Bitter Taste Study in a Sardinian Genetic Isolate Supports the Association of Phenylthiocarbamide Sensitivity to the TAS2R38 Bitter Receptor Gene

D.A. Prodi¹, D. Drayna², P. Forabosco¹,³, M.A. Palmas¹, G.B. Maestrale¹,³, D. Piras³, M. Pirastu¹,³ and A. Angius¹,³

¹Shardna Life Sciences, Cagliari, Italy, ²National Institute of Deafness and Other Communication Disorders, Rockville, MD, USA and ³Institute of Population Genetics, CNR, Alghero, Italy

Abstract

Recently, a major locus on chromosome 7q was found in association with the taste sensitivity to phenylthiocarbamide (PTC) in humans. This region contains the TAS2R38 gene that encodes a member of the TAS2R bitter taste receptor family. Three SNPs within this gene demonstrated a strong association with taster status in Utah families and in an additional sample of 85 unrelated individuals. We studied a small isolated village in eastern Sardinia and carried out a genome-wide scan to map the genetic basis of PTC perception in this population. We performed both qualitative and quantitative PTC-taste linkage analysis. Qualitative analysis was carried out by defining a cut-off from the bimodal distribution of the trait and classifying subjects as tasters and non-tasters (75 and 25%, respectively). Linkage analysis on 131 subjects belonging to a unique large multi-generation pedigree comprising 239 subjects confirmed significant evidence for linkage at 7q35 also in our population. Haplotype analyses of the three SNPs inside the PTC gene allowed us to identify only two haplotypes that were associated with the non-taster phenotype (80% AVI homozygous) and to taster phenotype (40% PAV homozygous and 56% PAV/AVI heterozygous). Sex, age and haplotype effect explained 77.2 % of the total variance in PTC sensitivity.

Key words: genetic isolates, genomewide search, haplotypes, 7q35

Introduction

Bitter is a well-characterized taste modality in humans and variation in this ability may influence food selection and nutritional status (Drewnowski and Rock, 1995; Tepper, 1998; Keller et al., 2002). The perception of bitter taste is mediated by G-protein coupled receptors, located in taste cells within taste bud of the tongue (Wong et al., 1996; Adler et al., 2000; Chandra shekar et al., 2000), that interact with tastants and initiate signaling cascades that culminate in neurotransmitter release.

Among the best-studied bitter substances are phenylthiocarbamide (PTC) and related compounds containing the C–N=S moiety, because of the remarkable occurrence of a differential ability to taste these substances in human populations worldwide. The inability to taste PTC and related compounds has been known for >70 years (Fox, 1931; Blakeslee and Salmon, 1935). Several studies on this trait showed an autosomal recessive transmission, but other genetic mechanisms have also been suggested (Olson et al., 1989; Bartoshuk et al., 1994; Reed et al., 1995). Genetic linkage and gene mining studies in mice have shown the presence of three gene clusters (Capeless et al., 1992; Lush et al., 1995; Blizard et al., 1999; Adler et al., 2000; Chandrashekar et al., 2000; Matsunami et al., 2000) on murine chromosomes 15 and 6, associated with the sensitivity to many bitter substances such as cyclohexamide, sucrose octaacetate, raffinose undecacetate and quinine. In humans, Reed et al. (1999) have identified the presence of a major locus for 6-n-propyl-2-thiouracil (PROP) sensitivity on chromosome 5p15. Subsequently, homology studies identified the TAS2R1 gene on 5p15.2 and 2 clusters of additional genes on human chromosomes 7 and 12 respectively, homologous to the gene cluster present on murine chromosome 6. These genes are grouped in a family, called TAS2R, that contains ~24 members in humans. This is consistent with the great variety of bitter compounds and the high discriminatory capability of human bitter receptors.

The perception of PTC is most precisely measured by administering a series of solutions of different concentrations to determine the minimal PTC concentration detected by an individual. The cut-off value used to separate tasters...
from non-tasters has differed from study to study and a high
diversity in the frequency of these two classes has also been
observed (Jones and McLachlan, 1991; Guo et al., 1998).
Recently, a small region on chromosome 7q (2.6 Mb)
(Drayna et al., 2003) was found in association with PTC
taste ability. This region was narrowed to a 150 kb interval
using the Utah CEPH families (Dausset et al., 1990) and, in
this interval, Kim et al. (2003) identified the gene responsible
for PTC taste ability, which is the TAS2R38 bitter receptor.
This gene encodes a 7-transmembrane domain, guanine
nucleotide-binding protein (G-protein)-coupled receptor
that shows 30% amino acid identity with human TAS2R7,
the most closely related member of this family. This gene
contains a single coding exon 1002 base pairs in length.
Three common SNPs within this gene, all of which result in
amino acid changes in the protein (A49P, V262A and
I296V), demonstrated a strong association with taster status
in their Caucasian Utah sample and in a replication sample
from a multi-racial population enrolled at the National
Institutes of Health (NIH).

