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Research report

Variation in the gene *TAS2R38* is associated with the eating behavior disinhibition in Old Order Amish women

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ABSTRACT

Insensitivity to the bitter-tasting compound 6-n-propylthiouracil (PROP) has been proposed as a marker for individual differences in taste perception that influence food preference and intake. The principal genetic determinants of phenotypic variation in PROP taste sensitivity are alleles of the *TAS2R38* gene, which encodes a chemosensory receptor sensitive to thiourea compounds including PROP and phenylthiocarbamide. Members of the *TAS2R* family are expressed in the gustatory system, where they function as bitter taste receptors, and throughout the gut, where their physiological roles in prandial, gut-derived hormone release are beginning to be elucidated. To better understand the relationship between *TAS2R* function and ingestive behaviors, we asked if *TAS2R38* variants are associated with one or more of three eating behaviors: restraint, disinhibition, and hunger. We genotyped a single nucleotide polymorphism (SNP) located within the *TAS2R38* gene, rs1726866 (T785C, Val262Ala) in 729 nondiabetic individuals (381 females, 348 males) within the Amish Family Diabetes Study. Eating behaviors were assessed using the Three-Factor Eating Questionnaire. An association analysis between rs1726866 and these three traits revealed a significant association of the PROP-insensitive “T” allele with increased disinhibition ($p = 0.03$). Because eating behaviors differ substantially between males and females, we subsequently performed sex-stratified analyses, which revealed a strong association in females ($p = 0.0002$) but not in males. Analyses with other SNPs in close proximity to rs1726866 suggest that this locus is principally responsible for the association. Therefore, our results indicate that a polymorphism in *TAS2R38* is associated with differences in ingestive behavior.

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Introduction

Taste strongly affects ingestive behavior and nutrient intake. An individual's sensitivity to the tastes evoked by certain compounds in foods (i.e., sweet, salty, sour, bitter) is strongly affected by genetic variation (Bachmanov & Beauchamp, 2007; Bufe et al., 2005; Fushan, Simons, Slack, Manichaikul, & Drayna, 2009; Kim et al., 2003; Kim, Wooding, Riaz, Jorde, & Drayna, 2006; Pronin et al., 2007). The best studied human taste phenotype is the relative sensitivity of individuals to the bitter taste evoked by any variety of compounds that containing a N–C=S (thiourea) moiety, such as phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (see Wooding, 2006, for a review). A moderate perception of bitterness can be appealing and expected in a variety of foods (e.g., alcohol and cheeses). However, compounds perceived as intensely bitter are usually

rejected by humans and other mammals, likely because bitter taste is closely associated with the presence of toxins (e.g., Ames, Profet, & Gold, 1990; Bravo, 1998; Fenwick, Heaney, & Mullin, 1983; Tepper, 2008). For example, a large body of research indicates that adults who possess an enhanced perception of PROP avoid certain bitter-tasting foods, including specific fruits and vegetables (Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006; Drewnowski, Henderson, Hann, Berg, & Ruffin, 2000; Drewnowski, Henderson, Levine, & Hann, 1999; Drewnowski, Henderson, & Shore, 1997; Drewnowski, Henderson, Shore, & Barratt-Fornell, 1998; Fischer, Griffin, England, & Garn, 1961; Glanville & Kaplan, 1965; Jerzsa-Latta, Kronld, & Coleman, 1990; Tepper, Williams, Burgess, Antalis, & Mattes, 2008b) (although see Drewnowski, Henderson, & Cockroft, 2007; Mattes & Labov, 1989). Thus, taste sensitivity to PROP is often used as a marker for individual differences in taste perception that influences food preferences and intake (e.g., Dinehart et al., 2006; Duffy & Bartoshuk, 2000; Tepper, 2008).

The principal genetic determinants of phenotypic variation in PROP/PTC taste sensitivity are alleles of the gene *TAS2R38* (Bufe

