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Variant in a common odorant-binding protein gene is associated with bitter sensitivity in people

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Abstract

Deeper understanding of signaling mechanisms underlying bitterness perception in people is essential for designing novel and effective bitter blockers, which could enhance nutrition and compliance with orally administered bitter-tasting drugs. Here we show that variability in a human odorant-binding protein gene, OBPIIa, associates with individual differences in bitterness perception of fat (oleic acid) and of a prototypical bitter stimulus, 6-n-propylthiouracil (PROP), suggesting a novel olfactory role in the modulation of bitterness sensitivity.

Keywords

Bitter taste; olfaction; smell; olfactory binding protein; individual differences; PROP

The ability to taste bitterness likely evolved to serve a critical function for survival allowing animals, humans included, to discriminate safe from potentially harmful foods. Because of its innate negative hedonic value, bitterness perception deters feeding, and differences in bitter taste sensitivity influence human dietary behavior. Many healthy foods and medicines have a bitter taste, presenting a serious challenge for the food and pharmaceutical industries. Efforts to reduce bitterness perception, to date, involve mainly the gustatory system, such as the addition of sweet or salty compounds and the use of antagonist or inverse agonist of bitter taste receptors (T2Rs) [1]. There are 25 human *T2R* genes with many associated polymorphisms and a wide range of individual differences in bitter sensitivity. This helps explain why thousands of chemically dissimilar compounds can elicit a single taste quality and contributes to the elusiveness of finding a universal bitter-blocker. Among the best-

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studied individual differences in taste perception, is the inherited ability to taste (or not) compounds containing an –N-C=S group, such as phenylthiocarbamide (PTC) and PROP. Around 1930, Arthur Fox serendipitously discovered that PTC concentrations that tasted intensely bitter for some people were virtually tasteless for others [2]. More recently, we learned that most of this phenotypic disparity is explained by genetic variants in one bitter gene, TAS2R38 [3]. Akin to the bimodal distribution of PROP sensitivity, we serendipitously discovered, while studying fat sensory perception, that ~ 45% of subjects (19 out of 42) perceived bitterness in milkshakes containing very dilute concentrations of oleic acid (Fig. 1a), and that bitter perception was dramatically reduced when subjects tasted the milkshakes wearing noseclips. Thus, eliminating retronasally perceived odorants remarkably blunted bitter “taste” perception of oleic acid, suggesting a critical role of olfactory stimulation in the process (Fig. 1b) (for experimental details, see below Materials and Methods). While retronasal odors can enhance taste intensity [4], it is unknown why some people would perceive bitterness from oleic acid odor whereas others would not.

Odorants, like fats and many bitter compounds, are hydrophobic and rely on protein binding to effectively travel hydrophilic mediums, such as nasal mucus or saliva, to reach their receptors [5]. We hypothesized that genetic variation(s) in an olfactory binding protein (OBPIIa) highly expressed in the olfactory cleft and with high affinity for long chain fatty acids [6] might help explain our observed bitter “taste” phenotype. To test this, we genotyped subjects for a common single nucleotide polymorphism (rs2590498) in the OBPIIa gene and reanalyzed their sensory data. We found that subjects who were homozygous for the rs2590498 A-allele, but not the G-allele or heterozygous, perceived bitterness in milkshakes containing oleic acid when noseclips were omitted (Fig. 1c), but not when they were included (Fig. 1d). For validation of our observation that olfactory binding proteins could affect bitterness perception, we tested a new cohort of 96 participants. Here we evaluated whether *OBPIIa* gene variation could explain differences in PROP bitterness sensitivity independently of the *TAS2R38* genotype. Indeed, we found that subjects homozygous for the *rs2590498* A-allele perceived PROP as more bitter than those homozygous for the G-allele in each *TAS2R38* genotype groups (Figs. 1e–f and Table 1). Finally, as proof of concept, we investigated the response of cultured human olfactory cells [7] to PROP and oleic acid by measuring changes in intracellular calcium. Both oleic acid and PROP elicited increases of intracellular calcium (Fig. 2). Out of 148 cells, 54.8% responded to oleic acid, 10.8% to PROP and 7.4% to eugenol, an odorant stimulus used as control.

