

TAS2R38 Predisposition to Bitter Taste Associated with Differential Changes in Vegetable Intake in Response to a Community-Based Dietary Intervention

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ABSTRACT Although vegetable consumption associates with decreased risk for a variety of diseases, few Americans meet dietary recommendations for vegetable intake. *TAS2R38* encodes a taste receptor that confers bitter taste sensing from chemicals found in some vegetables. Common polymorphisms in *TAS2R38* lead to coding substitutions that alter receptor function and result in the loss of bitter taste perception. Our study examined whether bitter taste perception *TAS2R38* diplotypes associated with vegetable consumption in participants enrolled in either an enhanced or a minimal nutrition counseling intervention. DNA was isolated from the peripheral blood cells of study participants (N = 497) and analyzed for polymorphisms. Vegetable consumption was determined using the Block Fruit and Vegetable screener. We tested for differences in the frequency of vegetable consumption between intervention and genotype groups over time using mixed effects models. Baseline vegetable consumption frequency did not associate with bitter taste diplotypes ($P = 0.937$), however after six months of the intervention, we observed an interaction between bitter taste diplotypes and time ($P = 0.046$). Participants in the enhanced intervention increased their vegetable consumption frequency ($P = 0.020$) and within this intervention group, the bitter non-tasters and intermediate-bitter tasters had the largest increase in vegetable consumption. In contrast, in the minimal intervention group, the bitter tasting participants reported a decrease in vegetable consumption. Bitter-non tasters and intermediate-bitter tasters increased vegetable consumption in either intervention more than those who perceive bitterness. Future precision medicine applications could consider genetic variation in bitter taste perception genes when designing dietary interventions.

KEYWORDS

taste perception
bitter taste
taste receptors
gene-diet
interaction

Few Americans consume the recommended amount of dark green and orange vegetables, despite the association between vegetable consumption and reduced risk of chronic diseases (Kimmons *et al.* 2009). Public health practitioners and researchers aim to increase vegetable consumption through dietary interventions, but the impact of interventions on fruit and vegetable intake yields mixed results. For example, some interventions resulted in increased vegetable consumption by participants (Emmons *et al.* 2005; Bowen *et al.* 2009; Djuric *et al.* 2010), whereas others did not significantly affect vegetable consumption (Dzewaltowski *et al.* 2009). In instances where interventions increase vegetable intake,

the effects are generally small and participants often do not reach recommended intake levels (Pomerleau *et al.* 2005; Thomson and Ravia 2011).

One possible explanation for the mixed results of dietary intervention studies is heterogeneity of participants regarding characteristics that strongly influence vegetable intake, such as taste preferences. Taste is an important determinant of fruit and vegetable intake in adults and children in the United States (US) (Rasmussen *et al.* 2006; Guillaumie *et al.* 2010). While phytonutrients in vegetables, such as phenols, flavonoids, isoflavones, terpenes, and glucosinolates, seem to be protective

against certain cancers, their bitter taste can be a deterrent to consumption (Drewnowski 1997). Vegetable sweetness and bitterness were found to be independent predictors of more or less preference for sampled vegetables and vegetable intake, respectively, and the ability to detect a bitter tasting compound called propylthiouracil (PROP) was related to vegetable taste preferences (Dinehart *et al.* 2006).

Identified in 2003 (Kim *et al.* 2003), the *TAS2R38* gene encodes a G protein coupled receptor that functions as a taste receptor, mediated by ligands such as PROP and phenylthiocarbamide that bind to the receptor and initiate signaling that can confers various degrees of taste perception (Kim and Drayna 2005). Vegetables in the brassica family, such as collard greens, kale, broccoli, cabbage, and Brussels sprouts, contain glucosinolates and isothiocyanates, which resemble PROP, and therefore much of the perceived “bitterness” of these vegetables is mediated through *TAS2R38* (Bufe *et al.* 2005). Bitter taste receptors in the TS2R family are also found in gut mucosal and pancreatic cells in humans and rodents. These receptors influence release of hormones involved in appetite regulation, such as peptide YY and glucagon-like peptide-1, and therefore may influence caloric intake and the development of obesity (Rozengurt 2006). Thus, bitter taste perception may affect dietary behaviors by influencing both taste preferences and metabolic hormonal regulation.

Three variants in the *TAS2R38* gene – rs1713598, rs1726866, and rs10246939 – are in high linkage disequilibrium in most populations and result in amino acid coding changes that lead to a range of bitter taste perception phenotypes (Kim *et al.* 2004; Genick *et al.* 2011). The PAV haplotype is dominant; therefore, individuals with at least one copy of the PAV allele perceive molecules in vegetables that resemble PROP as tasting bitter, and consequently may develop an aversion to bitter vegetables. In contrast, individuals with two AVI haplotypes are bitter non-tasters. PAV and AVI haplotypes are the most common, though other haplotypes exist that confer intermediate bitter taste sensitivity (AAI, AAV, AVV, and PVI) (Boxer and Garneau 2015). This taste aversion may apply to vegetables in general (Duffy *et al.* 2010). Therefore, dietary interventions aiming to increase vegetable intake may have different outcomes depending on individuals’ perceptions of the taste.

While many studies have examined whether certain participant and intervention characteristics influence differential response to dietary interventions, such as age, sex, race, education, disease state, and intervention delivery methods (Ammerman *et al.* 2002; Carcaise-Edinboro *et al.* 2008), we are not aware of studies examining whether genes associated with bitter taste perception moderate participants’ responses to dietary interventions. The Heart Healthy Lenoir (HHL) Project offers a unique opportunity to test a concept that the genetic predisposition to bitter taste perception may associate with a differential response to a dietary intervention among a diverse, community-based

study population (Keyserling *et al.* 2016; Cené *et al.* 2017). In this paper we tested the following two hypotheses:

1. Participants with the *TAS2R38* bitter non-taster diplotype will consume more servings of vegetables per day at baseline than participants with intermediate or bitter taster diplotypes.
2. The *TAS2R38* diplotype will moderate the effect of the HHL intervention on vegetable consumption such that participants with a bitter taster diplotype will have a lower increase in reported vegetables intake than participants with a bitter non-taster diplotype after 6 months of the intervention.

