RELATIONSHIPS BETWEEN THE HTAS2R38 GENOTYPE, FOOD CHOICE, AND ANTHROPOMETRIC VARIABLES IN NORMAL-WEIGHTED AND OVERWEIGHT ADULTS

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Aim: Taste is a major determinant of food choice; however, there is a great lack of knowledge about how taste perception affects human nutrition. Bitter taste perception presents unique opportunities for investigating this subject. The aim of this study was to determine whether polymorphisms on the bitter taste receptor gene hTAS2R38 affect an individual’s food choices and some anthropometric variables. Subjects and Method: In this study, the possible relationship between food preferences, body weight, and polymorphisms on hTAS2R38 was investigated in healthy volunteers (n=178) who weighed within the normal range (BMI: 20-24.9 kg/m², n=90) and those who were overweight, but otherwise healthy (BMI ≥ 25.0 kg/m², n=88). Descriptive information about the subjects was collected via a questionnaire, and anthropometric measurements were taken by the researcher. Records of three consecutive days of food consumption were collected to determine each subject’s macronutrient intake. For identification of the hTAS2R38 genotype, samples were taken from each participant's in-mouth epithelial cell line, and the genetic material was analyzed at the laboratory for Rs713598. Results: The percentage of “non-tasters” (n=42) among the whole population was 23.6% (C-Homozygote: 23.6%) while “tasters” (n=136) comprised 76.4% (CG-Heterozygote: 46.6%, G-Homozygote: 29.8%). When group-wide and between-group comparisons were made, it was revealed that taster status didn’t affect differences in anthropometric measures. Detected differences in macronutrient intake were due to gender. Discussion:
Polymorphisms on hTAS2R38 bitter taste receptor gene had no effect on variables such as body weight, anthropometric variables, body fat percentage, or food choices within the study population.

**Key words**: Single Nucleotide Polymorphism, TAS2R38 protein, Food preferences, Body weight and measurements

**INTRODUCTION**

In 1931, Arthur L. Fox accidentally discovered differences in phenylthiocarbamide (PTC) bitterness perception. He determined that individuals may fall into two different categories: those who are able to taste PTC’s bitterness, even at very low concentrations, may be classified as “tasters”, and those who are unable to taste PTC’s bitterness, except at very high concentrations may be classified as “non-tasters”. This discovery led researchers to identify new families for “taste blindness”. Many studies have hypothesized that PTC bitter taste perception may well be explained with general Mendelian genetics; however, more recent studies have shown that mechanisms other than general Mendelian genetics are more efficient in explaining variations for PTC taste perception (Kim & Drayna, 2005; Wooding, 2006).

After the discovery of the bitter taste receptor genes (T2R or TAS2R genes) for humans, approximately 25 potentially functional T2R genes have been reported. Each of the bitter receptors recognizes a specific set of cognate bitter compounds (Behrens et al., 2007; Bufo et al., 2002; Sainz et al., 2007).

Naturally occurring alleles of the TAS2R38 receptor gene have been found to be responsible for individual differences in the ability to taste PTC and its chemical relative, propylthiouracil (PROP)(Reed et al., 2006). Common single nucleotide polymorphisms (SNPs) of the TAS2R38 gene (proline or alanine at position 49, Reference SNP number 713598; alanine or valine at position 262, Rs1726866; valine or isoleucine at position 296, Rs10246939) constitute five different haplotypes (PAV, AVI, and the less common haplotypes AAI, PVI and AAV), and these haplotypes have accounted for 55% to 85% of the variance in the PTC/PROP taste sensitivity. The two most common haplotypes, the PAV and AVI forms, are highly correlated with taster status. The PAV form is considered to be a major taster haplotype, and the AVI form is considered to be a major non-taster haplotype (Kim et al., 2003; Reed et al., 2006; Tepper et al., 2008).

PTC/PROP taster status has been extensively investigated for its relationship to some chronic diseases and health-related issues, such as cardiovascular diseases (Timpson et al., 2005), diabetes (Dotson et al., 2008), dental caries (Hedge & Sharma, 2008), schizophrenia (Moberg et al., 2005), depression (Joiner & Perez, 2004), emotional status (Macht & Mueller, 2007), smoking (Cannon et al., 2005; Enoch et al., 2001; Snedecor et al., 2006) and malignant tumors (Basson et al., 2005). In addition, the correlation between PTC taster status and alcoholism has been another important point of research (Driscoll et al., 2006; Duffy et al., 2004; Guo & Reed, 2001).

