Combined Influence of TAS2R38 Genotype and PROP Phenotype on the intensity of Basic Tastes, Astringency and Pungency in the Italian Taste Project

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Abstract

The combined influence of TAS2R38 genotype and PROP phenotype on oral sensations is still to be clarified. The present work investigates their influence on the intensity of basic tastes and somatosensory stimuli (capsaicin, aluminium sulphate), using a large cohort of 1117 individuals. The possible influences of gustin genotype and fungiform papillae density were also assessed. PROP phenotype was mainly associated with TAS2R38 genotype with AVI/AVI individuals reporting the lowest mean bitterness intensity (12.6±1.26), and PAV/AVI individuals rating PROP lower (46.53±0.93) than PAV/PAV individuals (54.14±1.33). However, 25% of AVI/AVI subjects reported PROP bitterness perception higher than 'moderate' and small percentages of both PAV/PAV and PAV/AVI responded very little to PROP stimulation. PROP phenotype significantly affected ratings to all the tastant solutions with ST subjects giving the highest ratings and NT the lowest. An unexpected systematic effect of TAS2R38 diplotype on perceived intensity was found, with AVI/AVI individuals rating tastant solution intensity higher than PAV/AVI and PAV/PAV for all the stimuli. Recursive partitioning analysis was used to determine the influence of the explanatory variables

(TAS2R38 diplotype, PROP status, age and gender) on intensity for each tastant solution. Regression trees indicated that TAS2R38 genotype is the most important variable for explaining differences in intensity of basic tastes and astringency, when compared to PROP responsiveness, gender, and age. Gender was the primary determinant of heightened perception of pungency. PROP status was the second most influential variable in all the models, with limited influence only on sweetness and umami perception. No significant variations of intensity of taste and somatosensory sensations were found in association to gustin polymorphism or fungiform papillae density. These findings call for a re-examination of the notion that the TAS2R38 gene uniquely controls PROP tasting and for future research devoted to a more in-depth genetic characterization of the AVI/AVI group and its possible associations with other polymorphisms.

Key words: *TAS2R38*, PROP phenotype, tastes, astringency, pungency, recursive partitioning

Introduction

It is well established that the capacity to perceive bitter thiourea compounds such as PROP (6-*n*-propylthiouracil) varies among individuals. Three distinct groups of individuals can be distinguished: 1) those with very high PROP intensity perception or 'super tasters'; 2) those with low PROP intensity perception or 'non tasters'; 3) those who perceive moderate intensity from PROP, named 'medium tasters' (Bartoshuk et al., 1994). These individual differences in PROP perception are mainly due to genetic variation in the *TAS2R38* gene (Kim et al., 2003). Three Single Nucleotide Polymorphisms (SNPs) within *TAS2R38* give rise to three amino acid substitutions (A49P, A262V and V291I) that define two common haplotypes: PAV (considered the "taster haplotype") and AVI (considered the "non taster" haplotype). PROP non tasters are typically homozygous for the AVI haplotype, while large genotypic overlap has been found between medium and super-tasters. Thus, being homozygous for the PAV haplotype is not necessarily a PROP supertaster, although they may perceive greater PROP bitterness than heterozygotes (Bufe et al., 2005; Duffy et al., 2004; Hayes et al., 2008).

Responsiveness to thiourea compounds has been connected to the evolutionary advantage for individuals carrying the taster haplotype variant to taste anti-thyroid plant toxins and thereby avoid poisoning (Dinehart et al., 2006; Duffy et al., 2010; Wooding et al., 2010). However, frequencies of haplotype distribution across the human population, with almost 50% of individuals carrying the non-taster AVI haplotype (Guo & Reed, 2001), support the hypothesis that balancing natural selection has acted on *TAS2R38,* thus maintaining the AVI haplotype at the same worldwide frequency as the PAV haplotype. Based on this hypothesis, it has been suggested that the AVI haplotype may encode for a functional receptor, possibly responsible for sensitivity to other groups of bitter compounds even

outside the thiourea family (Bufe et al., 2005; Wooding, 2006; Wooding et al., 2004). Moreover, since bitter receptors are expressed in the respiratory and gastrointestinal system, and the *TAS2R38* gene has been linked to susceptibility to upper respiratory infections, pathogens may be considered the real target of natural selection (Lee et al., 2012). However, recently a revised hypothesis for the evolution of *TAS2R38* gene was proposed as evidence for a relaxation of recent selective forces acting on this gene emerged (Wang et al. 2004). A recent study has suggested that the existing high frequencies of the PAV and AVI haplotypes could have resulted from demographic or population stratification events (i.e., various genetic bottlenecks contributing to modify genetic structure) (Risso et al., 2016).

Although *TAS2R38* polymorphisms account for the most of the phenotypic variance in PROP taste responsiveness (Kim et al., 2003; Wooding et al., 2004; Bufe et al. 2005), additional factors other than *TAS2R38* polymorphisms have been evoked to explain the variability in responsiveness to PROP. These factors include, among others, differences in anatomy of the peripheral taste system (i.e. fungiform papillae density) (Bartoshuk et al., 1994; Essick et al., 2003; Piochi et al., 2019; Tepper & Nurse, 1998), age (Mennella et al., 2010; Tepper et al., 2017), gender (Barragán et al., 2018; Tepper et al., 2017), salivary protein composition (Melis et al., 2017), psychological traits such as alexithymia (Robino et al., 2016) and disgust sensitivity (Ammann et al., 2019), polymorphisms in the gustin (*CA6*) gene (involved in taste bud growth and maintenance) (Calò et al., 2011; Padiglia et al., 2010), and variation in *TAS2R38* mRNA expression (Lipchock et al., 2017).

One reason for the continued interest in individual differences in PROP perception is that they have frequently been correlated with the intensity of other oral stimuli. Several studies have reported increased responsiveness to the five basic tastes (bitterness, salt, sour, sweet and umami) in those with higher ratings of PROP bitterness (Bajec & Pickering, 2008a; Bartoshuk et al., 1992; Dinnella et al., 2018; Drewnowski et al., 1998; Hayes et al., 2008; Ly & Drewnowski, 2001; Nolden et al., 2020; Piochi et al., 2021; Prescott et al., 2001). Moreover, responsiveness to PROP has been related to higher intensities of irritants, alcohol, fat or creaminess sensations (Duffy et al., 2004; Dinehart et al., 2006; Keller et al., 2002; Prescott et al., 2004; Prescott & Swain-Campbell, 2000; Shen et al., 2016; Tepper, 2008; Tepper & Nurse, 1998). The possible consequences of such individual variation in chemosensory perception related to PROP phenotypes on food preferences and dietary outcomes have been investigated and there is conflicting evidence on the relationship between PROP responsiveness and anthropometric and adiposity measures, such as Body Mass Index (BMI) (see Tepper et al. 2014 for review).

Given the strong positive associations that have been reported between PROP bitterness and the intensity of other oral stimuli, it has often been assumed that similar associations exist between *TAS2R38* genotypes and these same perceptions. However, several studies show weak or no associations between *TAS2R38* variants and oro-sensory perceptions (Fischer et al., 2014; Hayes et al., 2008; Nolden et al., 2020). Interestingly, two studies reported relatively weak associations between PROP bitterness and the perception of other oral qualities, but if subjects were grouped by *TAS2R38* genotypes, the strength of these associations improved (Fischer et al., 2014; Nolden et al., 2020). For example, Nolden et al. (2020) recently reported better associations between PROP bitterness and the intensity of sucrose, quinine, and capsaicin when subjects were classified by *TAS2R38* genotypes. In a large cohort of 1670 participants, Fisher et al. (2014) also reported that the strength of the association between PROP and the basic tastes differed by *TAS2R38* diplotypes, with significantly stronger relationships in PAV homozygotes. These data suggest that *TAS2R38* variation plays a critical role in the perception of PROP, but it may have far less direct influence on the perception of other oral stimuli. These findings lend additional support to the so called '2nd receptor hypothesis'(Hayes et al., 2008) that other genetic loci on chromosomes 5 (Reed et al., 1999) and 16 (Drayna et al., 2003) may be involved in PROP tasting. Based on this evidence, the combined influence of PROP and *TAS2R38* genotype on other oral sensations is still to be clarified.

The present work addresses this question by utilizing recursive partitioning analysis to evaluate the influence of both PROP responsiveness and TAS2R38 genotype on the intensity of basic tastes (sodium chloride, sucrose, caffeine, citric acid, monosodium glutamate) and somatosensory (capsaicin, aluminium sulphate) stimuli, using a large cohort of 1117 individuals. A large number of predictor variables can be dealt with in recursive partitioning analysis, even in the presence of complex interactions, thereby obtaining a hierarchical and graphical representation of these interactions between variables. Recently, this approach has been used to analyze variation in taste intensity, demonstrating that it can be a valid tool to study complex sensory data (Robino et al., 2016; Torri & Salini, 2016; Yang et al., 2020). The possible influence of gustin genotype, fungiform papillae density, gender and age was also assessed.

Material and methods

The data analysed in the present study represent a selection from the Italian Taste Project (Monteleone et al., 2017). Only data on demographic characteristics, anthropometric measures, injuries and pathologies potentially compromising taste functioning, saliva collection and sensory evaluations of basic tastes, astringency, pungency, and PROP solutions are considered here. The

experimental plan included two independent sessions in a sensory lab held in different days. From 1 to 7 days were left between the two sessions, according to subject availability. PROP ratings were collected at the end of the first session, saliva collection and ratings of tastant solution were collected in the second session. Participants completed an online questionnaire including information on demographics, anthropometric measures and information on injuries and pathologies in the days preceding the first lab session.

1. Participants

Participants were recruited on a national basis by means of announcements published on research unit websites and newspapers, emails, pamphlet distribution and by word of mouth. The exclusion criteria were pregnancy and breastfeeding at the time of the test, and not having lived in Italy for at least 20 years. Data presented here are a subset from the Italian Taste project of 1166 subjects (age range: 18-60 years; gender: 59.5% women) on whom genetic analysis was performed. Only subjects with the 3 common diplotypes (PAV/PAV, PAV/AVI and AVI/AVI) were included in the present study (n=1125). Subjects with missing data for only one of the sensory variables analyzed were retained, while eight subjects were excluded from the analysis due to incomplete sensory tests, leaving a final N of 1117 subjects.

