

Short Communication

Normal taste acuity and preference in female adolescents with impaired 6-n-propylthiouracil sensitivity

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This study was conducted to determine the relationship between 6-n-propylthiouracil sensitivity and taste characteristics in female students at Nara Women's University. Participants (n=135) were screened for 6-n-propylthiouracil sensitivity using a taste test with 0.56 mM 6-n-propylthiouracil solution, and the sensitivity was confirmed by an assay for the bitter-taste receptor gene, *TAS2R38*. Based on the screening results, 33 6-n-propylthiouracil tasters and 21 non-tasters were enrolled. The basic characteristics that are thought to influence taste acuity, including body mass index, saliva volume and serum micronutrient concentrations (iron, zinc and copper), were similar between the two groups. In an analysis using a filter-paper disc method, there were no differences in the acuity for four basic tastes (sweet, salty, sour and bitter) between 6-n-propylthiouracil tasters and non-tasters. In addition, the taste preference for the four basic tastes as measured by a visual analogue scale was also comparable between the two groups. This is the first study to demonstrate that 6-n-propylthiouracil non-tasters have taste sensitivity for the four basic tastes similar to that in 6-n-propylthiouracil tasters, at least in female adolescents, as measured by the gustatory test using a filter-paper disc method.

Key Words: 6-n-propylthiouracil, *TAS2R38*, taste acuity, filter-paper method, visual analogue scale

INTRODUCTION

Phenylthiocarbamide and its related compound, 6-n-propylthiouracil (PROP), were discovered in the 1930s as substances inducing a strong bitter taste in humans.¹ The PROP bitter-taste phenotype, as determined by the PROP solution test, is associated with allelic variation of the specific bitter-taste receptor gene, *TAS2R38*.² The two common alleles are AVI (alanine, valine, isoleucine) and PAV (proline, alanine, valine). AVI/AVI homozygotes have been proven to be less sensitive to PROP bitterness than PAV/PAV homozygotes or AVI/PAV heterozygotes. On the other hand, the bitter-taste receptor gene for quinine is considered to be different from that for PROP. The quinine taste perception is related to common genetic variants in a bitter receptor cluster on chromosome 12 (*TAS2R7*, *10*, *14*, *19*, *43* and *46*).^{3,4} The estimated prevalence of PROP non-tasters by the solution test is ~30% among Caucasians and 10-20% in China or Japan.⁵ The prevalence of PROP non-tasters by genotype analysis is roughly consistent with the solution test data-i.e., genotype analysis yielded non-taster prevalence of 7.3% in Asians, 31.9% in Caucasians, and 35.1% in South Asians. A strong association between the phenotype and the genotype of PROP sensitivity, especially in women, has been reported.⁶ Individuals can be classified into three PROP taster categories by the phenotype: non-tasters, medium-tasters, and super-tasters based on PROP supra-threshold measures.^{7,8} The percentage of non-tasters does not differ by gender in young children.⁹ However, at or near puberty, comparatively more males are non-tasters

and more females are tasters,¹⁰ and this dichotomy persists into adulthood.¹¹

Several studies have indicated an association between PROP sensitivity and food preference.^{12,13} Previous investigators reported that greater PROP sensitivity was associated with lower acceptance of coffee, cruciferous vegetables, tart citrus fruit, and dark breads in adults.¹⁴ In addition, PROP non-tasters preferred a significantly higher concentration of sucrose.^{15,16} In these previous studies, however, the food preference was tested by using either a food preference checklist or a food frequency questionnaire. On the other hand, studies that have compared the acuity of the four basic tastes by means of objective gustatory tests are limited. Based on such previous knowledge, we here attempted to determine the sensitivity to the basic four tastes by using a filter-paper disc (FPD) test in PROP tasters and non-tasters.

METHODS

Study subjects

The study subjects were female students at Nara Women's University and the study was conducted between

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May and July of 2012. First, we recruited 135 applicants for the PROP (Wako Pure Chemical Industry, Ltd., Osaka, Japan) solution test described below. Among them, 111 (82.2%) and 24 (17.8%) applicants were found to be PROP tasters and PROP non-tasters, respectively. In the next step, we asked the applicants to participate in a genetic analysis. Since the number of PROP non-tasters was limited, we strongly encouraged them to participate as subjects in the subsequent experiments. Among the 33 tasters and 22 non-tasters who agreed to participate in the further studies, there was one PROP non-taster whose genetic analysis was discordant with the solution test. After excluding this applicant, 33 subjects as PROP tasters and 21 subjects as PROP non-tasters were finally enrolled. This project was approved by the ethical and epidemiological committees at Nara Women's University.