Studies on the relationships between the perceived fat content of foods, acceptance of high-fat foods, variations on Body Mass Index (BMI) and their connection with PROP taster status have produced conflicting results. While some studies strongly support these relationships (Duffy et al., 1996; Tepper & Nurse, 1997; Tepper & Nurse, 1998), others have found a very weak association or no association at all (Drewnowski et al., 1998; Yackinous & Guinard, 2001).
The aim of this study was to evaluate whether polymorphisms on the TAS2R38 bitter taste receptor gene have any effect on an individual’s body weight and food choices.

MATERIALS AND METHODS

Procedure and Individuals
The study population (n=178) consisted of a normal-weighted (BMI 20-24.9 kg/m², n=90) control group and an overweight group (BMI ≥25 kg/m², n=88). Volunteers were at least 19 years old, had never smoked, only drank socially, didn’t take any medications that might affect food intake, were not pregnant or lactating, didn’t have any chronic, hereditary or psychological diseases, didn’t use vitamin or mineral supplements, didn’t engage in vigorous physical activities, and hadn’t deviated from their habitual diet for the previous 3 months. An informed consent form was signed by all participants, and the study was approved by the Medical, Surgical, and Pharmacological Studies Ethic Committee of the Hacettepe University Faculty of Medicine (Approval date: March 21st, 2007, Approval number: LUT 07/1-44).

This study had three sections: the questionnaire and food consumption records, analysis of genetic material, and application of the PROP taste test.

The questionnaire and records of three consecutive days of food consumption
The first part of the questionnaire evaluated primary demographic variables (age, gender, marital status, education level, and job status) and anthropometric measurements. Waist circumference, hip circumference, and upper mid-arm circumference measurements were taken by the researcher, and body weight and height were self-reported. The percentage of body fat was calculated via Omron® BF-306. This device uses the hand-to-hand bioelectrical impedance method which is compatible with dual-emission X-ray absorptiometry (DXA) measurements, currently considered to be the “gold standard” for this type of measurement (LINTSI et al., 2004). The end of the questionnaire contained available space for recording the three consecutive days (two week days and one weekend day) of food consumption. These records were analyzed for macronutrient content via packaged software (BeBIS, Nutrition Information System Version 7, Germany).

Collection of Genetic Material and Analysis
In this study we choose hTAS2R38 bitter taste receptor gene because the polymorphisms on the gene is well-described and extensively investigated for relationships with food choice and intake, some chronic diseases, fat perception and obesity (TEPPER, 2008). For identification of the hTAS2R38 genotype, the samples taken from each participant’s in-mouth epithelial cell line via buccal swabs and genetic material analyzed at the GENAR Biotechnology and Molecular Genetics Research and Application Laboratories (www.genar.gen.tr) for Rs713598 were used. Rs713598 identifies the G-C exchange at position 229 of mRNA that encodes hTAS2R38. Although C-homozygotes express Alanine aminoacid at the position 49 of hTAS2R38, CG-heterozygotes and G-Homozygotes express Proline aminoacid at the same position (KIM et al., 2003). It is assumed that C-Homozygotes have AVI, CG-heterozygotes and G-Homozygotes have PAV haplotype and rare haplotypes (AAI, PVI and AAV) does not exist in the study population. Due to very limited frequency of rare haplotypes within populations this assumption has been adopted (PEMBERTON et al., 2008).
The subjects were asked to not eat or drink anything for at least one hour prior to taking the samples. Two samples were taken from each individual in case of insufficient DNA extraction. The collected samples were stored at +4°C until the analysis. For identification of Rs713598, the laboratory used the matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) method.

**The Application of the PROP Taster Test**

The PROP taster test is frequently used in the literature and is based on the comparison of perceived taste intensity of PROP and NaCl. The taster status of PROP doesn’t affect taste intensity assessments for NaCl; thus, the psychophysical functions for NaCl have become the standard by which comparisons with PROP functions are made. The detailed methodology of this test may be found elsewhere in the literature (BARTOSHUK et al., 1994; DRENOWSKI et al., 1997a; DRENOWSKI et al., 1997b; DRENOWSKI et al, 1998).

**Statistical Analyses**

Statistical Package for the Social Sciences (SPSS, version 16.0) was used for data analysis. All raw data obtained within this study was evaluated for each working group (control and overweight) along with gender and taster status. Because of some sub-groups didn’t have proper number of individuals to conduct parametric tests, Kruskal-Wallis H Test used to compare sub-groups. The Mann-Whitney U Test has been used to analyze the specific sample pairs for possible significant differences. Chi-square test was used for analyze the categorical datas. A p value of 0.05 was considered to be statistically significant.

