = 11)
Education	
<high school	7%
High school diploma or GED	31%
Some college	26%
Bachelor's degree or higher	36%
Age	60.7 ± 13.4

Table 2 SNP genotyping statistics and results of association analyses[#]

Chromosome, position (kb)	SNP ID	Associated/ nearest gene	Call rate (%)	HWE *P* Value	Major/minor allele	MAF	SNP type	Six or more	Per day	Drink containing alcohol
7, 122625	rs1308724	***TAS2R16***	93.3	0.055	G/C	0.38	Noncoding	0.49	0.87	0.08
7, 122630	rs846672	***TAS2R16***	93.8	0.561	C/A	0.35	Noncoding	0.61	0.52	0.91
7, 141464	rs765007	***TAS2R3***	97.3	0.613	C/T	0.47	5′ UTR	0.11	0.15	0.42
7, 141479	rs2234001	***TAS2R4***	95.1	0.554	C/G	0.48	V96L	0.35	0.69	0.28
7, 141490	rs2234012	***TAS2R5***	96.4	0.032	G/A	0.13	5′ UTR	0.46	0.24	0.37
7, 141673	rs10246939	***TAS2R38***	96.4	0.067	T/C	0.46	I296V	0.72	0.76	0.87
7, 141673	rs1726866	***TAS2R38***	98.2	0.266	T/C	0.46	V262A	0.10	0.34	0.57
7, 141673	rs713598	***TAS2R38***	99.6	0.095	C/G	0.43	P49A	0.03	0.68	0.0008
7, 142881	rs4726600	***TAS2R39***	97.3	0.518	G/A	0.23	Noncoding	0.32	0.93	0.12
7, 142920	rs10260248	***TAS2R40***	95.1	0.012	C/A	0.09	S187Y	0.86	0.79	0.95
7, 142921	rs534126	***TAS2R40***	97.8	0.934	C/T	0.35	Noncoding	0.51	0.43	0.19
7, 143168	rs12666496	***TAS2R41***	97.3	0.019	A/T	0.26	Noncoding	0.90	0.77	0.37
7, 143175	rs1404635	***TAS2R41***	96.0	0.255	G/A	0.29	T63T	0.21	1.00	0.08
12, 10954	rs619381	***TAS2R7***	98.7	0.910	C/T	0.12	M304I	0.69	0.13	0.34
12, 10959	rs1548803	***TAS2R8***	97.8	0.138	T/C	0.42	L183L	0.01	0.04	0.55
12, 10978	rs10845219	***TAS2R10***	97.8	0.244	C/T	0.42	Noncoding	0.02	0.05	0.72
12, 10982	rs4763216	***TAS2R10***	98.7	0.236	G/C	0.42	Noncoding	0.00	0.02	0.38
12, 11061	rs1015443	***TAS2R13***	98.2	0.027	C/T	0.42	S259N	0.0002	0.001	0.06
12, 11092	rs7138535	***TAS2R14***	95.5	0.021	T/A	0.24	G38G	0.26	0.42	0.41
12, 11139	rs1376251	***TAS2R50***	95.5	0.055	C/T	0.31	C203Y	0.56	0.49	0.65
12, 11150	rs10845281	***TAS2R20***	97.3	0.235	T/C	0.33	I236V	0.49	0.95	0.23
12, 11174	rs10772420	***TAS2R19***	94.6	0.230	A/G	0.47	C299R	0.06	0.08	0.18
12, 11182	rs11612527	***TAS2R31***	95.5	0.078	T/A	0.22	Noncoding	0.81	0.18	0.78
12, 11000	rs1047699*	***PRR4***	91.1	0.481	C/T	0.21	G43R	0.62	0.45	0.86
12, 11000	rs1063193*	***PRR4***	90.0	0.434	T/C	0.47	Q109R	0.98	0.65	0.72
12, 11036	rs10492098*	***PRH1***	91.3	0.009	A/G	0.43	intronic	0.003	0.005	0.06

kb, kilobases; MAF, minor allele frequency.

*SNPs genotyped after the first 23 SNPs; used for LD analysis.