Classification of PROP tasters and PROP non-tasters

PROP sensitivity was measured by using a method developed by Keller et al.⁹ Briefly, the subjects tasted 10 mL of 0.56 mM PROP solution in distilled water. They were then asked the question "Do you taste anything?" Subjects giving responses that included the words *bitter*, *bad*, or *like medicine* were classified as tasters, whereas those using the words *like water* or *nothing* were classified as non-tasters. For the genetic analysis, the samples were taken by scraping the buccal mucosa 15 times. After extraction of DNA, a TaqMan MGB probe (Applied Biosystems Co., Tokyo, Japan) (3SNP:*TAS2R38* A49P rs713598, *TAS2R38* V262A rs1726866, *TAS2R38* V296I rs10246939) was used as a tag SNP to distinguish between the AVI and PAV haplotypes.^{17,18} The genetic assay was performed by Genesis Healthcare Co. (Tokyo, Japan).

Testing method for taste acuity

The FPD method was used for evaluating gustatory functions. The gustatory tests were carried out in the morning under a fasting state by a single well-trained dietitian. During the tests, the participant's mouth was rinsed with distilled water before testing the next concentration. Test discs of 5 mm in diameter (Taste Disc, Sanwa Chemical Inc., Nagoya, Japan) were placed on the left lateral part of the tongue at approximately 2 cm from the proglossis, which is thought to be innervated by the chorda tympani nerve. Sucrose, sodium chloride, tartaric acid, and quinine hydrochloride were used to test the taste acuity for the four primary tastes, sweet, salty, sour, and bitter, respectively. The concentrations used to test the four tastes were slightly modified from our previous study to evaluate the threshold more accurately.¹⁹ Namely, each test solution was serially two-fold diluted with distilled water from the highest concentration used in the original study, i.e., 9 solutions of sucrose (9.1-2336 mM), 7 solutions of sodium chloride (53.4-3420 mM), 9 solutions of tartaric acid (2.1-532 mM), and 13 solutions of quinine hydrochloride (0.024-100 mM). The concentrations at each taste were serially scored from disc number 1 (lowest) to numbers 7, 9, and 13 (highest). When the subject could not detect the taste at the highest concentration, the score was given as the highest score plus one. The lowest concentration at which a particular taste was correctly identified was de-

finied as the recognition threshold. In our previous study, the cut-off concentrations used to define hypogeusia were >438 mM for sucrose, >1283 mM for sodium chloride, >199.5 mM for tartaric acid, and >7.5 mM for quinine hydrochloride.¹⁹ These concentrations are almost equal to the disc numbers of ≥ 7 for sucrose, ≥ 6 for sodium chloride, ≥ 8 for tartaric acid, and ≥ 10 for quinine hydrochloride in the present study.

Visual analogue scale (VAS)

The VAS is a scale of 100 mm in length with the phrases "most dislike" at the left end and "most like" at the right end. To determine a subject's personal preference for eating sweet, salty, sour or bitter foods, she was asked the question "Do you like sweet food?" The subject answered by pointing to the appropriate place on the scale, and then the distance from the left end was measured.²⁰

Measurement of serum micronutrients and saliva volume

Fasting blood samples were drawn in the morning to measure micronutrients, including iron (Fe), zinc (Zn), and copper (Cu). To evaluate serum Zn levels, blood was collected in trace-element-free tubes, and put on ice immediately. Serum Zn was determined by an atomic absorption method. Serum Fe and Cu levels were determined by a colorimetric method. The assays were done by the Mitsubishi Chemical Medience Corporation (Tokyo, Japan). The reference values of three micronutrients were established by the company using more than several hundred healthy adult volunteers. Saliva volume was determined according to bioimpedance value measured by an oral moisture-checking device with sensor of capacitance (Life Co., Saitama, Japan). The sensor assembly of the oral moisture-checking device was pressed onto the centre part of the tongue at approximately 1 cm from the proglossis. The result of measurement was indicated immediately on the display screen as the relative amount of water in the mucosal epithelia. The reference values for these variables are shown in Table 1.

Statistical analysis

Differences in the demographic features, serum concentrations of micronutrients, saliva volume, four basic tastes expressed by the disc numbers, and VAS scores between PROP tasters and non-tasters were analyzed by Mann-Whitney U test. The prevalence of hypogeusia was compared between the two groups by Chi-square test. All statistical analyses were carried out using Excel Statistics, Version 2007. Values of *p* less than 0.05 were considered significant.

RESULTS

Basic characteristics of PROP tasters and PROP non-tasters

Several factors, including the presence of obesity as measured by body mass index (BMI),²¹ saliva volume²² and serum micronutrients,²³ have been reported to influence taste acuity. Therefore, we compared these factors in order to ascertain the similarity of the two groups. Table 1 indicates that these background factors exhibited similar levels of statistical significance between PROP tasters

and non-tasters.