The study was conducted in agreement with the Italian ethical requirements on research activities and personal data protection (D.L. 30.6.03 n. 196). The study protocol was approved by the Ethics Committee of Trieste University. The respondents gave their written informed consent at the beginning of the test according to the principles of the Declaration of Helsinki.

2. Saliva collection and genetic analysis

Saliva collection was performed in individual booths. Subjects were instructed to rinse their mouth with water, wait 10 minutes, and then to spit saliva into a graduated test tube until a volume of 2 ml was collected. A square of parafilm (3*3 cm) to chew while spiting was provided to help saliva production if necessary. Saliva was preserved at room temperature using the Norgen Saliva DNA collection and preservation devices until DNA was extracted. DNA extraction was then performed using a saliva DNA isolation kit, according to the manufacturer's instructions (Norgen Biotec Corp, Ontario, Canada; Isohelix, Cell Projects, Kent, UK).

All subjects were genotyped with an Illumina high density SNPs-array (Illumina, Inc., San Diego, CA, USA) and three SNPs (rs1726866, rs713598, and rs10246939) at base pairs 145, 785 and 886 of the *TAS2R38* gene and the rs2274333 SNP at *CA6* gene were extracted and analyzed.

3. Sensory evaluations

Stimuli

Seven solutions corresponding to the basic tastes (bitterness, sourness, sweetness, saltiness, and umami), plus astringent and pungent sensations were prepared. The following tastant concentrations were selected to reflect moderate/strong intensity of target sensations on the general Labelled Magnitude scale (Bartoshuk et al., 2004) : citric acid, 4 g/kg (sourness); caffeine, 3 g/kg (bitterness); sucrose, 200 g/kg (sweetness); sodium chloride, 15 g/kg (saltiness); monosodium glutamate (MSG), 10 g/kg (umami); capsaicin, 1.5 mg/kg (pungent); and aluminium sulphate, 0.8 g/kg (astringency).

Procedure

Before tasting the stimuli, subjects were instructed on the use of the gLMS (0-not detectable, 1- barely detectable, 6- weak, 17- moderate, 35- strong, 53- very strong, 100- strongest imaginable sensation of any kind) (Bartoshuk et al., 2004). Subjects were told that the top of the scale represented the most intense sensation that they could ever imagine experiencing and they were asked to recall a variety of remembered sensations from different modalities (loudness, oral pain/irritation, and tastes) to familiarise themselves with the scale anchors (Bajec & Pickering, 2008b; Kalva et al., 2014; Webb et al., 2015). For practice on the use of the gLMS, subjects were asked to rate the intensity of the brightest light they had ever seen. The criterion to conclude that the subjects correctly used the scale was that their ratings were higher than "very strong" and lower than "the strongest imaginable sensation of any kind". Ratings out of this range were individually discussed and the scale use clarified (Dinnella et al., 2018).

The samples for evaluation were presented as aqueous solutions (10 mL) in 80 cc plastic cups identified by a 3-digit code. The seven solutions were presented in random order for the tastes and astringent solution, while the capsaicin solution was always evaluated as the last sample to avoid carryover effect of the pungency. During tasting, subjects were instructed to hold the whole sample in their mouth for 3 s, expectorate, then wait a further 3 s (5 s in the case of bitterness, umami, astringency, and pungency), and evaluate the intensity of the target sensation. After each sample, subjects rinsed their mouth with water for 30 s, ate some plain crackers for 30 s, and finally rinsed their mouth with water for a further 30 s. Evaluations were performed in individual booths under white lights. Data were collected with the software Fizz (ver. 2.51. A86, Biosystèmes).

4. PROP taster status

A 3.2 mM PROP solution was prepared by dissolving 0.5447 g/L of 6-*n*-propyl-2-thiouracil (Sigma-Aldrich) into deionized water (Prescott et al., 2004). Subjects were presented with two samples (10 mL) coded with 3-digit codes and were instructed to hold each sample in their mouth for

10 s, expectorate, and then wait 20 s before evaluating the intensity of bitterness using the gLMS (Masi et al., 2015). Subjects had a 90 s break between samples. During the break, subjects rinsed their mouth with water for 30 s, ate some plain crackers for 30 s, and finally rinsed with water for a further 30 s before they evaluated the second PROP sample. The arbitrary cut-offs used in previous studies were used to categorize subjects as non-tasters (NT: PROP bitterness on gLMS < moderate, 17), medium tasters (MT: PROP bitterness on gLMS > moderate, 17 and < very strong, 53), super tasters (ST: PROP bitterness on gLMS > very strong, 53) (Hayes et al. 2010; Fischer et al. 2013). The average bitterness score across the two PROP samples was used for each subject.

5. Fungiform papillae density

The density of fungiform papillae (FPD) on the tongue was assessed according to Dinnella et al., 2018. The anterior portion of the dorsal surface of the tongue was swabbed with household blue food coloring and digital pictures of the tongue were recorded using a digital microscope (MicroCapture, version 2.0 for ×20 to ×400) (Masi et al., 2015). For each participant, the clearest image was selected, and the number of FP was counted in two 0.6 cm diameter circles, one on right side and one on left side of tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline. The number of FP was manually counted by two researchers independently according to the Denver Papillae Protocol (Nuessle et al., 2015). The mean of FP number was used for each image and expressed as density (FP/cm^2: FPD). Limits of 25th and 75th percentiles were used as empirical cutoffs to classify subjects in low FPD (L-FPD), medium (M-FPD) and high FPD (H-FPD).

6. Data analysis

The body mass index (BMI) was computed according to the Quetelet formula and categorized into 5 levels according to WHO classification. Given the very low prevalence of subjects with BMI >30, the original three obese classes were collapsed into one single category (Obese).

Chi-square tests were used to test differences in gender, age (three classes C1: 18-30 y.o.; C2: 31-45 y.o.; C3: 46-60 y.o.), BMI (three classes: under-weight BMI<18.5; normal weight BMI= 18.5-24.99; obese BMI≥30), smoker status (three classes: never; quit; current) distribution by *TAS238* diplotype and PROP status. Fisher's exact test was run to test the significance by cell (significance level fixed at p=0.05). Fixed ANOVA models with two-way interactions were applied to test the *TAS2R38* diplotype (three levels: AVI/AVI, PAV/AVI, PAV/PAV) and PROP status (three levels: NT, MT and ST) effect on intensity ratings from tastant solutions. Correlations between FPD and intensity ratings of PROP and tastant solutions were tested by Pearson correlation, with significance level fixed at p≤0.05. One-way ANOVA models were independently applied to test for 1) gustin genotype effect (three levels AA, AG, GG) on intensity of tastant and PROP solutions and on FPD

and 2) FPD effect (three levels H-FPD, M-FPD, L-FPD) on intensity of tastant solutions and PROP bitterness.

Conditional inference-based recursive partitioning (implemented in the R "party" package) (Hothorn et al., 2006) was used to determine the influence of the explanatory variables (*TAS2R38* diplotype and PROP status) on intensity for each water solution. Age and gender were also included as explanatory variables in the analysis. A regression tree was created by recursive partitioning, in which the variable with highest predictive power (the lowest p-value after Bonferroni correction) is represented as the first node of the decision tree, and then two subgroups are created (I and II). For subgroup I, the variable with lowest p-value (if there is one) is taken as the second or third node. The same is done for subgroup II. The final model is based on the splitting variables in each node with the highest statistical significance (Strobl et al., 2009). The size of the split was fixed to five percent of the whole sample as the smallest acceptable size of a subgroup. The branching stops when splits are not significant. Thus, the regression tree shows data broken down in smaller subsets significantly differing in level of response variable (intensity of tastants in the present study). Nodes and branches are represented accordingly to the hierarchical order of predictive power of the relevant explanatory variable. Thus, the higher is the node position in the tree, the more powerful is the influence of the variable on response variability. Here, the regression tree was generated from the intensity ratings for each modality, with the four factors as independent variables (*TAS2R38* diplotype, PROP status, gender and age class).

Results

1. Participants

Demographic characteristics of the population considered in the present work are reported in Table 1. The sample showed a mean age of 37.6 years (SD = 13.1; 18–60 years old range). The age class distributions of the men and women groups were not significantly different (chi-square = 0.433; chi-square critical value = 5.99; $P = 0.805$). The majority of respondents were normal weight (BMI= 18.5-24.99, 65.6%) and 23.95% were overweight (BMI=25-29.99). Only a minority of the population was represented by underweight (BMI<18.5, 3.86%) and obese (BMI≥30, 6.55%) individuals. Most respondents did not smoke (76%). The vast majority of participants did not report infections and traumas that would impair perceptive abilities (83.0%), food allergies and intolerances (92.0%), chronic diseases requiring long-term diet restrictions (99.3%). The sample can therefore be considered representative of the Italian healthy adult population and it is reasonable to hypothesize

that intensity response to oral stimuli explored in this article are not affected by specific environmental insults as confounding factors.

2.TAS2R38 genotype and PROP responsiveness

The haplotype distribution was consistent with its theoretical distribution for Caucasian populations (Guo & Reed, 2001), with AVI/AVI representing 25.8% of the sample, and PAV/AVI and PAV/PAV, 49.9% and 24.35%, respectively. No significant differences were found in gender, age class, BMI and smoker status distribution by diplotypes (diplotype/gender: chi-square=5.497; chi-square critical value=5.991, p=0.064; diplotype/age class: chi-square = 4.003; chi-square critical value=9.488; p=0.406; diplotype/BMI chi-square = 3.336; chi-square critical value = 9.488; p=0.503; dipotype/smoker status chi-square = 3.025; chi-square critical value = 9.488; p=0.554).