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et al., 2005; Kim et al., 2003). This gene, which contains a single coding exon approximately 1 kb in size, encodes a chemosensory receptor sensitive to PROP, PTC, and many other thiourea-containing compounds (Bufe et al., 2005; Kim et al., 2003; Prodi et al., 2004). Three nonsynonymous coding SNPs within *TAS2R38* (rs713598 – G145C, Ala49Pro; rs1726866 – T785C, Val262Ala; rs10246939 – A886G, Ile296Val) give rise to several haplotypes, only two of which (Pro-Ala-Val (PAV) and Ala-Val-Ile (AVI)) are commonly found in human populations (Kim, Wooding, Ricci, Jorde, & Drayna, 2005; Wooding et al., 2004). Individuals who possess at least one copy of the PAV allele are significantly more sensitive to PROP (Bufe et al., 2005) or PTC (Kim et al., 2003) than are those who are homozygous for the AVI allele. These haplotypes are also correlated with bitterness perception of plants, (e.g., turnips, broccoli, watercress) that synthesize glucosinolates, a class of compounds that also contain the thiourea moiety (Sandell & Breslin, 2006). These associations suggest that variation within *TAS2R38* may also influence the ingestion of certain foods. Indeed, individuals who possess at least one copy of the PROP sensitive allele of *TAS2R38* eat fewer cruciferous vegetables than do adults who are homozygous for the PROP insensitive allele (Sacerdote et al., 2007).

Members of the *TAS2R* family are expressed in the gustatory system, where they function as bitter taste receptors. However, it is now clear that *TAS2Rs* and other chemosensory receptors are also expressed in cells of the gastrointestinal (GI) tract and associated organs, suggesting that they may be involved in the nutrient-dependent regulation of metabolism. Components of the taste transduction cascade, including *TAS2Rs*, are expressed in the cells of the stomach and small intestine (e.g., Bezencon, le Coutre, & Damak, 2007; Rozengurt & Sternini, 2007; Rozengurt et al., 2006; Wu et al., 2002), where they can mediate insulin release from the pancreas (Nakagawa et al., 2009), the incretin response to sweet- and bitter-tasting compounds (Dotson et al., 2008; Jang et al., 2007; Jeon, Zhu, Larson, & Osborne, 2008), glucose assimilation (Mace, Affleck, Patel, & Kellett, 2007; Mace et al., 2009; Margolskee et al., 2007) and the secretion of cholecystokinin (CCK) from enteroendocrine cells (Jeon et al., 2008). Thus, because both the gustatory and digestive systems use *TAS2Rs* to detect substances present in food, polymorphisms in these receptors could impact ingestive behaviors either by affecting taste sensitivity or by influencing the postprandial response to nutrients.

When attempting to maintain a healthy lifestyle, an individual's eating patterns are as important as what they eat (e.g., Greenwood & Stanford, 2008; Haines & Neumark-Sztainer, 2006). These patterns are known to be heritable (de Krom, Bauer, Collier, Adan, & la Fleur, 2009; Steinle et al., 2002; Tholin, Rasmussen, Tynelius, & Karlsson, 2005) and related to weight gain and BMI (Bryant, King, & Blundell, 2008; Hays & Roberts, 2008; Keskitalo et al., 2008). The Three-Factor Eating Questionnaire assesses three behavioral traits related to the control of food intake: restraint, disinhibition, and hunger (Stunkard & Messick, 1985; Stunkard & Wadden, 1990). Restraint is an avoidance of eating to control body weight. Disinhibition is loss of restraint that results in overeating. Hunger measures the perceived need for food. It has been suggested that variation in taste function could influence eating behaviors (de Krom et al., 2009; Murphy & Kemmotsu, 2009). Interestingly, the behavioral trait restraint mitigates the relationship between PROP taster status and adiposity in women (Tepper et al., 2008a). However, it remains unclear whether a direct relationship exists between eating behaviors and *TAS2R38* genotype. To investigate this issue, we examined whether differences in restraint, disinhibition and hunger are associated with *TAS2R38* variants in an Old Order Amish population.

Methods

Subjects

The University of Maryland School of Medicine's Institutional Review Board approved all studies. The Amish Family Diabetes Study (AFDS) is an ongoing effort to identify genetic contributors to obesity, diabetes, cardiovascular disease and related disorders (Dammott et al., 2006; Hsueh et al., 2000; Steinle et al., 2002). Detailed descriptions of the population (the Old Order Amish of Lancaster County, Pennsylvania, USA), study design, recruitment methods, phenotypic characterization, clinical characteristics of the subjects and statistical methods have been published previously (see Hsueh et al., 2000 for details). Briefly, the mean age of subjects was 46 ± 15 years for men and 45 ± 15 years for women. The mean BMI was 26.4 ± 3.7 kg/m² for men and 28.1 ± 5.4 kg/m² for women ($p < 0.001$); the mean leptin level was 3.7 ± 3.2 µg/L for men and 16.2 ± 11.1 µg/L for women ($p < 0.001$). The Old Order Amish of Lancaster, Pennsylvania, have a common lifestyle and socioeconomic status, and possess detailed genealogical records dating to the period of their early migration from Europe in the 1700s (Hsueh et al., 2000). Currently, the AFDS includes over 1300 subjects.