Comparison of the four basic taste acuities between PROP tasters and PROP non-tasters

The thresholds of the four taste acuities as demonstrated by the disc numbers were not statistically different between PROP tasters and non-tasters (Table 2). However, PROP tasters were slightly, but not significantly, more sensitive to the sweet taste than non-tasters ($p=0.10$). When we divided all subjects into normogeusia and hypogeusia groups, the prevalence of hypogeusia was found to be equivalent between PROP tasters and non-tasters for each of the four taste acuities (Table 2).

Comparison of the four basic taste preferences by VAS between PROP tasters and PROP non-tasters

Table 3 summarizes the responses given by the subjects regarding their preferences for the four basic tastes. There were no significant differences in this examination between PROP tasters and non-tasters.

DISCUSSION

Dietary habits play an important role in maintaining and promoting a healthy lifestyle. Since differences in sweet, salty, sour or bitter taste perception are thought to be closely associated with dietary habits,²⁴ the identification of taste perception in individuals is an important nutritional concern. Sensitivity to PROP-induced bitterness in particular has been extensively investigated in relation to food preferences.^{5,25} Although PROP itself is a synthesized substance, PROP-related compounds such as phytochemicals are found in many vegetables and fruits.^{26,27} Therefore, numerous investigators have investigated the relation between PROP sensitivity and acceptance of these foods in daily life, and have reported that the higher sensitivity to PROP was linked with lower acceptance of these foods.^{5,25} Furthermore, individuals not sensitive to PROP have been shown to prefer sweeter or higher-fat foods than those with PROP-sensitivity.^{7,14,15} However, these results were highly dependent upon the age, gender, race, and health status of the study populations. In con-

Table 1. Comparison of basic characteristics between PROP tasters and non-tasters

	Tasters	Non-tasters	<i>p</i> value [†]	Reference values
Number	33	21		
Age (year)	21 (19-24)	20 (18-22)	<0.01	
Height (cm)	157 (145-171)	159 (152-170)	0.22	
Weight (kg)	51 (31-62)	52 (42-59)	0.32	
Body mass index (kg/m ²)	19.5 (13.4-24.7)	20.3 (15.4-23.5)	0.41	
Saliva volume (%)	31.5 (22.0-37.5)	29.9 (23.9-35.3)	0.42	<30.0
Serum Fe (µg/dL)	88 (28-216)	100 (23-168)	0.34	40-180
Serum Zn (µg/dL)	84 (64-113)	87 (57-113)	0.47	64-111
Serum Cu (µg/dL)	93 (53-204)	90 (70-103)	0.54	70-132

Values are shown as the median and range. PROP: 6-n-propylthiouracil.

[†]Mann-Whitney U test.

Table 2. Comparison of taste acuity between PROP tasters and non-tasters by the filter-paper disc method

	Tasters (n=33)	Non-tasters (n=21)	<i>p</i> value
A) Tube number			
Sweet	6 (1-10)	8 (2-10)	0.10 [‡]
Salty	5 (1-8)	3 (1-8)	0.86 [‡]
Sour	6 (2-10)	7 (2-10)	0.48 [‡]
Bitter	7 (1-13)	7 (1-14)	0.98 [‡]
B) Proportion of hypogeusia [†]			
Sweet	15 (45.5)	11 (52.4)	0.62 [§]
Salty	15 (45.5)	9 (42.9)	0.85 [§]
Sour	10 (30.3)	7 (33.3)	0.82 [§]
Bitter	10 (30.3)	9 (42.9)	0.35 [§]

Tube numbers are shown as the median and range. PROP: 6-n-propylthiouracil.

The proportion of hypogeusia is presented as the number and percentage of subjects (in parenthesis).

[†]Hypogeusia is a reduced ability to taste things (to taste sweet, sour, bitter, or salty substances), see Methods.

[‡]Mann-Whitney U test.

[§]Chi-square test.

Table 3. Comparison of taste preferences between PROP tasters and non-tasters by visual analogue scale

	Tasters (n=33)	Non-tasters (n=21)	<i>p</i> value [†]
Sweet	8.7 (3.7-9.9)	8.3 (3.6-10.0)	0.71
Salty	6.8 (4.6-10.0)	7.1 (2.1-9.8)	0.92
Sour	6.2 (0-9.3)	5.9 (1.4-9.2)	0.61
Bitter	3.3 (0.1-7.4)	3.3 (0.3-7.2)	0.66

Values are shown as the median and range. PROP: 6-n-propylthiouracil.