PROP ratings tended to a normal distribution (W=0.959; p<0.001) but skewed on the right (skewness=0.342) (Fig.1S). The frequency of distribution of PROP ratings showed that first and third quartile limits (1st quartile limit=17; 3rd quartile limit=59) were very close to the arbitrary cut-off proposed for subject classification according to their PROP status as NT (gLMS<moderate, 17) and ST (gLMS>very strong, 53) (Hayes et al. 2010; Fischer et al. 2013). Thus, the population sample was classified as NT, MT and ST according to the arbitrary cut-offs. Significant differences were found in gender distribution by PROP status (PROP status/gender: chi-square=20.70; chi-square critical value=5.991, p<0.0001). The proportion of NTs was lower in women than in men, while ST were more abundant in women than in man. No significant differences were found in age class distribution by PROP status group (PROP status/age class: chi-square = 7.27; chi-square critical value=9.49; p=0.122).

As expected, ratings of PROP bitterness significantly differed by *TAS2R38* diplotype (F=315.0; p=0.0001), with AVI/AVI individuals showing the lowest mean bitterness intensity (12.6±1.26), and PAV/AVI individuals rating PROP lower (46.53±0.93) than PAV/PAV individuals (54.14±1.33). PAV/AVI and PAV/PAV women rated PROP bitterness significantly higher than did PAV/AVI and PAV/PAV men, respectively (F=18.3; p<0.0001) (Figure1a). Age significantly affected PROP bitterness ratings (F=5.0; p=0.007) (Figure1b). Among PAV/PAV individuals, the C3 (46-60 y) group rated PROP bitterness lower than the C1 group, but the C2 group did not differ from the other two groups. Among PAV/AVI individuals, the C3 group rated PROP bitterness lower than the C1 or C2 group. No differences by age were found among AVI individuals. No significant interactions diplotype*gender and diplotype*age class were found.

The composition of *TAS2R38* diplotype groups in terms of PROP NT, MT and ST was computed and expressed as percentages (Figure 2). Seventy-three percent of AVI/AVI subjects were

classified as NT, 23.3% as MT and 3.1% as ST. The PAV/AVI group consisted of 53.7% of MT, 37.9% of ST and 8.4% of NT. The PAV/PAV group was composed by 51.5% of ST, 45.2% by MT and 3.3% by NT.

3. Effect of TAS2R38 diplotype and PROP status on tastant intensity

Mean values of ratings of taste solutions were on average close to 'strong' on the gLMS (bitterness: 32.5, SE=0.65; sourness: 34.4, SE=0.61; sweetness: 40.1, SE=0.58; saltiness: 37.8, SE=0.61; umami: 26.9, SE=0.58). Astringency was rated close to 'moderate' intensity (20.3, SE=0.56) and pungency between 'strong' and 'very strong' (48.4, SE=0.68).

Significant effects of TAS2R38 diplotypes were found on all tastant solutions (bitterness: $F=29.02$, $p\leq0.0001$; sourness: $F=20.34$, $p\leq0.0001$; sweetness: $F=6.21$, $p=0.002$; saltiness: $F=21.09$, $p\leq0.0001$; umami: $F=12.22$, $p\leq0.0001$; astringency: $F=20.87$, $p\leq0.0001$; pungency: $F=12.29$, $p<0.0001$). AVI/AVI subjects consistently rated tastes, astringent, and pungent solution intensities higher than both PAV/AVI and PAV/PAV groups; PAV/AVI and PAV/PAV groups did not significantly differ in the response to any solution (Figure 3).

A significant effect of PROP status (NT, MT and ST) was found on bitterness ($F=10.91$, $p\leq0.0001$), sourness ($F=8.45$, $p=0.000$), sweetness ($F=4.15$, $p=0.002$), saltiness ($F=9.10$, $p=0.000$), umami ($F=7.21$, $p=0.001$), astringency ($F=7.00$, $p=0.001$ and pungency ($F=16.30$, $p\leq0.0001$) (Figure 4). ST subjects gave the highest ratings to all the tastant solutions and NT the lowest, with the exceptions of sweetness, where ST and MT did not significantly differ, and pungency, where MT and NT did not significantly differ. Interactions between *TAS2R38* diplotype*PROP status were never significant ($p\geq0.29$).

4. Fungiform papillae density PROP responsiveness and tastant solutions

FPD tended toward a normal distribution ($W \geq 0.971$; $P \leq 0.001$) (Figure 5). Subjects were classified into three groups as low (L-FPD) (1st quartile limit; <13.27 FP/ cm^2), medium (M-FPD) (interquartile limits; >13.27 and < 30.08 FP/cm^2) and high (H-FPD) (3rd quartile limit; 30.08 FP/cm^2) (Shen et al., 2016; Dinnella et al., 2018).

FPD class did not affect the intensity of tastant solutions and PROP bitterness ($p\geq0.086$). No significant correlations were found between FPD and intensity ratings of PROP ($p=0.346$), and tastant solutions (sour $p=0.576$; bitter $p=0.999$; sweet $p=0.372$; salty $p=0.264$; umami $p=0.412$; astringent $p=0.362$; pungency $p=0.06$).

5. Gustin genotype, PROP responsiveness, tastant solutions and papillae density

A majority (54.08%) of individuals for the rs2274333 SNP in *CA6* were AA, 38.30% AG and 7.62% GG, showing a G allele frequency of 26.8% in agreement with data on European populations (https://www.ncbi.nlm.nih.gov/snp/rs2274333#frequency_tab.).

No association emerged between rs2274333 genotypes (AA, AG, GG) and PROP bitterness, or with the intensity of other oral stimuli or fungiform papillae density. No significant results emerged when AG and GG individuals were combined and compared with AA individuals (Tab.1 supplementary).

6. Partitional tree analysis of responsiveness to tastant solutions

Recursive partitioning tree modeling was applied to the intensity ratings of the oral stimuli to further explore the results. These models take into account, as explanatory variables, all factors showing a significant effect according to independent ANOVA models (*TAS2R38* diplotype, PROP status, gender and age).

TAS2R38 diplotype was the most important factor explaining the variance in intensity of all sensations measured (Figure 6 A-F), with the exception of pungency (Figure 7) which is described in the next section. The first partition (node 1) for sour, salty, astringent, bitter (caffeine), umami and sweet split the population in two groups: (1) AVI/AVI and (2) PAV/AVI + PAV/PAV, with the AVI/AVI group rating stimulus intensity higher than the PAV/AVI + PAV/PAV group. In all cases, the subsequent partition showed the significant effect of PROP status, with slightly different patterns for the different stimuli. In the case of sourness, saltiness, bitterness and astringency (Figures 6 A-D, respectively), node 2 split AVI/AVI subjects into two groups: a PROP taster (MT+ST) group (node 3) that rated intensities higher than the NT group (node 4). Node 5 divided the PAV/AVI + PAV/PAV group into STs (node 6) who rated intensities higher than the MT+NT group (node 7). In the case of umami and sweetness (Figure 6 E-F, respectively), PROP status significantly affected the intensity only in the PAV/AVI+PAV/PAV group, with STs rating intensities higher than the NT+MT group (node 3). Additionally, for sweetness, the NT-MT group was further divided into MTs and NTs (node 5), with MTs giving higher ratings than NTs.

A significant effect of gender was only observed for caffeine bitterness in the AVI/AVI NT group, with women rating bitterness intensity higher than men.

The regression tree obtained for pungency (Figure 7) showed a different pattern of impact by the explanatory variables. In this case, gender was the most relevant variable (node 1), with women rating pungency higher than men. PROP status was the second most important variable, splitting women (node 2) and men (node 9) into ST and MT+NT groups, with STs rating pungency higher than the MT+NT group. Diplotype showed a significant effect only in women. ST women were split

into two groups consisting of AVI/AVI+PAV/AVI women who rated pungency higher than the PAV/PAV women (node 3). MT+NT women were divided in two groups, AVI/AVI and PAV/AVI+PAV/PAV (node 6), where the AVI/AVI group rated pungency higher than PAV/AVI+PAV/PAV group.

Age did not significantly affect the variance of intensity for any oral stimulus.

Discussion

The data described in the present work show that the *TAS2R38* genotype is strongly associated with individual variability in intensity ratings of PROP and other oral stimuli (tastes, astringency, and pungency). Mean ratings of bitterness from PROP are higher in individuals carrying the PAV haplotype than in AVI/AVI individuals. On the other hand, homozygous individuals for the recessive allele (AVI/AVI) show heightened response to all other stimuli with respect to both heterozygous (PAV/AVI) and homozygous for dominant allele form (PAV/PAV).

Overall, results from the present study are in line with already existing data on distribution of haplotypes and PROP phenotypes and their association with demographic factors, confirming the reliability of both genetic and phenotype characterization. PROP phenotype distribution was also in general agreement with results from previous studies (Fischer et al., 2013; Hayes et al., 2010). The distribution of the three most common *TAS2R38* haplotypes tend to agree with results from previous large sample studies on Caucasian populations (Barragán et al., 2018; Feeney & Hayes, 2014; Fischer et al., 2013) and was not significantly affected by age and gender (Barragán et al., 2018). Moreover, we confirmed the negative effect of aging on PROP ratings in PAV/PAV and PAV/AVI individuals, with significant lowering of PROP bitterness ratings in individuals aged 46-60 in respect to the younger age classes, as well as the heightened responsiveness of PAV/PAV and PAV/AVI women to PROP bitterness (Barragán et al., 2018; Fischer et al., 2014; Tepper et al., 2017). BMI was not significantly associated with TAS2R38 diplotype or PROP phenotypes. The influence of responsiveness to PROP on dietary habits and healthy outcomes has been previously investigated with conflicting findings (see Tepper 2014 for review) and the role of factors other than taste responsiveness has been suggested in defining food preference pattern and dietary behaviour.

As expected, PROP responsiveness was mainly associated with *TAS2R38* genotype, with the greater response showed by PAV homozygotes and the lowest by AVI homozygotes, on average. However, around 25% of AVI/AVI subjects reported PROP bitterness perception higher than ‘moderate’ and small percentages of both PAV heterozygotes and homozygotes responded very little to PROP stimulation. Fluctuation in PAV haplotype expression has been hypothesized as one possible

explanation of phenotypic expression. Substantial variations have been reported in PAV-*TAS2R38* mRNA expression in different tissues of heterozygous individuals (Douglas et al., 2019) and its variation in taste cells positively correlated with bitterness perception of PROP and glucosinolate-containing natural compounds (Bufe et al., 2005; Lipchock et al., 2017). Thus, heterozygous individuals might tend to a show a PROP phenotype more similar to NTs or to STs as a function of their expression of the PAV allele.