Three-Factor Eating Questionnaire

Assessment of members of the AFDS cohort using the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985; Stunkard & Wadden, 1990) has been described previously (Steinle et al., 2002). In the Amish, responses to each question were distributed across the full range of possible responses. Furthermore, eating behavior scores spanned all possible values for each of the three behaviors, and the configuration of frequency of scores was compatible with a Gaussian distribution. Mean scores from the Amish are comparable to those reported in other populations (e.g., Stunkard & Wadden, 1990).

Genotyping

Genotyping of SNPs in the AFDS has been described previously (Dotson et al., 2008; Hsueh et al., 2007). Briefly, SNPs were identified from the Entrez SNP database (Sherry et al., 2001), as well as from published reports (Kim et al., 2005). Three nonsynonymous coding SNPs in the gene *TAS2R38* comprise a haplotype that is associated with phenotypic variation in PROP/PTC taste sensitivity. We selected the nonsynonymous coding SNP rs1726866 to assess the effects of variations in *TAS2R38* on eating behaviors. This SNP accounts for ~97% of the variation in *TAS2R38* in Caucasian populations (Kim et al., 2003). Additional SNPs were genotyped both upstream and downstream of rs1726866 to determine the extent of linkage disequilibrium (LD) that exists around this locus.

SNPs were genotyped using the TaqMan (Applied Biosystems) or GeneChip Human Mapping 100K Set (Affymetrix) platforms according to manufacturer's protocols. SNPs found to be monomorphic in the AFDS ($n = 2$) were not analyzed further. Genotypes were checked for Mendelian consistency; inconsistencies, which were detected in <0.5% of genotypes, were removed from analysis. Genotype frequencies of all SNPs were tested for consistency with Hardy–Weinberg expectations by the χ^2 test; no SNPs showed extreme deviation from Hardy–Weinberg Equilibrium ($p < 0.001$). SNPs with call rates <90% ($n = 4$) were eliminated from further analysis. In total, twenty SNPs were found to be polymorphic in the AFDS, passed quality control filters (see below) and were subsequently analyzed (Table 1).

Table 1
 SNP genotyping statistics.

| Chromosome, position (kb) | SNP ID | Platform | Associated gene | Call rate (%) | HWE <i>p</i> value | Major/minor allele | MAF | SNP type |
|---------------------------|------------|------------|-----------------|---------------|--------------------|--------------------|-------|-----------|
| 7, 141059 | rs1476640 | Affymetrix | WEE2 | 97.8 | 0.59 | C/T | 0.42 | Intronic |
| 7, 141089 | rs2013816 | Affymetrix | SSBP1 | 94.6 | 0.1 | T/C | 0.27 | Intronic |
| 7, 141090 | rs6957284 | Affymetrix | SSBP1 | 93.7 | 1.0 | G/A | 0.007 | Intronic |
| 7, 141095 | rs10485836 | Affymetrix | SSBP1 | 99.1 | 1.0 | C/T | 0.001 | Intronic |
| 7, 141109 | rs11763979 | Taqman | TAS2R3 | 98.4 | 0.227 | G/T | 0.27 | Noncoding |
| 7, 141111 | rs2233998 | Taqman | TAS2R4 | 92.7 | 0.052 | T/C | 0.23 | F7S |
| 7, 141125 | rs2234001 | Taqman | TAS2R4 | 97.0 | 0.073 | G/C | 0.23 | V96L |
| 7, 141137 | rs2227264 | Taqman | TAS2R5 | 95.8 | 0.103 | G/T | 0.23 | S26I |
| 7, 141319 | rs1726866 | Taqman | TAS2R38 | 97.0 | 0.430 | T/C | 0.24 | V262A |
| 7, 142592 | rs4726600 | Taqman | TAS2R39 | 97.7 | 0.279 | G/A | 0.25 | Noncoding |
| 7, 142592 | rs1298582 | Affymetrix | MGAM | 93.3 | 0.39 | T/A | 0.36 | Intronic |
| 7, 142592 | rs1527304 | Affymetrix | MGAM | 99.8 | 0.05 | G/A | 0.34 | Intronic |
| 7, 142592 | rs2204607 | Affymetrix | MGAM | 99.4 | 1.00 | C/T | 0.15 | Intronic |
| 7, 142592 | rs1358304 | Affymetrix | MGAM | 98.1 | 0.27 | G/A | 0.34 | Intronic |
| 7, 142592 | rs1527307 | Affymetrix | MGAM | 99.3 | 0.31 | C/T | 0.19 | Intronic |
| 7, 142630 | rs10260248 | Taqman | TAS2R40 | 97.7 | 0.928 | C/A | 0.04 | S187Y |
| 7, 142631 | rs534126 | Taqman | TAS2R40 | 98.0 | 0.622 | C/T | 0.38 | Noncoding |
| 7, 142852 | rs4595035 | Taqman | TAS2R60 | 97.7 | 0.616 | C/T | 0.35 | R310R |
| 7, 142885 | rs1404635 | Taqman | TAS2R41 | 100 | 0.577 | G/A | 0.16 | T63T |
| 7, 142885 | rs10278721 | Taqman | TAS2R41 | 97.7 | 0.653 | C/T | 0.16 | P127L |

