

SHORT REPORT

Open Access



Rare haplotypes of the gene *TAS2R38* confer bitter taste sensitivity in humans

Emma E. Boxer^{1,2} and Nicole L. Garneau^{1*}

Abstract

Background: The *TAS2R38* gene is widely responsible for the well-known bimodal response to a family of bitter chemicals which includes 6-*n*-propylthiouracil (PROP). There are two common haplotypes of this gene, the recessive AVI and the dominant PAV, both of which are well studied. Conversely, the role of rare *TAS2R38* haplotypes on bitter taste sensitivity has been notoriously difficult to study due to small sample sizes. Here we present PROP sensitivity data of 97 individuals that have been observed to carry rare haplotypes (AAV, AAI, PAI, PVI) of the *TAS2R38* gene.

Results: Participants rated their bitter taste perception to a PROP filter disc then provided a buccal DNA sample from which the *TAS2R38* gene was sequenced and analyzed. We found the prevalence of the PAV haplotype to be 42.3 %, AVI 53.1 %, AAV 2.5 %, AAI 1.2 %, PAI 0.8 % and PVI 0.1 %. We found that the AAI, AAV, and PAI haplotypes present intermediate taste sensitivity.

Conclusions: These data are further evidence that bitter taste sensitivity to PROP exists as a broad range, and not exclusively as nontasters, medium tasters, and supertasters.

Keywords: *TAS2R38*, Rare haplotype, PROP, Bitter, Taste, SNP

Background

The ability of humans to detect bitter compounds begins with the 25 known genes that code for bitter receptors expressed by taste cells. These ligand-specific receptors constitute a family of proteins called T2R (Chandrashekar et al. 2000). Two ligands, PROP and phenylthiocarbamide (PTC), cause a well-studied bimodal response of a bitter sensation in some and “taste blindness” in others (e.g., Fox 1932).

This taste phenomenon was found to be primarily due to the *TAS2R38* gene. A 2003 report by Kim et al. shows that the two common haplotypes of the gene account for about 85 % of the bimodal taste response observed. Composed of 1002 nucleotides, *TAS2R38* contains three missense-coding single nucleotide polymorphisms (SNPs) at positions 145 (proline or alanine); 785 (alanine or valine), and 886 (valine or isoleucine) (Kim et al. 2003). Despite the eight possible amino acid combinations, only six haplotypes have been observed in the context of taste sensitivity; PAV and AVI are the common haplotypes, AAI and AAV

are rare, PAI and PVI are extremely rare, and AVV and PVV have only been observed in disease-based population studies with no associated taste sensitivity data (Kim et al. 2003; Bufe et al. 2005; Mennella et al. 2011; Pemberton et al. 2009; Carrai et al. 2011). Typically, individuals with at least one copy of the dominant PAV haplotype have the ability to taste PTC/PROP, AVI homozygotes do not, and the rare haplotypes AAI and AAV have both been observed to confer an intermediate sensitivity (e.g., Kim et al. 2003; Bufe et al. 2005; Mennella et al. 2011, Garneau et al. 2014). Beyond these reports, taste sensitivity analysis of individuals with rare diplotypes has been scarce, with sample sizes too small to conduct statistics. Moreover, even when studies are large enough to capture rare haplotypes, they often use methodology that does not sequence all three SNPs, thus complicating the ability to interpret the haplotypes observed. To overcome this limitation in sequence analysis, we sequenced all three SNPs to confidently assess each haplotype. These data were then analyzed in the context of an individual's PROP bitterness ratings. Here we report both the haplotype and diplotype frequencies observed, as well as the sensitivity of rare haplotypes relative to the

*Correspondence: ngarneau@dmns.org

¹ Genetics of Taste Lab, Health Sciences Department, Denver Museum of Nature & Science, 2001 Colorado Blvd., Denver, CO 80205, USA
Full list of author information is available at the end of the article

common ones. As *TAS2R38* has been reported to contribute to nutritional choices (Duffy et al. 2010; Sandell et al. 2014; Hoppu et al. 2015), BMI (Tepper et al. 2014), cigarette and alcohol consumption (Keller et al. 2013; Hayes and Pickering 2012) and immunity (Lee et al. 2012), the data presented further advance the conversation on the role that rare haplotypes may be playing in not only taste, but in these important areas of human health.