AVI homozygotes responding to PROP has already been reported (Bufe et al., 2005; Feeney & Hayes, 2014; Fischer et al., 2014; Hayes et al., 2008; Nolden et al., 2020; Shen et al., 2016), confirming the incomplete correspondence between *TAS2R38* genotype and PROP phenotype in some studies. The existence of an additional PROP receptor(s) has been suggested to explain the higher than expected responses of AVI homozygotes to PROP supra-threshold stimulation (Galindo-Cuspinera et al., 2009; Hayes et al., 2008; Nolden et al., 2020). Psychophysical functions for PROP bitterness show clear differences between taster (PAV/AVI and PAV/PAV) and non-taster (AVI/AVI) individuals at intermediate concentrations (around 0.32 mM) while intensity response overlap is observed at higher concentration levels (3.2 mM) (Bufe et al., 2005; Galindo-Cuspinera et al., 2009; Hayes et al., 2008). It has been hypothesized that responses to PROP from AVI/AVI individuals are due to the activation of lower affinity receptors at high PROP concentrations (Galindo-Cuspinera et al., 2009; Hayes et al., 2008). Thus, the observed distribution across *TAS2R38* diplotypes of bitterness ratings of 3.2 mM PROP possibly reflects the activation of receptors under the control of genetic factors other than *TAS2R38* polymorphisms.

Previously, linkage studies suggested that other loci on chromosome 5 and 16 may influence taste perception of PTC, another thiourea compound (Drayna et al., 2003; Reed et al., 1999). These findings were not confirmed for PROP perception in a recent genome-wide association (GWA) study, that identified a possible additional locus on chromosome 2 within the *DIRC3* gene (Hwang et al., 2018). However, also for this locus results were not confirmed in further studies (Drayna et al., 2003; Hwang et al., 2018; Reed et al., 1999) . Therefore, the possible contribution of further loci, other bitter receptors and the possible interaction among them remain to be elucidated.

Results from univariate linear models used here showed an unexpected systematic effect of *TAS2R38* genotype on intensity of all the stimuli, with AVI/AVI individuals rating tastant solution intensity higher than PAV/AVI and PAV/PAV. Moreover, regression trees indicated that *TAS2R38* diplotype is the most important variable for explaining differences in intensity of basic tastes and astringency, when compared to PROP responsiveness, gender, and age. These findings seem

counterintuitive to the widely held notion that *TAS2R38* diplotypes underlie general differences in oro-sensory responses in the same manner observed for PROP.

That these findings are at odds with those from other studies requires consideration of potential artifacts in our data. The most obvious candidate is in the ratings of the stimuli. One possibility is the existence of reversal artifacts in the ratings. These refer to distortion in scale use in subject groups differing in sensory acuity that might lead to draw wrong conclusions when the groups' intensity ratings are compared. Bartoshuk and co-workers (Bartushuk et al., 2003; 2004) showed that the meaning of the "very strong" label on the scale varied across NT, MT and ST PROP groups. This might lead to the reversal artifacts, namely the less sensitive subjects appear to rate intensity higher than more sensitive subjects in the case of small effect of their biological differences on the intensity response According to Bartoshuk (2004), this effect is at least partially controlled when using the gLMS in which the upper scale anchor "the strongest imaginable sensation of any kind" is not systematically different across groups and allow the comparison of their intensity responses. Reversal artifacts are also seen in "contextual shifts" in which moderate intensity stimuli are rated lower in the context of stronger intensity items and higher in the context of weaker intensity items (Diamond and Lawless, 2001).

We believe that such reversal artifacts do not account for our findings. In the present study, intensity ratings of PROP and tastant solutions were collected on the gLMS in two independent sessions at least 1 day apart. Thus, experimental conditions appear appropriate to control for reversal artifacts to the best of our knowledge. Furthermore, if severe reversal artifacts would have affected the results reported here, the same "inverse" relation observed for AVI/AVI and PAV/* groups should have also been observed for PROP phenotype groups. This was not the case, and consistent with many previous studies, ST rated oral stimuli as significantly more intense than NT.

Another explanation based on experimental artifacts lies in the method of defining PROP phenotypes. Here, these were determined using the one-solution standard method that provides equivalent group separation to methods using multiple PROP solutions (Tepper et al., 2001; Prescott et al., 2004). The adopted water/cracker/water rinsing procedure for a total of 90 s was proven effective to control for carryover effect of long-lasting sensations (Monteleone et al., 2004) and lasted longer than other widely employed time break between subsequent PROP sample evaluation (30 s, Nolden et al., 2020). Thus, phenotypes determined in the present paper are unlikely to be affected by potential artifacts due to PROP bitterness carryover effect.

More pertinent in helping to explain the findings of genotype/oral stimulus intensity relationships that are inconsistent with previous data are several recent findings that conflict with the

assumption that differences in oro-sensory responses overlap between *TAS2R38* diplotypes and PROP phenotypes. For example, Hayes et al. (2008) reported no difference by *TAS2R38* genotype in intensity of sweetness, sourness, saltiness or quinine bitterness, while Nolden et al. (2020) found no differences in sweetness, pungency and quinine bitterness. Importantly, Nolden et al. (2020) reported that individual ratings were scattered along the scale and that around 40% of AVI/AVI subjects rated quinine bitterness and pungency from capsaicin at 'strong' or even higher intensities. Studies examining responses to foods and beverages have had similar findings, showing, for example, that AVI homozygotes perceived more sourness and astringency from berries (Laaksonen et al., 2016) and more bitterness from grapefruit juice (Hayes et al., 2013). Adding further support to our findings, Fisher et al. (2014), in a study of 1258 individuals, comparable in size to the present study, noted that the non-tasters or weak tasters of PROP are not necessarily weak tasters of other taste qualities, and that AVI/AVI non-tasters were more responsive to salt, sweet, sour, and quinine bitterness than were other haplotype combinations.

Even accepting the veracity of the current data, the mechanism that underlies the higher intensity ratings among AVI homozygotes nevertheless remains to be elucidated. The existence of an additional TAS2R receptor explaining strong bitterness from PROP in some AVI homozygotes has been previously proposed (Hayes et al. 2008; Nolden et. al 2020). Similarly, we can speculate that further genetic variations in other TAS2R genes or in genes encoding taste signaling molecules (e.g. GNAT3, PLCB2, TRPM5) could explain the elevated oral perception we observed in AVI homozygotes. In this light, further research analyzing additional polymorphisms and their combined effect should be devoted to better exploring their role possibly related to increased oral acuity of AVI/AVI subjects.

In the present study, PROP status was strongly associated with heightened responsiveness to different oral stimuli thus confirming the widely accepted notion that responsiveness to PROP bitterness is a general marker of oral responsiveness (Tepper et al., 2017). However, despite research efforts aimed at elucidating the possible reasons explaining this association, the specific mechanism remains unclear.

The association of PROP responsiveness to anatomical differences in the peripheral receptor system (fungiform papillae density) has been extensively investigated based on the idea of spatial summation (the higher the number of stimulated receptors the higher the signal intensity) (Delwiche et al., 2001; Linschoten & Kroeze, 1991). A number of studies on relatively small samples clearly show positive relationships between PROP responsiveness, fungiform papillae density and the intensity of different oral stimuli, including tactile and irritant sensations (Essick et al., 2003; Tepper

& Nurse, 1998). In contrast, studies on large population samples, including results from the present work, tend to agree on the lack of direct association between responsiveness to PROP bitterness and variation in fungiform papillae density (Feeney & Hayes, 2014; Fischer et al., 2013; Garneau et al., 2014). These relationships are unlikely to be straightforward. A complex interplay between PROP responsiveness and FPD in modulating the intensity to suprathreshold tastes and somatosensory stimuli has been observed (Dinnella et al., 2018), and there is evidence of different associations between FPD and super-tasting phenotypes in different *TAS2R38* diplotypes (Hayes et al., 2008). Moreover, methodological differences in papillae counting techniques and sensory data collection might at least partially account for the inconsistent results (Piochi et al., 2018). Furthermore, FPD was found to correlate with amount and composition of saliva in response to gustatory reflex (Gardner & Carpenter, 2019). Specific changes characterize the salivary protein profiles of individuals with varied responsiveness to PROP and modulate the intensity response to astringent stimuli of medium and super tasters (Melis et al., 2017). Thus, FPD could, even indirectly, affect ~~the~~ stimulus intensities by modulating the salivary changes induced by oro-sensory stimulation.

The role of gustin, the major zinc-containing salivary protein implicated in the growth and development of taste buds, in determining the perception of oral stimuli remains unclear. The polymorphism rs2274333 (A/G) of the gustin gene modulates the protein structure affecting the zinc binding capacity and its full functionality. Studies in the Sardinian population showed that the functional AA genotype was associated with PROP ST phenotype whereas G allele was more frequent in NT (Calò et al., 2011; Padiglia et al., 2010). These findings support the hypothesis that the heightened responsiveness of ST individuals to a wide range of oral stimuli can be mediated by a larger number of fungiform papillae. In the present study, the rs2274333 polymorphism in the gustin gene was not associated with intensity responses to chemosensory stimuli, including PROP. Thus, our results support findings that failed to replicate this association (Bering et al., 2014; Feeney & Hayes, 2014). Ethnic differences may be one possible factor responsible for these differences among studies. Another possible explanation is that, in the present work and in other studies that failed to identify this relationship, there was a low percentage of GG individuals, compared to the studies on Sardinian samples (Calò et al., 2011; Padiglia et al., 2010).

Recent measures of the activation of the peripheral gustatory system by electrophysiological recordings from the tongue show robust positive association with taste intensity as a function of PROP status and papillae density (Melis et al., 2020; Sollai et al., 2017). Thus, it appears that coupling biopotential recording and sensory measures would provide further insights into mechanisms underlying the relationships of genetic variation of gustatory system functioning and intensity responses to oral stimulation.

