ORIGINAL ARTICLE

Genetic variation in the hTAS2R38 taste receptor and brassica vegetable intake

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Abstract
The human TAS2R38 receptor is believed to be partly responsible for the ability to taste phenylthiocarbamide (PTC), a bitter compound very similar to the bitter glucosinolates found in brassica vegetables. These vegetables and their active compounds have chemo-protective properties. This study investigated the relationship between genetic variation in the hTAS2R38 receptor and the actual consumption of brassica vegetables with the hypothesis that taster status was associated with intake of these vegetables. Furthermore, secondary intake information on alcohol, chocolate, coffee, smoking, BMI and waist-circumference was analysed for association with the hTAS2R38 receptor polymorphisms. The subjects were selected from the Diet, Cancer and Health (DCH) study, which is an ongoing prospective Danish cohort study. Two groups, each consisting of 250 healthy subjects were selected based on their brassica vegetables intake from the upper quartile (>23 g/day) and the lower quartile (<7 g/day) daily intake of brassicas from a randomly selected sub-cohort of DCH. DNA was analysed for three functional SNPs in the hTAS2R38 gene. The hTAS2R38 bitter taste receptor haplotypes were not associated with the daily intake of brassica vegetables in our study, and no association between the haplotypes and any of the other variables tested was found. We have demonstrated that the hTAS2R38 haplotypes are not associated with brassica vegetable intake and that results from experimental setups cannot be applied directly to the everyday situation. This indicates that non-genetic factors may have more influence on dietary choice than genetics.

Key Words: Alcohol, genetics, obesity, smoking, taste, vegetables

Introduction
The ability to taste bitter substances is believed to have evolved in humans to avoid food that contain toxins, since these often have common chemical properties that can be recognized by taste receptors. Taste is very important in food selection and therefore foods that are perceived as bitter are often excluded from the diet despite their nutritional and disease protective qualities.

An important and well-studied human trait is the sensitivity to the bitterness of the thioureas phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), a trait that shows large inter-individual differences [1,2]. The underlying gene that is responsible for PTC/PROP taste sensitivity is hTAS2R38, a bitter taste receptor that binds the thiourea moiety [3–5]. Although the inheritance pattern for the PTC/PROP trait is not fully determined, polymorphisms in the hTAS2R38 gene are believed to account for up to 85% of the phenotypic variance of PTC/PROP sensitivity [3,6–8].

The human TAS2R38 gene has three missense single nucleotide polymorphisms that change the taste receptor's affinity for thioureas. The three corresponding amino acid substitutions are situated at positions 49, 262 and 296, resulting in two major phenotypic forms. The major taster form has proline at position 49, alanine at position 262, and valine at position 296, constituting the PAV haplotype. The major non-taster form contains alanine, valine and...
vegetables were assessed from this questionnaire. The
elsewhere [17,18]. Frequencies of intake of brassica
questionnaire has been validated and the results published
times per day or more. The food-frequency question-
within 12 categories ranging from never to eight
participants completed a detailed food-frequency
diagnosis accepted and were examined. At enrolment
consisting of 2614 persons with ≥23 g/day intake of
of brassicas. Individuals for the high brassica intake
group were randomly selected from the upper quartile
of 2409 persons with ≤7 g/day intake.

Blood samples and PCR
DNA was extracted from frozen blood samples accord-
ing to the procedure described by Miller et al. [19]. A
NanoDrop ND1000 spectrophotometer (Nanodrop
Technologies Inc., Rockland, USA) was used to assess
DNA quantity. DNA samples were diluted to 10 ng
per well and analysed for the hTAS2R38 Ala49Pro
(rs713598), Val262Ala (rs1726866) and Ile296Val
(rs10246939) polymorphisms using the fluorogenic
5-nuclease assay (TaqMan® SNP Genotyping Assay
Made to Order on an ABI 7900HT, Applied Biosys-
tems, Foster City, USA) Genotypes were determined
in a 25 μl reaction mix containing 11.25 μl diluted
DNA sample and 13.75 μl master mix solution
(0.625 μl DNase/RNase free water, 0.625 μl Taq-
Man® SNP Genotyping Assay, and 12.5 μl TaqMan
Universal PCR Master mix (Applied Biosystems)
according to the manufacturer’s instructions). PCR
amplification was performed with an initial step of
95°C for 10 min followed by 45 cycles of 92°C for
15 sec and 60°C for 1 min (Applied Biosystems
7900HT Sequence Detection System). The fluores-
cence profile of each well was measured in an Applied
Biosystems 7900HT Sequence Detection System,
and the results were analysed with Sequence Detec-
tion Software (SDS 2.3, Applied Biosystems). Con-
trols were included on each plate. Reproducibility
was checked by re-genotyping 10% of the cases with
100% agreement. The hTAS2R38 genotyping was
unsuccessful for 14 samples.