Our results suggest a novel olfactory role in the modulation of bitter taste sensitivity that might have physiological implications. Involvement of olfaction in bitterness perception suggests that the age-related declines in olfactory sensitivity [8] and those in perception of PROP [9] and other bitter compounds [10] are likely to be related. Furthermore, bitter receptors expressed in the nose and upper airways contribute to epithelial innate defense by helping detect invading pathogens and toxins [11]. A better understanding of the signaling mechanisms underlying bitterness perception could have important therapeutic value not only for ingestive behavior, but also for respiratory immune responses [12].

Materials and Methods

1. Subjects

1.1 Study with oleic acid—Forty-two adult volunteers (34.2 (SD 9.1) years of age, 27.3 (SD 6.9) kg/m^2, 38 females, 48% Caucasian, 38% African American, 14% Asian) participated in the fat sensory study and provided authorization to use their blood for genetic studies of flavor related genes. At recruitment, subjects were selected based on their rs1761667 (A/G) CD36 genotype (22 subjects were homozygous for the rs1761667-A allele and 20 subjects were homozygous for the rs1761667-G allele, which associates with lower and higher CD36 expression, respectively). Subjects who smoked cigarettes in the last 6 months, had chronic sinus problems, previous malabsorptive or restrictive intestinal surgery, or diabetes or who were pregnant, breastfeeding or taking any medication that might affect taste perception were excluded. All procedures were approved by the Human Research Protection Office at the University of Washington University in St. Louis and each subject gave informed written consent before participation.

1.2 Study with PROP—Ninety-six non-smoking, Caucasian healthy, young subjects (27.2 (SD 6.5) years of age, 22.0 (SD 2.3) kg/m^2, 55 females) from Sardinia, Italy, participated in the PROP study. Potential subjects who were following weight-loss diets or were taking medications that might affect taste perception were excluded. All procedures were approved by the Ethical Committee of the University Hospital of Cagliari in Italy and each subject gave informed written consent before participation.

2. Genotyping

We genotyped subjects for the OBPIIa gene polymorphism rs2590498 (A/G) by using a custom TaqMan® SNP Genotyping Assay (Step One; Life Technologies, in USA and Applied Biosystems by Life-Technologies Italia, Europe BV) and ABI 7000 sequence detector system (Applied Biosystems) according to manufacturers' instructions. The following primers set were used: Forward PCR Primer 5′-GCCAGGCAGGGACAGA-3′ and Reverse PCR primer 5′-CTACACCTGAGACCCCACAAG-3′ and two TaqMan probes were designed according to the OBPIIa gene (bold and underlined), probe/reporter 1: VIC-TCGGTGAC**A**TGAACC and probe/reporter 2: FAM–TCGGTGAC**G**TGAACC. Following PCR, the resulting fluorescence of plates was read (60 °C for 1 min) by the sequence detector system, and the results analyzed by allelic discrimination of the sequence detector software (Applied Biosystems). Replicates and positive and negative controls were included in all reactions.

In addition, subjects in the PROP study were genotyped for three single nucleotide polymorphisms (SNPs) at base pairs 145 (C/G), 785 (C/T), and 886 (G/A) of the TAS2R38 locus by using previously described methods [13]. The three SNPs of TAS2R38 give rise to 3 non-synonymous coding exchanges (proline to alanine at residue 49, alanine to valine at residue 262 and valine to isoleucine at residue 296) and result in two major haplotypes, PAV, the dominant taster variant and AVI, the recessive non-taste variant. Three additional subjects with rare haplotypes (AAI, AAV and PVI) were excluded.

3. Sensory evaluation of genotyped subjects

3.1 Study with oleic acid—Before testing, and after an overnight fast, each subject was trained in the use of the general Labeled Magnitude Scale (gLMS), [14] with the top of the scale described as the “strongest imaginable” sensation of any kind. The gLMS is a computerized psychophysical tool that requires subjects to rate the perceived intensity along a vertical axis lined with adjectives that are spaced semilogarithmically, based upon experimentally determined intervals to yield ratio-quality data. To determine the role of retronasal volatiles in overall flavor perception, subjects were evaluated both without and with noseclips (which eliminated retronasal perceived volatiles) on two separate days as previously described [15]. The test sessions were performed in the Clinical Research Unit. Sample presentation order and condition (with or without noseclips first) was randomized using a computer program. Subjects tasted each sample for five seconds and without swallowing, used the gLMS to rate the intensity of creaminess, sweetness, bitterness, and sourness. Each subject evaluated all the samples in duplicated over each testing session. Nine- out of 42 subjects did not complete the visit where they tasted milkshakes while wearing noseclips.

