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Research Paper

A Study of Phenylthiocarbamide Polymorphism Among Basrah Population/ Iraq

Biomedical

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ABSTRACT

The present study deals with the distribution of PTC tasting ability as a marker to study the genetic structure of Basrah population; as no detailed information is available. To investigate the prevalence and gene frequencies of PTC taste sensitivity among male and females in this region and its correlation with body weight, height and BMI. Individuals' body weight, height and BMI were also calculated. Genetic and phenotypic analysis was done on collected data. Frequency of taster among female, male and overall were 64.96, 62.90 and 64.32% respectively. Males revealed significant differences between observed and expected values. However, females were under HWE. Observed and expected heterozygosity was 41.20% and 48.74% respectively. Correspondence values for male and female were 31.12% and 66.46% respectively. Genetic breeding coefficient (Fis) was high (around 16%) indicate that there are sort of relative marriages. It was very high (48.8%) for male and was very low (0.7%) for female. However, allele frequency-based correlation between male and female (Fst) was very low (0.0003). With regarding PTC as quantitative traits genetic portion was more than 70% (heritability=0.739) the rest (less than 30%) was environmental factor. PTC scored show positive genetic and additive value (breeding value) with low dominant effect as dominant deviation of Tt genotype was 0.18. BMI showed negatively significant phenotypic correlation (-0.40) with PTC threshold. There is negative and significant regression between BMI and genotype (-2.2671 kg/m3 per 1 unit increase of PTC). PTC genotype determined around 14.8% of body weight variation, whereas, it determined 87.6% of PTC threshold. The genotypes TT and Tt showed lower (P<0.05) BMI than that of tt genotype. Body weight of the Tt genotype was the lowest (P<0.05) among other genotypes and that of TT genotype came in the middle.

Introduction

The ability to taste phenylthiocarbamide (PTC) is considered as an important tool in the study of human diversity (Campbell et al, 2012). Studies went on to estimate the frequency of presumed "taster" and "non-taster" alleles in hundreds of populations worldwide (Guo& Reed 2001). These studies showed that the frequency of the non-taster allele in human populations (estimated under the assumption that the inability to taste PTC is attributable to the recessive allele in a one-locus, two- allele system) varies around a mean of ~50% (Wooding, 2006).

Although PTC itself has not been found in nature is associated with perception of a broad range of basic tastes and textures in the diet, the ability to taste PTC is correlated strongly with the ability to taste other naturally occurring bitter substances found in cruciferous vegetables like cabbage and broccoli, many of which are toxic (Tepper*et al*, 2008). Furthermore, variation in PTC taste sensitivity has been correlated with dietary preferences that may have significant health effects (Bartoshuk*et al.* 2005). Variable aversions to these compounds have been implicated in the variable rates of thyroid-deficiency disease in PTC tasters and non-tasters, with non-tasters being more susceptible (Kim and Prayan, 2004).

The ability to taste phenylthiocarbamide (PTC), a bitter chemical has longbeen known to be a bimodal autosomal trait inherited in a simple Mendelian recessive pattern which is being widely used for both genetic and anthropological studies. The present study deals with the distribution of PTC tasting ability as a marker to study the genetic structure of Basrah population; as no detailed information is available. To investigate the prevalence and gene frequencies of PTC taste sensitivity among male and females in this region and its correlation with body weight, height and body mass index (BMI).

Material and Methods

Levels of PTC bitter taste sensitivity were measured for 199 individuals (137 female, 62 male aged 18-30 years) from Basrah populations using a modification of the classic recognition threshold method described by Harris and Kalmus (1949). Specifically, the test was administered to subjects using serial dilutions of PTC labeled 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 and 1, with solution 13 containing the most dilute solution of PTC and 1 containing the most concentrated solution. Raw PTC score represents the point in the successive dilution taste test at which individuals were able to detect the bitter taste of PTC. Individual whose score 13-10 or 9-4 or 3-0 consider as TT, Tt and tt genotypes of PTC taste respectively. Genetic, additive and dominant values and variations were calculated as described by Falconer and MacKay (1996). Body weight, height and BMI of each individual were also measured.

Statistical procedures: Binomial $\chi 2$ was used to test for differences in dichotomous variables (e.g., sex) between tasters and non-tasters. Independent samples *t*-tests were done to test for differences in BMI *z*-score as a function of PTC status (tasters vs. non-tasters). Two-way ANOVA were used to test the interaction of PTC phenotype with sex (independent variables) on BMI *z*-score, body weight and height (dependent variables). For all statistical tests, a *P* value of <0.05 was the cutoff for significance. All hypotheses were two-tailed. Descriptive statistics are reported as means ± S.D. Primary analyses were done using SPSS, version 20.0 (SPSS, Chicago, IL).