Subjects and methods

Study population
The subjects were selected from the prospective Diet,
Cancer and Health (DCH) study, which is an ongo-
ing Danish cohort study. Detailed information about
the DCH study design is available elsewhere [16].
Briefly, from 1993 to 1997 a random population of
160,725 individuals aged 50–64 years, born in
Denmark, living in the greater Copenhagen and Aar-
hus areas was invited to participate in the study. A
total of 57,053 individuals with no previous cancer
diagnosis accepted and were examined. At enrolment
the participants completed a detailed food-frequency
questionnaire containing 192 different food items
within 12 categories ranging from never to eight
times per day or more. The food-frequency question-
naire has been validated and the results published
elsewhere [17,18]. Frequencies of intake of brassica
vegetables were assessed from this questionnaire. The

Glucosinolates, also containing thiourea moieties,
are present in large quantities in brassica vegetables
and are responsible for their bitter taste. In western
countries, the primary dietary sources of brassica
vegetables come from the species Brassica oleracea
including broccoli, Brussels sprouts, cabbage and cau-
liflower. Tasters (PAV haplotype carriers) are believed
to perceive bitterness from brassica vegetables and
avoid intake, while non-tasters (homozygous AVI
haplotype carriers) are believed to consume larger
amounts of these vegetables. An inverse relationship
between high intake of brassica vegetables and sev-
eral types of cancer has been observed in some epi-
demiological studies [9–11]. Experimental studies
on brassicas have shown that their active compounds
block tumorigenesis in animal models and in humans,
even though these studies are small scale [12–15].
The present study was designed to investigate the
association of the hTAS2R38 receptor haplotypes
with food preference and dietary intake of brassica
vegetables by testing the hypothesis that the intake
of brassica vegetables is associated with different
hTAS2R38 haplotypes. We compared two groups of
individuals with high (>23 g/day) and low (<7 g/day)
daily intake of brassica vegetables in relation to their
hTAS2R38 status in a cohort of healthy Danes. In
addition the hypothesis that hTAS2R38 receptor
haplotypes might also be associated with BMI and
body type, smoking status, alcohol habits and choco-
late and coffee consumption was tested.
Ethics

The Diet, Cancer and Health study and this sub-study was approved by the Danish Data Protection Agency, and by the regional Ethics Committees of Human studies in Copenhagen and Aarhus (File: H-KF-01-345/93, notification number: 19739).

Statistical methods

The primary endpoint was haplotype frequency difference between brassica high-intake vs. low-intake. Our study had 250 case patients and 250 control patients. Prior data indicate that the proportion of non-tasters among controls is around 30%. We will be able to detect true odds ratios for low brassica intake of 1.719 in non-tasters relative to tasters with probability (power) 80%. The Type I error probability associated with this test of the null hypothesis that this odds ratio equals 1 is 0.05.

Secondary end-points were alcohol intake, coffee intake, chocolate intake, smoking, BMI and waist-circumference. The Cochrane-Armitage trend test was used to detect possible gene-dose effects. Logistic regression was used for obtaining unadjusted and adjusted estimates of haplotype effects on brassica intake and secondary end-points.