kb, Kilobases; HWE, Hardy–Weinberg Equilibrium; MAF, minor allele frequency.

Statistical analysis

Associations with SNP genotype and the various phenotypes were performed using pedigree-based analysis by regressing the effect of the marker genotype while accounting for residual familial correlations among related individuals using age, age², and sex as covariates (for sex stratified analyses, age and age² were the only covariates used). Phenotypic data were transformed by their natural logarithms to normalize the data distributions. To account for the relatedness among family members, we employed the measured genotype approach, in which we estimated the likelihood of specific genetic models given the pedigree structure. Parameter estimates were obtained by maximum likelihood methods and the significance of association was tested by likelihood ratio tests. All analyses were carried out using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software program (Almasy & Blangero, 1998). Bonferroni corrections were preformed to control for inflation of the type I error rate due to the number of comparisons. Pairwise LD between the SNPs and haplotype block analysis was computed using Haploview 4.1 (Barrett, Fry, Maller, & Daly, 2005). Haplotype blocks were defined by 95% confidence bounds on *D'* (Gabriel et al., 2002).

Results

We first asked whether a variant in the taste receptor gene *TAS2R38* was associated with eating behaviors as assessed by the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985; Stunkard & Wadden, 1990). We performed association analysis of the SNP rs1726866, a nonsynonymous coding SNP within *TAS2R38* (T785C; Val262Ala), and the eating behaviors hunger, restraint and disinhibition. The “C” allele of rs1726866, the minor allele in the AFDS, is strongly associated with increased taste sensitivity to PROP/PTC (Bufe et al., 2005; Kim et al., 2003). We observed no significant association between SNP genotype and the eating behaviors hunger or restraint. However, there was a significant association of the minor allele with decreased disinhibition (*p* = 0.03; Table 2). Because eating behaviors differ substantially between males and females (e.g., Burton, Smit, & Lightowler, 2007), we subsequently performed sex-stratified analyses. Stratification of the cohort by gender revealed a strong association in females (*p* = 0.0002, Tables 2 and 3) but not in males (*p* = 0.86; Tables 2 and 3). This significance value survived our correction to control for the

Table 2
 Disinhibition *p* values for rs1726866.

| | <i>p</i> value | <i>p</i> value (leptin adjusted) |
|-------------------|----------------|----------------------------------|
| Males and females | 0.03 | ND |
| Males | 0.86 | ND |
| Females | 0.0002 | 0.004 ^a |

ND, Not determined. Covariates: age, age², with adjustments for family structure. An additive inheritance model was assumed.

^a Plasma leptin added as an additional covariate.

increase in type I error associated with multiple comparisons (*p* = 0.02 Bonferroni corrected).

To define the extent of LD around rs1726866, we examined previously genotyped haplotype-tagging SNPs (Dotson et al., 2008) both upstream and downstream of *TAS2R38* (Fig. 1). We identified two LD blocks. The first (LD Block 1) contains four SNPs (rs11763979, rs2233998, rs2234001, and rs2227264), is found ~182 kb upstream of rs1726866 and extends for 27 kb (Fig. 1). SNP rs1726866 (*TAS2R38*) displays moderate LD with the SNPs in this block (*r*² = 0.48–0.61; *D'* = 0.73–0.80), but well less than the threshold for inclusion in the LD block (*D'* ≥ 0.95) (Gabriel et al., 2002). These SNPs were also significantly associated with disinhibition in female subjects, though the *p*-values of their association (ranging from *p* = 0.002 to *p* = 0.004) were at least an order of magnitude higher than that observed for rs1726866 (Table 4). Six other SNPs located ~1.3 mb downstream of *TAS2R38* show no LD with rs1726866, although two of them form a small LD block at the distal end of this cluster (LD Block 2; Fig. 1).