Methods

Participants

Data from 1156 participants (age range of 18–93, 61 % female) were previously collected as part of a population study in the Genetics of Taste Lab at the Denver Museum of Nature & Science (Museum). Briefly, the citizen scientists in the original study facilitated data collection from a convenience sample of Museum guests, including buccal swabbing for DNA extraction and analysis, and standardized taste sensitivity ratings to a 0.453 M PROP filter disk (Garneau et al. 2014). The taste test was scored through trained use of a general Labeled Magnitude Scale (Green et al. 1996). The experiment took place in a sensory lab, and participants gave written informed consent as well as volunteered their time. All procedures were approved by the Western Institutional Review Board (Study No. 1109386, Protocol No. 2009 1028).

***TAS2R38* SNP analysis and haplotype determination**

Participant DNA was extracted from the buccal swabs. The 1002 nucleotide (nt) *TAS2R38* gene was amplified from nt 94 through nt 925 using PCR primers (Forward ACCAATGCCTTCGTTTCTTGTTGA, Reverse TCA-CAGCTCTCCTCAACTTGGCA, Invitrogen). Sanger sequencing using the forward primer (High Throughput Genomics Center, Seattle, WA, USA, <http://www.htseq.org>) was conducted and the results were analyzed at the Museum using the software program Geneious. The forward read allowed for analysis of SNPs at positions 145 and 785. From this first assessment we could determine all samples that were AVI/AVI, PAV/AVI and PAV/PAV carriers. The remaining samples contained possible rare haplotypes. These were sequenced using both the forward and reverse primers, creating two reads that together fully captured all three SNPs. This analysis revealed 97 of our 1156 participants to be a carrier of a rare haplotype.

Statistical analysis

Haplotype phase was resolved using the haplo.score package in R version 3.1.3. To normalize the taste sensitivity data, we transformed each raw PROP score using the log base 10. These normalized PROP score data were analyzed by one-way analysis of variance (ANOVA) and Tukey’s post hoc test to determine the differences in

PROP ratings between all observed *TAS2R38* diplotypes. Analyses were performed using SYSTAT 13.

Results

Diplotype frequencies

The frequencies of each observed diplotype and haplotype are shown in Table 1.

Table 1 a *TAS2R38* haplotype, b diplotype and c frequencies and diplotype comparisons (ANOVA and Tukey’s Post Hoc)

	n	Frequency (%)	
a Haplotype			
AVI	1227	53.1	
PAV	978	42.3	
AAV	59	2.5	
AAI	28	1.2	
PAI	18	0.8	
PVI	2	0.1	
b Diplotype			
PAV/AVI	461	39.9	
AVI/AVI	356	30.8	
PAV/PAV	242	20.9	
AAV/PAV	27	2.3	
AAV/AVI	24	2.1	
AAI/PAV	5	0.4	
AAI/AVI	15	1.3	
PAI/AVI	15	1.3	
AAV/AAV	3	0.3	
AAI/AAV	2	0.2	
AAI/AAI	2	0.2	
AAI/PAI	2	0.2	
PVI/PAI	1	0.1	
PVI/PAV	1	0.1	
Total	1156	100.0	
Diplotype	AVI/AVI	PAV/AVI	PAV/PAV
c Significantly different (p < 0.05) than			
PAV/AVI	✓		
AVI/AVI		✓	
PAV/PAV	✓		
AAV/PAV	✓		
AAV/AVI	✓	✓	✓
AAI/PAV	✓		
AAI/AVI	✓		✓
PAI/AVI	✓		
AAV/AAV			✓
AAI/AAV			
AAI/AAI			
AAI/PAI			
PVI/PAI			
PVI/PAV			

PROP sensitivity

The range of PROP scores (\log_{10}) of all subjects are shown in Fig. 1a. We hypothesized that rare haplotypes of the gene *TAS2R38* would fall within this wide range of sensitivity to the tastant PROP. Using ANOVA and Tukey's post hoc test, we conducted genotype/phenotype relational analysis from participants with usable PROP data ($n = 1051$) to assess the role of three rare *TAS2R38* haplotypes, AAI (Fig. 1b) and AAV (Fig. 1c), and PAI (Fig. 1d) in bitter taste sensitivity. We included some additional sensitivity comparisons in Table 1c.