The continuous variables were changed to dichotomous variables by using median as cut-off. Haplotypes and haplotype frequencies were inferred by using Phase v.2.1.1. Software [20,21]. Hardy-Weinberg equilibrium was assessed using Haploview v4.1 (www.broad.mit.edu/mpg/haploview/). All reported \( p \) values are two sided. The analyses were performed using the SAS Statistical Package Version 9.1.3 (SAS Institute Inc, Cary, NC).

Results

Genotype and haplotype distribution

Genotype data and demographic data are summarized in Table I. All genotypes were in Hardy-Weinberg equilibrium. Genotyping was successful in 98% of the samples. Taster haplotypes are defined in the introduction and presented in Table II along with the distribution. Fourteen persons out of the 500 persons had incomplete genotyping data and they were excluded from the analysis. As expected the three polymorphisms were tightly linked with \( r^2 \) values ranging from 0.82–1.00 (Figure 1).

Haplotypes

Associations between haplotypes and intake of brassica vegetables and secondary diet intakes were analysed using the non-taster (AVI/AVI) and taster (PAV/AVI or PAV/PAV) classification. All other haplotypes were excluded from the initial analysis since their taster status has not been conclusively determined [3,4,22,23]. There was no association between taster status and brassica intake (see Table III). The absolute difference in non-taster proportions between the low and high brassica intake groups was \( \left( p_{\text{low}} - p_{\text{high}} \right) \pm 1.96 \times se\left( p_{\text{low}} - p_{\text{high}} \right) = 1.3 \% \pm 9.1 \% \), indicating that the real difference lies between –8 and 10% in non-taster haplotype distribution.

Our population was selected on the basis of brassica intake information, so analyses were only carried out on those secondary intake or demographic variables that showed the same degree of associations in the two intake groups separately or to rephrase where brassica intake was not an effect modifier of the association of taster status with secondary endpoints. All of the secondary endpoints fulfilled this assumption and could be analysed. Logistic regression analysis showed similar results both unadjusted and adjusted for sex and age (Table III).

These analyses were repeated using 3-group classification into taster (PAV/PAV), intermediate taster (PAV/AVI or PAV/AVI) and non-taster (AVI/AVI) classification. All other haplotypes were excluded from the analysis since their taster status has not been conclusively determined [3,4,22,23]. There was no association between taster status and brassica intake (see Table III).

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Table I. Demographic data and hTAS2R38 genotype distribution.

<table>
<thead>
<tr>
<th>Brassica vegetables intake</th>
<th>Low: ( \leq 7 ) g/day</th>
<th>High: ( \geq 23 ) g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>56 (50–65)</td>
<td>57 (50–65)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>128 (51%)</td>
<td>122 (49%)</td>
</tr>
<tr>
<td>Female</td>
<td>122 (49%)</td>
<td>128 (51%)</td>
</tr>
<tr>
<td>Ala49Pro (rs713598)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>97 (39%)</td>
<td>104 (42%)</td>
</tr>
<tr>
<td>Pro/Ala</td>
<td>110 (44%)</td>
<td>115 (46%)</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>36 (14%)</td>
<td>28 (11%)</td>
</tr>
<tr>
<td>Missing</td>
<td>7 (3%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Val262Ala (rs1726866)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>87 (35%)</td>
<td>93 (37%)</td>
</tr>
<tr>
<td>Ala/Val</td>
<td>114 (46%)</td>
<td>116 (46%)</td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>43 (17%)</td>
<td>38 (15%)</td>
</tr>
<tr>
<td>Missing</td>
<td>6 (2%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>lle296Val (rs10246939)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lle/lle</td>
<td>87 (35%)</td>
<td>92 (37%)</td>
</tr>
<tr>
<td>Val/lle</td>
<td>113 (45%)</td>
<td>115 (46%)</td>
</tr>
<tr>
<td>Val/Val</td>
<td>43 (17%)</td>
<td>38 (15%)</td>
</tr>
<tr>
<td>Missing</td>
<td>7 (3%)</td>
<td>5 (2%)</td>
</tr>
</tbody>
</table>

Table II. hTAS2R38 haplotype distribution in groups with high and low brassica vegetables intake.