Because of the large intergenic intervals between rs1726866 and some of these other SNPs, we sought to further refine the extent of LD around rs1726866 by examining several additional SNPs upstream and downstream of *TAS2R38* in a subset of the AFDS

Table 3
 Mean disinhibition scores (±s.e.m.) stratified by sex.

| | rs1726866 genotype | | |
|---------------------------|----------------------------------|----------------------------------|---------------------------------|
| | TT | CT | CC |
| Males (<i>n</i> = 348) | 4.45 ± 0.12 (<i>n</i> = 193) | 4.39 ± 0.12 (<i>n</i> = 131) | 4.71 ± 0.12 (<i>n</i> = 24) |
| Females (<i>n</i> = 381) | 6.13 ± 0.14 (<i>n</i> = 225) | 4.92 ± 0.14 (<i>n</i> = 138) | 4.55 ± 0.13 (<i>n</i> = 18) |

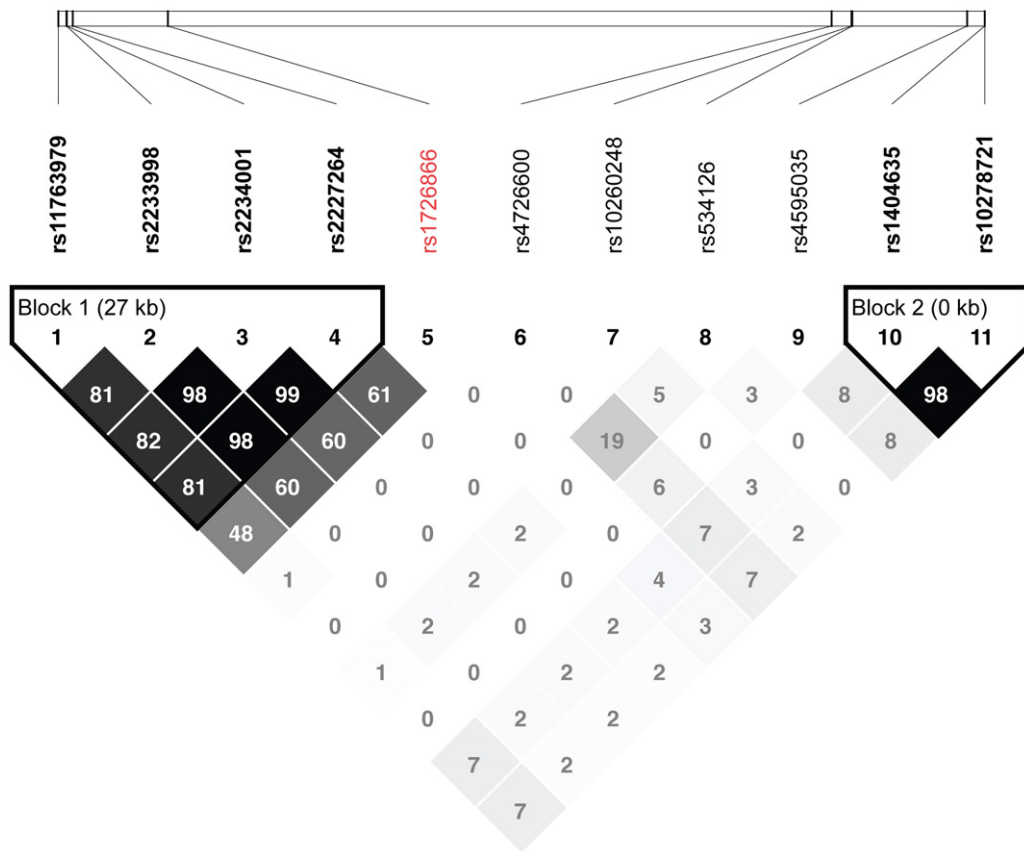


Fig. 1. Pairwise LD (r^2) among 11 SNPs located both upstream and downstream of rs1726866 on chromosome 7 in the AFDS ($n = 1330$). r^2 values $\times 100$ are indicated within squares, with darker shades indicating higher r^2 values.

