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

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## 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; Chandrashekar *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 octacetate, 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 genealogy 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 culturally homogeneous population, a large proportion of individuals 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 informativeness, 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 population 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 individuals 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 ($1.04 \times 10^{-6}$ 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 individuals 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 Genotyping 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 polymorphism (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 population 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 penetrance (90%) and a gene frequency of 0.5 estimated from prevalence of the non-taster phenotype in the Talana population. 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 neighbouring markers at each location of the genome, thus

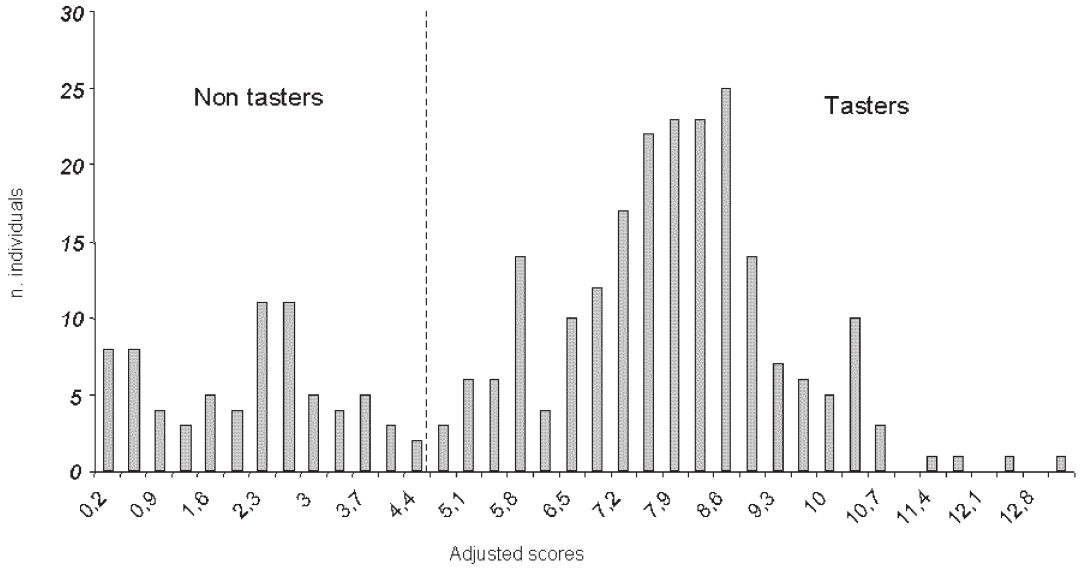


**Figure 1** Distribution of PTC sensitivity in Talana. The *x*-axis indicates the PTC scores, adjusted for age and sex using the following formula: $n + [(a - a_M)/20] - 0.73$female, where $n$ is the PTC tasted solution, $a$ is the age of the individual and $a_M$ is the average age of participants. 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.

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

| Marker | cM | Two-point lod scores | Multipoint lod scores[a] |
|---|---|---|---|
| D7S486 | 0.0 | 0.45 | 0.88 |
| D7S3061 | 4.3 | 0.84 | 1.01 |
| D7S530 | 10.5 | 1.00 | 1.77 |
| D7S1804 | 12.9 | 1.54 | 1.25 |
| D7S640 | 13.8 | 2.16 | 1.21 |
| D7S2560 | 21.3 | 0.57 | 0.93 |
| D7S684 | 23.1 | 0.84 | 0.87 |
| D7S1824 | 25.8 | 2.10 | 2.73 |
| D7S2513 | 27.2 | 2.82 | 3.08 |
| D7S661 | 31.0 | 3.33 | 3.17 |
| GATA104 | 31.0 | 3.27 | 3.17 |
| D7S3070 | 39.0 | 0.51 | 0.29 |

[a]Multipoint lod scores obtained at the location of the marker.

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).