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Classification [3]</th>
<th>Brassica vegetables intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low: ( \leq 7 ) g/day</td>
</tr>
<tr>
<td>AVI/AVI</td>
<td>Non-taster</td>
<td>86 (34%)</td>
</tr>
<tr>
<td>PAV/AVI</td>
<td>Taster/Intermediate</td>
<td>100 (40%)</td>
</tr>
<tr>
<td>PAV/PAV</td>
<td>Taster</td>
<td>33 (13%)</td>
</tr>
<tr>
<td>PAV/AVI</td>
<td>Unknown</td>
<td>8 (3%)</td>
</tr>
<tr>
<td>PAV/PVI</td>
<td>Unknown</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>PVI/AVI</td>
<td>Unknown</td>
<td>1</td>
</tr>
<tr>
<td>AAV/AVV</td>
<td>Unknown</td>
<td>0</td>
</tr>
<tr>
<td>AAV/AVV</td>
<td>Unknown</td>
<td>11 (4%)</td>
</tr>
<tr>
<td>AAV/AVV</td>
<td>Unknown</td>
<td>11 (4%)</td>
</tr>
</tbody>
</table>
other variable tested in the two brassica vegetables intake groups. Our study is a cross-sectional study based on a healthy prospective cohort where the present study population was chosen based on brassica intake balanced with regards to age and sex. Out of the total 500 individuals in our study group 14 were excluded due to incomplete genotyping. The association between intake of different brassica vegetables and the phenotypic PTC/PROP taster status has been studied earlier with inconclusive results [24–26]. Taster status, tested by genetic variation in PROP/PTC or hTAS2R38, has been associated with higher bitterness and lower acceptance scores for brassica vegetables when compared to non-tasters, although these studies were also inconclusive [5,27–29]. However, only two studies in the past few years have examined the brassica intake in relation to the hTAS2R38 haplotypes. One study of 3383 British women found no association with the hTAS2R38 receptor when testing the cumulative green vegetable intake [30]. They did not examine the brassica vegetables independently, making their results incomparable with ours. Another study was performed on the Italian population from the European Prospective Investigation into Cancer and Nutrition (EPIC) including 634 subjects. They found a significant association between intake of brassicas and the hTAS2R38 haplotypes [22]. Our study differs from this study in that we carried out a full genotyping whereas only Ala49Pro (rs713598) and Val262Ala (rs1726866) were genotyped in the Italian study. The intake amounts seem to be different as well so the studies are not completely comparable, opening the possibility that the genetic effects may only be relevant depending on average population intake amount. Our results do not support these findings that the hTAS2R38 haplotypes are associated with brassica vegetable intake. The present lack of association between taster status and brassica vegetable intake indicates that bitterness perception results found in the laboratory setting do not modify intake in population studies.

Discussion

The results of our study clearly show that the hTAS2R38 haplotypes were not associated with brassica intake in the Danish diet in a design where subjects from a high intake and a low intake group were compared. Our results indicate that the difference in non-taster-haplotypes is around 1% (–10 to 10%) between high and low brassica intake groups making them unlikely determinants of brassica vegetable intake. Furthermore no association was found between hTAS2R38 receptor haplotypes and any