Table 4
 Significant disinhibition p values for SNPs in LD Block 1.

| Chromosome, position (kb) | SNP ID | SNP type | Disinhibition p value |
|---------------------------|------------|-----------|-------------------------|
| 7, 141109 | rs11763979 | Noncoding | 0.004 |
| 7, 141111 | rs2233998 | F7S | 0.002 |
| 7, 141125 | rs2234001 | V96L | 0.002 |
| 7, 141137 | rs2227264 | S26I | 0.003 |

kb, Kilobases. Covariates: age, age², with adjustments for family structure.

($n = 550$). These SNPs were genotyped previously using the Affymetrix GeneChip Human Mapping 100K Set (Hsueh et al., 2007). Four upstream SNPs showed little LD with rs1726866 ($r^2 = 0.0-0.44$; Fig. 2). The five SNPs located downstream of *TAS2R38* also display little LD with rs1726866 ($r^2 = 0.0-0.18$; Fig. 2). Collectively, these data again suggest that variation in the bitter taste receptor gene *TAS2R38* is principally responsible for the disinhibition association observed in female AFDS participants.

The eating behavior disinhibition is strongly related to BMI, particularly in women (e.g., Hainer et al., 2006; Hays et al., 2002; Lindroos et al., 1997; Savage, Hoffman, & Birch, 2009; Williamson et al., 1995). However, we saw no association between variation at rs1726866 and BMI ($p = 0.27$). These results are consistent with previous reports indicating a lack of association between BMI and variation at *TAS2R38* (Sausenthaler, Rzehak, Wichmann, & Heinrich, 2009; Tepper et al., 2008a; Timpson et al., 2005).

Discussion

TAS2R38 genotype may influence the types of food an individual prefers or consumes (Sacerdote et al., 2007; Sandell & Breslin, 2006), or possibly an individual's postprandial response to

ingested nutrients (Chen, Wu, Reeve, & Rozengurt, 2006). However, it was not known whether *TAS2R38* genotype influences how one eats. Here we have identified an important link between chemosensory receptor gene variation and the control of eating. Our study indicates an association between the major allele (i.e., the PROP-insensitive allele) of rs1726866, a nonsynonymous coding SNP located within *TAS2R38*, and increased disinhibition in female Amish individuals.

We previously reported linkage peaks for eating behaviors in the Amish (Steinle et al., 2002), none of which contained *TAS2R38*. However, it is not surprising that this previous study failed to capture the association with the *TAS2R38* locus: the marker coverage on chromosome 7 in that study was extremely sparse (the average interval between markers was 10 cM). In fact, the closest marker to *TAS2R38*, D7S636, was located approximately 5.2 MB downstream of that gene. Furthermore, that study did not stratify the population by gender, suggesting that an association would have been missed even if markers close to *TAS2R38* had been used.

How might genetic variation in *TAS2Rs* influence eating behavior? One possibility is that variation in receptor function can alter the perceived qualities of food (i.e., their taste) and thereby impact nutrient intake. It is well known that orosensory variation strongly affects ingestive behavior and, as such, nutrient intake (Duffy, 2007). Orosensory variation is strongly affected by taste and by genetic variation in chemosensory receptors (Bachmanov & Beauchamp, 2007). *TAS2Rs* show a high level of allelic variation across species as well as across human populations (Kim et al., 2005; Shi & Zhang, 2006; Shi, Zhang, Yang, & Zhang, 2003; Wang, Thomas, & Zhang, 2004), and could be subject to positive evolutionary selection (e.g., Soranzo et al., 2005). Therefore, individual *TAS2R* alleles may encode receptors specialized to detect food compounds prevalent in local environments.

