

Updating the Taste Test for the A& P Laboratory

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Biography

Karen A. McMahon is an instructor at The University of Tulsa where she has taught the introductory Human Anatomy & Physiology course for the last 10 years. She is indebted to the many students who have enthusiastically swabbed tongues blue and suffered other indignities in the pursuit of an education in science.

Introduction

Fox (1931) discovered that phenylthiocarbamide (PTC) was extremely bitter to certain individuals but completely tasteless to others. Additional studies identified individuals in all age, gender, and ethnic groups as either tasters (approximately 75%) or nontasters (approximately 25%). It was hypothesized that the ability to taste PTC was due to the presence of at least one dominant allele and the pattern of inheritance followed Mendelian genetics. Later researchers (Bartoshuk *et al.* 1994) observed that not all tasters were alike. Some tasters reacted more strongly and characterized PTC as very bitter. It was hypothesized that the homozygous dominant TT genotype characterized supertasters and the heterozygous Tt correlated with medium tasters. Many researchers have now abandoned PTC in taste experiments because it emits a detectable sulfurous odor and there were concerns about its toxicity. PROP (6-*n*-propylthiouracil) is chemically similar to PTC and is now the standard for in research on the discrimination of bitter taste.

In 2003, Kim *et al.* located and sequenced the *TAS2R38* or *PTC* gene on chromosome 7 responsible for the PTC reaction. This gene encodes for one of the estimated 25 bitter taste receptor proteins present in taste buds. Three common SNPS (single nucleotide polymorphisms) resulting in three amino acid substitutions have been identified in the *TAS2R38* gene and account for five different haplotypes found in human populations. The two most common are PAV (proline-alanine-valine) identified as the major taster haplotype and AVI (alanine-valine-isoleucine) as the major nontaster haplotype. Individuals with two copies of the AVI haplotype are largely nontasters whereas either one or two copies of the PAV haplotype were mostly tasters. PAV homozygotes are more sensitive to PTC/PROP than PAV/AVI heterozygotes (Kim and Drayna 2004, Minella *et al.* 2005, Reed *et al.* 2006).

Miller and Reedy (1990) developed a method using methylene blue solution to stain the anterior tongue. Filiform papillae which do not contain taste buds stained a deep blue whereas fungiform papillae which have taste buds stained lightly and could be counted against the dark blue background of filiform papillae. They discovered that there were variations in both the number of fungiform papillae and the number of taste buds on the papillae among test subjects, and suggested that these differences might account for the observed variations in taste sensitivity among individuals. Several studies (Bartoshuk *et al.* 1994, Delwiche *et al.* 2001) confirmed that the perceived bitterness of PROP tended to increase with the density of fungiform papillae.

In 1991, the National Cancer Institute launched the 5-A -Day-Program to encourage people to eat 5-9 servings of fruits and vegetables daily to promote consumption of phytochemicals as a dietary strategy for disease prevention. For most, taste is the main determinant in food selection and perceived bitterness in a food is often the primary reason for its rejection. Many phytochemicals, such as the flavonoid naringin in grapefruit juice and glucosinolates in cruciferous vegetables (broccoli, cabbage, kale, etc.) are bitter-tasting. Several studies reported that supertasters showed a tendency to avoid certain foods which they perceive as very bitter (Drewnowski *et al.* 1997, Dinehart *et al.* 2006). The consequences of diet choice to health may be significant. A study of men over 65 who had been identified as supertasters had a significantly higher number of colon polyps, a finding which is associated with a higher risk of colon cancer (Milius, 2003). The supertasters reported that they avoided strong vegetable tastes. The diet of a supertaster appeared to be deficient in both

protective phytochemicals and fiber which led to the higher formation of polyyps, raising the risk of colon cancer.

This study will investigate if taste reaction to unsweetened grapefruit juice can predict PROP taster status and the density of fungiform papillae. It will also examine how identified PROP taster status correlates to the expected haplotype.

Student Outline

Learning Objectives

1. Learn about the differential response of supertasters, medium tasters, and nontasters to the bitter compound PROP ((6-*n*-propylthiouracil).
2. Distinguish fungiform papillae (with taste buds) from filiform papillae (lacking taste buds) on the anterior tongue.
3. Understand how SNPs in the *TAS2R838* gene may explain differing sensitivities to PROP.
4. Understand how sensitivity to bitter taste may influence food choice and health.