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Unadjusted OR (95% CI, p-value)</th>
<th>Adjusted OR (95% CI, p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brassica intake</td>
<td>0.95 (0.65 – 1.38, 0.77)</td>
<td>0.92 (0.62 – 1.34, 0.65)</td>
</tr>
<tr>
<td>Beer</td>
<td>0.92 (0.63 – 1.34, 0.66)</td>
<td>0.79 (0.52 – 1.22, 0.29)</td>
</tr>
<tr>
<td>Spirits</td>
<td>1.21 (0.83 – 1.78, 0.32)</td>
<td>1.17 (0.79 – 1.73, 0.44)</td>
</tr>
<tr>
<td>Wine</td>
<td>1.26 (0.86 – 1.85, 0.24)</td>
<td>1.23 (0.83 – 1.80, 0.30)</td>
</tr>
<tr>
<td>Total alcohol</td>
<td>0.89 (0.61 – 1.38, 0.55)</td>
<td>0.80 (0.54 – 1.20, 0.29)</td>
</tr>
<tr>
<td>Coffee</td>
<td>1.09 (0.72 – 1.63, 0.70)</td>
<td>1.06 (0.70 – 1.60, 0.80)</td>
</tr>
<tr>
<td>Smoking*</td>
<td>1.06 (0.75 – 1.51, 0.74)</td>
<td>1.05 (0.74 – 1.50, 0.79)</td>
</tr>
<tr>
<td>Chocolate</td>
<td>0.94 (0.63 – 1.40, 0.75)</td>
<td>0.92 (0.61 – 1.38, 0.68)</td>
</tr>
<tr>
<td>BMI</td>
<td>1.02 (0.70 – 1.50, 0.92)</td>
<td>0.98 (0.66 – 1.44, 0.90)</td>
</tr>
<tr>
<td>Waist-circumference</td>
<td>1.14 (0.78 – 1.67, 0.50)</td>
<td>1.05 (0.67 – 1.66, 0.83)</td>
</tr>
</tbody>
</table>

*Results from ordinal logistic regression.

*Sex and age.
The reasons for this might seem evident when considering the complexity of determinants for food preferences. In the everyday situation other factors like economy, sex, age, cultural heritage, deviating taste preferences, prior experience, sociocultural variables and health-awareness influence human food selection [31,32]. Furthermore, laboratory testing is done using pure bitter compounds while in real life the mixing of foods (and beverages) may mask bitter compounds and produce additional taste nuances that are not present in the laboratory setting.

An association between bitter taste, defined by taste thresholds for PROP/PTC), and alcohol consumption, dependence and family history has been investigated in several studies. The results from these studies are contradictory showing general associations [33–35], associations for subgroups only [36,37] or no associations at all [30,38,39] with alcoholism or alcohol intake. Studies specifically studying the association of hTAS2R38 with alcohol intake have also been contradictory, but in the largest study including more than 3000 women no association was found. We found that the PTC taster status, determined by the hTAS2R38 haplotypes, was not associated to total alcohol intake.

We also investigated the possible relation between PTC perception and BMI in our sample. Positive results have been shown in phenotype studies with mainly small population samples [40,41]. It has been suggested that the underlying reason for differences in BMI measures for the taster and non-taster individuals is the different perception of fat in the diet and thus their intake of fat-containing foods, but this theory is questionable [42,43]. More studies point in the opposite direction, reporting no influence of PTC taste ability on BMI-measure, waist-hip ratio and waist-circumference when testing for an association with either PTC/PROP sensitivity or the hTAS2R38 receptor haplotypes [7,8,43,44]. Our results are in accordance with these negative studies rejecting the theory that the hTAS2R38 receptor should have any influence on BMI or waist circumference.

Because PTC perception has been linked to several bitter compounds, a relation between the bitterness of chocolate and taster status has been suggested. Ly and Drenowski examined the sensory response to three kinds of chocolate (white/milk/dark) and their intake, but found no significant difference between the phenotypic taster and non-taster groups [45]. We examined chocolate consumption in our sample with the hypothesis that non-tasters would consume more chocolate because they would not be able to detect its bitterness but did not find any correlation between the hTAS2R38 haplotypes and chocolate intake.

Conclusions

Our results do not support previous findings of an association between brassica intake and hTAS2R38 haplotypes, indicating that non-genetic factors may be of greater importance than genetics in determining food selection. In conclusion, our study indicates that the determinants of bitter food intake, specifically brassica vegetables, may be principally independent of genetic profiles of the hTAS2R38 bitter taste receptor. Additionally hTAS2R38 haplotypes were not associated with intake of alcohol, chocolate or body type measured by BMI and waist circumference.

Acknowledgments

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


[40] Tepper BJ, Ullrich NV. Infl uence of genetic taste sensitivity to 6-n-propylthiouracil (PROP), dietary restraint and disinhibition on body mass index in middle-aged women. Physiol Behav 2002;75:305–12.