MATERIALS AND METHODS

The Heart Healthy Lenoir (HHL) Project Overview

The overall goal of the HHL Project was to reduce Cardiovascular Disease (CVD) risk and disparities in CVD risk among Lenoir County, North Carolina residents, as previously described (Pitts *et al.* 2013; Halladay *et al.* 2013). It was conducted in Lenoir County because of its location in the “stroke belt” (Howard *et al.* 2007) of eastern North Carolina, where rates of CVD are higher than state and national averages (Centers for Disease Control and Prevention 2014) and because it has a large minority population (40% African American) that experiences disproportionately higher rates of CVD (Mozaffarian 2016). The overall Project included three coordinated studies: a lifestyle intervention study focusing on diet and physical activity (Keyserling *et al.* 2016) a study to improve high blood pressure management at local clinical practices (Cené *et al.* 2017) and a study examining associations between genetic markers and change in CVD risk factors. The project was designed and conducted with input from a local Community Advisory Committee and approved and monitored by the University of North Carolina at Chapel Hill’s Institutional Review Board, with data collected from September 20, 2011 to November 7, 2014 and analyzed in 2017. This trial is registered as # NCT01433484 at clinicaltrials.gov. All study participants gave verbal consent for administration of the study screening questionnaire (to assess eligibility) and written consent before study data were collected.

Heart Healthy Lenoir (HHL) Interventions

Participants in the HHL Project (N = 664 in total) could take part in the lifestyle study (N = 339), the high blood pressure study (N = 525) or both (N = 200). All participants were invited to take part in the genomics study. We utilized the data collected at baseline and at the 6-month follow-up that included participants with complete data for the variables of interest in this study, including bitter taste perception phenotype characterized by three SNPs on the *TAS2R38* gene, vegetable intake frequency, and model covariates (N = 497). Twelve participants of the 509 genotyped (2%) were missing data (other than household income) and therefore removed from the analysis. The lifestyle intervention is described in detail elsewhere (Keyserling *et al.* 2016). Briefly, during the first 6 months, the dietary component of this intervention included four counseling sessions that focused on improving dietary fat and carbohydrate quality, consistent with a Mediterranean dietary pattern. The primary focus of the second counseling session was on increasing fruit and vegetable consumption with a goal of seven total servings per day. The high blood pressure intervention is also described in detail elsewhere (Halladay *et al.* 2013; Cené *et al.* 2017). Participants in the high blood pressure study received limited dietary counseling by phone, with only 13 receiving a counseling phone call before the 6-month follow-up measurement visit. Accordingly, in this paper, the dietary intervention given to lifestyle study participants is considered the “enhanced”

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intervention, while the intervention given to those who only participated in the high blood pressure study is considered the “minimal” intervention.

Genotyping procedure

SNP status was obtained from 505 HHL participants at baseline via DNA isolated from peripheral blood cells using the Infinium Human Omni Express Exome+ BeadChip (Illumina). Genotypes were generated from genomic DNA using the Infinium workflow essentially as described by the manufacturer. DNA was amplified, fragmented, precipitated with isopropanol, and resuspended prior to hybridization onto BeadChips containing 50mer probes. After hybridization, enzymatic single base extension with fluorescently labeled nucleotides was conducted to distinguish alleles. Hybridized BeadChips were imaged using an Illumina iScan to determine intensities for each probe. Corresponding genotypes were extracted from intensity data and called using a standard cluster file within Illumina Genome Studio software.

Imputing SNPs

All DNA samples identified as either African American (AA, N = 304) or Caucasian American (CAU, N = 201) were imputed for a total of 505 samples. The array data were exported into plink format converted into chromosome-specific variant call format, applying the following filters: merge replicate probes, switch the alternate (ALT) or reference (REF) sequence if deemed necessary by reference, exclude markers where neither REF nor ALT matches the reference, exclude markers where REF is not AGCT. Additionally, in preparation for imputing the following filters were further applied: remove markers not in the reference, fill ALT values in from reference where genotype is entirely homozygous for reference. Samples were imputed twice, once with the Michigan imputation server (Das *et al.* 2016) and once with Beagle (v4.1) (Browning and Browning 2016). All 505 samples imputed with Beagle were run against the 2504 sample reference panel from 1000 genomes. The Haplotype Reference Consortium (HRC, 65k haplotypes) reference panel was used to run the CAU samples on the Michigan imputation server, and the Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA) reference panel was used to run the AA samples on the imputation server. A brief summary of coverage regarding the panels and how they performed with the target marker set (the markers from the genotyping array) is provided (Table S1). However, the Illumina genotyping arrays are sparse compared to the reference panels. We filtered our array data for conformity and the markers remaining used for the variant call formatted files (VCF) are indicated (Table S2).

Phased genotype, haplotype, and diplotype analysis

The phased genotyping data on chromosome 7 for the three *TAS2R38* SNPs (rs713598, rs10246939, and rs1726866) were used to extract the haplotypes of each study subject using the public server at usegalaxy.org (Afgan *et al.* 2016) to analyze the data with the VCFgenotype-to-haplotype tool (v1.0.0). VCFtools (v0.1.15) was used to generate all genotype and haplotype frequencies as well as the linkage disequilibrium analyses (Danecek *et al.* 2011). The resulting diplotype consisting of the three substitution mutations was used to determine the bitter taste sensitivity phenotype using previously published PROP taste responsiveness with a single PAV haplotype conferring bitter taste (Boxer and Garneau 2015).

Outcome variable

We used the Block Fruit and Vegetable Screener (Block *et al.* 2000) to assess vegetable consumption in two mutually exclusive categories:

green salads and other types of vegetables. The Block F&V screener is valid for assessing high and low vegetable intake and has been used in African American and White populations (Block *et al.* 2000; Gary *et al.* 2004). Frequency scores were calculated by adding the frequency categories (0 = less than once/week; 1 = once/week; 2 = 2-3 times/week; 3 = 4-6 times/week; 4 = once/day; 5 = 2 or more/day) for the two questions. Frequency scores ranged from 1-10. A score of four is equivalent to about one serving of vegetables per day and a score of five is equivalent to two or more servings per day.

Covariates

The following covariates were included in the models: sex, age, household income, education, and current smoking status. Taste perception diminishes with age (Mennella *et al.* 2005) and females are typically more taste sensitive than males (Bartoshuk *et al.* 1994). Smoking reduces taste perception (Peterson *et al.* 1968). Race, income, smoking status, and education levels are associated with vegetable consumption (Serdula *et al.* 2004; Drewnowski 2004; Grimm *et al.* 2012). Sex, smoking status (currently smoking, non-smoker), race (African American or Caucasian), household income (reported in \$5,000 incremental categories), and highest year of education achieved, were included as categorical variables. Income was defined as total combined income of participants' household in the past year, including income from all sources such as wages, salaries, Social Security or retirement benefits, and help from relatives. The mean household income was imputed when data were missing (Table 1). Age was used as a continuous variable.