Preparation of oleic acid emulsions. The procedure to prepare emulsions followed Chalé-Rush et al 2007 [16] with some modifications. Food grade oleic acid (Sigma Aldrich, St Louis, MO) were stored in opaque bottles below 4°C. Oleic acid was added at two concentrations (0.045%w/v and 0.090 %w/v) to skim milk containing 8% (w/v) sucrose and 2 drops of vanilla. In addition, all preparations were mixed with 5% (w/v) Gum Arabica (AEP Colloids, Hadley, NY) and white food colorant to produce perceptually identical viscosity and color between oil and control samples. All samples were sonicated for 6–9 min using a Branson 250 digital sonicator (Branson Ultrasonic Corporation, Danbury, CT) at 50% power with 30 second on, 60 second off. An ice bath was used during sonication to control for temperature. Samples were stored in opaque polypropylene cylinders and used for testing within 48 hours of preparation. Control samples were prepared in the same way, but without added oil.

3.2 Study with PROP—Subjects were requested to refrain from eating, drinking (except water), and using oral care products or chewing gum for at least 8 h prior to testing. Taste intensity ratings for a single suprathreshold PROP (50 mM) solution were collected using paper disks impregnated with PROP (50 mmol/l). Briefly, subjects were instructed to rinse with spring water at room temperature before tasting the paper disk. Each subject was asked to place the paper disk with the taste stimulus on the tip of the tongue for 30 seconds or until the disk was thoroughly wet with saliva, and then spit it out. After tasting each sample, the subject placed a mark on the LMS scale corresponding to his/her perception of the stimulus [13].

The research team conducting all sensory testing was blinded to subject’s genotype.

4. Primary cultures of human olfactory epithelial cells

Human olfactory cells were derived from healthy, adult subjects as previously described [17]. Briefly, biopsy tissue was cut into small pieces and incubated in HBSS w/o Ca+2 and

Mg+2 for 20 min, then the suspension triturated in fire-polished glass pipettes to further break up large pieces. The dissociated tissue was centrifuged at 600 g for 5 min with slow acceleration and deceleration. The pellet was suspended in Iscove's culture medium supplemented with 10% FBS and penicillin/streptomycin, and transferred to a 25 cm^2 culture flask. The flask was incubated in humidified 5% CO2 at 37 °C and allowed to grow for 1 – 2 weeks until cell growth was sufficient for transfer to continuous culture. Cells were maintained in 75 cm^2 culture flasks and grown to confluence (about 4 wk). Cultured olfactory cells were plated and grown on untreated sterile glass coverslips for Ca+2 imaging.

5. Single cell calcium imaging

Cultured human olfactory cells were seeded on 15 mm coverslips and then reached 70–80 % confluence, cells were loaded with the calcium sensitive dye Fura-2AM by incubating the cells in Ringer's solution (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ 1 mM $CaCl_2$,1 mM Na-pyruvate, and 20 mM Hepes-Na, pH 7.2) supplemented with 1 mM Fura-2 AM (Molecular Probes Inc. Eugene, OR) and 10 mg/ml Pluronic F127 (Molecular Probes Inc.) for 60 min at 37°C. The cells were exposed to stimuli by switching the superfusion to stimuli solutions, which allowed for a complete change of bath solutions in the chamber within 20 s. PROP (50 mM) was dissolved in Ringer's solution, and pH and osmolarity were readjusted when needed. Oleic acid was dissolved in DMSO as 1000X concentration and diluted to 3.2 mM concentration in Ringer's solution. Eugenol was of the highest purity available (98% pure) (Sigma, St. Louis, MO). Eugenol was made up at 1 M in DMSO stocks and diluted to a final concentration of 500 μM in Ringer's solution. Stimuli were bath applied for 60 s using a peristaltic pump-controlled perfusion system. Each stimulus application was followed by an approximately 2 min wash out period with Ringer's solution.

Calcium imaging recordings were performed using standard imaging techniques. Illumination was via an LSR SpectraMASTER monochromator coupled to the microscope. Cells were illuminated with light emitted by a 75 W Xenon lamp alternately filtered with narrow bandpass filters at 340 nm and 380 nm. The light emitted from the Fura-2 AM in the cells under 200× microscopic magnification was filtered at 510 nm and passed through an image intensifier coupled with a cooled CCD camera (Olympix, Perkin Elmer Life Sciences, Bethesda MD). Exposure times were minimized and the light was shuttered between acquisitions to minimize photobleaching. Cells remained viable in the recording setup without visible effects of dye bleaching. Ratio data for the cells were subsequently analyzed in Excel to determine which cells had responded with a significant change in intracellular calcium.

