

Original Article

Taste Perception of *Antidesma bunius* Fruit and Its Relationships to Bitter Taste Receptor Gene Haplotypes

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Editorial Decision 30 May 2018.

Abstract

It was shown more than 40 years ago that the ability to perceive the bitterness of the fruit of the *Antidesma bunius* tree is inversely correlated with the ability to perceive the well-studied bitter tastant phenylthiocarbamide (PTC). To determine if variants of the *TAS2R38* gene, which encodes the PTC taste receptor, or variants in any of the other *TAS2R* bitter or *TAS1R* sweet receptor genes account for *Antidesma* taste perception, we recruited an independent subject sample and examined associations between these taste receptor gene haplotypes and *Antidesma* perception. Consistent with previous findings, almost none of our subjects who reported *Antidesma* juice as bitter was a PTC “responder” by previous definitions (i.e. a PTC taster). In our study, of the 132 individuals who perceived PTC as bitter, 15 perceived *Antidesma* as bitter, although these 15 subjects had very weak bitterness perception scores. Examination of *TAS2R38* gene haplotypes showed that, of the subjects who perceive *Antidesma* as bitter, all carried at least one copy of the *TAS2R38* AVI (PTC non-taster) haplotype. However, 86 subjects carried at least one AVI haplotype and failed to perceive *Antidesma* as bitter. No other *TAS2R* or *TAS1R* gene variants showed an association with *Antidesma* bitter, sweet, or sour perception. Our results show that *TAS2R38* haplotypes are associated with differential perception of *Antidesma* berry juice bitterness, and that all those who perceive this bitterness carry at least one AVI haplotype. This indicates that the AVI haplotype is necessary for this perception, but that additional variable factors are involved.

Keywords: *Antidesma*, balancing selection, fruit, *TAS2R38*, *TAS2Rs*, *TAS1Rs*

Introduction

The ability to taste phenylthiocarbamide (PTC) and the structurally related compound 6-*e*-propylthiouracil (PROP) is one of the best investigated human phenotypes. These compounds are well-known because of their ability to generate a bitter taste in some individuals

but not in others, dividing individuals into those who cannot perceive any taste at all (“non-tasters”), versus others that perceive a strong bitter taste (“tasters”) (Fox 1932). Since then, many family, twin and population studies have shown that the inability to taste PTC is inherited in a nearly Mendelian recessive manner due to a

locus on chromosome 7 now designated *TAS2R38* (Drayna et al. 2003; Kim et al. 2003). Common variants in this gene explain all of the bimodality in the distribution of PTC taste perception thresholds, and approximately 75% of the total variation in PTC taste sensitivity (Blakeslee 1932; Drayna et al. 2003; Knaapila et al. 2012). *TAS2R38* is a member of the *TAS2R* bitter taste receptor gene family (Adler et al. 2000) which in humans consists of 25 functional genes and 11 pseudogenes, many of which have undergone some form of natural selection (Wooding et al. 2004; Dong et al. 2009; Campbell et al. 2012; Risso et al. 2014; Risso et al. 2017). Like many other genes encoding G protein-coupled receptors, *TAS2R38* has a single coding exon ~1000 bp in length, and within this coding sequence there are 3 single nucleotide polymorphisms (*rs714598*, *rs1726866*, *rs10246939*), at positions encoding amino acids 49, 262 and 296 respectively, which are the most common variant alleles. Two common haplotypes of these polymorphisms encode the “taster” PAV (Proline, Alanine, Valine) and “non-taster” AVI (Alanine, Valine, Isoleucine) forms of the receptor. Two uncommon haplotypes (frequency <5%, AAV and AAI) and 4 rare haplotypes (frequency <1%, PAI, PVI, AVV, and PVV) carrying different combinations of these 3 variant amino acids have also been identified (Wooding et al. 2004; Carrai et al. 2011; Risso et al. 2016).