Statistical analysis

We used mixed effects models with repeated measures using STATA (v15.0. StataCorp). The margins command was used to estimate the adjusted predicted vegetable consumption score for participants within each intervention group and phenotype group at baseline and 6-months follow up. We tested two-way interactions (phenotype group: intervention group and phenotype group: time) and a three-way interaction (phenotype group: intervention: time). Adjusted predicted margins estimate the means for each group of interest, adjusting for the covariates in the mixed effects models (Williams 2012). Predicted margins for vegetable consumption scores were contrasted to test whether there were significant differences between participants by intervention group and phenotype group over time. Statistical significance was defined as $P \leq 0.05$. Statistical analyses were conducted in STATA. Principal components analysis and the p value of individual SNPs or the SNP: time interaction using mixed effects models with repeated measures was conducted in JMP Pro (v13.2.0, SAS).

Data availability

A MAIME-compliant dataset of the microarray data generated is available at the NCBI database of Genotypes and Phenotypes (dbGaP, study ID phs001471) and additional demographic, social, and clinical variable data are available through the National Heart, Lung, and Blood Institute's Biologic Specimen and Data Repository Information Coordinating Center (<http://biolincc.nhlbi.nih.gov/studies/hhl/>). The following supplementary data files are available online (<https://doi.org/10.6084/m9.figshare.6130748>), Table S1: Comparison of the coverage of reference panels used for SNP imputation; Table S2: SNP imputation results; Table S3: Allele frequencies of *TAS2R38* SNPs by each ancestral group and time point; Table S4: Linkage disequilibrium analysis of *TAS2R38* SNPs at each time point of the intervention; Table S5: Haplotype distribution of *TAS2R38* alleles at each time point of the

■ **Table 1 Study participant demographics at baseline and after 6-months of dietary intervention**

	Baseline characteristics (N = 497)		p^{\dagger}	Characteristics at 6-month follow-up (N = 387)		p^{\ddagger}
Intervention intensity	Minimal	Enhanced		Minimal	Enhanced	
Total participants	238 (48%)	259 (52%)		176 (46%)	210 (54%)	
Phenotype						
Bitter non-taster	45 (19%)	48 (19%)	0.203	31 (18%)	42 (20%)	0.987
Intermediate taster	21 (9%)	36 (14%)		14 (8%)	29 (14%)	
Bitter taster	172 (72%)	175 (68%)		131 (74%)	139 (66%)	
*Sex						
M	78 (33%)	62 (24%)	0.029	62 (35%)	45 (21%)	0.883
F	160 (67%)	197 (76%)		114 (65%)	165 (79%)	
**Race (ancestry)						
White (CAU)	110 (46%)	90 (35%)	0.009	81 (46%)	71 (34%)	0.795
Black (AA)	128 (54%)	169 (65%)		95 (54%)	139 (66%)	
Age (y)						
18-29	3 (1%)	4 (2%)	0.078	1 (1%)	3 (1%)	0.258
30-44	31 (13%)	33 (13%)		12 (7%)	23 (11%)	
45-65	134 (56%)	171 (66%)		103 (59%)	137 (65%)	
> 65	70 (29%)	51 (20%)		60 (34%)	47 (22%)	
***Education						
Grade 12 or less	171 (72%)	148 (57%)	0.003	121 (69%)	113 (54%)	0.628
1- 2 y post high school	35 (15%)	46 (18%)		27 (15%)	36 (17%)	
3- 4 y post high school	20 (8%)	46 (18%)		19 (11%)	42 (20%)	
≥ 5 y post high school	12 (5%)	19 (7%)		9 (5%)	19 (9%)	
Total household income						
≤ \$14,999	70 (29%)	79 (31%)	0.409	49 (28%)	59 (28%)	0.900
\$15,000 – 29,000	53 (22%)	62 (24%)		41 (23%)	54 (26%)	
\$30,000 – 49,000	33 (14%)	30 (12%)		22 (13%)	24 (11%)	
≥ \$50,000	41 (17%)	63 (24%)		26 (15%)	56 (27%)	
Did not report	41 (17%)	25 (10%)		38 (22%)	17 (8%)	
**Smoking status						
Never	180 (76%)	220 (85%)	0.009	144 (82%)	179 (85%)	0.221
Some days or everyday	58 (24%)	39 (15%)		32 (18%)	31 (15%)	

Data presented as the frequency in each category for the indicated time point and intervention: *, **, and *** correspond to $P < 0.05$, < 0.01 , or < 0.001 via a chi-squared comparing intervention intensity at baseline (†). The p value of a chi-squared test comparing baseline to 6-month follow-up is also indicated (‡).

intervention; Table S6: T2R gene family member SNPs used in multivariate analysis; Table S7: Full factor loadings of the principal components analysis; Table S8: Selected factor loadings of the principal components analysis; Table S9: T2R gene family member SNPs used in multivariate analysis.

RESULTS

Study Population

Demographics: Participant characteristics at baseline and after 6-months are shown in Table 1. There were several differences between participants in the minimal vs. the enhanced intervention groups. More women, Caucasians, highly educated, and non-smokers participated in the enhanced intervention compared to the minimal intervention at baseline. Despite attrition, there were no significant differences in participant characteristics within each intervention group at baseline and after 6-months.

TAS2R38 genetic characterization: All three alleles located in the TAS2R38 gene are common variants in both African and Caucasian American populations (Risso *et al.* 2016) similar to our sample enrolled in HHL (Table S3). In our CAU participants the three alleles had similar frequencies and were in high linkage disequilibrium (Table 2). The linkage disequilibrium was not as high across the pairwise allele comparisons in the AA participants (R^2 range 0.46 – 0.95, D' > 0.98) in part due to the difference in allele frequency of rs1726866 (Table 2).

Therefore, we used the phased genotypes to determine the haplotypes found in our population. In our AA population, PAV was the most frequent haplotype, followed by AVI, that encode the bitter and bitter non-taster haplotypes, respectively (Table 2). This distribution was reversed in our CAU population. Demonstrating the genetic diversity between AA and CAU populations, nearly one-third the AA haplotypes were AAI (intermediate-taster phenotype) whereas the CAU haplotypes were almost exclusively PAV (bitter tasters) or AVI (bitter non-tasters) (96%).

The PAV is a dominant allele, therefore instead of relying on an index SNP or haplotypes, we used a dominant model to derive a bitter taste phenotype score based on the diplotype (Table 3). Contingency analysis of the bitter taste phenotype revealed that the percentage of bitter-tasting participants was similar between AA and CAU (Figure 1). However, among those not falling into the bitter tasting category, we observed a higher proportion of bitter non-tasters in CAUs (29%) vs. AAs (12%) and three times as many intermediate tasters in AAs vs. CAUs (Figure 1), likely due to the prevalence of the AAI (intermediate-taster) haplotype in our AA population (Table 2).