[#]Raw, unadjusted ***p*** values are presented in the table.

drinking. We evaluated the impact of genetic variation in taste receptor SNPs on responses to three questions from the AUDIT: "How often do you have a drink containing alcohol?" (DRINKSCONTAININGALCOHOL); "How many drinks containing alcohol do you have on a typical day when you are drinking?" (PERDAY); and "How often do you have six or more drinks on one occasion? (SIXORMORE)."

Analysis

The genetic association analyses were performed using SNP & Variation Suite 7.4 (Golden Helix, Bozeman). All SNPs were analyzed for extreme deviation from Hardy–Weinberg equilibrium ($p < 0.001$) using chi-square test. SNPs with less than 90% complete genotyping information were excluded from analyses. Single marker associations were tested using linear regression including age, sex, and smoking status (i.e., 1 = never smoked, 2 = past smoker, 3 = current smoker) as covariates in an additive model (i.e., heterozygotes expressing intermediate phenotypes: xx = 0, Xx = 1, XX = 2).

Genetic association can be confounded by population stratification (Hamer and Sirota 2000). Therefore, our data was corrected by principal component analysis to control for the possibility of stratification (Price et al. 2006). For

our initial association analyses, in addition to the observed *p* values, we present Bonferroni adjusted values for the interested reader. There is, however, an active debate as to whether multiple comparison adjustments are appropriate for exploratory studies (Perneger 1998; Bender and Lange 2001). Analysis of associations related to *TAS2R16* (i.e., rs1308724 and rs846672) and *TAS2R38* (i.e., rs10246939, rs1726866 and rs713598) were based on a priori hypotheses (Duffy et al. 2004; Hinrichs et al. 2006; Wang et al. 2007; Hayes JE et al. 2011). Thus, no multiple comparisons adjustments were made. Pairwise LD between the SNPs and haplotype block analysis was computed using Haploview 4.2 (Barrett et al. 2005). Haplotype blocks were defined by 95% confidence bounds on *D*' (Gabriel et al. 2002).

Results

We first asked whether any variants in and around select *TAS2R*s, previously associated with measures of alcohol consumption, were correlated with reported alcohol intake in our cancer patients. We observed that a nonsynonymous coding SNP located in the gene *TAS2R38* showed significant association with the measure DRINKSCONTAININGALCOHOL ($p = 0.0008$) but not with PERDAY or SIXORMORE. The C allele of rs713598, the major allele in the cohort, is strongly associated with increased taste sensitivity to PROP (Kim et al. 2003; Bufe et al. 2005) and decreased alcohol consumption in our head and neck cancer cohort.

Next we asked whether any other *TAS2R* SNPs were associated with measures of alcohol consumption from the AUDIT. We found one SNP, located on chromosome 12, that showed significant associations with the measures PERDAY and SIXORMORE. Variation in the nonsynonymous coding SNP rs1015443, located in the gene *TAS2R13*, was significantly associated with the measure PERDAY ($p = 0.0011$; Bonferroni value = 0.03 after correction for multiple comparisons; Table 3) and with SIXORMORE ($p = 0.000231$; Bonferroni value = 0.005; Table 3). By and large, subjects homozygous for the major allele (CC carriers) consumed alcoholic beverages less frequently relative to heterozygotes and those homozygous for the minor allele (Table 4).

To define the extent of LD around rs1015443, we used the other taste receptor-associated SNPs that we genotyped both upstream and downstream of *TAS2R13* on chromosome 12 (Figure 1). We identified three LD blocks. The first (LD Block 1) contains four SNPs (rs619381, rs1548803, rs10845219, and rs4763216) and is found ~79 kb upstream of rs1015443, extending for ~28 kb (Figure 1). SNP rs1015443 (*TAS2R13*) displays moderate LD with the SNPs in this block ($r^2 = 0.13$–0.65) but significantly less than the threshold for inclusion in the LD block. Indeed, none of these SNPs were associated with any of the measures of alcohol intake. The five other SNPs in this region show low levels of LD with rs1015443, although four of them form two small LD blocks at the distal end of this cluster (LD Blocks 2 and 3; Figure 1).

Table 3 PERDAY and SIXORMORE *p* values for rs1015443* and rs10492098#

SNP/Genotype	PERDAY	SIXORMORE
rs1015443	0.001	0.0002
rs10492098	0.005	0.003

*Number of subjects per rs1015443 genotype (TT = 42; CT = 61; CC = 66).