Probably the most important contributions of the present study are the results from the regression trees, which revealed deeper insights into the links between *TAS2R38* genotype and PROP phenotype, and their impact on oral sensations. *TAS2R38* diplotype was the primary factor driving heightened chemosensory responses to all stimuli (except pungency), followed by PROP classification. When grouped by PROP status, AVI homozygotes classified as MTs or STs consistently gave higher intensity ratings to sour, salty, astringent, bitter and umami stimuli than PAV/PAV and PAV/AVI individuals regardless of their PROP classification (Figure 6 A-E). The analysis for sweet taste (Figure 6 F) showed a slightly different pattern. Here, the regression tree was also dominated by *TAS2R38* diplotypes showing a major division between AVI/AVI individuals and PAV/AVI+PAV/PAV individuals into separate nodes. However, AVI homozygotes consistently gave higher sweetness ratings regardless of their PROP status while the PAV/AVI + PAV/PAV group showed further separation into three nodes corresponding to NTs, MTs and STs.

Together, these novel findings suggest that the pathways linking *TAS2R38* genotype to oral sensations are complex and are unlikely to be explained by *TAS2R38* polymorphisms alone. This is further underscored by the results for pungency (capsaicin) perception (Figure 7) where gender was the primary determinant of heightened perception, followed by PROP status with STs rating pungency higher than MTs and NTs. *TAS2R38* diplotype affected pungency perception only in women. Specifically, a small group of women STs who were in the AVI/AVI group (node 4) gave higher ratings to capsaicin than women MTs and NTs, regardless of their *TAS2R38* diplotype (nodes 7 and 8). In men, PROP status, but not *TASR38* diplotype, was the defining feature of their perception of pungency. These findings confirm previous reports on heightened responsiveness to capsaicin in women in comparison to men that have been associated to previous exposure to spicy foods (intake/familiarity), responsiveness to PROP and personality traits (Byrnes & Hayes, 2013, 2015; Spinelli et al., 2018). They also suggest a gender dichotomy in the effects of PROP phenotypes and *TAS2R38* diplotypes on the perception of capsaicin pungency which has been reported before for astringency (Melis et al., 2017) and for other outcome variables (e.g., body mass index) (Tepper, 2008).

We can conclude from these results that multiple factors are likely to play a role in shaping the super taster phenotype defined as heightened responsiveness to oral stimuli including PROP. One relatively recent contribution to understanding responses to oral stimuli has been the “central gain” theory, which hypothesizes that differences both at peripheral and central levels can define the chemosensory responses to oral suprathreshold stimulations. Heightened excitability of brain regions where oro-sensory stimuli converge would produce a higher “gain” in the afferent system and a stronger response to a given level of suprathreshold stimulation (Green, 1993; Green & George,

2004). This theory appears a reasonable explanation for the existence of the supertaster phenotype irrespective of *TAS2R38* genotype (Nolden et al., 2020). That is, individuals with high central gain would show heightened responsiveness to a wide range of chemosensory stimuli which would include bitterness from PROP, but only for those carrying the PAV haplotype. Recent findings indicate the inhibitory role of inputs from amygdala on the gustatory network during tasting (Veldhuizen et al., 2020). This further corroborates the hypothesis that individual differences in signal central processing might contribute to individual variations in taste responsiveness. Top-down influences related to learning, attention and memory have proved to be highly significant in perceptual responses to visual and olfactory stimuli, while they are largely unexplored in their influence on tastes and other oral stimuli, thus representing a potential fruitful area of investigation (White et al., 2020). Moreover, individual differences in personality and psychological traits might modulate chemosensory responses thus helping to explain differences in intensity responses particularly to warning sensory cues such as bitterness, sourness, astringency and pungency (Laureati et al., 2018; Spinelli et al., 2018).

Strengths and Limitations

A strength of the present study was the large number of respondents and the balanced composition of the population sample in terms of age and gender. These features suggest the study sample was highly representative of the adult Italian population. Moreover, the study collected responses to a wide array of stimuli encompassing several sensory modalities including taste, touch (astringency) and chemesthesis (capsaicin).

The main weakness of the study was the collection of sensory data at a single concentration for each stimulus rather than for a range of concentrations. In the latter case, we would have been able to track concentration-dependent, individual differences in psychophysical responses that are well known to occur. On the other hand, the complexity and the length of the experimental plan prevented the testing of more than one sample per stimulus. Nevertheless, it is notable that the concentration of capsaicin was the only one eliciting on average a strong/very strong response and this was the only stimulus that produced a regression tree that was unique from the other sensations and more complex.

Conclusions

The present study confirms that PROP status was associated with the expected variation in perceived intensity of prototypical oral stimuli representing sour, salty, bitter, umami, and sweet, tastes as well as astringency and pungency. However, our findings showed that AVI homozygotes (mainly ‘non-tasters’) gave higher ratings to these stimuli than PAV homozygotes or heterozygotes.

Regression tree analysis allowed to further clarify diplotype-phenotype relationships and their complex effects on oral perceptions, showing a combined influence of TAS2R38 genotype and PROP phenotype.

These findings raise many questions in need of additional investigation on the role of the *TAS2R38* gene on taste perception. Future research should be devoted to a more in-depth genetic characterization of the AVI/AVI group to explore the possible associations with other polymorphisms and the ancient balancing evolutionary selection that maintained both PAV and AVI alleles at roughly the same frequency (Risso et al., 2016). Studies investigating the combined influence of multiple *TAS2R* genes or taste signal transduction on PROP responsiveness and other oral sensations seem warranted.

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Author Contributions

Conceptualization: A.R., C.D.; formal analysis, A.R., C.D., M.P.C.; investigation: A.R., C.D., M.P.C., S.S., L.P., P.G., E.M., T.G.T., L.T., E.P., F.G.; methodology: A.R., C.D. and M.P.C.; supervision, C.D.; visualization, L.P., M.P.C.; writing - original draft: A.R., C.D. and M.P.C.; writing, review and editing, S.S., B.J.T., P.G., J.P., E.M.; Review and editing: T.G.T., L.T., E.P., F.G. All authors read and agreed to the published version of the manuscript.

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Figure captions

Figure.1: Effects of TAS2R38 diplotype, gender and age on PROP mean ratings.
Total observation n=1117; AVI/AVI: n=288, W n= 156, M n=132; C1 n=111, C2 n=79, C3 n=98; PAV/AVI: n=557, W n= 348, M n=209, C1 n=200, C2 n=164, C3 n=193; PAV/PAV: n=272, W n=160, M n=112; C1 n=117, C2 n=70, C3 n=85.
Different letters indicate significantly different values ($p \leq 0.05$).

Figure 2: Percentage composition of *TAS2R38* diplotype groups in terms of Non Tasters (NT), Medium Tasters (MT) and Super Tasters (ST).
Total observations: n=1117; AVI/AVI: n=288, PAV/AVI: n=557; PAV/PAV n=272

Figure 3: Effect of *TAS2R38* diplotype on stimulus intensity ratings.
Saltiness, bitterness, sweetness, umami: total observations n=1117, AVI/AVI n=288, PAV/AVI=557, PAV/PAV=272; Sourness and pungency : total observations n=1112, AVI/AVI n=287, PAV/AVI n=554, PAV/PAV n=271; Astringency: total observations n=1107, AVI/AVI n=286, PAV/AVI n=551, PAV/PAV n=270
Different letters indicate significantly different values ($p \leq 0.002$).

Figure 4: Effect of PROP status on stimulus intensity ratings.
Saltiness, bitterness, sweetness, umami: total observations n=1117, NT n=268, MT n=489, ST n=360; Sourness: total observations n=1112, NT n=267, MT n=488; ST n=357; Astringency: total observations n=1107, NT n=265, MT n=486; ST n=356; Pungency: total observations n=1112, NT n=266, MT n=487; ST n=359.
Different letters indicate significantly different values ($p \leq 0.002$).

Figure 5: Distribution of fungiform papillae density values (FPD). Red line represents the best fitting normal distribution
Total observations: n=1117

Figure 6a: Partitioning analysis for the responsiveness to sourness form citric acid (n=1112). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 3-6 <0.001; nodes 3-7 <0.001; nodes 4-6 NS; nodes 4-7 <0.001.

Figure 6b: Partitioning analysis for the responsiveness to saltiness from NaCl (n=1117). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 3-6 <0.001; nodes 3-7 <0.001; nodes 4-6 NS; nodes 4-7 <0.001.

Figure 6c: Partitioning analysis for the responsiveness to bitterness from caffeine (n=1117). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 3-5: <0.01; nodes 3-6 <0.001; nodes 3-8 <0.001; nodes 3-9 <0.001; nodes 5-8 <0.05; nodes 5-9 <0.001; nodes 6-8 NS; nodes 6-9 <0.001

Figure 6d. Partitioning analysis for the responsiveness to sweetness from sucrose (n=1117). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 2-6: <0.01; nodes 2-7 <0.001; nodes 4-6 <0.001; nodes 4-7 <0.001

Figure 6e: Partitioning analysis for the responsiveness to umami from MGS (n=1117). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 2-4 NS; nodes 2-5 <0.001

Figure 6f: Partitioning analysis for the responsiveness to astringency from alum sulphate (n=1107). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 3-6 <0.001; nodes 3-7 <0.001; nodes 4-6 NS; nodes 4-7 <0.001.