We have studied a small isolated village (Talana, 1200
inhabitants) in eastern Sardinia. We reconstructed the gene-
alogy of each inhabitant using archival data and, identifying
maternal and paternal lineages, we showed that 80% of the
present-day population descended from <20 founder
couples (Angius et al., 2001). In this genetically and cultur-
ally homogeneous population, a large proportion of individ-
uals presenting a given trait are likely to share the same trait-
predisposing gene inherited from a common ancestor.
Furthermore, inbreeding, typical of small communities such
as Talana, reduces genetic heterogeneity and increases
homozygosity, providing greater power for detection of
recessive susceptibility genes. On the other hand, increased
homozygosity expected in Talana compared to outbred
populations is likely to affect only slightly marker informa-
tiveness, as highly polymorphic microsatellite markers are
used in the linkage analysis. Previous studies in the
Sardinian population have shown variation in PTC taste
sensitivity (Maxia et al., 1975), which suggested this popula-
tion may be useful for refining our understanding of the
contribution of the TAS2R38 gene to PTC taste ability.

Materials and methods
To define the sensitivity to PTC in the village of Talana, we
initially tested 228 random individuals using a filter paper
impregnated with 1 µg of dried PTC (Lab-aids Inc.).
Subjects were asked to place the paper in their mouths and
to rate the bitterness of taste. Subsequently, these individ-
uals and their relatives were submitted to refined testing
based on an abbreviated version of the classic Harris–
Kalmus method (Harris and Kalmus, 1958). This test
employed seven (rather than the original 14) scalar PTC
solutions, starting from the most dilute (1.04 × 10^{-6} M) and
rising four-fold in concentration at each step to a maximum
concentration of 4.27 mM. When a subject perceived the
bitter taste, he or she was submitted to a blind sorting test
that required distinguishing PTC solutions at the perceived
concentration versus natural water in order to confirm the
tasted score. All together, we tested 280 persons in Talana
and calculated age- and sex-adjusted PTC scores using the
corrections of Harris and Kalmus (1958), whose distribution
showed a typical bimodal curve (Figure 1). From the
minimum node in frequency between tasters and non-tasters
in the distribution of scores, we fixed a cut-off value of 4.5
( representing a PTC concentration of 795 µM) and classified
70 individuals (25%) as non-taster (NT) and 210 (75%) as
taster (T). Among the phenotyped subjects, we identified
131 individuals clustering in a unique large multi-generation
pedigree comprising a total of 239 individuals. These indi-
viduals were used in the linkage analysis.

All individuals participating in the study signed informed
consent forms and all samples were taken in accordance with
the Declaration of Helsinki. Genomic DNA was extracted
from 7 ml of EDTA-treated blood, as described by Ciulla
et al. (1988). Genotyping was done by the Mammalian Geno-
typing Service of Marshfield Laboratory, directed by Dr
James Weber. A genome-wide scan (GWS) for linkage was
performed using a set of 376 short tandem repeat polymor-
phism (STRP) markers with average spacing of 9.5 cM.
Mean marker heterozygosity in Talana was high (0.70),
although slightly lower than in the CEPH families (0.75).
We selected additional markers from the genome databases
to better investigate the regions that produced the highest
scores for linkage in the initial GWS. STRP genotyping
products were analyzed using an ABI PRISM 3100 DNA
Analyzer (Applied Biosystems, Foster City, CA). Single
nucleotide polymorphism (SNP) genotyping was done by
direct DNA sequencing using the Big Dye Terminator Cycle
Sequencing method (Applied Biosystems). Primer sequence
were selected according to Kim et al. (2003). Marker allele
frequencies were estimated from the entire Talana popu-
lation and the Marshfield genetic maps were used in pairwise
and multipoint linkage analysis.

In order to confirm the locus on chromosome 7 and/or to
identify additional regions associated to PTC perception, we
performed qualitative and quantitative linkage analysis
across the whole genome. Qualitative analysis was carried
out under a recessive genetic model with incomplete pene-
trance (90%) and a gene frequency of 0.5 estimated from
prevalence of the non-taster phenotype in the Talana popu-
lation. Two-point analysis was performed using Fastlink
4.1P (Cottingham et al., 1993) splitting the whole pedigree
into eight more tractable sub-families. Multipoint analysis
was performed with Genehunter 2.1 (Kruglyak et al., 1996)
on 10 smaller families due to computational constraints and
with Simwalk2 (Sobel and Lange, 1996), which allowed us to
analyze the extended eight families, in specific suggestive
regions. The main advantage of multipoint linkage analysis
is that it allows retrieval of phase information from neigh-
bouring markers at each location of the genome, thus
increasing the probability that at least one of these is heterozygous.