[†]Mann-Whitney U test.

trast, studies on the relation of PROP sensitivity to the taste thresholds of the four basic tastes are limited. Pasquet *et al* demonstrated that PROP super-tasters exhibited significantly lower thresholds for sucrose and fructose than PROP medium- and non-tasters.²⁸ In addition, Chang *et al* have found that the thresholds for the sweet and bitter tastes were lowest in PROP super-tasters, followed in order by medium-tasters and non-tasters by a whole mouth gustatory test.¹⁶

In the present study, we investigated the relation of PROP sensitivity to the taste acuity and preference for the four basic tastes. To evaluate the taste threshold, we used our recently developed variation of the FPD method. We failed to detect any differences in the thresholds of all four basic tastes between PROP tasters and non-tasters. This result was in contrast with the previous studies of Chang *et al*¹⁶ and Pasquet *et al*.²⁸ Possible reasons for the discrepancy include the following: (i) differences of age, gender, and races between the studies; (ii) the difference in the methods used for the gustatory tests, i.e., the FPD method vs. the whole mouth method; and (iii) the classification of PROP sensitivity, since we did not use the category of super-tasters in our study. Although we did not determine umami taste in this study, Hong *et al* showed that the threshold for monosodium glutamate was not different between PROP tasters and non-tasters.²⁹ With respect to food preference, we used the VAS, which has been well validated as a single measure for appetite sensations.²⁰ Here again, there was not any difference in taste preference between PROP tasters and non-tasters. This result was well correlated with our present FPD study, but was not in line with most previous studies.

It has been hypothesized that sensitivity to PROP could serve as a marker for various health outcomes, including obesity and chronic disease risks.⁵ Baranowski *et al* documented that PROP super-tasters had the largest BMI and BMI z-scores within a group of subjects with high socio-economic status.³⁰ Another study in 242 Plains American Indians found that PROP tasters were less likely to become smokers than PROP non-tasters.³¹ In a study on alcohol intake, PROP non-tasting was a predictor for a higher alcohol intake.⁸ Finally, Timpson *et al* reported that subjects with the non-taster haplotype had a lower risk of diabetes mellitus than those with the taster haplotype, although the difference was statistically marginal.³²

There are several limitations in the present study. First, the number of subjects was limited. In addition, we cannot rule out the possibility of a selection bias in the participants, since we recruited them as volunteers. Second, we used only the FPD method for evaluating the taste acuity. Ideally, other methods such as the whole mouth method or taste strip method would be used concurrently.¹⁹ Furthermore, we adopted the VAS method for evaluating the food preference. For the comparison with previous studies, it would have been preferable to use food checklists or food frequency questionnaires simultaneously.

On the other hand, the present study also has several strengths. First, the PROP sensitivity of enrolled subjects was confirmed by both the phenotype and the genotype. Second, several background factors associated with the taste acuity, i.e., BMI, saliva volume, and serum micronutrients, were checked and found to be similar among the

PROP taster and non-taster groups. Finally, the FPD method was used for the first time, as far as we know, to determine the thresholds of the four basic tastes related to the PROP sensitivity. Taking these limitations and strengths into consideration, we consider that a new study using several gustatory tests and food check lists, and a cohort with a wide variety of ages and a large number of subjects is merited, and such a study is currently underway in our laboratory.

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AUTHOR DISCLOSURES

The authors declare that they have no conflicts of interests. This work was supported by JSPS KAKENHI Grant Number 23700903.

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6-N-丙基硫氧嘧啶敏感性受损青少年女性的正常味觉敏感度和味觉偏好

本研究测定了奈良女子大学女生 6-N-丙基硫氧嘧啶的敏感性和味觉特征之间的关系。135 名参与者的 6-N-丙基硫氧嘧啶的敏感性是用 0.56 毫摩尔 6-N-丙基硫氧嘧啶溶液的味觉试验测试进行筛选的，再用苦味受体基因 TAS2R38 来确认其敏感性。根据筛选结果，33 位 6-N-丙基硫氧嘧啶尝出者和 21 位未尝出者入选。那些被认为影响味觉敏感度的基本特征，包括体质指数、唾液量和血清微量元素的浓度（铁、锌和铜）两组之间相似。在使用滤纸片法的分析中，6-N-丙基硫氧嘧啶尝出者和未尝出者四种基础味觉（甜、咸、酸和苦）的敏感度没有差异。此外，通过视觉模拟评分测量两组之间四种基础味觉的味觉偏好也相似。首次研究证明：至少在用滤纸片法作为尝味测试的青少年女性中，6-N-丙基硫氧嘧啶未尝出者与尝出者对四种基础味觉的味觉敏感度相似。

关键词：6-N-丙基硫氧嘧啶、TAS2R38、味觉敏感度、滤纸法、视觉模拟评分