6. Statistical Analysis

The statistical significance of values between groups and conditions was evaluated by using general linear mixed models (PROC MIXED) and factorial ANOVAs. Significant interactions were further analyzed using Fisher least significance difference tests. Summary residuals and fit statistics were examined for marginal and conditional raw and standardized residuals. Convergence criteria for the repeated measures analysis were met. In addition, a quantitative analysis of the contribution of TAS2R38 A quantitative analysis of the contribution of TAS2R38 and OBPIIa gene loci to PROP (50mM) bitterness was performed

using the Generalized Linear Model (GLM) method. This method provides regression analysis and analysis of variance for one dependent variable by one or more independent variables. The analysis was carry out through a backward stepwise procedure, starting with the full model, including independent variables and second order interactions between them, and deleting step by step non-significant interactions and variables. The interaction between the two genes was included in the model, and the different effects between the homozygous and the heterozygous condition were analyzed for both genes. The Partial Eta Squared represents the strength of the association of each independent variable (genotypes of TAS2R38 and OBPIIa gene loci) with the dependent variable (PROP bitterness), after the effects of all other independent variables were accounted for. The GLM procedure was used to estimate the expected bitterness mean value for each combination of TAS2R38 and OBPIIa gene genotypes. The degree of overlap between the expected and the observed mean values defined to what extent PROP bitterness is explained by the two loci. The global R squared offers a measure of their contribution (see Supplementary table). All analyses were two-tailed tests, performed with SAS (9.4) and STATISTICA 8.0 (StatSoft, Tulsa OK), and criterion for statistical significance was $P < 0.05$. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those generally employed in the field.

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ABBREVIATIONS

PROP	6-n-propylthiouracil
T2Rs	bitter taste receptors
PTC	phenylthiocarbamide
OBPIIa	olfactory binding protein
gLMS	Labeled Magnitude Scale

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Highlights

- We show that olfaction plays an important role on people's bitter "taste" sensitivity
- Variability in an odorant-binding protein gene associates with oleic acid bitterness
- Variability in an odorant-binding protein gene associates with PROP bitterness

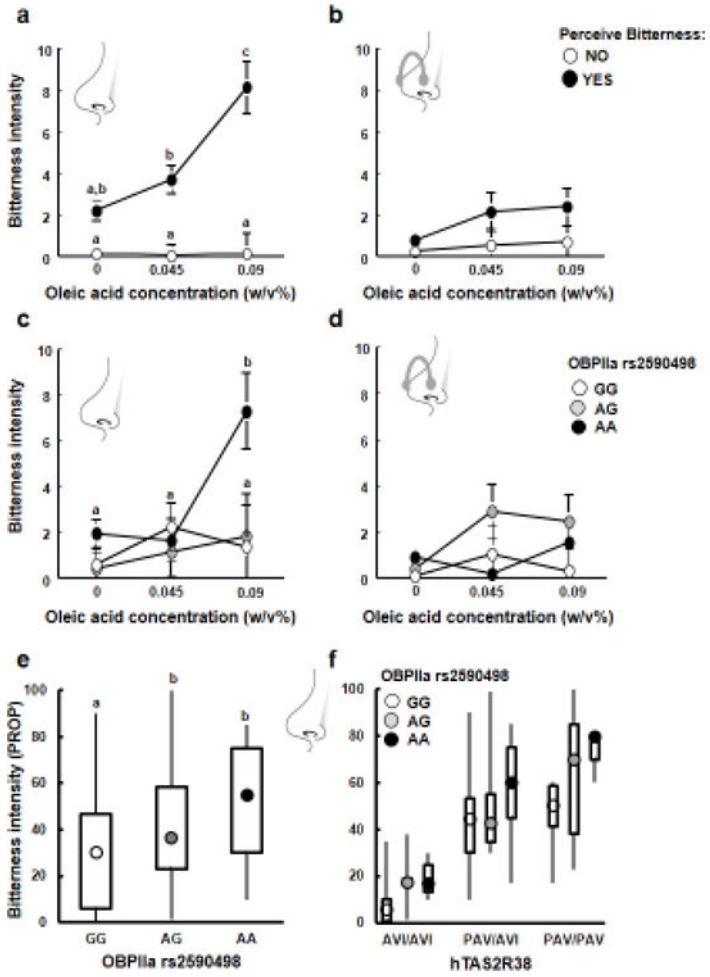

Figure 1. Retronasal odors affect bitterness perception and a common SNP (rs 2590498) in the human odorant-binding protein gene (OBPIIa) associates with variability in bitter taste perception of oleic acid and 6-n-propylthiouracil (PROP)