**RESULTS**

The study population (n=178) consisted of 59 men (33.1%) and 119 women (66.9%) with a mean age of 28.72±9.35 years. In general, 19-35 years age group has greater percentage in both groups (control group n=75, 83.3%; overweight group n=69, 78.4%, p=0.403). The control group (n=90, 50.6%) and overweight group (n=88, 49.6%) were almost equally distributed within the study population. Study groups were similar in gender [control group n=90, men=24 (26.7%), women=66 (73.3%); overweight group n=88, men=35 (39.8%), women=53 (60.2%) (p=0.063)].

The proportion of Rs713598 polymorphisms within individuals was 23.6% for C-homozygote subjects (n=42), 46.6% for CG-heterozygote subjects (n=83), and 29.8% for G-homozygote subjects (n=53). “Non-tasters” comprised 23.6% of the study population while “tasters” made up 76.4%. (Table 1).

“Tasters” had a mean BMI of 26.22±5.16 kg/m² while the mean BMI for “non-tasters” was 25.33±5.04 kg/m². In addition to BMI, weight (73.49±16.59 kg vs. 69.34±17.12 kg, respectively), waist circumference (89.07±15.59 cm vs. 86.17±16.48 cm, respectively), hip circumference (102.80±11.92 cm vs. 101.38±11.52 cm, respectively), waist/hip ratio (0.86±0.08 vs. 0.84±0.08, respectively), upper mid arm circumference (28.79±4.85 cm vs. 28.07±5.17 cm, respectively), and body fat percent (30.50±8.50% vs. 30.53±8.68%, respectively) were analyzed and did not differ significantly among taster groups. When gender and BMI status were taken into account, statistically significant differences were revealed for anthropometric variables. Further statistical analyses showed that these differences depended on gender instead of taster status (Table 2).
Table 1: Distribution of Rs713598 polymorphisms and taster status according to study groups.

<table>
<thead>
<tr>
<th>Rs713598 Genotype</th>
<th>CONTROL (n=90)</th>
<th>OVERWEIGHT (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n=24)</td>
<td>Women (n=66)</td>
</tr>
<tr>
<td>C Homozygote</td>
<td>5 (20.8%)</td>
<td>17 (25.8%)</td>
</tr>
<tr>
<td>CG Heterozygote</td>
<td>9 (37.5%)</td>
<td>30 (45.5%)</td>
</tr>
<tr>
<td>G Homozygote</td>
<td>10 (41.7%)</td>
<td>19 (28.8%)</td>
</tr>
<tr>
<td>Taster Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-tasters</td>
<td>5 (20.8%)</td>
<td>17 (25.8%)</td>
</tr>
<tr>
<td>Tasters</td>
<td>19 (79.2%)</td>
<td>49 (74.2%)</td>
</tr>
</tbody>
</table>

Table 2: Evaluation of primary anthropometric measures for study groups according to gender and taster status.

After the three-day food consumption records for the study groups were analyzed according to gender and taster status, the differences in macronutrient intake levels between the groups were found to be statistically significant (p<0.05). Detailed statistical analyses revealed that the source of these differences was gender. The individual’s taster status did not affect macronutrient intake levels (Table 3).
Due to unwillingness of the participants to join PROP taster test, only 20 subjects completed the test. Results didn’t reach a level of statistical importance because of unbalanced numbers of the taster and non-taster groups (results not shown).

DISCUSSION

After genetic analyses, it was shown that 23.6% of the study population was C-homozygote, 46.6% was CG-heterozygote, and 29.8% was G-homozygote. The calculated allele frequency was C/G: 46.6/53.4%. This distribution is consistent with Northern and Western European samples of genotype frequency distribution. Genetic analyses conducted on individuals who lived in Utah and who have Northern and Western European ancestors (CEPH group) within the International HapMap project showed that C-homozygotes were 18.0%, CG-heterozygotes were 48.0%, and G-homozygotes were 34.0% of the total population (KIM et al., 2003).

Mangold et al. (MANGOLD et al., 2008) found that the allele frequency (C/G) of Rs713598 within African Americans is 41.9/59.0% while it is 47.0/53.0% for European Americans. In a similar study which investigated relationships between TAS2R38 haplotypes and alcohol dependence, the Rs 713598 allele frequency (C/G) for European Americans was 42.0/58.0% (WANG et al., 2007).