#Number of subjects per rs10492098 genotype (GG = 41; GA = 57; AA = 60).

Table 4 Phenotype averages* by rs1015443 genotype#

Phenotype	TT	CT	CC
PERDAY	1.50 ± 0.93	1.57 ± 1.01	1.41 ± 0.85
SIXORMORE	1.75 ± 1.32	1.66 ± 1.09	1.57 ± 1.06

*Mean ± standard deviation. These values are unadjusted for the variance associated with the covariates used in the regression models (age, sex, and smoking status).

#Number of subjects per genotype (TT = 42; CT = 61; CC = 66).

Because of the large intervals between rs1015443 and the SNPs located in LD block 1, we sought to further refine the extent of LD in this region by examining several additional SNPs in the genes located directly upstream of *TAS2R13*. The coding sequences of two genes, proline-rich protein HaeIII subfamily 1 (*PRH1*) and proline rich 4 (*PRR4*), are located in this region. Thus, we identified haplotype-tagging SNPs within each of these genes; two SNPs were identified for *PRR4* (rs1047699 and rs1063193) and a single SNP was selected for *PRH1* (rs10492098).

The addition of these three SNPs upstream of *TAS2R13* slightly redefined the LD structure we observed between the genotyped SNPs. After inclusion of these SNPs, we observed four LD blocks. Three of the LD blocks were the same as that defined in Figure 1. A new LD block was formed that consisted of two of the three SNPs that were introduced in this later analysis (rs1047699 and rs1063193). These SNPs showed low to moderate LD with rs1015443 ($r^2 = 0.10$ and 0.65, respectively; Figure 2). The other SNP included in this LD analysis, rs10492098, was in tight LD with rs1015443 ($r^2 = 0.96$; Figure 2). rs10492098 was also significantly associated with the measures PERDAY and SIXORMORE, though the *p*-values of its association were higher than that observed for rs1015443 (Table 3). When taken together, these data suggest that variation in the bitter taste receptor gene *TAS2R13* is principally responsible for the association observed in our head and neck cancer cohort.

Discussion

Our results indicate that genetic variation in the gene *TAS2R13* is associated with a self-reported increase in

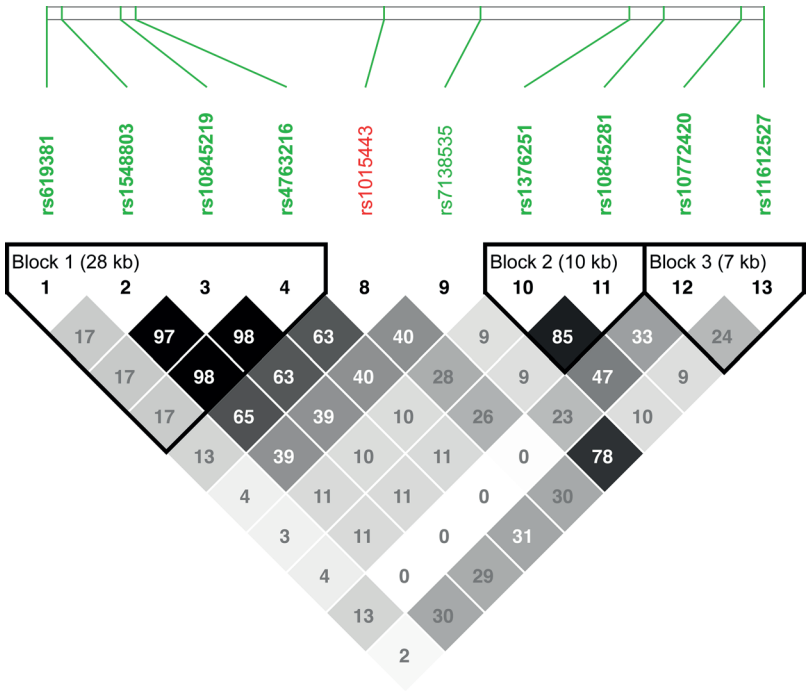

Figure 1 Pairwise LD (r^2) among nine SNPs located both upstream and downstream of rs1015443 on chromosome 12. r^2 values × 100 are indicated within squares, with darker shades indicating higher r^2 values.