Here we show that AAI, AAV, and PAI confer taste sensitivity to PROP, supporting our hypothesis and confirming an earlier reported trend (Mennela et al. 2010).

The following logic supports these conclusions. First, we found AAI/AVI has significantly greater sensitivity to PROP than the nontaster AVI/AVI ($p = 0.02$). It is clear that the AAI haplotype must confer this taste sensitivity in the AAI/AVI diplotype, since AVI does not. Second, AAI/AVI is significantly different from PAV/PAV ($p = 0.03$), but not AVI/PAV, suggesting that AAI has an intermediate taste sensitivity similar to that of AVI/PAV. Unsurprisingly due to the small sample size ($n = 1$), we could not infer any conclusions from AAI/AAI.

For the rare haplotype AAV, we found AAV/AVI to have a significantly greater sensitivity than AVI/AVI ($p = 0.01$), but lower sensitivity than AVI/PAV ($p = 0.0$) and PAV/PAV ($p = 0.0$), suggesting AAV confers an

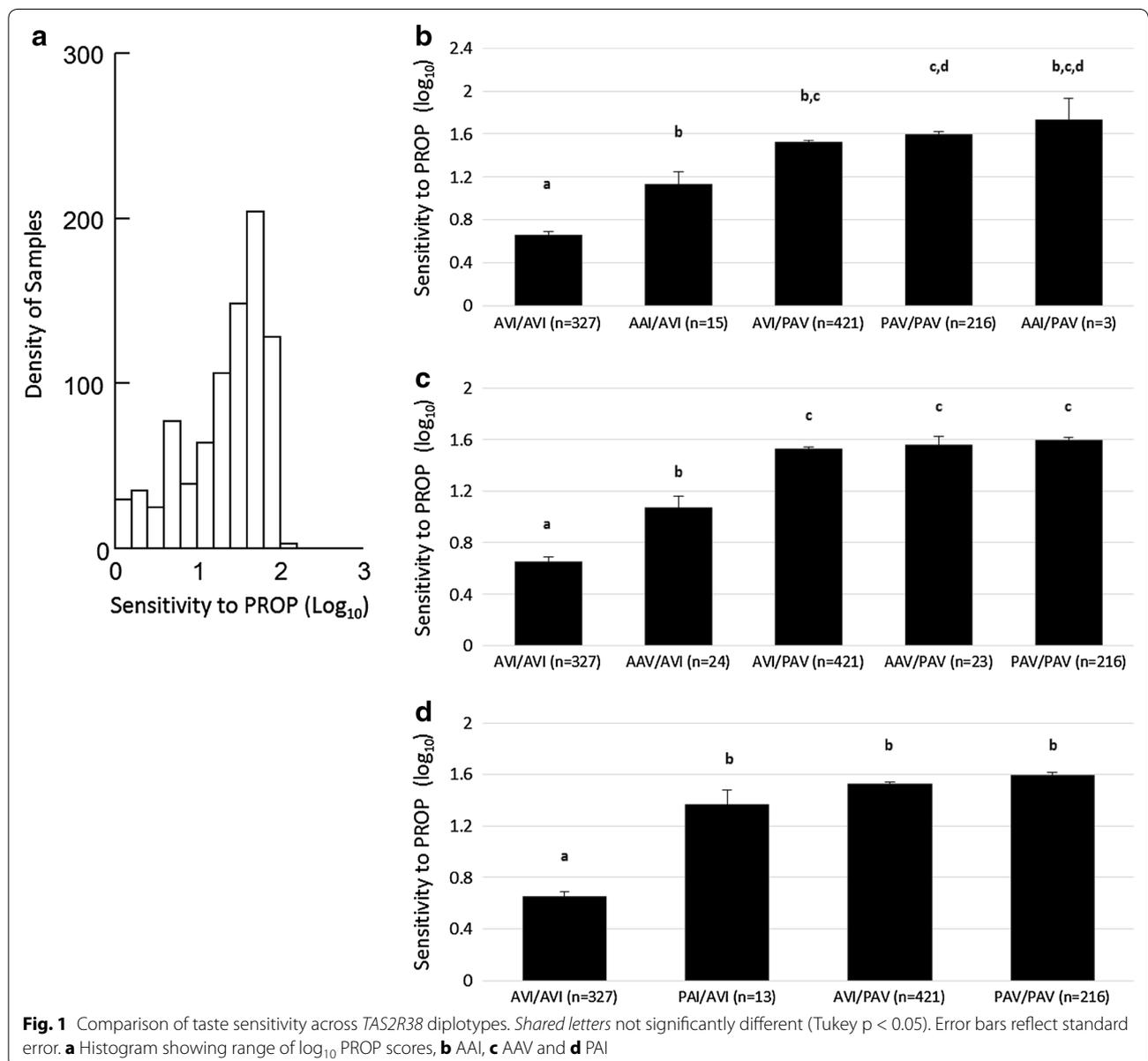


Fig. 1 Comparison of taste sensitivity across *TAS2R38* diplotypes. Shared letters not significantly different (Tukey $p < 0.05$). Error bars reflect standard error. **a** Histogram showing range of \log_{10} PROP scores, **b** AAI, **c** AAV and **d** PAI

intermediate taste sensitivity as well, perhaps a weaker one than AAI since AAV/AVI is significantly less sensitive than AVI/PAV, but AAI/AVI is as sensitive as AVI/PAV. AAV/AAV ($n = 3$) had too small a sample size to infer any conclusions from.

For the haplotype PAI, we observed that PAI/AVI has a significantly greater sensitivity than AVI/AVI ($p = 0.0$), but no difference from AVI/PAV or PAV/PAV. This suggests that PAI confers a strong sensitivity, perhaps stronger than AAI and AAV, since these rare haplotypes did have significantly weaker sensitivity than PAV/PAV. Thus we propose a tentative relationship between these three haplotype sensitivities as $AAV < AAI < PAI$. The final rare haplotype, PVI, had sample sizes too small to analyze.

It should be noted here that although *TAS2R38* is known to be the primary influencer of PROP perception, there may be other cofactors not accounted for here, including other genes that have yet to be associated with taste, which may confound the data presented and should be examined in future studies.