Figure 7: Partitioning analysis for the responsiveness to pungency from capsaicin (n=1112). Mean and standard deviation for each node are reported. p-values at Wilcoxon test, used to compare the subgroups indicated by nodes, are: nodes 4-7 NS; nodes 4-8 <0.001; nodes 4-10 <0.001; nodes 4-11 <0.001; nodes 5-7 NS; nodes 5-8 NS; nodes 5-10 NS; nodes 5-11 <0.001; nodes 7-10 <0.01; nodes 7-11 <0.001; nodes 8-10 NS; nodes 8-11 <0.01

Author Contributions

Conceptualization: A.R., C.D.; formal analysis, A.R., C.D., M.P.C.; investigation: A.R., C.D., M.P.C., S.S., L.P., P.G., E.M., T.G.T., L.T., E.P., F.G.; methodology: A.R., C.D. and M.P.C.; supervision, C.D.; visualization, L.P., M.P.C.; writing - original draft: A.R., C.D. and M.P.C.; writing,

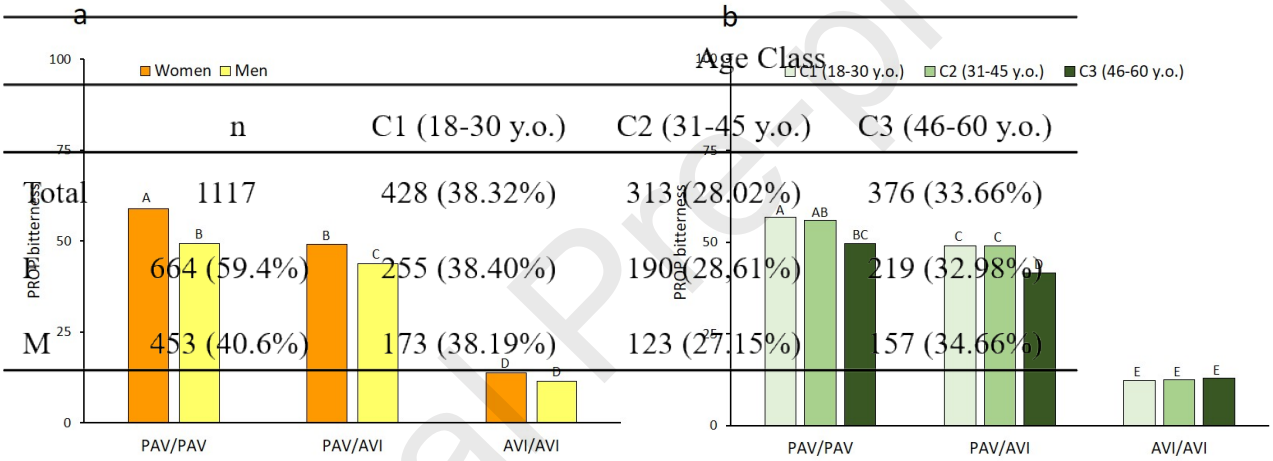
review and editing, S.S., B.J.T., P.G., J.P., E.M.; Review and editing: T.G.T., L.T., E.P., F.G. All authors read and agreed to the published version of the manuscript.

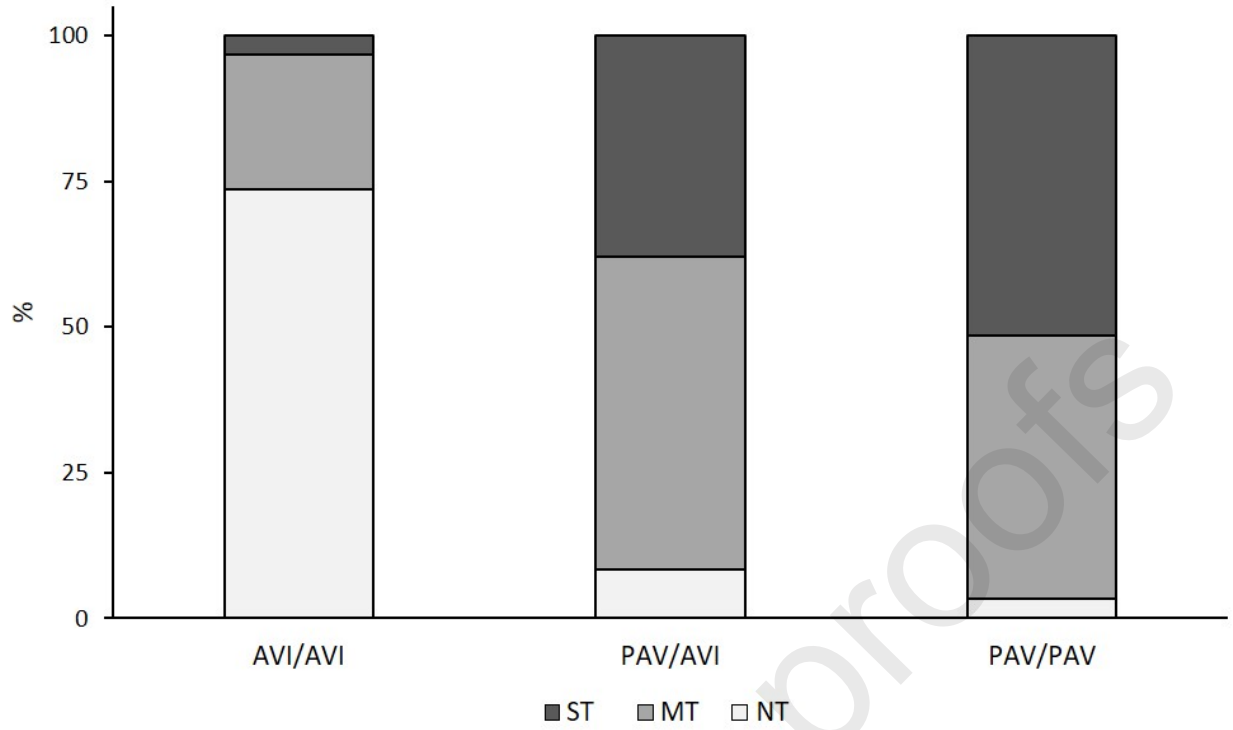
Highlights

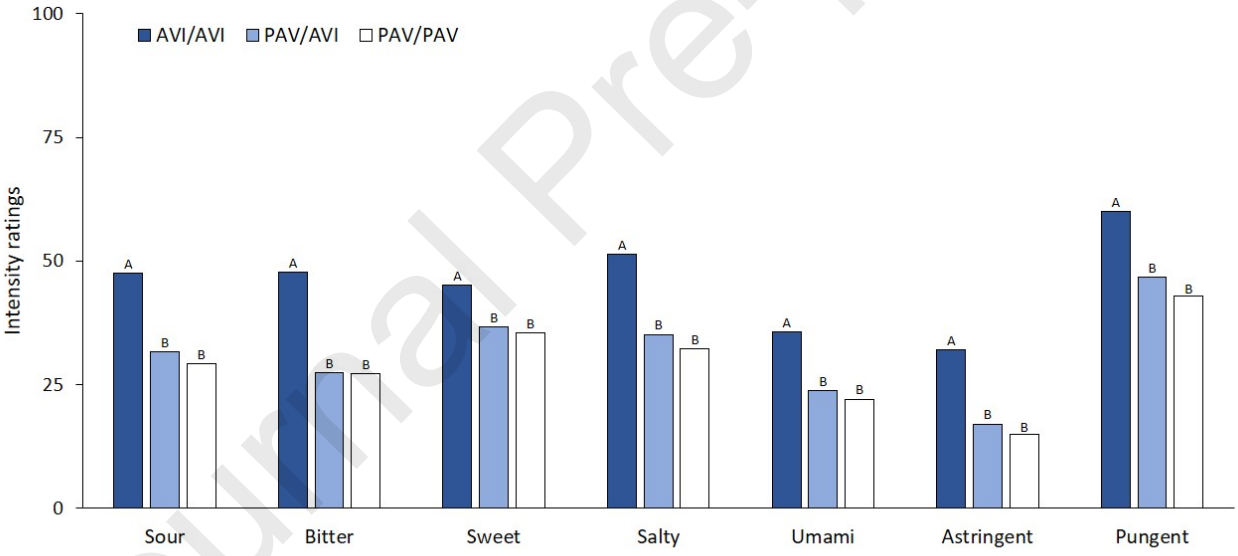
- *TAS2R38* genotype differently associates with intensity ratings of PROP and tastants
- Response to PROP is higher in PAV/PAV and PAV/AVI than in AVI/AVI individuals
- Response to tastants is higher in AVI/AVI than in PAV/AVI and PAV/PAV individuals
- TAS2R38 is the primary determinant of intensity of tastes and astringency
- Gender is the primary determinant of intensity of pungency followed by PROP status

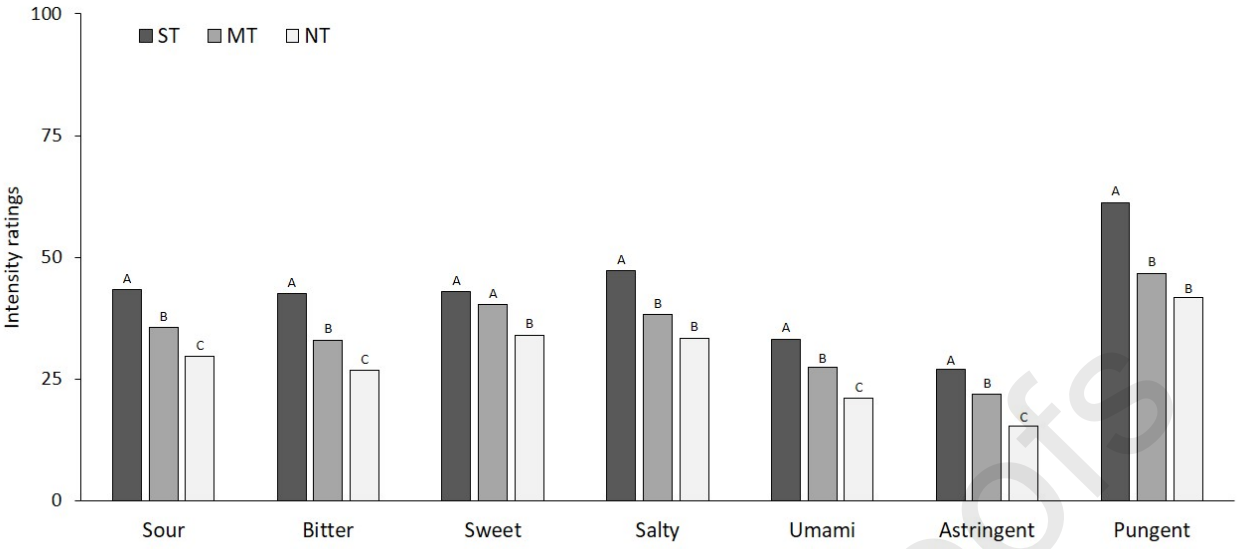
Table 1. Demographic characteristics of respondents

	n	C1 (18-30 y.o.)	C2 (31-45 y.o.)	C3 (46-60 y.o.)
Total	1117	428 (38.32%)	313 (28.02%)	376 (33.66%)
F	664 (59.4%)	255 (38.40%)	190 (28.61%)	219 (32.98%)
M	453 (40.6%)	173 (38.19%)	123 (27.15%)	157 (34.66%)