Materials Needed

Blue Food Coloring

Cotton-tipped Swab Applicators

Magnifying (10x) Hand Mirrors

Paper Cups, Small (3 ounce/88ml)

PROP Test Paper (6-*n*-propylthiouracil)

Reinforcement Labels (adhesive)

DNALC (Dolan DNA Learning Center) Kit-Using a single Nucleotide Polymorphism (SNP) to Predict Bitter Tasting Ability

Protocol

1. Examine slides of different types of papillae. Look for taste buds which appear as lighter-stained oval structures in the epithelium of the papillae. Which types of papillae have taste buds? Which do not? What would be the function of papillae which lack taste buds?
2. Taste unsweetened grapefruit juice and record your taste reaction as *dislike*, *neutral*, or *like*. Rinse mouth with water.
3. Take one PROP taste paper and place on your tongue. Identify your reaction as *strongly bitter*, *bitter*, or *no taste* which is indicative of a supertaster, medium taster, or nontaster respectively. Record your taster status. Rinse mouth with water.
4. Dab some blue food color on a cotton swab. Using the mirror for guidance, swab the tip of the tongue with blue food color. Fungiform papillae which contain taste buds will not stain and appear pink against the blue background of filiform papillae which do not contain taste buds and stain blue. If the color is too dark, rub the tongue on the roof of the mouth. If too light, dab on a little more blue dye.



Figure 1. Anterior tongue stained with blue food coloring. Non-staining fungiform papillae appear pink against the blue background of stained filiform papillae. The adhesive reinforcement label is placed on the tongue tip. Papillae are counted in the encircled area.

5. Place a reinforcement label (Fig. 1) over the blue stained area (BBC, 2003). Stick out your tongue to cover the lower lip. Gently close your mouth and use your teeth to hold your tongue in place. Shine a flashlight on the exposed tongue. With the 10x magnifying mirror, count the number of pink fungiform papillae in the center hole of the reinforcement label. Have your lab partner verify the count by looking over your shoulder into the mirror. Record the number (Table 1).

Table 1. Record your PROP status, reaction to unsweetened grapefruit juice, number of fungiform papillae, density of fungiform papillae, and genotype.

| PROP Status | Reaction to unsweetened grapefruit juice | Count of fungiform papillae | Density of fungiform papillae (/cm ²) | Genotype |
|-------------|--|-----------------------------|---|----------|
| | | | | |

6. Calculate the density of the fungiform papillae per cm² in the area encircled.

7. Calculate the class mean for both the number and density of fungiform papillae for each PROP taster status: supertaster, medium taster, and nontaster.

8. How does PROP taster status correspond to the mean number and density of fungiform papillae? How does the taste reaction to grapefruit juice correspond to PROP taster status? Is the hypothesis, that a subject's taste response to grapefruit juice will predict both PROP status and the relative density of fungiform papillae, supported? Explain.

9. Follow the instructions in the *DNALC Kit-Using a SNP to Predict Bitter*

Tasting Ability to obtain human cheek cells with a saline mouthwash, extract the DNA, amplify a 221 nucleotide base region of *TAS2R38* by PCR, and digest it with the restriction enzyme *Hae* III which will cut PAV but not AVI. After the restriction fragments are separated on a 2% agarose gel, score your genotype as PAV/PAV, PAV/AVI, or AVI/AVI and record the result in Table 1. Does your scored genotype correspond to your PROP status? Explain.

Notes for Instructor

The following results are from laboratory classes in the fall '07 and spring '08 semesters at The University of Tulsa. PROP testing identified 19 supertasters, 28 medium tasters, and 10 nontasters. Most PROP supertasters disliked the taste of unsweetened grapefruit juice. Medium tasters were divided in their taste response. Nontasters made up the highest percentage (30%) of those who liked the taste and also the lowest percentage (30%) of those who disliked unsweetened grapefruit juice (Fig 2.)