**Results**

In the qualitative analysis, the strongest evidence for linkage (Table 1) was obtained on chromosome 7 with a peak two-point lod score of 3.27 and a multipoint lod of 3.10 at GATA104 (155.1 cM). Adding supplementary markers in the region yielded an increased two-point lod score of 3.33 at D7S661 and a multipoint peak of 3.50 between markers D7S2513 and D7S661 (151.25–155.1 cM), a location <1 cM from the highest lod score peak previously reported (D7S498-AFM183ya3; Drayna et al., 2003). We did not observe significant genetic heterogeneity in the sample of families we analyzed, indicating that the locus on chromosome 7q accounted for all of the linkage signal observed in this very large extended family.

On chromosome 6 we identified an interval of 26.7 cM flanked by markers D6S942 and D6S1006 (0–26.7 cM) with multipoint lod scores >2 and a peak two-point lod score of 2.46 at marker SE30 (9.2 cM). On chromosome 17 a two-point lod score of 3.09 was obtained at marker D17S974 (22.2 cM). Additional markers typed in these regions allowed us to exclude the involvement of these loci; on chromosome 6 multipoint lod scores were <2 over the whole region, while on chromosome 17 we observed a two-point lod score of −2.58 at marker D17S1852 located at 0 cM from D17S974. Notably, no significant evidence for linkage was obtained for the loci previously identified on chromosome 5 and on chromosome 12.

In order to capture all variation of PTC taste ability, we also performed quantitative linkage analysis using a variance components approach with SOLAR (Almasy and Blangero, 1998) on the large multigeneration pedigree. The maximum quantitative genome-wide lod score confirmed qualitative analysis showing a peak multipoint lod of 4.73 between markers D7S661 and D7S3070 (155.1–163.0 cM).

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**Figure 1** Distribution of PTC sensitivity in Talana. The x-axis indicates the PTC scores, adjusted for age and sex using the following formula: \( n + \frac{(a - a_M)}{20} - 0.73 \) female, where \( n \) is the PTC tasted solution, \( a \) is the age of the individual and \( a_M \) is the average age of participants. The value of 0.73 was deducted in females since women are 0.73 dilution steps more sensitive than men at all ages. Distribution of corrected PTC scores showed significant bimodality with a cut-off value of 4.5 that separates the group of tasters from the group of non-tasters, with estimated means of 2.19 (SD = 1.12) and 7.99 (SD = 1.44), respectively.

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**Table 1** Results of linkage analysis for the chromosome 7q35 region; underlined markers have been subsequently added

<table>
<thead>
<tr>
<th>Marker</th>
<th>cM</th>
<th>Two-point lod scores</th>
<th>Multipoint lod scores*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S486</td>
<td>0.0</td>
<td>0.45</td>
<td>0.88</td>
</tr>
<tr>
<td>D7S3061</td>
<td>4.3</td>
<td>0.84</td>
<td>1.01</td>
</tr>
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<td>D7S530</td>
<td>10.5</td>
<td>1.00</td>
<td>1.77</td>
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<td>D7S1804</td>
<td>12.9</td>
<td>1.54</td>
<td>1.25</td>
</tr>
<tr>
<td>D7S640</td>
<td>13.8</td>
<td>2.16</td>
<td>1.21</td>
</tr>
<tr>
<td>D7S2560</td>
<td>21.3</td>
<td>0.57</td>
<td>0.93</td>
</tr>
<tr>
<td>D7S684</td>
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<td>0.84</td>
<td>0.87</td>
</tr>
<tr>
<td>D7S1824</td>
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<td>2.10</td>
<td>2.73</td>
</tr>
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</tr>
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<td>3.33</td>
<td>3.17</td>
</tr>
<tr>
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<td>31.0</td>
<td>3.27</td>
<td>3.17</td>
</tr>
<tr>
<td>D7S3070</td>
<td>39.0</td>
<td>0.51</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*Multipoint lod scores obtained at the location of the marker.
No other lod scores were significant (all lod < 2.0) in the rest of the genome. We next investigated two locus inheritance running a second GWS conditional on the QTL on 7q. No other regions showed significant linkage and the only lod score that increased in this scan did not reach significance (two-locus lod = 1.60 at 54.4 cM on 18q12.1). This locus has not been associated with any taste perception function. These linkage results confirm in Sardinia the previously reported locus on chromosome 7q containing the TAS2R bitter receptor gene responsible for the PTC taste ability.