Associations Between Vegetable Consumption and Genetic Predisposition to Bitter Taste

Bitter taste diplotypes did not associate with differences in baseline vegetable intake: We first measured associations between baseline vegetable intake and TAS2R38 phenotypes using model 1. Sex, education, and household income were positively associated with reported

■ Table 2 *TAS2R38* linkage disequilibrium and haplotype frequencies

AA (N = 304)					
LD analysis	SNP1	SNP2	R ²	D	Dprime
	rs10246939	rs1726866	0.49	-0.16	-1.00
	rs10246939	rs713598	0.95	0.24	0.99
	rs1726866	rs713598	0.46	-0.16	-0.98
HAPLO	C:G:G:307 PAV	T:A:C:190 AVI	T:G:C:104 AAI	C:G:C:5 AAV	T:A:G:2 PVI
CAU (N = 201)					
LD analysis	SNP1	SNP2	R ²	D	Dprime
	rs10246939	rs1726866	0.98	-0.25	-0.99
	rs10246939	rs713598	0.84	0.23	1.00
	rs1726866	rs713598	0.84	-0.23	-1.00
HAPLO	C:G:G:170 PAV	T:A:C:214 AVI	T:G:C:1 AAI	C:G:C:16 AAV	C:A:C:1 AVV

Statistical analyses of linkage disequilibrium (LD) are represented by R-squared (R²), D, and Dprime values of the pairwise comparisons of the indicated SNPs from the AA and CAU participants. The plus strand haplotype sequence (HAPLO), the count of each haplotype, and the resulting amino acid sequence of the allele are indicated from the AA and CAU participants.

vegetable consumption frequency scores, as expected (Table 4). Participants reported similar vegetable consumption frequency independent of their genetic predisposition toward bitter taste sensitivity, $P = 0.937$ (Figure 2A, 2B, Table 4). Thus, we rejected our first hypothesis that participants would report different vegetable consumption frequency scores at baseline according to their *TAS2R38* diplotype. These data suggest that within our HHL population, the *TAS2R38* polymorphisms were not associated with vegetable intake prior to the intervention. This finding is consistent with another study examining the association between self-reported vegetable intake and PROP sensitivity in a community-based population (Drewnowski *et al.* 2001).

Participants with bitter non-taster or intermediate-bitter taster diplotypes increased vegetable intake after the intervention: Using model 2, we incorporated variables to measure the impact of the different interventions over time and to measure interactions between *TAS2R38* diplotypes, intervention intensity, and time (Table 4). We observed the same associations between reported vegetable consumption frequency scores and sex, education, and household income. Consistent with our second hypothesis, we observed an interaction between phenotype and time (Figure 2A, 2B). Bitter non-tasters had higher vegetable intake compared to bitter tasters at the 6 month time point (Figure 2B) and bitter non-tasters reported 0.65 higher vegetable intake frequency scores, or about 0.20 servings of green salads or other vegetables per day, after 6 months of the intervention (Figure 2C). Vegetable intake frequency scores also increased by 0.55 among intermediate bitter tasters (Figure 2A, 2C). However, intake scores only increased 0.04 among bitter tasters at the end of the intervention (Figure 2A, 2C). Importantly, we did not see differences in participant demographics (Table 1) or allele frequencies, linkage disequilibrium, or haplotype distributions (Tables S3, S4, S5) due to intervention attrition at the 6-month time point.

Vegetable intake increased in the enhanced dietary intervention: Given the enhanced intervention included tailored dietary goals and behavior change strategies, we hypothesized that participants in the enhanced intervention would have a greater increase in vegetable intake. As expected, the change in vegetable intake frequency scores was higher

in the enhanced intervention group compared to the minimal group over time (Figure 3A, 3B). In fact, participants in the minimal intervention group reported a decrease of 0.19 in vegetable intake frequency scores (Figure 3B), whereas participants in the enhanced intervention group increased their reported scores by 0.58 (Figure 3B), suggesting that the enhanced intervention contributed to dietary changes regarding vegetable intake.

Bitter taste perception and the intensity of the dietary intervention may influence vegetable intake: Although the enhanced intervention associated with increased reported vegetable intake (Figure 3A, 3B), could this response be modified by the *TAS2R38* phenotype? Despite

■ Table 3 *TAS2R38* diplotype frequencies and associated phenotype

AA (N = 304)		
Diplotype	Freq	Phenotype
PAV / PAV	0.286	bitter
PAV / AVI	0.270	bitter
AAI / PAV	0.155	bitter
AVI / AVI	0.118	non
AAI / AVI	0.115	intermediate
AAI / AAI	0.033	intermediate
AAV / PAV	0.013	bitter
AVI / PVI	0.007	intermediate
AAV / AAI	0.003	intermediate
CAU (N = 201)		
Diplotype	Freq	Phenotype
AVI / PAV	0.438	bitter
AVI / AVI	0.289	non
PAV / PAV	0.184	bitter
AAV / AVI	0.040	intermediate
AAV / PAV	0.040	bitter
AVI / AAI	0.005	intermediate
AAV / AVI	0.005	intermediate

The distribution of diplotypes within the AA and CAU participants with the indicated bitter tasting phenotype for each diplotype indicated.

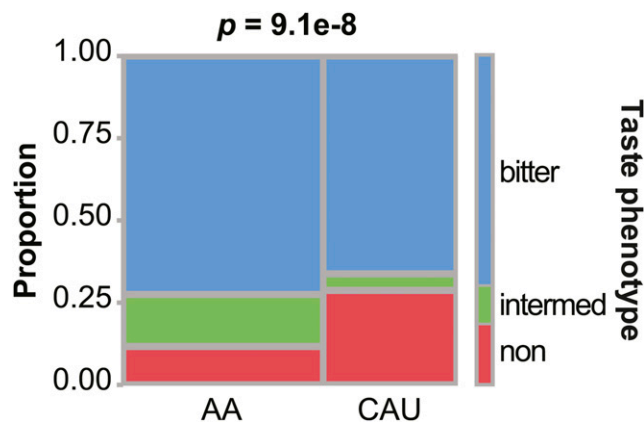


Figure 1 *TAS2R38* bitter taste phenotype distribution in the HHL cohort. Contingency plot and *p* value of the Fisher's Exact Test in comparing the distribution (proportion) of taste phenotypes in the AA and CAU group.

significant main effects, the three-way interaction between intervention group, phenotype, and time was not statistically significant, $P = 0.392$. Still, the 3-way interaction analysis trended similar to that seen in the 2-way interactions (Figure 4A). Bitter non-tasters and intermediate-bitter tasters in the enhanced intervention increased their vegetable

intake frequency score the most ($\Delta = 0.71$ and 0.89 , respectively, Figure 4B). Consistent with our hypothesis, bitter tasting participants in the minimal intervention were the only group that decreased their vegetable intake ($\Delta = -0.44$, Figure 4B), however there was an increase among bitter tasting participants in the enhanced intervention ($\Delta = 0.50$, Figure 4B). Our data suggest that these *TAS2R38* alleles and resulting phenotypes may impact a person's response to dietary interventions regarding vegetable intake.