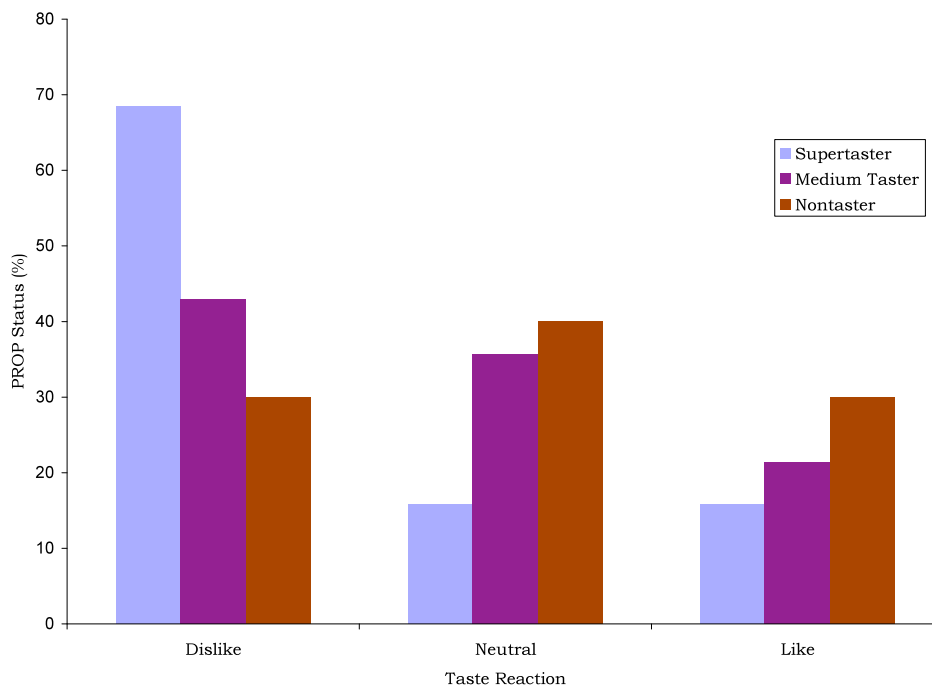


Figure 2. Taste reaction to unsweetened grapefruit

juice according to PROP status. N=57.

Supertasters had the highest mean and density of fungiform papillae and nontasters the lowest (Table 2).

Table 2. Fungiform papillae mean and density according to PROP taster status. (+/- standard deviation)

| PROP status | Mean | Density /cm² |
|----------------------|----------------|--------------------------------|
| Supertaster | 24.16 +/- 7.07 | 75.14 +/- 22.00 |
| Medium Taster | 19.50 +/- 7.33 | 60.65 +/- 22.08 |
| Nontaster | 17.20 +/- 6.18 | 53.50 +/- 19.22 |

Thirty-two (56%) of the cheek DNA samples were successfully amplified and digested. PAV/PAV genotype was identified in more than half of supertasters, 13 % of medium tasters, but in none of the nontasters. PAV/AVI genotype was identified in most medium tasters and less than half of the supertasters, and 25% of nontasters. The AVI/AVI genotype was found in majority of the nontasters but in none of the supertasters (Fig. 3).

Taste response to the bitter flavonoid naringin in unsweetened grapefruit juice is a fairly good predictor of PROP taster status. Mean number and density of fungiform papillae were also generally reflected by PROP taster status with supertasters having the highest means and densities whereas nontasters had the lowest, but some individual means and densities overlapped taster classes. A supertaster identified by PROP and confirmed by genotype, had a low papillae count and density and was indifferent to the taste of the juice. All PROP identified supertasters were either homozygous (56%) or heterozygous (44%) for the PAV allele. Medium tasters were predominantly heterozygotes. Seventy-five percent of nontasters were AVI/AVI, 25% were heterozygotes and none carried the PAV allele.