(**a–d**) Bitterness intensity at various oleic acid concentrations in milkshakes (0–0.090% w/v). (**a–b**) General linear mixed models (PROC MIXED) including subject (n=42) as a random effect show that bitterness increased as oleic acid concentration increased for subjects perceiving bitterness when noseclips were omitted (**a**) [F(2,80)= 6.81, P=0.0019], but not when included, which eliminates retronasally perceived odors (**b**), all P' values>0.15 (8 subjects, three who perceived bitterness, did not complete testing wearing noseclips). (**b–c**) The OBPIIa rs2590498 (A/G) genotype associated with bitterness ratings when noseclips were omitted (**c**) [F(4,77)= 3.02, P= 0.023; n=16 AA,13 AG, and 13 GG], but not when included (**d**), all P'values>0.19 (2 AA, 2 AG, and 4 GG subjects did not completed testing wearing noseclips). (**e–f**) Bitterness of a paper disk impregnated with 50 mM PROP (n=96). (**e**)Two-way ANOVA shows that variations in both genes independently associated with bitterness ratings (OBPIIa: F(2,87)= 5.10; n=21 AA, 30 AG, and 45 GG; TAS2R38: F(2,87)=37.73; n= 18 PAV/PAV, 45 PAV/AVI, and 33 AVI/AVI; both P' values <0.01), rs2590498 allele A (OBPIIa) associated with increased perception of bitterness at all TAS2R38 genotypes (**f**). Values (**a–d**) are means±SEM. The box-and-whisker plots (**e–f**)

show minimum, first quartile, median, third quartile, and maximum of each set of data. Values that do not share a subscript differ by LSD post-hoc testing.

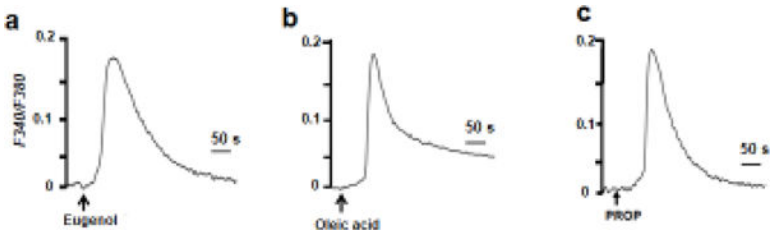

Figure 2. Human olfactory cells respond to oleic acid, PROP, and eugenol (odorant control)

Changes in intracellular calcium concentrations [Ca^{2+}] were measured at least in duplicated in 96-well plates using fura-2AM. Arrows indicate time of stimulus delivery (**a**) oleic acid: 3.2 mM, (**b**) PROP:50mM, (**c**) eugenol:500uM. Graphs illustrate representative changes in $[Ca^{2+}]_I$ concentrations in individual cells (148 cells included).

Table 1

PROP bitterness intensity according to *TAS2R38* and *OBPIIa* gene genotype

	PROP bitterness	
	Obs	**Exp**
Genotype		
PAV/PAV-AA	73.33 ± 10.87	64.73
PAV/PAV-AG	63.15 ± 6.28	59.27
PAV/PAV-GG	46.20 ± 7.69	49.55
PAV/AVI-AA	58.47 ± 5.22	59.07
PAV/AVI-AG	50.24 ± 5.44	53.60
PAV/AVI-GG	43.96 ± 4.21	43.88
AVI/AVI-AA	19.08 ± 10.28	22.70
AVI/AVI-AG	17.20 ± 6.28	17.24
AVI/AVI-GG	8.48 ± 4.32	7.51
Global R Squared	0.548	

Mean values of bitterness intensity of PROP (50 mM) observed (Obs) in the trials (± SEM) and expected (Exp) through General Lineal Model analysis (n = 96). The model showed that having the allele A in the OBPIIa gene increased bitterness intensity in each TAS2R38 group; the progression across groups was nearly perfect.