Vegetable intake associated specifically with *TAS2R38* variants and not other variants in related *T2R* genes: Other genes in *T2R* family are also implicated in taste perception, neuroendocrine function, appetite, and satiety (Bachmanov and Beauchamp 2007) as well as human aging (Campa *et al.* 2012). We extracted the genotypes of these related family members (Table S6) and along with the *TAS2R38* variants we used principal components analysis with the adjusted predicted vegetable intake as a supplementary variable to determine if other *T2R* genes associate with the responsiveness to our dietary interventions (Table S7). The first four components accounted for 51% or 59% of the total variance in AA (Figure 5) and CAU (Figure 6) subjects, respectively. Next, we identified the components that corresponded to the highest loading for vegetable intake. Not surprisingly, this resulted in segregation of the *TAS2R38* bitter taste phenotypes and revealed that the three *TAS2R38* alleles were highly correlated to the variance of PC4 or PC2 in the AA or CAU groups, respectively (Figure 5, Figure 6, Table

Table 4 Regression coefficients for vegetable intake frequency at baseline (Model 1) and mixed effects coefficients at 6 months (Model 2)

MODEL 1						
Variables	Coefficient	SE	t	$P > t $	95% CI	
Intermediate taster	-0.10	0.337	-0.28	0.777	-0.76 - 0.57	
Bitter taster	0.01	0.231	0.03	0.979	-0.45 - 0.46	
Non-smoker	0.14	0.231	0.58	0.562	-0.32 - 0.59	
**Female	0.63	0.199	3.15	0.002	0.24 - 1.02	
Age	0.01	0.008	1.88	0.061	-0.001 - 0.03	
*Education	0.08	0.037	2.08	0.038	0.004 - 0.15	
***Income	0.14	0.034	4.11	<0.001	0.07 - 0.21	
Race	0.14	0.195	0.74	0.459	-0.24 - 0.53	
Constant	0.80	0.766	0.97	0.335	-0.77 - 2.25	

MODEL 2						
Variables	Coefficient	SE	z	$P > z $	95% CI	
Intermediate taster	0.06	0.501	0.13	0.899	-0.92 - 1.05	
Bitter taster	0.37	0.313	1.17	0.242	-0.25 - 0.98	
Enhanced intervention group	0.19	0.387	0.49	0.621	-0.57 - 0.95	
Inter.: Enhanced	-0.17	0.644	-0.27	0.791	-1.43 - 1.09	
Taster: Enhanced	-0.70	0.434	-1.54	0.123	-1.52 - 0.18	
6-month follow-up	0.46	0.338	1.36	0.174	-0.20 - 1.12	
Inter.: 6-months follow-up	-0.26	0.601	-0.43	0.671	-1.43 - 0.92	
*Taster: 6-months follow-up	-0.89	0.376	-2.38	0.018	-1.63 - -0.16	
Enhanced: 6-months	0.25	0.450	0.56	0.573	-0.063 - 1.13	
Inter.: Enhanced: 6-month follow-up	0.40	0.758	0.53	0.598	-1.09 - 1.89	
Taster: Enhanced: 6-month follow-up	0.68	0.505	1.35	0.177	-0.31 - 1.67	
Non-smoker	0.30	0.198	1.54	0.123	-0.08 - 0.69	
***Female	0.70	0.166	4.22	<0.001	0.38 - 1.02	
Age	0.01	0.007	1.55	0.122	-0.003 - 0.02	
**Education	0.09	0.031	2.89	0.004	0.03 - 0.15	
***Income	0.14	0.028	4.93	<0.001	0.08 - 0.19	
Race	-0.01	0.164	-0.01	0.994	-0.32 - 0.32	
Constant	0.75	0.164	1.12	0.264	-0.56 - 2.05	

The coefficient of variation, standard error (SE), t statistic (Model 1), z score value (Model 2), 2-tailed *p* values ($P > |t|$ or $P > |z|$), and 95% confidence intervals (CI) are provided: *, **, and *** correspond to $P < 0.05$, < 0.01 , or < 0.001 .

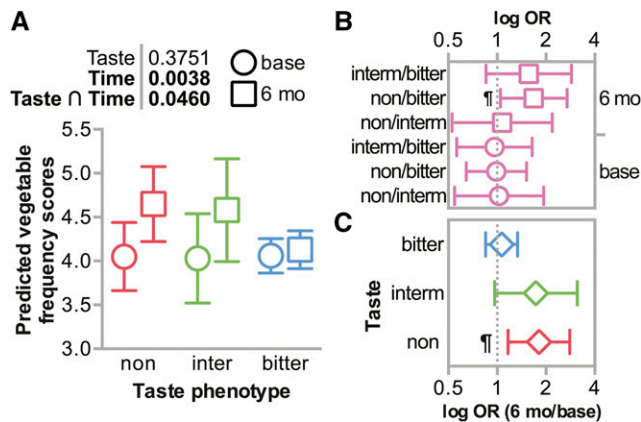


Figure 2 Vegetable intake at baseline and after 6 months categorized by *TAS2R38* bitter taste phenotype. (A) Predicted vegetable intake adjusted for sex, ancestry, age, education, income, and smoking status are represented by box plot and summarized by the mean \pm 95% confidence intervals at either the onset of the study (base) or at the 6-month follow up (6 mo), grouped by phenotype: bitter non-tasters (non), intermediate-bitter tasters (inter), or bitter tasters (bitter). The *p* values of the main effects (time and taste phenotype) and the interaction between taste phenotype and time are indicated. The log odds ratios (OR) of vegetable intake comparing (B) 6 months vs. baseline for each taste phenotype or (C) the indicated taste phenotypes at either baseline (base) or at the 6-month follow up (6 mo) are represented by box plot and summarized by the mean \pm 95% confidence intervals: ¶ indicates the ratios where the 95% confidence interval does not contain the value of 1.

S8). We also identified another associated locus common to both AA and CAU populations that harbors *TAS2R20* and *TAS2R50* (Table 5, Figure 5, Figure 6, Table S8). However, when we used a mixed model approach to look at the association of these individual SNPs or the SNP: time interaction and reported vegetable intake, we only observed an association with two *TAS2R38* alleles, rs713598 and rs10246939 (Table 5). Another locus of interest included the *TAS2R3*, *TAS2R4*, and *TAS2R5* genes that had high correlation in PC2 in the CAU group (Figure 6, Table S8). However, like the other loci we analyzed, we did not find any association with vegetable intake either analyzed with both populations or only within the CAU group (Table S9). These data suggest that *TAS2R38* is likely the largest genetic contributor to our association analysis. The other SNPs we identified in this analysis, however, may play other roles that contribute to taste perception and diet.