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 (Scheneider *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, rs1285895 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

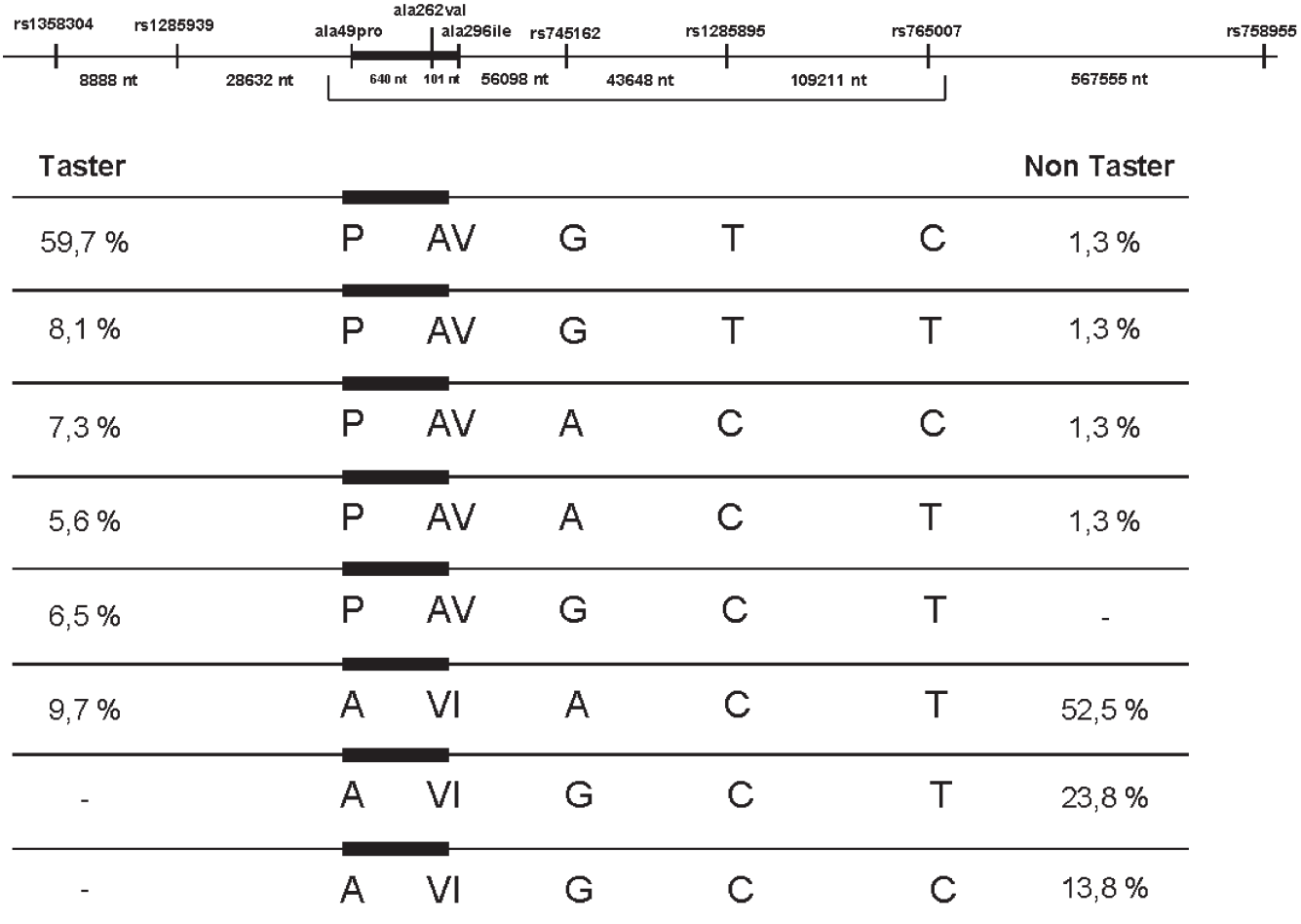


**Figure 2** Physical map of the SNPs analyzed and haplotype association with taste phenotypes. The bold line indicates the region covered by the TAS2R gene.

**Table 2** Haplotype association with taste phenotype; the total number of samples was 207 in Talana, 180 in Utah and 84 in NIH samples (Kim *et al*., 2003)

| Haplotype | No. of subjects | |
|---|---|---|
| | Non-tasters | Tasters |
| Talana | | |
| AVI/AVI | 40 | 6 |
| AVI/PAV | 7 | 89 |
| PAV/PAV | 3 | 62 |
| Utah | | |
| AVI/AVI | 38 | 14 |
| AVI/AAV | 10 | 7 |
| */PAV[a] | 3 | 108 |
| NIH | | |
| AVI/AVI | 21 | 0 |
| AVI/AAV | 1 | 3 |
| */PAV[a] | 1 | 58 |

[a]The asterisk signifies any haplotype found in the sample.

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 culturally and genetically homogenous population, which suggests that the contribution of this gene to the phenotype is between these two values, closer to the upper end of the range.

## 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).

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.

## Acknowledgements

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## Electronic 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

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