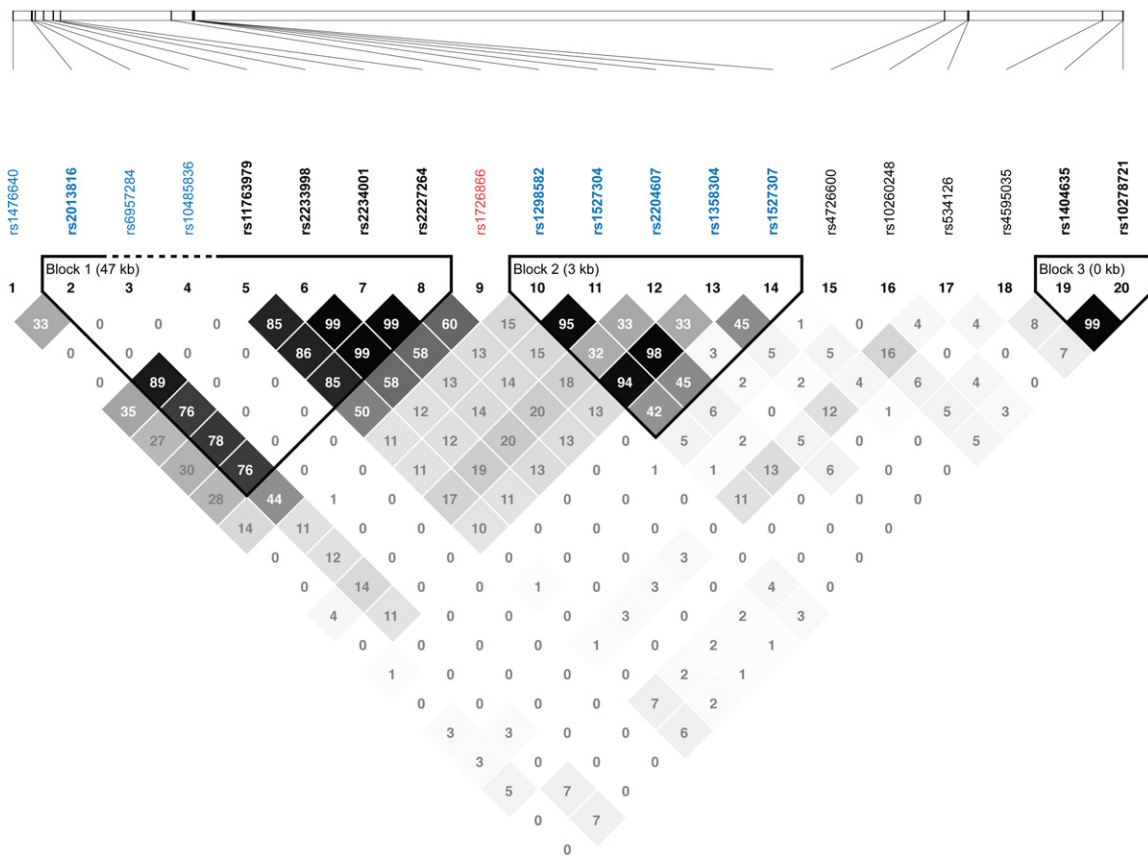


Fig. 2. Pairwise LD (r^2) among 11 SNPs located both upstream and downstream of rs1726866 and 9 additional SNPs adjacent to *TAS2R38* in a subset of the AFDS ($n = 550$). r^2 values $\times 100$ are indicated within squares, with darker shades indicating higher r^2 values.

In this context, polymorphisms in individual *TAS2Rs* are strong candidates to influence food intake. However, several groups have reported that *TAS2R38* genotype is not associated with body mass index (BMI) (Kim et al., 2003; Prodi et al., 2004; Tepper, 2008). Consistent with these findings, we saw no association between variation at rs1726866 and BMI in the Amish despite the observation that disinhibition is significantly correlated with BMI in Amish females (Table 5). Thus, *TAS2R38* genotype affects that portion of disinhibition that is not related to changes in BMI (e.g., moving from Q3 to Q4; Table 5). In contrast, taste sensitivity to PROP is associated with BMI (Duffy, 2004; Goldstein, Daun, & Tepper, 2005; Tepper & Ullrich, 2002; Tepper et al., 2008a). This seeming contradiction can be explained by the fact that while *TAS2R38* genotype accounts for the majority of PROP taste sensitivity variation (estimates range from 55 to 85%) (Kim et al., 2003; Prodi et al., 2004), *TAS2R38*-independent factors (e.g., fungiform papillae density) also impact PROP taster status (Hayes, Bartoshuk, Kidd, & Duffy, 2008). It is quite possible that these *TAS2R38*-independent factors are responsible for the relationship between PROP taste sensitivity and BMI. Thus, the absence of an association between *TAS2R38* and BMI suggests that the mechan-

isms by which *TAS2R38* impacts disinhibition is independent of taste sensitivity to PROP and related compounds (Fig. 3). However, because the relationship between PROP taste sensitivity and eating behaviors is unknown, this hypothesis is somewhat provisional.