DISCUSSION

The primary goal of HHL was to reduce CVD-related health disparities in a rural population in North Carolina. In this study, we tested the concept that participants in a dietary intervention designed to promote heart healthy eating patterns may respond differently according to their genetic predisposition of bitter taste perception mediated by the *TAS2R38* gene and allelic variants that can affect receptor signaling and hence, perception of bitter taste compounds found in many vegetables. Our HHL sample was represented by two ancestral populations, African and Caucasian Americans, and we were cognizant of the genetic population structure of our cohort. When we analyzed the diplotypes and corresponding phenotypes of our cohort, we observed similar proportion of bitter tasters in the AA and CAU groups (Figure 1). There was a striking difference, however, in the proportion of bitter non-tasters and intermediate bitter tasters such that the CAU group

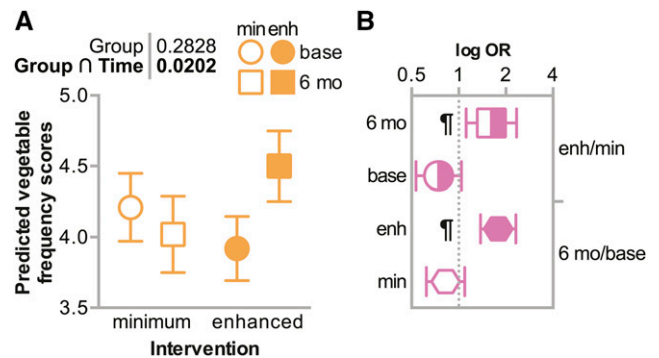


Figure 3 Vegetable intake at baseline and after 6 months categorized by intervention intensity. (A) Predicted vegetable intake adjusted for sex, ancestry, age, education, income, and smoking status are represented by box plot and summarized by the mean \pm 95% confidence intervals at either the onset of the study (baseline) or at the 6-month follow up (6 mo), grouped into the minimal (min) or enhanced (enh) intervention group. The *p* values of the main effect (group) or the interaction between intervention intensity and time are indicated. (B) The log odds ratios (OR) of vegetable intake comparing the enhanced vs. minimum intervention at either 6 months or baseline (top) or at 6 months vs. baseline in either the enhanced or minimal intervention (bottom) are represented by box plot and summarized by the mean \pm 95% confidence intervals: ¶ indicates the ratios where the 95% confidence interval does not contain the value of 1.

had nearly triple the frequency bitter non-tasters (Figure 1), consistent with a recent study on the natural selection of *TAS2R38* haplotypes (Risso *et al.* 2016). Although we lacked the power to stratify our HHL cohort for robust, focused analyses within each ancestry group, we accounted for ancestry in our analyses and the variable accounting for ancestry in either of our models did not approach our defined level of statistical significance (Table 4). Although these data suggest that ancestry did not associate with changes in reported vegetable consumption in our cohort, future studies should consider and seek to define differences in allele frequency and interactions with other biological factors that contribute to taste perception in distinct ancestral populations to determine the applicability of precision medicine to dietary interventions.

We found differences in vegetable consumption frequencies between intervention participants at follow-up according to their bitter taste perception phenotype characterized by common coding variants in the *TAS2R38* gene (Figure 2A, 2C). Participants with bitter non-taster *TAS2R38* diplotypes increased vegetable consumption more than participants whose genotypes associate with bitter taste perception (Figure 2C). Our findings are consistent with other studies that observed differential vegetable preferences according to the presence of bitter taste perception SNPs (Dinehart *et al.* 2006; Bell and Tepper 2006). Moreover, women who were PROP non-tasters lost more weight on a low carbohydrate diet compared to a low fat diet (Burgess *et al.* 2017). In contrast, there was no difference in weight loss when comparing low carbohydrate or low fat diets in PROP tasters, suggesting that the bitter non-taster phenotype may influence the responsiveness to certain diets (Burgess *et al.* 2017). However, other studies suggest that bitter taste sensitivity is not associated with food selection due to other factors such as attitudes toward foods, cultural norms, and one's food environment (Tepper 2008; Tepper *et al.* 2009). More research is needed to better understand how genetic taste variation and other factors influence vegetable selection and consumption (Tepper 2008), and importantly, how this information can help inform dietary interventions.

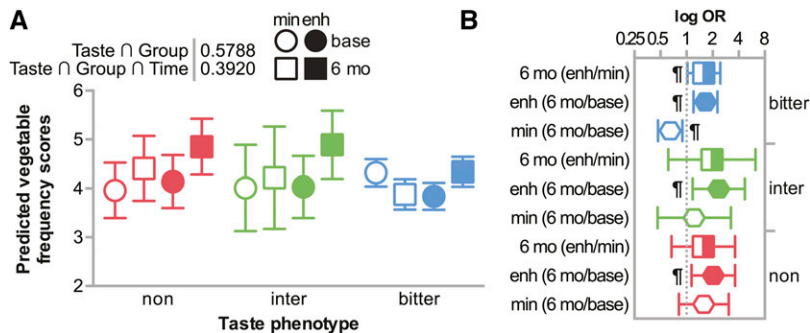


Figure 4 Vegetable intake at baseline and after 6 months in either intervention group categorized by *TAS2R38* bitter taste phenotype. (A) Predicted vegetable intake adjusted for sex, ancestry, age, education, income, and smoking status are represented by box plot and summarized by the mean \pm 95% confidence intervals at either the onset of the study (baseline) or at the 6-month follow up (6 mo), grouped by phenotype: bitter-non taster (non), intermediate-bitter taster (inter), or bitter taster (bitter) within each intervention: minimum (min) or enhanced (enh). The *p* value of the two-way interaction between taste phenotype and intervention intensity and *p* value of the three-way interaction between taste

phenotype, time, and intervention intensity is indicated. (B) The log odds ratios (OR) within each taste phenotype of vegetable intake comparing the enhanced vs. minimum intervention at either 6 months or baseline as well as the comparison of vegetable intake at 6 months vs. baseline in either the enhanced or minimal intervention are represented by box plot and summarized by the mean \pm 95% confidence intervals: ¶ indicates the ratios where the 95% confidence interval does not contain the value of 1.