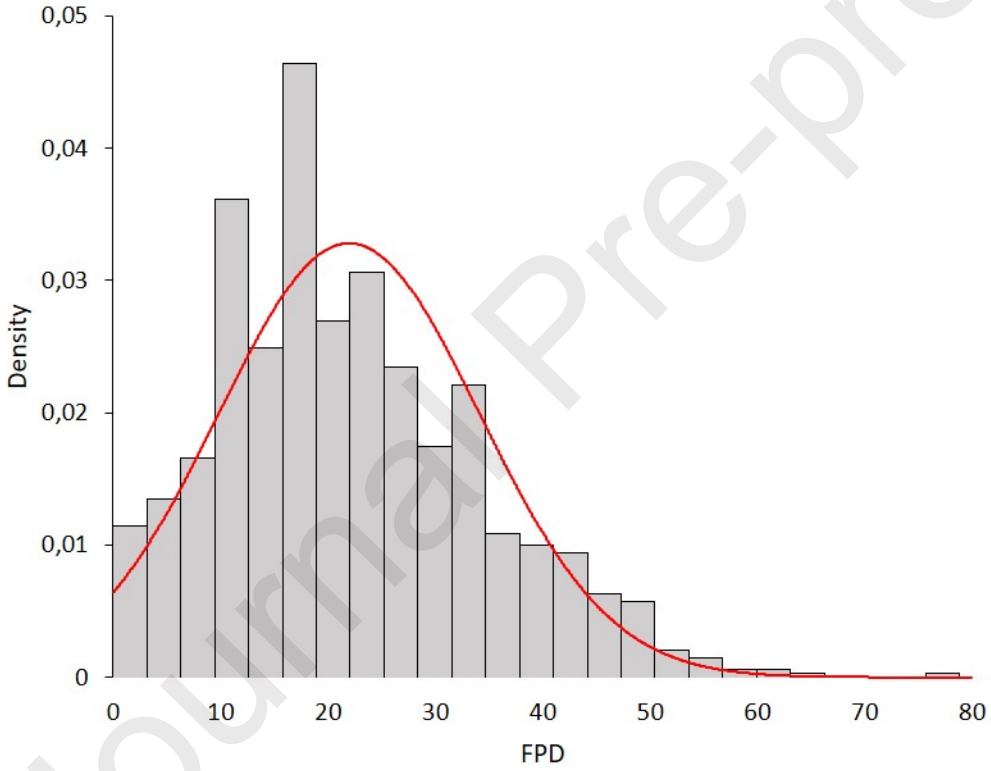


Figure 6a

Sour

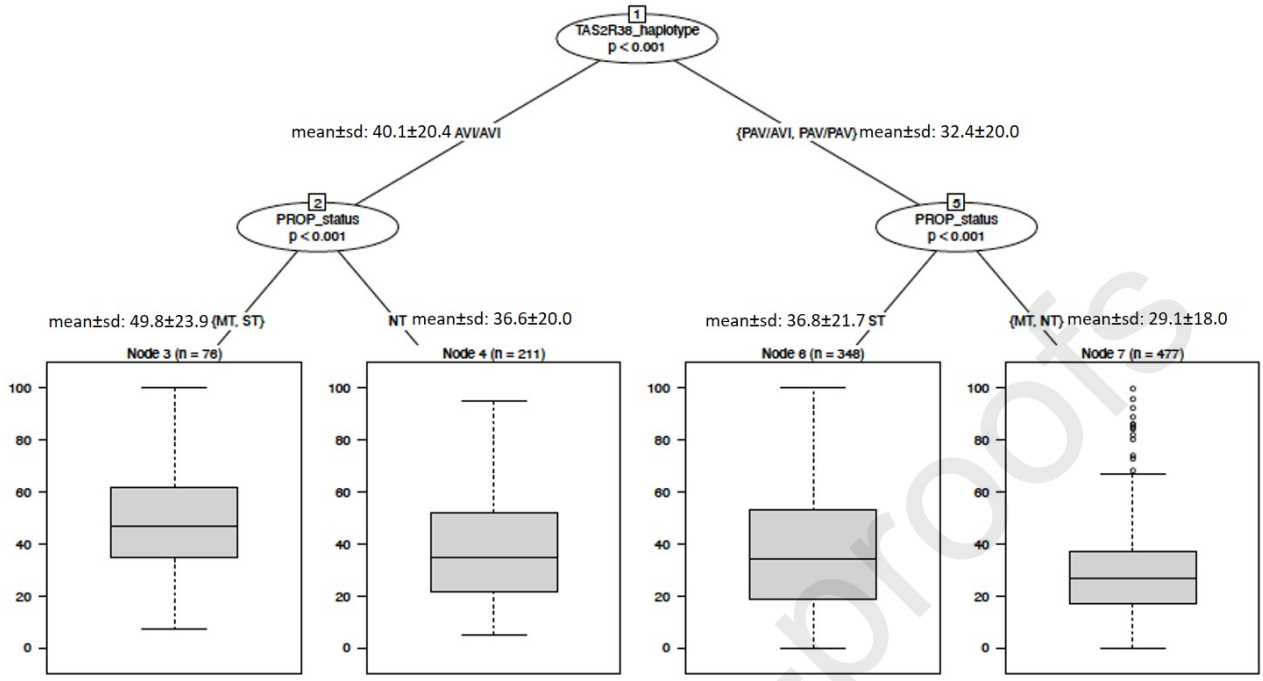


Figure 6b

Salty

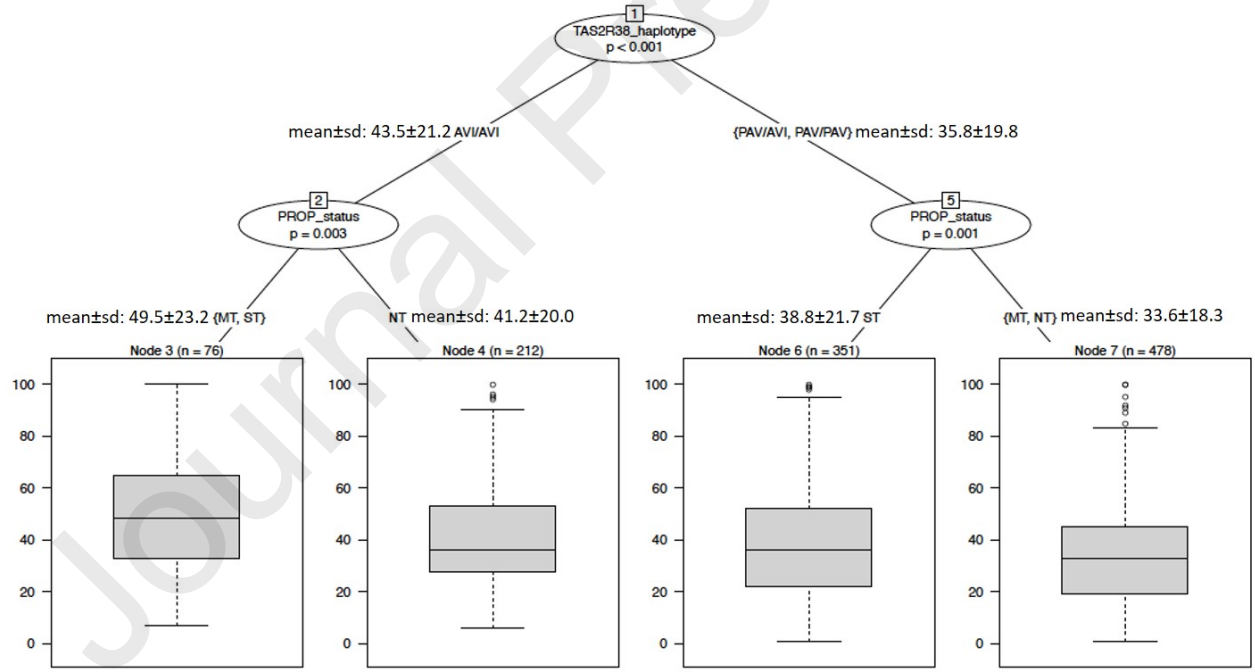


Figure 6c

Bitter

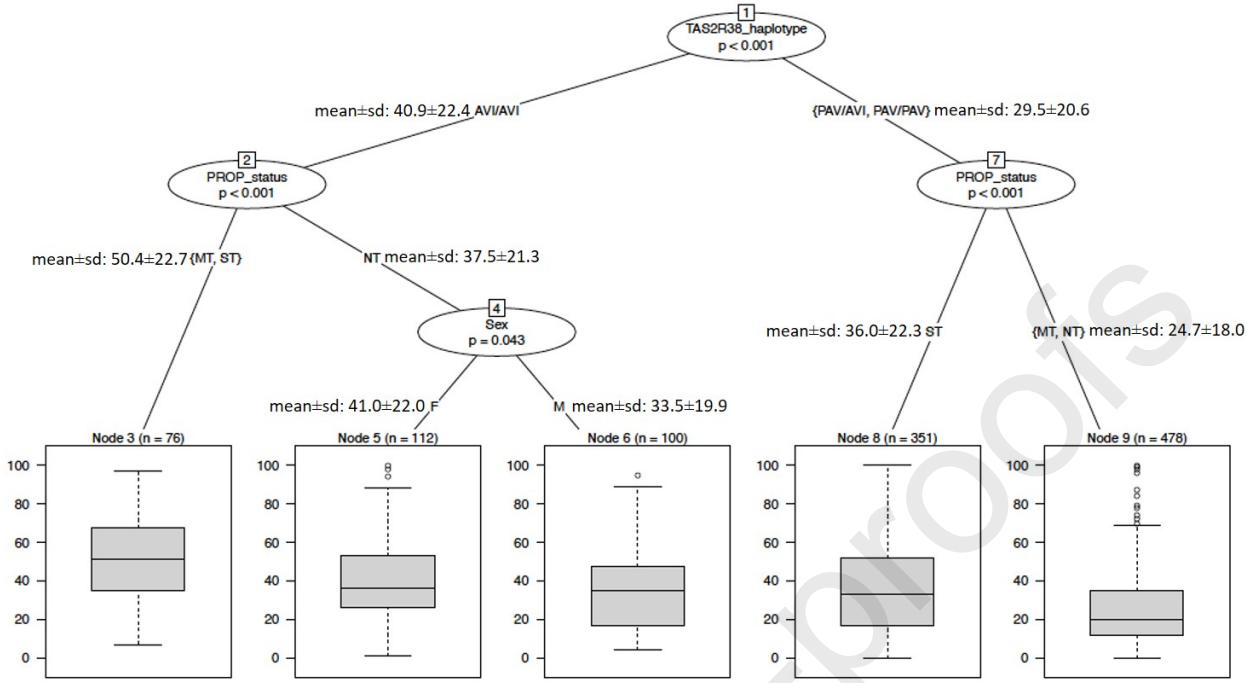