The fact that approximately 40% of individuals worldwide are AVI-carriers represents a paradox, since it is thought that bitter taste has evolved to prevent ingestion of potentially toxic or harmful compounds mostly found in plants (Wooding et al. 2004; Antinucci and Risso 2017). In addition, it has recently been suggested that an important function of the *TAS2R38* receptor is to recognize and protect individuals from bacterial infections of the respiratory tract. For example, bacterial acyl-homoserine lactones (AHLs), quorum-sensing molecules produced by *Pseudomonas aeruginosa* and other gram negative bacteria, have been demonstrated to be ligands for the PAV form of *T2R38* (Lee et al. 2012). Recently Verbeurgt et al. (2017) demonstrated that *T2R38* is much more broadly tuned for bacterial compounds than previously thought, and that PAV is the functional allele of the receptor. Therefore, it is unclear how a presumably non-functional allele rose to a high frequency in the worldwide population.

Two major hypotheses have been presented to explain this paradox. One explanation is that genetic drift could be responsible for the present day distribution of both *TAS2R38* forms, which reached their present population frequencies because of random fluctuations and therefore could be due to demographic events, rather than selective ones (Wang et al. 2004; Risso et al. 2016). However, most authors have hypothesized that both haplotypes have been maintained by balancing natural selection (Fisher et al. 1939; Wooding et al. 2004; Campbell et al. 2012). Some of these balancing selection hypotheses have also suggested that the AVI non-taster allele could encode a fully functional receptor for another, as yet unidentified bitter substance (Wooding et al. 2004).

A phenomenon that may shed light on this hypothesis occurs in the taste perception of the fruit of *Antidesma bunius*, a fruit tree of the Phyllanthaceae family native to Southeast Asia and northern Australia. The human taste perception of this fruit varies greatly among individuals, and it was reported more than 40 years ago that a perception of bitterness in this fruit is inversely correlated with the ability to taste PTC. In particular, individuals who perceive *Antidesma* fruit as bitter were reported to be uniformly PTC non-tasters and conversely, those who perceived PTC as bitter were uniformly *Antidesma* bitterness non-tasters (Henkin and Gillis 1977). A more recent abstract reported a similar finding in a smaller sample, and suggested that *TAS2R38* gene haplotypes may also be related to *Antidesma* perception (Tharp et al. 2005).

To test this hypothesis and to shed more light on the evolutionary history of *TAS2R38*, we examined the associations between PTC perception, *TAS2R38* haplotypes and *Antidesma* perception in an independent subject sample of 169 individuals. We also performed complete sequencing of the coding region of all the other 24 functional *TAS2R* bitter receptor genes in these individuals, and of the *TAS1R2* and *TAS1R3* sweet receptor genes, and we tested variants in these genes for association between PTC and *Antidesma* perception.

Materials and methods

Participants

Following pre-screening to ensure study eligibility, a total of 169 individuals were enrolled. Subjects were eligible if they were age 18 or older, reported themselves to not be pregnant, did not smoke, and had no medical defects related to their sense of taste. Written informed consent was obtained from all participants. The study was approved by the National Institutes of Health Combined Neurosciences/Blue Panel Institutional Review Board (National Institutes of Health protocol 01-DC-0230), and all procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. Most of the participants ($N = 114$) were Caucasians, and the remaining subjects were Asian Americans ($N = 36$) or African Americans ($N = 19$). 57% of the subjects were females and the average age was 30.02 (± 10.27 SD). Additional details on enrolled subjects are shown in Supplementary Table 1.

DNA collection and sequencing

Saliva samples were collected from all participants using Oragene saliva collection kits (Genotek Inc., Kanata, Ontario, Canada) and genomic DNA was purified following the manufacturer's protocol (<http://www.dnagenotek.com/US/pdf/PD-PR-006.pdf>). A dedicated set of primers was designed to completely sequence the single coding exon of the *TAS2R38* gene, as previously published (Risso et al. 2015). In addition, PCR primers for all the human functional *TAS2R* bitter taste receptor genes and the *TAS1R2* and *TAS1R3* sweet taste receptor genes, were designed to fully sequence the coding regions of these genes (Supplementary Table 2) which were sequenced using dideoxy Sanger sequencing (Sanger et al. 1977). DNA chromatograms were individually analyzed with the Lasergene suite (DNASTAR, Madison, Wisconsin; <http://www.dnastar.com/t-all-products.aspx>), to evaluate the presence of calling errors and document genotypes at variant sites in these genes.