Alternative physiological mechanisms may link *TAS2R* function to the modulation of eating behavior. In addition to influencing taste perception, *TAS2Rs* and *TAS1Rs* (which function as sweet or amino acid taste receptors in the gustatory system) help to mediate both nutrient uptake and gut hormone release. For example, *TAS1R*-dependent glucose stimulation increases the amount of the glucose transporter GLUT2 in apical membranes of enterocytes and other intestinal cells (Mace et al., 2007, 2009). In addition, both *TAS2Rs* and *TAS1Rs* impact the release of GLP-1 from gut enteroendocrine L cells (Dotson et al., 2008; Jang et al., 2007; Jeon et al., 2008). Because of the considerable influence that

Table 5
 Mean BMI (\pm s.e.m.) stratified by disinhibition scores.

| | |
|------------------|------------------|
| Q1 ($n = 109$) | 25.56 \pm 0.49 |
| Q2 ($n = 117$) | 26.97 \pm 0.43 |
| Q3 ($n = 108$) | 29.10 \pm 0.45 |
| Q4 ($n = 73$) | 30.47 \pm 0.65 |

Disinhibition scores are significantly correlated with BMI in female AFDS participants; $r = 0.34$, $p < 0.0001$; Q(x) = quartiles.

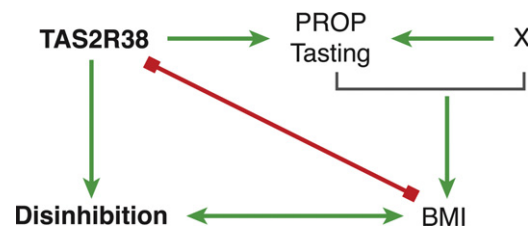


Fig. 3. Schematic of putative relationships between *TAS2R38*, disinhibition, PROP taste sensitivity and BMI. *TAS2R38*-independent factor(s) that impact PROP taste sensitivity are indicated by "X." Green indicates an association, while red indicates the absence of an association. Arrowheads indicate the likely directionality of the association. It is unclear if *TAS2R38*-independent factor(s) impacting PROP taste sensitivity or the taste phenotype itself is associated with BMI.

chemosensory receptors such as TAS2Rs have on the nutrient dependent regulation of metabolism, it is possible that genetic variation in these receptors could impact hormonal systems that contribute to the regulation of eating behaviors. For example, genetic variation in TAS2Rs could impact the secretion of GLP-1 (Dotson et al., 2008), CCK (Chen et al., 2006), leptin, insulin or other hormones that play a significant role in the regulation of eating patterns (Berthoud, 2002). Indeed, there is a strong relationship between plasma leptin levels and the eating behavior disinhibition (Blundell et al., 2008), and variation in the genes encoding both leptin and the leptin receptor have been associated with altered eating patterns (de Krom et al., 2007). The “PROP insensitive” allele of rs1726866 is significantly associated with increased plasma leptin levels ($p = 0.007$) in Amish females. Thus, the “T” allele of rs1726866 (i.e., the PROP-insensitive allele) is associated with increased plasma leptin and with increased disinhibition in female AFDS participants. Higher leptin levels are observed among the obese, especially obese women. Given the correlation between disinhibition score and BMI, it is possible that the higher leptin levels observed among individuals with the “T” allele of rs1726866 is reflective of their higher disinhibition score and higher BMI (see Table 5). Including leptin as a covariate decreases the significance of the association of rs1726866 and disinhibition by more than an order of magnitude (Table 2), suggesting that variation in leptin levels accounts for at least some degree of the variation observed in disinhibition levels in this female Amish cohort (see Baron & Kenny, 1986). It is also possible that TAS2R38 may influence eating behavior and satiety by modulating the hormones GLP-1, CCK and insulin, and the reduced leptin levels observed among individuals with the “C” allele are reflective of enhanced satiety mediated via these hormones. Further investigation is needed to establish if leptin levels are directly mediating this association, or if leptin is impacted indirectly via other factors such as secretion of other satiety hormones or adiposity.

Physiological or psychological variables can influence food preference (e.g., Ullrich, Touger-Decker, O’Sullivan-Maillet, & Tepper, 2004). It is possible that these factors may be more or less prominent in Amish females than in the general Caucasian population. For example, a reduced stigmatization of obese individuals among the Old Order Amish could diminish obesity associated eating behavior. In addition, genotype–phenotype associations can result for reasons other than a direct effect of gene on phenotype. For example, although the LD structure in the region of TAS2R38 strongly suggests that it is the causal locus, it remains possible that rs1726866 may tag variation at a distant locus that is actually the causal allele. Clearly, further studies of the physiological roles of TAS2R38 and the physiological basis of disinhibition are needed to answer these questions. Be that as it may, our studies support a role for a chemosensory receptor sensitive to bitter-tasting ligands in the modulation of eating behaviors, particularly among women.

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