Cervical Cancer Precursors, Diet and Bitter Taste (6-*n*-propylthiouracil 'PROP') Receptors

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Abstract: Prior studies suggest that 6-*n*-propylthiouracil (PROP) taste responsiveness is linked with reduced preferences for sweet and high-fat foods, lower adiposity, and favorable plasma lipid profiles. We hypothesize that women with more severe and persistent cervical intraepithelial neoplasia (CIN) have a greater sensitivity to PROP. To measure this, women with CIN II or III submitted a DNA blood sample and a food frequency questionnaire. Out of the samples tested, thirty-eight (47%) were classified as nontasters (AV) and 36 (44%) were classified as tasters (PA). There were no significant differences ($p<0.05$) between the taster groups with respect to demographic variables or food frequency data. Women with CIN II at baseline were significantly ($p<0.003$) more likely to clear the disease than women with CIN III at baseline. Genetic markers of PROP may indicate the likelihood of a woman's acceptance of bitter foods, but had no association with food consumption, body mass index, and persistence of CIN.

Keywords: Food preferences, taste threshold, propylthiouracil, women, cervical intraepithelial neoplasia.

INTRODUCTION

The gene involved in bitter taste receptors (TAS2R) for phenylthiocarbamide (PTC), a chemically related compound to 6-*n*-propylthiouracil (PROP), has two main haplotypes identified as nontaster (AVI) and taster (PAV) [1]. PROP taste responsiveness has been linked with reduced preferences for sweet and high-fat foods [2], lower adiposity [3], and favorable plasma lipid profiles [4]. Although a recent study reports no association between PROP responsiveness and diets, plasma profiles, and body mass index (BMI) in older adult women [5].

Several studies indicate a role for nutritional factors as independent contributors to cervical cancer risk [6-10]. We hypothesize that women genetically classified as bitter-tasters will have a significantly different self-reported dietary intake. In addition, we hypothesize that women with more severe and persistent cervical dysplasia will have a greater sensitivity to PROP.

MATERIALS AND METHODS

This report used data and specimens collected from a previously reported clinical trial of all-trans retinoic acid (atRA) [11]. Briefly, women ages 14 years and older were deemed eligible if they had histologically confirmed cervical intraepithelial neoplasia II or III with a fully delineated ecto-cervical lesion and adequate colposcopic evaluation. The study intervention consisted of four consecutive days of atRA (gift of Ortho-McNeil Pharmaceuticals, Raritan, NJ) with a cervical cap and sponge at one of three doses (0.16%, 0.28%, and 0.36%) or a placebo agent. The participants were examined 12 weeks after the last day of drug exposure. Prior to initiating the study intervention, baseline blood was collected for serum DNA extraction for future studies and a food frequency questionnaire was completed for this analysis.

Food Frequency Questionnaire

The Fred Hutchinson Cancer Research Center Food Frequency Questionnaire was used to estimate dietary intakes of selected nutrients [12]. Women reported the frequency and portion size of 122 foods and beverages consumed in the past month. Frequency responses ranged from 'never or less than once per month' to 'two or more times per day' for foods and up to 'six or more times per day' for beverages [12].

Genotype Analysis

Venous blood was collected from all participants prior to the study intervention. One 7 milliliter vacutainer tube containing ACD to prevent clotting was collected, spun for 10 minutes at 3300rpm and plasma was drawn off and aliquoted into 2 milliliter cryovials and stored at -70 degrees Celsius for archival. Ninety-six samples were available for DNA extraction and subsequent PROP genetic marker analysis.

DNA was extracted from the buffy coat preparation of the 3 ml whole blood sample by following the manufacturer protocol for DNA purification (Gentra, Minneapolis, MN). DNA was stored at -20 degrees Celsius until further analysis.

Once the DNA was stored, the sample was tested using a spectrometer for the DNA and protein concentration. The sample was tested for the gene known to affect PROP sensi-

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Table 1. Demographic Characteristics of Tasters and Nontasters

	Tasters n=36	**Nontasters n=38**
Age (years)	26.3	28.1
Race (percent) • Caucasian • African American	 64% 28%	 63% 26%
Married (percent)	67%	67%
Current Smoker (percent)	42%	47%
Body Mass Index (BMI) kg/m^2	23.7	24.3
Lifetime Sexual Partners (mean)	8.2	7.9
Age of Onset of Sexual Intercourse (mean)	15.6	14.9
Cervical Sample Positive for High Risk HPV (percent)	94%	95%
CIN II (percent)	69%	68%
CIN III (percent)	31%	31%