Taste blindness for PTC/PROP solutions is seen in almost all populations around the world. However, the frequency of “non-tasters” is highly variable due to racial and ethnic origins. The frequency of “non-tasters” in Caucasian populations is almost 30.0%. Generally, Chinese, Japanese, and African populations have lower frequencies (10.0-20.0%). An exception

Table 3: Evaluation of macronutrient intake levels (as grams and percentage of energy) for study groups according to gender and taster status.

<table>
<thead>
<tr>
<th>Anthropometric Variables</th>
<th>C-Hom (n=109)</th>
<th>TG-Het (n=117)</th>
<th>G-Hom (n=108)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)**</td>
<td>74.90 (22.00- 94.00)</td>
<td>76.00 (23.00- 98.00)</td>
<td>77.00 (24.00- 102.00)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Waist circum. (cm)**</td>
<td>94.90 (84.00- 110.00)</td>
<td>96.00 (86.00- 112.00)</td>
<td>97.00 (88.00- 114.00)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Waist/Hip Ratio**</td>
<td>0.82 (0.79-0.87)</td>
<td>0.85 (0.79-0.97)</td>
<td>0.85 (0.78-0.94)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Upper Mid-Arm circum. (cm)**</td>
<td>26.20 (26.00- 26.40)</td>
<td>26.20 (26.00- 26.40)</td>
<td>26.20 (26.00- 26.40)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (Kg/m²)**</td>
<td>22.20 (26.20- 24.20)</td>
<td>22.20 (26.20- 24.20)</td>
<td>22.20 (26.20- 24.20)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body fat %**</td>
<td>15.50 (8.50- 25.70)</td>
<td>15.50 (8.50- 25.70)</td>
<td>15.50 (8.50- 25.70)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**All values have shown as median (minimum-maximum)

*Significant at p<0.05 (gender)
is seen in some minority groups in India who have a frequency of more than 50.0%. The reason for these differences is still unknown (Guo & Reed, 2001).

In this study, the frequency of “non-tasters” was 23.6% while it was 76.4% for “tasters”. Taster status for gender in the control and overweight groups did not differ significantly.

When height, weight, BMI, body fat percentage, and other anthropometric variables (waist circumference, hip circumference, waist/hip ratio, upper mid-arm circumference) were evaluated for taster status, both groups had similar measurement results. In addition, when gender and taster status was taken into account in the study groups, statistically significant differences appeared. Further statistical analyses showed that BMI values and differences in body composition due to gender were responsible for this significant difference. This data revealed that the effects of taster status determined by the Rs713598 polymorphism on body weight, anthropometric variables, and body fat percentage was very limited or had no effect.

Tepper et al. (Tepper et al., 2008) found no relationship between hTAS2R38 haplotypes and BMI or waist circumference measurements among men. Besides, there is a tendency for an increase in the number of PAV alleles to result in a corresponding increase in BMI and waist circumference measurements.

A study conducted on a British Women’s Heart and Health Study cohort showed that no significant relationships existed between hTAS2R38 haplotypes, BMI (“non-tasters”: 27.55±4.96 kg/m², n=1065; “tasters”: 27.63±5.06 kg/m², n=2147), and waist/hip ratio (“non-tasters”: 0.81±0.06; “tasters”: 0.82±0.06) (Timpson et al., 2005).

Within the Amish Family Diabetes Study cohort, 729 non-diabetic individuals (381 women, 348 men; BMI: 28.1±5.4 kg/m² and 26.4±3.7 kg/m², respectively, p<0.001) were investigated for possible relationships between the hTAS2R38 genotype and eating behaviors. The results showed no significant relationship between the Rs1726866 polymorphism and BMI values (p=0.27) (Dotson et al., 2009).

In our study, the analysis of food consumption records showed significant differences in the macronutrient intake level and the percentage of contribution to total energy intake because of gender (not taster status) (p<0.05).

Drewnowski (Drewnowski et al., 1999; Drewnowski et al., 2000) found no significant differences between energy from carbohydrates (“non-tasters”: 55.6±0.6%, “tasters”: 54.5±1.1%) and energy from fat (“non-tasters”: 28.9±0.5%, “tasters”: 29.0±0.9%) when food consumption records of his two different study cohorts (total of 237 PROP tasters and 63 non-tasters) was analyzed. He also stated that the effects of taste genetics on food choice are very limited and easily affected by cultural, social, and behavioral factors and that PROP taster status did not affect chronic disease risk via a diet-mediated pathway.