We analyzed the entire sequence of the TAS2R gene, the three common SNPs (A49P, V262A and I296V) inside the gene and 6 additional SNPs (rs758955, rs765007, rs1285895, rs745162, rs1285939, rs1358304) to evaluate the extension of linkage disequilibrium in our population. P-values of allelic association were calculated using Markov-chain method (100 000 tables evaluated) by means of the ARLEQUIN 2.0 program (Schneider et al., 2000). Linkage disequilibrium test results indicated a point of high recombination between rs1285939 and A49P located at the 5′ of the gene. Beyond this, significant linkage disequilibrium (P-value < 0.05) was observed between A49P and rs765007 spanning a region of 260 561 basepairs. Haplotype analysis allowed the identification of two major extended haplotypes using the following markers A49P, V262A, I296V, rs745162, rs1285939 and rs765007. Haplotypes AVIACT and PAVGTC accounting for ~21 and 36%, respectively, of the total sample analyzed. The homozygotes AVIACT were present in 52.5% of non-tasters whereas the PAVGTC homozygotes account for the 59.7% of taster subjects (Figure 2).

Within the coding sequence, haplotype analyses of the three polymorphisms that previously demonstrated a strong association with taster status allowed us to identify only two haplotypes. Named in the order of the 3 SNPs (A49P, V262A and I296V), the AVI haplotype was associated with the non-taster phenotype (80% homozygous) and the PAV haplotype associated with the taster phenotype (40% homozygous and 56% heterozygous; Table 2). In Talana we found only three genotypes: AVI/AVI, AVI/PAV and PAV/PAV. No other haplotypes were found in our sample. Our results confirmed a model of a major recessive trait locus probably modified by other genetic factors that interact with

![Physical map of the SNPs analyzed and haplotype association with taste phenotypes.](https://academic.oup.com/chemse/article-abstract/29/8/697/326106/figure2)
PTC taste sensitivity. The high frequencies of AVI and PAV haplotypes in our population agreed with the frequencies found previously in other populations. The complete absence of the less frequent haplotypes (<3%) observed by Kim et al. (2003) was consistent with the low haplotype diversity and genetic drift typical of a founder population. Correlation of haplotypes with PTC scores showed the presence of many AVI/AVI homozygotes associated with low values of PTC scores, while there was a larger number of heterozygous individuals AVI/PAV and homozygotes, PAV/PAV, among the subjects whose scores were above the cut-off value of 4.5. Furthermore, we determined the effect of PTC haplotypes on the linkage results by including diplotypes as covariate in the quantitative analysis. Sex, age and haplotype effect explained 77.2% of the total variance in PTC scores. These results help refine estimates of the fraction of variance that this locus contributes to variation in PTC sensitivity. Previous estimates varied widely between populations, from 55% in the Caucasian Utah population to 85% in the multi-racial National Institutes of Health population. The contribution of haplotype alone was 75% in this population outside North America. We have also shown that this gene contributes a large amount of the total variance in this trait. The sequence conservation among the various G-protein-linked receptors in their cytoplasmic loops strengthens the hypothesis that the A49P, V262A and I296V variants may alter the domains that contain critical sites for proper coupling with G proteins on the intracellular side of the plasma membrane (O’Dowd et al., 1988).

We exploited the favorable characteristics of our genetic isolate that allowed us to use a relatively limited number of subjects and a rather coarse map of markers to locate the relevant gene and thus validate the feasibility of this isolated population for the study of a major recessive trait locus probably influenced by other genetic factors.

Discussion

We have confirmed the involvement of the TAS2R38 bitter receptor gene in the PTC sensitivity in the Talana genetic isolate in Sardinia. Our maximal lod scores at this locus range exceed the critical value of three for proof of linkage under analysis as a simple recessive trait. Given previous inconsistencies in linkage results (Guo and Reed, 2001), our results provide the first confirmation this gene linkage in a population outside North America. We have also shown that this gene contributes a large amount of the total variance in this trait. The sequence conservation among the various G-protein-linked receptors in their cytoplasmic loops strengthens the hypothesis that the A49P, V262A and I296V variants may alter the domains that contain critical sites for proper coupling with G proteins on the intracellular side of the plasma membrane (O’Dowd et al., 1988).

Electronice database information

Helsinki declaration: http://www.wma.net/e/policy/b3.html
Genetic maps Marshfield: http://research.marshfieldclinic.org/genetics/
The Genome Database: http://www.gdb.org

References


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