Collection of taste phenotypes

A 15-kg sample of *A. bunius* berries was collected at The Kampong National Tropical Botanical Garden (Miami, FL) and subsequently stored at -20°C before preparing the taste solutions. Berries were pressed, skin and seeds were removed, and an aqueous extract was derived as previously described (Henkin and Gillis 1977). All subjects tasted an aliquot from the same aqueous extract sample. To measure PTC taste sensitivity, a single supra-threshold PTC concentration of 256 $\mu\text{mol/L}$ was used because this concentration has been shown to provide the best discrimination between taster and non-taster status (Kim et al. 2003; Bufo et al. 2005). Volunteers were asked to refrain from eating and drinking for at least 3 h before the beginning of the session and to rinse their mouth with reverse osmosis water prior to the first and between the 2 samples. Subjects were first presented a 2-mL solution of *Antidesma* juice, followed by a 2-mL PTC solution, in 30 mL plastic cups. Subjects were asked to rate their perceptions after swishing them in their mouth for 10 s. The PTC

solution was presented within an interval of 1–5 min. To collect the perceived taste intensities of both solutions, the labeled magnitude scale (LMS), which ranges between 0 (“Barely detectable”) to 100 (“Strongest imaginable”), was used (Green et al. 1993). A training session was performed to explain the main aims of the study and to orient the participants on how to use the LMS scale. Subjects were asked to report the taste modalities elicited by the *Antidesma* juice (including bitter, sweet, and sour), after being informed that they may perceive more than one taste. This was done in accordance with previous studies and because sweet tastants can mask the perception of bitter (Henkin and Gillis 1977; Ley 2008).

Statistical analyses

Statistical analyses were performed using the R statistical analysis software (R Development Core Team 2011). We used PLINK v.1.0.7 to perform an initial quality control of genotypes and excluded variants with a call rate <90% or a deviation from Hardy–Weinberg equilibrium (HWE) ($P < 0.001$) (Purcell et al. 2007). PHASE v.2.1. was used to statistically infer *TAS2R* and *TAS1R* haplotypes, using individuals from the 1000 Genomes Project as a reference (Stephens et al. 2001; Abecasis et al. 2012). Only haplotypes with posterior probability of 90% or above were considered for further analyses. Differences in *TAS2R* and *TAS1R* haplotype distributions between taste phenotypes were examined using logistic regression with adjustments for demographic variables such as age, sex and ethnicity, assuming an additive model for the effect of haplotypes. The significance levels of the association tests were adjusted using the Bonferroni correction (adjusted $P = P$ value \times number of individual tests) and $P < 0.05$ was considered statistically significant.

Results

Distribution of taste phenotypes

As expected, most of the studied subjects ($N = 132$, 78.1%) perceived the PTC solution to be bitter and were therefore classified as “PTC responders”, according to Henkin and Gillis 1977. The remaining individuals ($N = 37$, 21.9%) were unable to perceive any taste when tasting the PTC solution, reporting a score of “0” on the LMS, and were classified as “PTC non-responders”.

Of the 37 PTC non-responders in our sample, 15, (40.5%) perceived *Antidesma* as bitter (i.e. were responders). This was similar to previous results in which 25 of 45 PTC non-responders (45.4%) perceived *Antidesma* as bitter (Henkin and Gillis 1977). Conversely, of the 132 PTC responders in our sample, 15 (11.4%) were *Antidesma* responders ($P < 0.001$), although they reported extremely low LMS scores (ranging from 2 to 5) for the perceived intensity of PTC bitterness. No differences in *Antidesma* LMS bitterness scores were found between PTC responders and non-responders (mean of 26.93 vs. 25.86, $P > 0.10$), and no significant correlation was found in responsiveness to either PTC or *Antidesma* with age, sex or ethnicity (All P 's > 0.10).