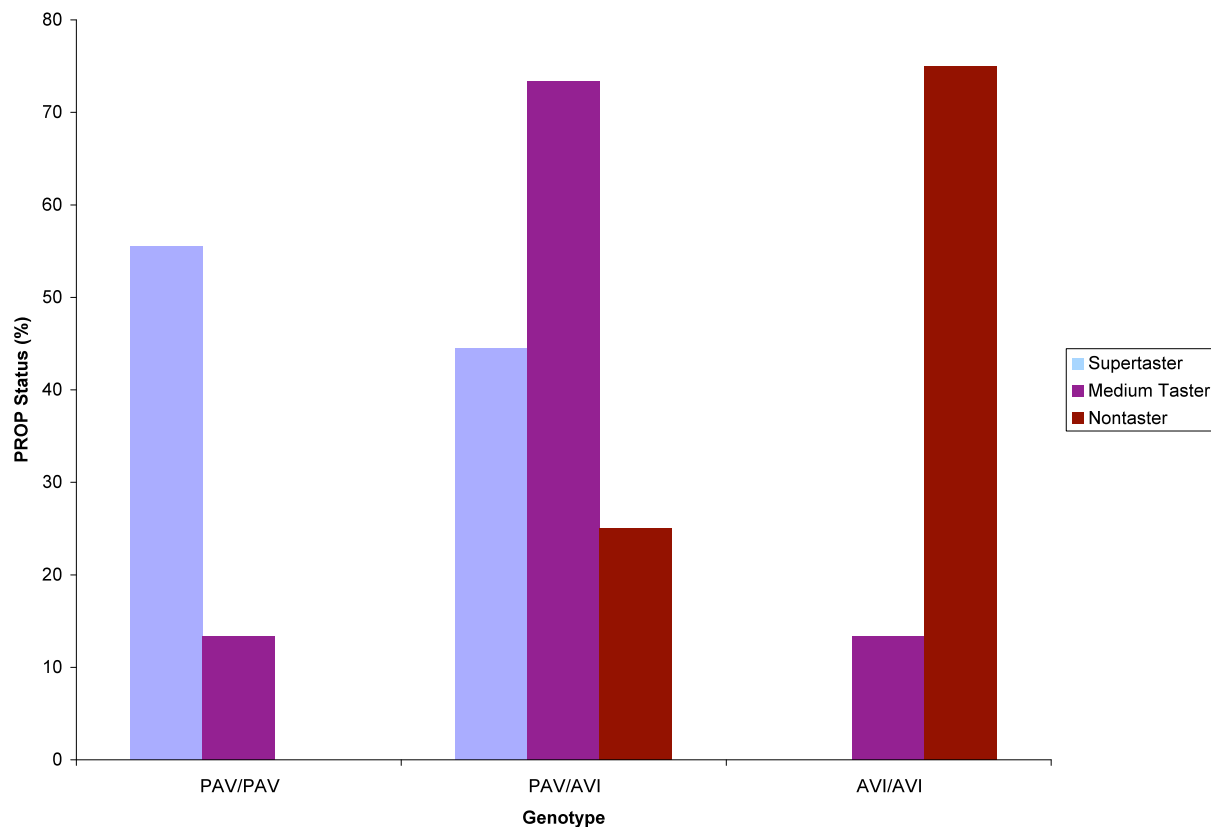


Figure 3. Taster genotype according to PROP status.

PROP taster status, reaction to grapefruit juice, number of fungiform papillae, and taster genotype can also be used to investigate:

- Gender differences. More women are supertasters than men.
- Smokers vs. nonsmokers. More smokers are nontasters than nonsmokers.
- Ethnic differences. More Africans and Asians are reportedly supertasters.
- Drinkers vs. non drinkers. Nontasters regularly consume more alcoholic beverages per year than suprtasters.
- Stabilizing selection. The selection for heterozygotes and the adaptive differences between the taster (reject bitter substances that may be poisonous) and the nontaster (tolerate bitter substances that may have a medicinal or health value) phenotypes.

PROP test paper can be purchased from Ward's. All other supplies (10x set margins magnifying mirrors, paper cups, food coloring, and reinforcement labels) are inexpensive and can be purchased locally at office supply stores, supermarket, or drug stores.

Fungiform papillae are found on the anterior of the tongue and are most abundant on the margins. It is important to sample the tongue in roughly the same region among individuals.

Ward's

800 962-2660

<http://wardsci.com>

PROP (*6-n-propylthiouracil*) Test paper (14 W 4104) VIAL OF 100

Carolina Biological Supply Company

800 334-5551

<http://www.carolina.com>

DNALC (Dolan DNA Learning Center) Kit-Using a single Nucleotide Polymorphism (SNP) to Predict Bitter Tasting Ability (FA-21-1379)

Acknowledgments

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