In conclusion, we found AAI, AAV, and PAI to confer sensitivity to PROP, and these data provide needed evidence to fill the gap in knowledge on the role of rare haplotypes of the gene *TAS2R38* in bitter taste perception. Further, they substantiate a growing number of studies showing PROP sensitivity to be a normal range of responses from low to high sensitivity, and not a categorical grouping. Finally, this work contributes to the broader implications of *TAS2R38* variation in nutritional choices, lifestyle choices, and immunity.

Abbreviations

PROP: 6-*n*-propylthiouracil; PTC: phenylthiocarbamide; SNP: single nucleotide polymorphism; Museum: Denver Museum of Nature & Science; nt: nucleotide.

Authors' contributions

EB participated in the sequence analysis, data analysis and interpretation, and drafted the manuscript. NG performed the statistical analysis, participated in data analysis and interpretation as well as study design, and helped to draft the manuscript. Both authors read and approved the final manuscript.

Author details

¹ Genetics of Taste Lab, Health Sciences Department, Denver Museum of Nature & Science, 2001 Colorado Blvd., Denver, CO 80205, USA. ² University of Colorado Denver, 1201 Larimer St., Denver, CO 80204, USA.

Acknowledgements

The authors wish to thank all current and previous members of the lab, including volunteer citizen scientists, Teen Science Scholars, interns, and staff members in both the Genetics of Taste Lab and on the Expedition Health core team for their support of the crowdsourcing and our citizen science research model. A special thanks to Stephanie A. Santorico, PhD, for her expertise in haplotype phasing software, Tiffany Nuessle, MA, for a careful reading of the manuscript, Diana Granados for support of the resequencing efforts, and Bridget C. Coughlin, PhD, for her leadership in establishing the Genetics of Taste Lab. This study was supported in part by a Science Education Partnership Award from the National Center for Research Resources, National Institutes of Health (R25RR025066).

Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

Ethical approval

All procedures performed were in accordance with the ethical standards governed by the Western Institutional Review Board, Puyallup, WA, USA (WIRB Study Number: 1109386; WIRB Protocol Number: 20091028).

Informed consent

Informed written consent was obtained from all individual participants included in the study.

Received: 4 May 2015 Accepted: 25 August 2015

Published online: 17 September 2015

References

- Bufe B, Breslin PA, Kuhn C, Reed DR, Tharp CD, Slack JP, Kim UK, Drayna D, Meyerhof W (2005) The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. *Curr Biol* 15:322–327
- Carrai M, Steinke V, Vodicka P et al (2011) Association between *TAS2R38* gene polymorphisms and colorectal cancer risk: a case-control study in two independent populations of Caucasian origin. *PLoS One* 6(6):e20464. doi:10.1371/journal.pone.0020464
- Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ (2000) T2Rs function as bitter taste receptors. *Cell* 100(6):703–711
- Duffy VB, Hayes JE, Davidson AC, Kidd JR, Kidd KK, Bartoshuk LM (2010) Vegetable intake in college-aged adults is explained by oral sensory phenotypes and *TAS2R38* genotype. *Chemosens Percept* 3–4:137–148
- Fox AL (1932) The relationship between chemical constitution and taste. *Proc Natl Acad Sci USA* 18:115–120
- Garneau NL, Nuessle TM, Sloan MM, Santorico SA, Coughlin BC, Hayes JE (2014) Crowdsourcing taste research: genetic and phenotypic predictors of bitter taste perception as a model. *Front Integr Neurosci* 8:33. doi:10.3389/fnint.2014.00033
- Green BG, Dalton P, Cowart B, Shaffer G, Rankin K, Higgins J (1996) Evaluating the 'labeled magnitude scale' for measuring sensations of taste and smell. *Chem Senses* 21:323–334. doi:10.1093/chemse/21.3.323
- Hayes JE, Pickering GJ (2012) Wine expertise predicts taste phenotype. *Am J Enol Vitic* 63:80–84. doi:10.5344/ajev.2011.1.1050
- Hoppu U, Laitinen K, Jaakkola J, Sandell M (2015) The h*TAS2R38* genotype is associated with sugar and candy consumption in preschool boys. *J Hum Nutr Diet* 28(s1):45–51. doi:10.1111/jhn.12249
- Keller M, Liu X, Wohland T, Rohde K, Gast M-T, Stummvoll M, Kovacs P, Tönjes A, Böttcher Y (2013) *TAS2R38* and its influence on smoking behavior and glucose homeostasis in the German sorbs. *PLoS One* 8(12):e80512. doi:10.1371/journal.pone.0080512
- Kim U, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D (2003) Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science* 299:1221–1225
- Lee RJ, Xiong G, Kofonow JM et al (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest* 122:4145. doi:10.1172/JCI64240
- Mennella JA, Yanina Pepino M, Duke FF, Reed DR (2011) Psychophysical dissection of genotype effects on human bitter perception. *Chem Senses* 36:161–167. doi:10.1093/chemse/bjq106
- Pemberton TJ, Mehta NU, Witonsky D et al (2009) Prevalence of common disease-associated variants in Asian Indians. *BMC Genet* 9:13. doi:10.1186/1471-2156-9-13
- Sandell M, Hoppu U, Mikkilä V, Mononen N, Kähönen M, Männistö S, Rönnemaa T, Viikari J, Lehtimäki T, Raitakari OT (2014) Genetic variation in the h*TAS2R38* taste receptor and food consumption among Finnish adults. *Genes Nutr* 6:433. doi:10.1007/s12263-014-0433-3
- Tepper BJ, Banni S, Melis M, Crnjar R, Tomassini Barbarossa I (2014) Genetic sensitivity to the bitter taste of 6-*n*-propylthiouracil (PROP) and its association with physiological mechanisms controlling body mass index (BMI). *Nutrients* 6(9):3363–3381. doi:10.3390/nu6093363