Regarding other *Antidesma* taste perception modalities, the majority of subjects ($N = 104$, 61.5%) reported sour to be the main taste they perceived when tasting the juice, while 35 (20.7%) perceived it as “mostly sweet”. In agreement with previous findings (Henkin and Gillis 1977), 30 (17.8%) of the individuals tested perceived the *Antidesma* juice as “mostly bitter”. Of these 30 subjects (“*Antidesma* responders”), 28 (93.3%) of them also reported perception of a sour taste and 20 (66.7%) described it also as sweet. Of the 139 individuals who could not identify a bitter taste, 133 (95.7%) and 99 (71.2%) perceived it as sour or

sweet, respectively. The difference in the distribution of either sour or sweet taste perception of *Antidesma* juice was not significantly different between individuals who perceived *Antidesma* taste as bitter or not, nor different between PTC responders and non-responders ($P > 0.10$).

Associations between *TAS2R*, *TAS1R* haplotypes and taste phenotypes

After phasing the genomic DNA sequence data, we identified 5 *TAS2R38* haplotypes in our subjects. The most common haplotype was PAV (51.5%), followed by AVI (46.1%), AAV (1.5%), AAI (0.60%), and PAI (0.30%). A total of 54 single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) > 0.01 , were identified in the other 24 functional *TAS2R* genes. In addition, 35 SNPs were identified in *TAS1R2* and *TAS1R3* genes.

As expected, PTC perceived bitterness was highly associated with the *TAS2R38* PAV taster haplotype ($P < 10^{-5}$), confirming the validity of our taste phenotypes collection. A detailed distribution and summary of PTC LMS scores, categorized by *TAS2R38* diplotype, are shown in Supplementary Table 3.

All the subjects who perceived *Antidesma* juice as bitter carried at least one AVI haplotype ($N = 30$, Figure 1, Supplementary Table 4). The majority of these *Antidesma* responders ($N = 16$, 53.3%) were AVI homozygotes, with the remaining being PAV/AVI ($N = 12$, 40%), AVI/AAI ($N = 1$, 3.3%), or AVI/AAV ($N = 1$, 3.3%) heterozygotes.

Overall, *TAS2R38* haplotypes showed a strong and significant correlation with the reported LMS scores of *Antidesma* bitterness perception. The non-taster AVI haplotype was directly correlated and the taster PAV inversely correlated with the reported *Antidesma* bitterness LMS scores ($P < 10^{-5}$, Table 1). No significant differences were found in the distribution of *Antidesma* bitterness LMS scores between AVI/AVI homozygotes and AVI/PAV heterozygotes ($P > 0.10$).

With similar testing, no significant correlations were found between any *TAS2R* or *TAS1R* SNPs with either *Antidesma* bitterness, sweetness, or sour perception (All P 's > 0.10). We additionally tested the hypothesis that variants in one of the other *TAS2R* or *TAS1R* genes could be present only in the AVI/AVI responders or AVI/AVI non-responders group. No variants were associated with *Antidesma* bitterness perception in either of these groups.

Discussion

The presence of 2 high-frequency *TAS2R38* haplotypes in the population has presented a longstanding question in chemosensory and evolutionary biology. Since bitter taste perception is thought to protect organisms from ingesting toxic substances, it is not clear how the presumably non-functional AVI haplotype came to such a high frequency worldwide. To address this question, some studies have shown that *TAS2R38* has undergone relaxation of selection in humans when compared with many other mammals, suggesting that the presence of both PAV and AVI haplotypes at high frequencies is due to demographic events, rather than selective ones (Wang et al. 2004; Risso et al. 2016). Other authors have noted the fact that bitter receptors are expressed in the respiratory and enteric systems and have suggested that pathogens may have been the real targets of natural selection. In support of this hypothesis, it has been shown that common polymorphisms in the *TAS2R38* gene are linked to significant differences in the ability of the upper respiratory cells to kill and clear bacteria and regulate innate immune responses (Lee et al. 2012; Gillis et al. 2015).