Table 2. Food Frequency Data

	Tasters n=36 Mean (95% Confidence Interval)	**Nontasters n=38 Mean (95% Confidence Interval)**
Energy (kcal)	1679.3 (1389.8 - 1968.9)	1447.9 (1173. 2 - 1722.7)
Total Protein (grams)	67.9 (56.3 - 79.5)	58.8 (47.3 - 70.3)
Total Fat (grams)	63.8 (50.6 - 77.2)	55.5 (42.3 - 68.7)
Vitamin C (mg)	78.4 (63.6 - 93.4)	78.5 (65.0 - 92.0)
Cholesterol (mg)	223.2 (175.7 – 270.7)	197.0 (139.7 - 254.5)
Beta Cryptocanthin (mcg)	85.9 (64.8 - 107.2)	86.4 (67.3 - 105.5)
Lycopene(mcg)	11,036.8 (7,838.7 - 14,234.8)	9744.7 (7392.7 - 12,096.6)
Luetein/Zeaxanthin (mcg)	1251.7 (987.6 - 1515.8)	1191.3 (976.5 - 1406.1)
Alpha Carotene (mcg)	716.2 (447.2 - 985.2)	753.4 (502.4 - 1004.4)
Beta Carotene (mcg)	2499.1 (1806.5 - 3192.8)	2626.8 (1962.8 - 3290.8)

tivity. A polymerase chain reaction (PCR) was performed by adding 2 µl of the DNA to the PCR mix and then run on a PCR thermocycler. A digest for two single-nucleotide polymorphism (SNP) markers, V262A and A49P, was performed using 10 µl of the PCR product with the digest mix and run on the PCR thermocycler. The digest product was viewed using gel electrophoresis, which allowed the haplotype and the ability to taste PROP to be determined.

STATISTICAL ANALYSIS

The study participants were classified as tasters and non-tasters based upon the genotype analysis described above. Comparisons were made between the two groups using T Test and Chi Square analysis as indicated. Transformations of the data were made to achieve normally distributed data with log transformation used for the food frequency data.

RESULTS

Out of the total 96 samples tested, the DNA product was not obtained from 14 of the samples. One sample did not visibly show A49P product upon gel electrophoresis. Genotypes were determined from the remaining 81 samples. Thirty-eight (47%) were classified as nontasters (AV) and 36 (44%) were classified as tasters (PA). Seven (9%) samples were homozygous for V262A and A49P.

There were no significant differences ($p<0.05$) between the two groups with respect to any of the demographic variables (see Table **1**). There were no significant differences between the two groups on any of the dietary variables obtained *via* food frequency questionnaire (see Table **2**). Women with CIN II at baseline were significantly ($p<0.003$) more likely to clear the disease than women with CIN III at baseline. Baseline characteristics of age, smoking status, number of lifetime partners, age of first intercourse, HPV status, race, BMI, and taste status were not predictive of disease clearance at week 12.

DISCUSSION

It has been hypothesized that a genetic predisposition to impaired bitter compound tasting ability should result in a preference for fatty foods and less intake of fruits and vegetables. With respect to cervical cancer, this should result in greater risk of pre-invasive cervical lesion and decreased clearance of these lesions over time. However, in this study, no significant differences were noted for BMI, dietary intake based on food frequency data and persistence of CIN over 12 weeks by tasting and non-tasting of PROP.

There are several possible explanations for a lack of differences between tasters and non-tasters. First, all of the women in the study had histological proven CIN II or III. As a result, the study population presents a rather homogenous group of women with respect to risk for CIN, including diet. Second, this is a relatively small study. With 40 women per arm there would be 85% power to detect a difference of 0.5 standard deviation in continuous variables such as BMI and dietary intake [5]. With about 35 per arm, the power is estimated to be reduced to 80%. So the lack of association may be an error. However, in a study of 358 women, there were no significant links between PROP responsiveness and food preferences, food or macronutrient intakes, BMIs, measures of body fatness, or plasma lipids [5]. Finally, the food frequency data may not reflect the diet over the period of developing the cervical lesion.

Though genetic markers of PROP may influence women's acceptance of bitter foods, there appears to be little influence on food consumption, BMI, and persistence of CIN. In conclusion, our findings add to the growing data that food choices are not determined by taste genetics alone.

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