Not surprisingly, we also found that participants in the enhanced dietary intervention increased their vegetable intake frequency scores more than those in the minimal intervention (Figure 3A, 3B). A review of behavioral interventions aiming to increase vegetable intake found that 17 of 22 studies reported small, but significant increases in vegetable intake (Ammerman *et al.* 2002). Many dietary intervention studies aim to change servings of total fruits and vegetables, while ours only examined a subset of vegetable intake (green salads and other vegetables) and likely explains the small changes we observed in daily servings of vegetables after the intervention. Moreover, the study participants reported very low intake of vegetables as baseline; in retrospect, participants may have benefitted from a more intensive vegetable consumption focus in the intervention than they received. In some cases, participants in the minimal intervention group reported lower vegetable intake frequency scores after 6 months than at baseline (Figure 3B).

Participants who took part in the enhanced intervention increased their vegetable intake over the course of the intervention, irrespective of the *TAS2R38* phenotype, whereas participants in the minimal intervention showed mixed results based on *TAS2R38* phenotype (Figure 4A). Bitter non-tasters in the minimal intervention group increased their vegetable intake while bitter tasters in the same intervention group decreased their vegetable consumption (Figure 4B). Our findings demonstrate that all participants in the enhanced condition, even those who are likely to perceive bitterness in some vegetables, increased vegetable consumption during the intervention. Biological sensitivity to bitter taste is likely one of many factors contributing to participants' decisions about vegetable consumption. Participants that perceive bitterness may choose to consume vegetables that are less bitter, such as carrots or cooked vegetables (Mikołajczyk-Stecyna *et al.* 2017) or utilize food preparation strategies that minimize the bitter taste. Participants may have also modified their preferences toward vegetable consumption over the course of the enhanced intervention; studies suggest that repeated exposure to foods and beverages can alter preferences for those foods and beverages (Pliner 1982; Stein *et al.* 2003; Anzman-Frasca *et al.* 2012). Since participants were receiving information about the benefits of a vegetable-rich diet, they may have been more willing to overcome taste aversions, and perhaps even modify their taste preferences during the 6-month enhanced intervention.

There were several limitations in this study. Frequency of vegetable intake questions used in this study did not specifically target vegetables that are high in bitter compounds (Dinehart *et al.* 2006; Mikołajczyk-Stecyna

et al. 2017). Additionally, cooking methods were not assessed, and cooking can affect consumers' vegetable preferences (Drewnowski and Gomez-Carneros 2000; Bongoni *et al.* 2014). Moreover, we did not include self-reported vegetable juice and vegetable soup intake in our outcome variable. These items were excluded because they are likely to have added salt or sugar, which suppresses bitterness (Breslin and Beauchamp 1997; Drewnowski and Gomez-Carneros 2000). Also, there was 22% attrition at the 6-month follow up; however, the haplotype frequencies were similar at baseline and follow-up (Table S5), so the differences seen between baseline and 6 months are not likely due to differences in genotypes. Additionally, our sample size limited our ability to detect a statistically significant interaction between genotype and intervention group at two time points and, given multiple comparisons, some nominally significant findings may be due to chance. Despite these limitations, the statistical significance of the main effects suggest that both genotype and intervention group influenced participants' vegetable consumption frequency (Figure 4). Future studies with larger sample sizes and more participants per phenotype and intervention group at each time point should be powered to identify additional three-way statistical interactions.

The T2R gene family represents a collection of 25 functional genes, along with 11 pseudogenes, found on chromosomes 5, 7, and 12 (Risso *et al.* 2017) that are expressed in taste bud cells. Given the ability of people to distinguish more distinct bitter tasting compounds than the number of receptors suggests T2R receptors likely respond to more than one bitter ligand (Behrens and Meyerhof 2006). We expanded our SNP-level analysis to cover 20 T2R genes to look for other taste receptors that may provide some insight into the phenotype of our HHL participants. Although our results at the individual SNP level in other T2R genes did not identify associations to changes in vegetable intake within our intervention (Table 5), our multivariate analysis (Figure 5) did identify other loci other than *TAS2R38* that should be considered in future studies, including *TAS2R50* that recognizes the naturally occurring bitter compounds amarogentin and andrographolide (Behrens *et al.* 2009), and *TAS2R20*, a receptor with no known natural ligand (Meyerhof *et al.* 2010). Within the CAU group our analysis identified SNPs from an additional locus containing three genes in chromosome 7, recently identified as having long-range haplotype structure with *TAS2R38* (Roudnitzky *et al.* 2015) that contains two receptors with undefined natural ligands, *TAS2R3* and *TAS2R5* (Meyerhof *et al.* 2010), and *TAS2R4*, a known receptor for quinine (Upadhyaya *et al.* 2016).