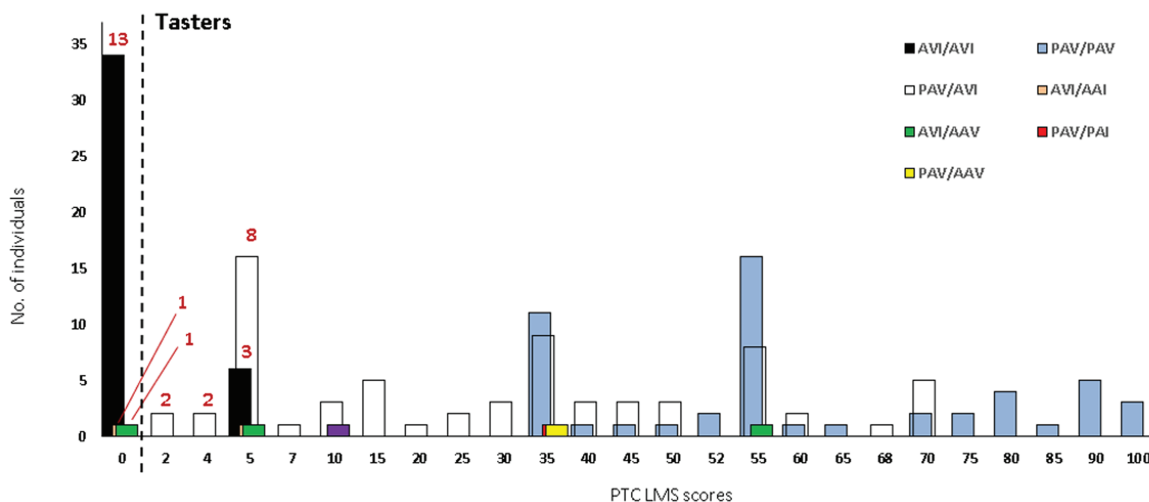


Figure 1. Distribution of PTC LMS scores by *TAS2R38* diplotypes for all 169 subjects tested. Numbers in red quantify the number of subjects, of a specific *TAS2R38* diplotype, who recognized a bitter taste in the *Antidesma* juice. The dotted line sets the threshold between PTC tasters and non-tasters.

Table 1. Distribution of bitterness LMS scores by *TAS2R38* diplotypes in the 30 *Antidesma* responders.

Diplotype	<i>Antidesma</i> bitter (%)	<i>Antidesma</i> bitterness LMS Score (SD)	Average PTC LMS score (SD)
AVI/AVI	16 (53.4)	26.81 (16.76)	0.94 (2.01)
AVI/AAI	1 (3.3)	29 (0)	0 (0)
AVI/AAV	1 (3.3)	20 (0)	0 (0)
PAV/AVI	12 (40)	23.17 (11.67)	4.33 (1.15)
PAV/PAI	/	/	/
PAV/AAV	/	/	/
PAV/PAV	/	/	/

It is notable that there is strong evidence that balancing selection maintained both PTC tasting and non-tasting haplotypes at high frequencies (Wooding et al. 2004; Campbell et al. 2012). Some of these balancing selection hypotheses have raised the possibility that the AVI non-taster allele encodes a fully functional receptor for other hypothetical bitter substances (Wooding et al. 2004), or for some not yet identified bacterial metabolites.

As one possible source of such a bitter substance, sensitivity to bitterness of the fruit of the *A. bunius* tree was previously reported to perfectly and inversely correlate with the ability to taste PTC bitterness: all subjects who perceived *Antidesma* extract as bitter found PTC not bitter, and none of the subjects who perceived PTC as bitter found *Antidesma* to be bitter. In that study, less than half of the PTC non-responders were able to detect *Antidesma* as bitter (45%) (Henkin and Gillis 1977). A more recent abstract reported a smaller study with a similar finding, again suggesting that *TAS2R38* gene haplotypes may be related to *Antidesma* perception even though the active substance responsible for the bitter taste responsiveness of *Antidesma* has not been identified, although it is water soluble and heat stable (Henkin and Gillis 1977; Tharp et al. 2005).

A number of structural modeling and functional studies of the *TAS2R38* receptor forms have been published that have shed light on possible receptor–ligand interactions in this system (Floriano et al. 2006; Miguet et al. 2006; Tan et al. 2011, 2012; Marchiori et al. 2013). Some of these studies have suggested that the AVI form presents steric hindrance or a lack of hydrogen bonding that would prevent ligand binding at the site used by PTC/PROP, while others

have suggested that the variant amino acids in the AVI form disrupt signal transduction rather than ligand binding. Identification of potential ligands for the *TAS2R38* AVI form in *A. bunius* followed by structural modeling studies could help clarify this issue.