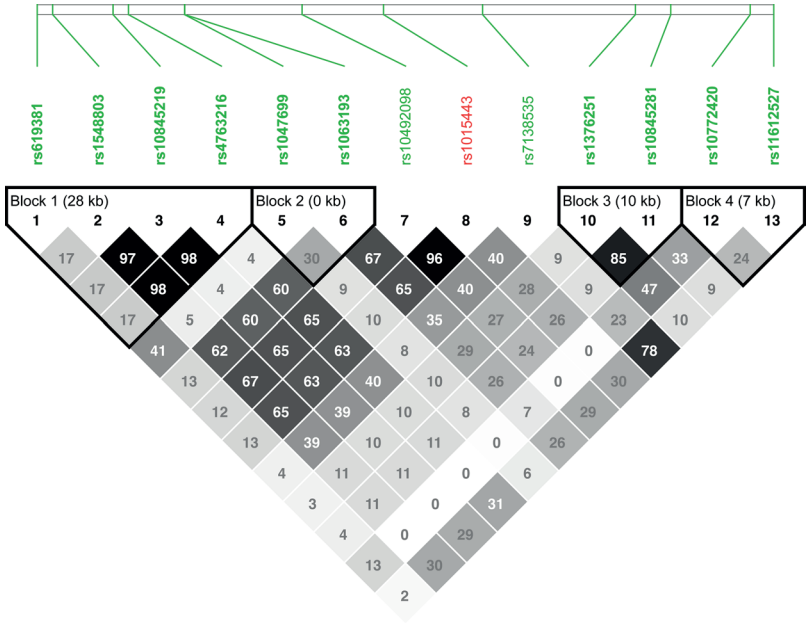

Figure 2 Pairwise LD (r^2) among the 9 SNPs located both upstream and downstream of rs1015443 on chromosome 12 and 3 additional SNPs located directly upstream of *TAS2R13*. r^2 values × 100 are indicated within squares, with darker shades indicating higher r^2 values.

alcohol consumption as assessed using two measures from the AUDIT: PERDAY and SIXORMORE. We also observed a significant association between genetic variation in the gene *TAS2R38* and a measure of alcohol consumption (DRINKSCONTAININGALCOHOL). Other measures of alcohol consumption have been associated with variation in *TAS2R38*. Variation in responses to the measure Maxdrinks (i.e., responses to the question: what is the largest number of drinks you have ever had in a 24-h period?) was significantly associated with genetic variation in the gene *TAS2R38* (Wang et al. 2007) in an African-American cohort. Hayes JE et al. (2011) reported associations with genetic variation in

TAS2R38 and a measure of the frequency of alcohol intake in a convenience sample of 96 healthy adults subjects. Thus, our results are consistent with previous studies that have reported that variation in bitter taste receptor genes is associated with alcohol consumption.

The AUDIT is an easily administered, widely used and referenced screening tool for the assessment of alcohol use. However, this instrument does not provide information on lifetime alcohol use/problems. Specifically, subjects who are lifetime abstainers are collapsed together with subjects who may be alcohol dependent but are abstaining from alcohol use. If such information were incorporated into our analyses, these data would likely have strengthened our results and, potentially, led to the discovery of other genotype–phenotype associations.

Taste abnormalities have been observed in patients with head and neck cancer, and it has been postulated that these defects are caused by treatment-based radiation exposure (Mirza et al. 2008). It is possible that any genetic or environmental factors that predominate in head and neck cancer patients may also allow the impact of *TAS2Rs* on alcohol intake to be more easily observed. More research is needed to elucidate any putative factors that may be influencing the relationship between genetic variation in *TAS2R* genes and alcohol intake. Nevertheless, our results provide new evidence supporting the link between gustatory functioning and the ingestion of alcoholic beverages.

Participants who were homozygous for the major allele of rs1015443 consumed alcoholic beverages less frequently relative to subjects that were heterozygotes or those subjects that were homozygous for the minor allele. It is unknown whether the amino acid change from asparagine (in receptors encoded by genes that possess the minor allele of rs1015443) to serine in those that possess the major allele would influence the functioning of *TAS2R13*. Based on the influence of other *TAS2Rs* on alcohol intake (i.e., alleles that decrease the sensitivity of a *TAS2R* receptor lead to increased alcohol consumption [Wang et al. 2007; Hayes et al. 2008]), we would predict that the minor allele of rs1015443 impacts negatively on the functioning of *TAS2R13*. The natural ligand or ligands for *TAS2R13* are unknown. However, the synthetic compounds diphenidol and denatonium benzoate are known *TAS2R13* agonists (Meyerhof et al. 2010). Experiments using diphenidol and/or denatonium benzoate designed to determine the impact of variation at rs1015443 will be needed to test this prediction.