Yackinous and Guinard (Yackinous & Guinard, 2002) investigated associations between PROP taster status and general markers of dietary intake and consumption of specific food groups, and they reported that PROP taster status does not affect the intake of macronutrients among men and women.

In our study, “tasters” rated second (0.032 mol/litre), third (0.1 mol/litre), and fourth (0.32 mol/litre) NaCl concentrations higher than “non-tasters”. While NaCl solutions were more intense compared with “non-tasters”, these differences did not reach statistically significant levels. When compared with “non-tasters”, “tasters” had higher intensity ratings for all PROP solutions.
A study on the TAS2R38 genotype, taste phenotype, and fungiform papillae count included 139 women and 59 men with European ancestry. For AVI/AVI individuals, increases in bitterness of 1.0-3.2 mM PROP solutions were almost parallel with PAV/AVI and PAV/PAV individuals. Researchers have reported that some receptors other than TAS2R38 may be functional in the perception of bitterness in a PROP solution. As a consequence of this, some non-taster homozygotes may be misclassified (Hayes et al., 2008). Bufe et al. (Bupe et al., 2005) showed that between haplotypes, PROP concentration intensity functions may not discriminate as powerfully as PTC. In addition, two AVI/AVI individuals responded to PROP at a high level, and two PAV/PAV individuals responded weakly to PROP solutions. This observation pointed out that supra-threshold sensitivity for PROP solutions may be under the control of additional genetic and environmental factors. In our study, after PROP taster test, two C-homozygote (non-tasters) individuals strongly perceived the PROP bitterness while one G-homozygote (taster) individual had lower intensity ratings for PROP. This observation is consistent with Bufe’s findings (Bupe et al., 2005).

CONCLUSION
At the end of the study, we concluded that the Rs713598 polymorphism on the hTAS2R38 bitter taste receptor gene and the relevant distribution of “tasters” and “non-tasters” were not significantly different between groups. Polymorphisms and taster status had no effect on the control or the overweight group’s choice of food. Polymorphisms on the hTAS2R38 bitter taste receptor gene explain the PROP phenotype to some degree, and bitter taste receptors other than TAS2R38 may be functional for the perception of PROP bitterness.

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REFERENCES


Izvod

Cilj: Ukus je glavna determinanta (odrednica) za izbor hrane; ali ipak postoji veliki nedostatak znanja o tome kako percepcija ukusa utice na ljudsku ishranu. Gorak ukus percepcije predstavlja jedinstvenu priliku za ispitivanje ove teme. Cilj ovog istraživanja je bio da se odredi da li polimorfizmi na gorak ukus receptora gena hTAS2R38 utiču na izbor hrane pojedinca i neke antropometrijske varijable. Predmeti i Metod: U ovoj studiji, moguća veza između preferiranja (izbora) hrane, telesne težine i polimorfizama na hTAS2R38 ispitivan je kod zdravih dobrovoljaca (n = 178), cija je težina bila u okviru normalnih granica (BMI: 20-24.9 kg/m², n = 90) i onih koji su bili gojazni, ali ipak zdravi (BMI ≥ 25.0 kg/m², n = 88). Opisne informacije o temama bile su prikupljene od ispitanika, i antropometrijskih merenja preuzeta od ispitivaca. Evidencija od tri uzastopna dana na bazi potrošnje ishrane prikupljeni su da se utvrdi makronutristicki unos svakog subjekta (cinioca). Za identifikaciju hTAS2R38 genotipa, uzorci su uzeti iz ustiju svakog učesnika sa epitela celija, a genetski materijal je analiziran u laboratoriji za Re713598. Rezultati: Procenat “ne-degustatora” (n = 42) nad celom populacijom 23,6% (C-homozigotnom: 23,6%), Dok je “degustatora” (n = 136) cinilo 76,4% (CG-heterozigot: 46,6%, G-homozigotnom: 29,8%). Kada je napravljeno poredjenje sirom grupa i medju grupom, pokazalo se da status degustatora ne utice na razlike u antropometrijskim merenjima. Otkriveni razlike medju makronutristickim uzimanjima pripisivane su polu. Diskusija: Polimorfizmi na hTAS2R38 na gen receptor gorkog ukusa ne utice na varijable kao što su težina tela, antropometričke varijable, procenat telesne masne, ili izbor hrane u okviru studije (proučavanja) populacije.

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