We sought to expand on previous studies by sequencing the entire coding sequence and determining haplotypes of the *TAS2R38* gene, as well as sequencing the entire coding sequence of all the other human bitter and sweet taste receptor genes, and by testing the association of variants in these genes with bitter, sweet and sour perception of *Antidesma* berry juice. Our results confirm that perception of the bitterness of *Antidesma* juice is highly correlated with the inability to perceive PTC as bitter. Similar to the previous results of Henkin and Gillis, who showed that approximately 15% of individuals are *Antidesma* responders, we found that approximately 18% of the participants of our study perceived *Antidesma* to be strongly bitter. Similarly, Henkin and Gillis reported that 45.4% of PTC non-tasters perceived *Antidesma* as bitter, which is consistent with our finding that 40.5% of PTC non-tasters perceived *Antidesma* as bitter.

However, our results differ from those of Henkin and Gillis in one aspect: while they found no individuals who were both PTC responders and *Antidesma* responders, we identified 15 PTC-responders who also perceived *Antidesma* to be bitter. However, it is important to note that these 15 subjects in our sample reported very low PTC bitterness LMS scores (ranging from 2 to 5). Thus, although not identical, our results are quite similar to those of Henkin and Gillis, and may be due to small differences in testing methodology or definition of cut-off values that define responders and non-responders.

We have also confirmed that *TAS2R38* haplotypes strongly contribute to the bitterness perception of *Antidesma*, as suggested by previous authors (Tharp et al. 2005). In our sample, all subjects who perceived *Antidesma* bitterness carried at least one AVI haplotype and no subjects without this haplotype perceived this bitterness, indicating this haplotype is necessary for this perception. However, because a large fraction of AVI carriers fail to perceive this bitterness, another factor or factors must be involved. Such a factor could be represented by differences in saliva composition, and for example, variable components of saliva could affect the binding of the bitter compounds present in *Antidesma* juice. Of the many possible candidates, our results show that differences in other bitter taste receptor

genes, or differences in sweet taste receptor genes, are unlikely to represent this additional contributor/s. Moreover, we showed that no coding sequence variants in any other bitter or sweet taste receptor gene contributed to differences in either *Antidesma* bitter, sweet or sour perceptions in our subject group. Our failure to identify any variants in any of the other *TAS2R* or *TAS1R* genes that could account for a lack of *Antidesma* bitterness perception in some carriers of an AVI haplotype could be due to other genetic differences in these individuals, or to the presence of components in saliva that vary between individuals that sequester the bitter component of *Antidesma*.

While our results strongly support previous hypotheses suggesting that *TAS2R38* variants contribute to variation in *Antidesma* bitter perception, they argue against the simple hypothesis that the AVI haplotype of this gene encodes a receptor that acts by itself to specify this perception of bitterness. Our data also indicate that another factor or factors, which may or may not be heritable, is clearly involved in *Antidesma* bitterness perception. Of the many possible candidates, our results show that differences in other bitter taste receptor genes, or differences in sweet taste receptor genes, are unlikely to represent this additional contributor/s.

Supplementary material

Supplementary material can be found at *Chemical Senses* online.

Funding

This work was supported by the National Institute on Deafness and Other Communication Disorders Intramural grant Z1A-000046-16, by the University of Gastronomic Sciences, and by the U.S. Food and Drug Administration through funds obtained under the Family Smoking Prevention and Tobacco Control Act (<https://www.fda.gov/TobaccoProducts/>) (DD, SW, DR). The content was not reviewed by the Food and Drug Administration, but underwent the standard manuscript clearance process for scientific papers published from the NIH intramural research program. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The authors would like to thank The Kampong National Tropical Botanical Garden (Miami, FL) for providing the *Antidesma* berries, Raffaello Verardi for help with berries lyophilization, Joanne Gutierrez for technical assistance, Thomas Friedman and Robert Morell for helpful comments on the manuscript, and Prof. Roberto Barale for helpful discussions.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

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