Figure 6d

Sweet

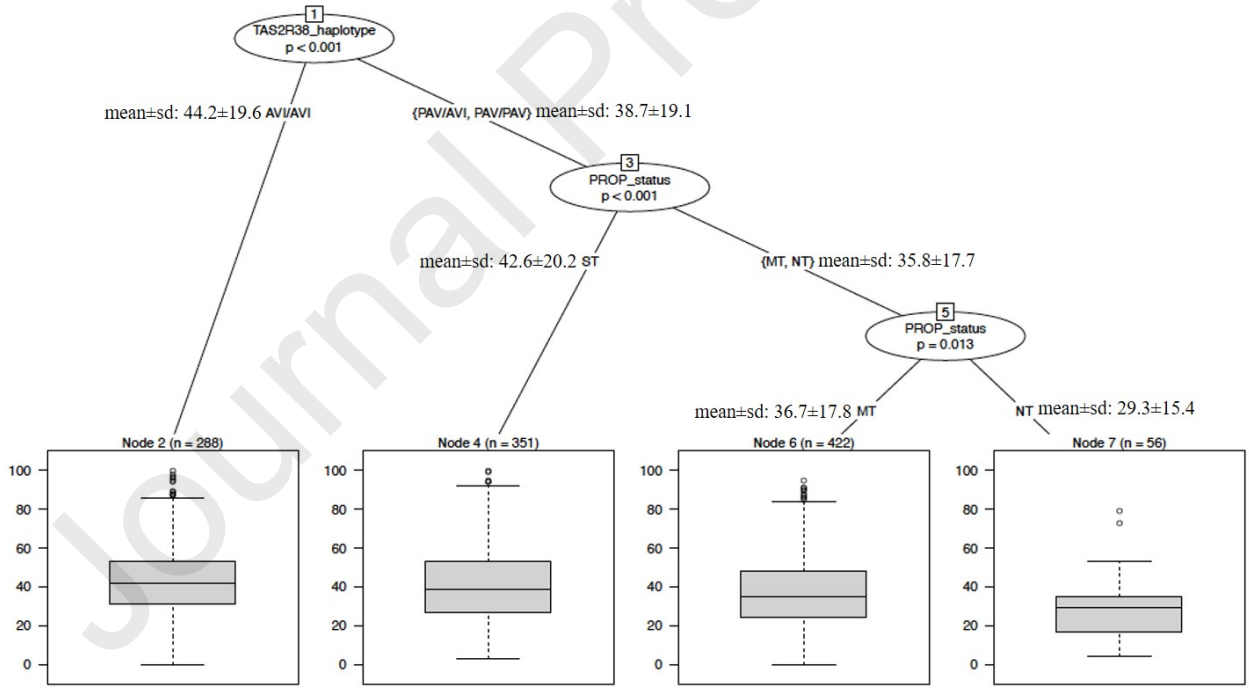


Figure 6e Umami

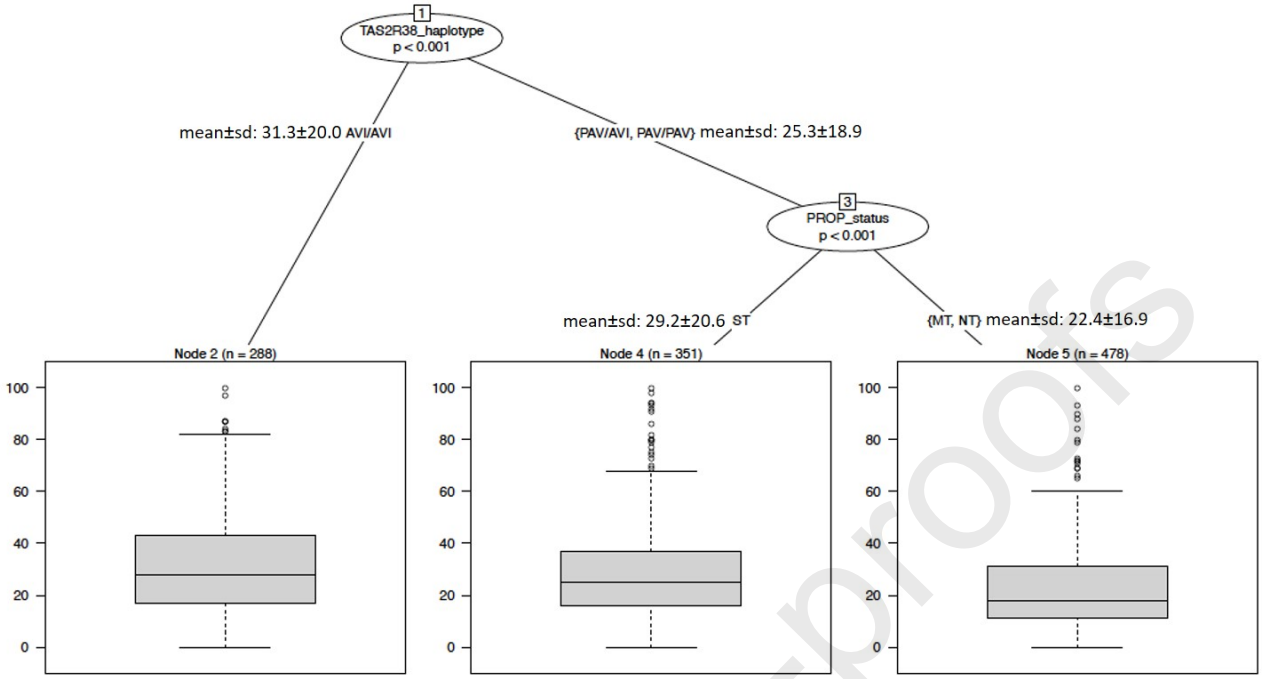
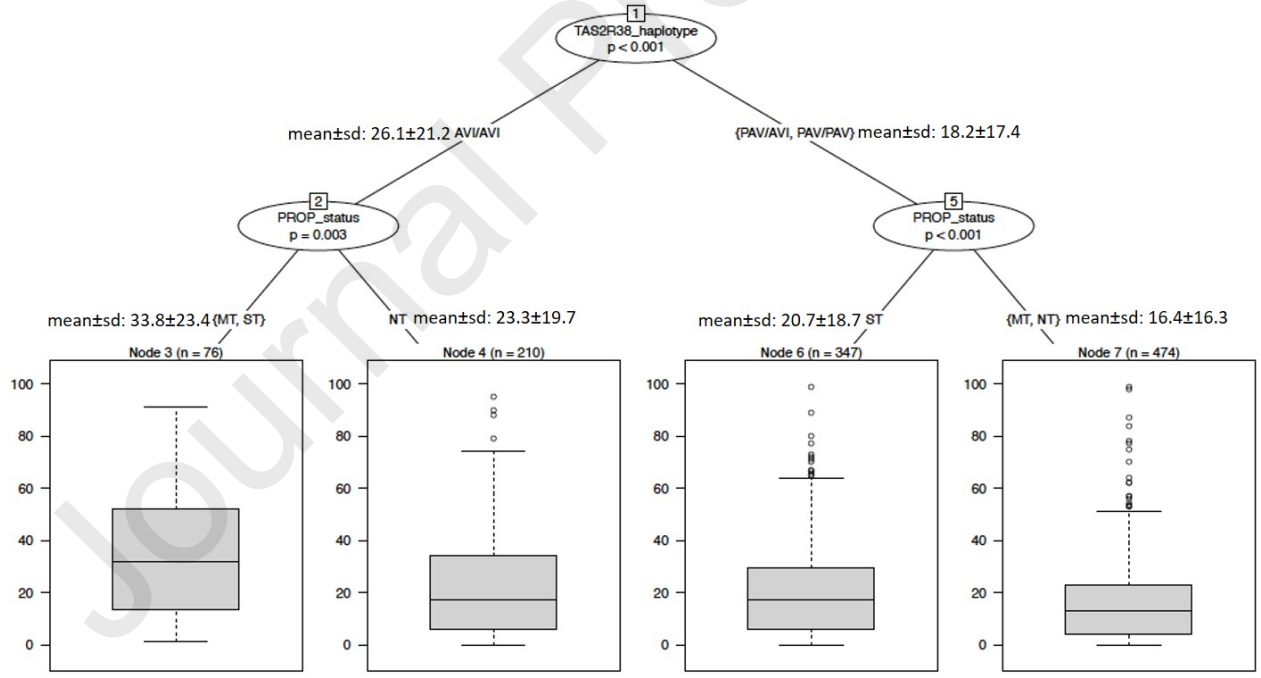


Figure 6f Astringent



Pungent