The analysis of LD surrounding *TAS2R13* revealed only a single other candidate locus that could be responsible for the association signal emanating from this genomic region. The SNP rs10492098, located in *PRH1*, is in extremely tight LD with rs1015443. *PRH1* encodes a member of the proline rich protein (PRP) family of peptides. These peptides are thought to play a role in oral mucosal defense by their involvement in maintaining calcium concentrations, as well as in retarding the aggregation capacity of microorganisms, thereby impacting their ability to colonize on tissue surfaces (Moreno et al. 1982; Gibbons et al. 1988; Ligtenberg et al. 1992; Fung et al. 2004). However, surprisingly, salivary levels of some of these peptides have been implicated in the modulation of bitter taste perception (Cabras et al. 2012). It should be noted that rs10492098 is on the borderline of Hardy Weinberg equilibrium in the sample (Table 2, HWE p value = 0.009). This fact presents a significant potential confound in the interpretation of the results based on data derived from the genotyping of this allele and, as such, adds to our confidence that rs1015443 is the true causal allele. Moreover, the observed *P*-values of the associations for rs10492098 were higher, and in one case, an order of magnitude higher, than that observed for rs1015443 (Table 3). Indeed, considering these factors, in addition to our a priori hypothesis regarding the impact of variation in bitter taste receptor genes on alcohol consumption, we conclude that rs1015443, located in the gene *TAS2R13*, is principally responsible for the association signal emanating from this genomic region. That said, based on the proximity of these two loci and the fact that in any population they are likely to be in tight LD, it will be exceedingly difficult to disentangle the influence of each locus on alcohol consumption using genotype/phenotype association testing alone. Additional *in vivo* experiments (e.g., Roudnitzky et al. 2011), and possibly the use of animal models, will be needed to determine the individual effects of these two genes on taste-related behavior.

It is well known that cigarette use is confounded with alcohol consumption (Bottoni et al. 1997; Koh et al. 2005), and some studies suggest that variation in bitter taste receptors may impact on cigarette usage (Cannon et al. 2005; Mangold et al. 2008). Although none of the SNPs in our head and neck cancer cohort was directly associated with smoking status (data not shown), we did control for cigarette use in our analyses. Thus, the use of cigarettes by our head and neck cancer patients does not appear to account for the observed associations between bitter taste receptor variants and alcohol consumption. Nevertheless, a more systematic evaluation of the relationship between bitter taste perception and its influence on the use of both cigarette and alcohol seems warranted.

In this report, we hypothesized that T2R receptor function influences alcohol consumption via mediation of bitter taste perception. However, it is also possible that bitter taste receptors affect alcohol intake via their expression and function in nonoral tissues. In addition to their expression in the oral–nasal cavity where they act as taste receptors, it is now well known that taste receptors, including T2Rs, are expressed in tissues all over the body, where they appear to act as general chemoreceptors (Behrens and Meyerhof, 2010, 2011). For example, T2Rs are expressed in tissues of the gastrointestinal tract (GI), where they appear to play a role in gastrointestinal hormone secretion as well as in the nutrient dependent regulation of metabolism (Sternini 2007; Kaji et al. 2009; Dotson et al. 2010; Janssen et al. 2011; Jeon et al. 2011). Thus, it is possible that it is the extraoral expression and function of T2Rs that is impacting on alcohol consumption. For example, it is known that plasma

levels of certain gastrointestinal hormones, which are known to be affected by taste receptor expression and function in the GI tract, are correlated with alcohol intake (Yeomans et al. 2003; Pravdova and Fickova 2006; Leggio et al. 2011).

In summary, alcohol intake is a complex trait influenced by numerous genes. We have observed an association between genetic variation in a bitter taste receptor gene and the consumption of alcohol. As alcohol consumption is a risk factor in developing head and neck cancer, taste perception may represent one of the many pathways that contribute to the development of these cancers of the oral cavity.

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