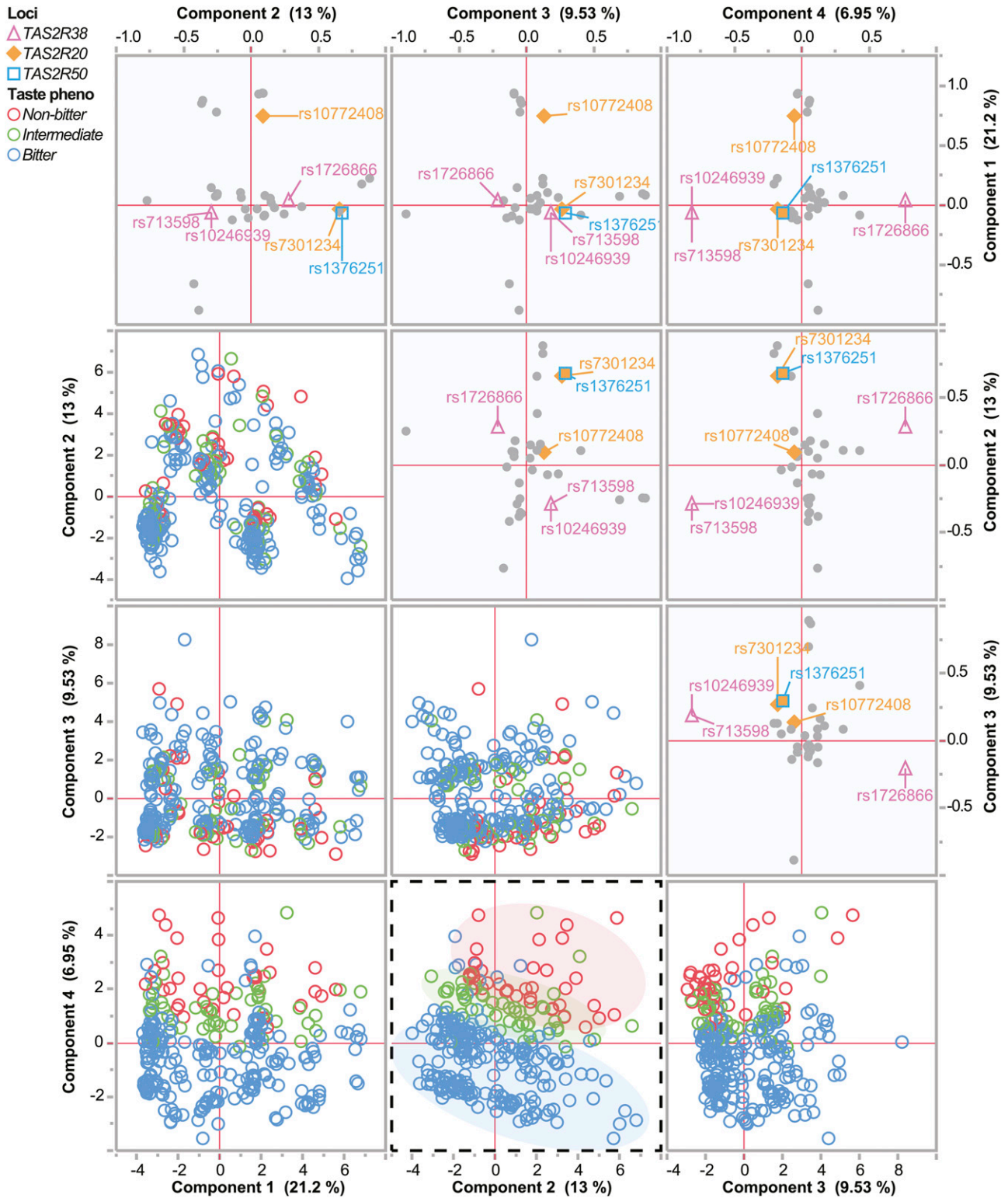


Figure 5 Multivariate analysis of T2R polymorphisms in the AA cohort. Analysis of polymorphisms using principal component analysis is represented by scatter plot matrix. The loadings plot representing SNPs (top) and score plot representing study subjects (bottom) for the first four components and the percent variance explained by each component are provided. Loci represented by SNPs of interest are indicated, and the study subjects are color coded by taste phenotype. The dashed plot highlights the taste phenotypes in component 2 vs. component 4.

■ Table 5 SNP and SNP-time associations with vegetable intake

SNP	Gene	Chr	Pos	p (SNP)	p (SNP:time)
rs713598	TAS2R38	7	141673345	0.0659	0.0147
rs10246939	TAS2R38	7	141672604	0.0659	0.0147
rs1726866	TAS2R38	7	141672705	0.1208	0.1452
rs10772408	TAS2R49	12	11151599	0.4936	0.7443
rs1376251	TAS2R49	12	11138852	0.3534	0.9068
rs7301234	TAS2R49	12	11150884	0.2838	0.7276

The *p* values of the association of either the indicated SNP or the SNP: time interaction with reported vegetable intake. The location of the gene is indicated by chromosome (Chr) and position (Pos).

glucosinolates and isothiocyanates (e.g., collard greens, broccoli, Brussels sprouts, kale), as well as vegetable preparation methods (e.g., cooked, fresh), could yield more robust associations between bitter taste perception alleles and consumption of bitter vegetables. Conducting a qualitative study among bitter tasters who consume vegetables to learn how and why they have overcome a genetic predisposition to perceive compounds in vegetables as bitter may yield strategies for vegetable consumption interventions. Future research could test whether personalizing diets to specific genetic-based taste profiles increases consumption of specific healthy foods more than generalized dietary advice. Supportive of this concept, a meta-analysis of behavioral interventions found that tailored nutrition interventions aiming to increase fruit and vegetable consumption were more successful than untailored interventions (Kroeze *et al.* 2006; Noar *et al.* 2007).

Nutrigenomics and other approaches to tailor nutrition advice and interventions based on genetic and metabolic profiles are increasing as scientists overcome technological and data challenges (Wittwer *et al.* 2011). In one study, genes associated with energy metabolism were used to personalize a low glycemic index weight management program informed by the Mediterranean diet for participants (Arkadianos *et al.* 2007). The authors observed greater diet adherence to the genetically tailored diets, as well as longer-term reductions in BMI and improved blood glucose levels compared to participants who received a low glycemic index weight management program informed by the Mediterranean diet that was not genetically-tailored (Arkadianos *et al.* 2007). A recent review of nutrigenomic studies did not report any studies that used genes associated with taste perception to inform dietary intervention strategies (Wittwer *et al.* 2011). Recognizing the important influence that taste perception has on diet and tailoring dietary interventions according to taste preferences may be a strategy for engaging participants and improving dietary intervention outcomes.

Reducing heart health disparities requires attention to the many factors driving the disparities. Despite high prevalence of cardiovascular disease among African Americans, this population is under-represented in GWAS studies (Lek *et al.* 2016). Likely explanations include mistrust between African American community members and researchers due to the legacy of unethical medical and genetic studies (Corbie-Smith 1999), and imbalances in information and power (Corbie-Smith *et al.* 1999), as well as persistent biases that influence research participation (Popejoy and Fullerton 2016). A strength of the HHL study was our community-based participatory research (CBPR) approach where we worked with a community advisory board, held focus groups with community members, and hired and trained community members as study staff (Halladay *et al.* 2013; Skinner *et al.* 2015). We believe these activities helped build trust between researchers and community-based participants, and helped the research team better understand and meet the expectations that community members had regarding their participation in the genomics portion of this study. Moreover, these activities

likely contributed to the high enrollment of African Americans in the genomics arm of the HHL study. In addition to the genomics and lifestyle counseling components of the study, HHL sought to address heart health disparities by increasing access to healthy foods; promoting knowledge of heart healthy choices through a collaboration with local restaurants that included information on healthful menu items and a coordinated monthly newspaper column with information on healthy eating (Thayer *et al.* 2017); and enhancing clinical care for hypertension in the Lenoir community (Halladay *et al.* 2013; Cené *et al.* 2017). These strategies were designed to address behavioral and environmental factors that drive heart health disparities in a rural NC population. Combining precision medicine insights to engage participants with CBPR principles and public health strategies that shape the context in which individuals live, work, and play may be a promising approach for reducing cardiovascular health disparities in the US.

This study demonstrates a concept that genes associated with bitter taste perception can influence frequency of vegetable intake in the context of a dietary intervention in a diverse, community-based study sample. The variability in frequency of intake according to participants' bitter taste perception phenotype could help explain why dietary change interventions report mixed results. Taste has a strong influence over individuals' dietary habits and should be considered when designing dietary change interventions and in developing novel precision medicine approaches to lifestyle interventions.

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