Results and Discussion

PTC threshold and phenotypes

Table 1 shows the mean PTC threshold value, standard deviation and Penrose index (D $\sqrt{S}$). These measures have been taken into account in order to minimize the amplitude of sampling error. The mean threshold values for males are calculated as 9.82±0.44 among the tasters and 2.09±0.30 among the non-tasters. Those for females were 7.62±0.26 and 2.38±0.28 respectively. Mean threshold values for the tasters and the non-tasters differ in both males and females. D $\sqrt{S}$ value of males and females are 10.77 and 7.46 respectively which imply a bimodal distribution.

Frequency of taster among female, male and overall were 64.96, 62.90 and 64.32% respectively. The values are consistence with other studies (e.g. Guo& Reed, 2001), where taster are more frequent than non-taster.

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Table (1) Mean± Standard Error of PTC due to male or female taster or non-taster, standard deviation (s) and difference between taster and non-taster (D)

Gender	No.	Non-Taster	Taster	S	D	Penrose index (D √S)
Female	137	2.38±0.28 (35.00%)	7.62±0.26 (64.96%)	2.03	5.24	7.46
Male	62	2.09±0.30 (37.10%)	9.82±0.44 (62.90%)	1.94	7.73	10.77
All	199	2.28±0.21 (35.68%)	8.29±0.25 (64.32%)	1.79	6.01	8.04

Figures between brackets are frequency of each genotype.

PTC genotypes

Number of observed and expected (calculated from Hardy-Weinberg equilibrium HWE) individuals belong to different genotypes are shown in table (2). The difference between observed and expected values of each genotype was tested by chi-square test. The test indicates there are significance differences ($P<0.05$). Significant test indicate that the studied population is not under HWE due to different causes (e.g. random drift, mutation, migration or natural selection).

Table (2) Number of observed and expected individuals of different genotypes

Genotype	Observed	Expected	X^2
TT	44	36	1.78
Tt	82	98	2.61
tt	73	65	0.98
Total	199	199	5.37
p-value			0.05

Numbers of male and female of different genotypes are shown in table (3). Males revealed significant differences between observed and expected values. However, females were under HWE. Although the PTC polymorphism has been regarded as a single locus trait, most investigators have pointed out its complex features, and have proposed that certain subject characteristics and environmental factors may alter the phenotype (Wooding, 2006). The most robust modifier of the genotype and phenotype relationship is sex (Keller et al, 2010); women are more likely to be tasters and can taste PTC at lower concentrations than can men.

Table (3) Number of observed and expected individuals of males and females of different genotypes

	Males			Females		
Genotype	Observed	Expected	X2	Observed	Expected	X2
TT	21	14	3.50	23	22.77	0.002
Tt	16	31	7.25	66	66.46	0.003
tt	25	17	3.76	48	47.77	0.001
Total	62		14.52**	137		0.006

PTC population parameters

Table (4) shows the frequency of PTC alleles (T Taster and t non-taster) and genetic breeding coefficient (Fis). Observed heterozygosity was 41.20%. The expected heterozygosity (48.74%) is equal to twice the cross of T and t frequencies (2pq). Correspondence values for male and female were 31.12% and 66.46% respectively. Different studies for different loci in human recorded an expected heterozygosity of 0.7- 0.9 (Biswas et al, 2009; GPC, 2010; Xing et al, 2010). Genetic breeding coefficient was high (around 16%) indicate that there are sort of relative marriages. It was very high (48.8%) for male and was very low (0.7%) for female. However, allele frequency-based correlation between male and female (Fst) was very low (0.0003), differences in gene frequency between male and female is around zero. The reason behind low heterozygosity and high Fis are number of allele (polymorphism in this locus), natural selection and isolation (close population). This also implies that a smaller effective population size and less informative markers would be needed for human whole genome association studies. To some extent, this situation may arise from the joint effects of migration, selection and genetic drift during long time. Genetic breeding coefficient or heterozygosity value suggests that natural selection may have been an important factor in evolution of this trait. Xing et al (2010) found that heterozygosity of PTC gene was significantly higher in African population than non-African population and decreases as geographic distance to east Africa increase (r=0.76). However, Khoisan individual showed low heterozygosity on SNP microarray genotypes (~ 22%, Schuster et al, 2010). These differences among populations could reflect unique attributes of population history. Wooding *et al* (2004) indicated that PTC haplotypes had similar frequencies across Africa, Asia and Europe and genetic differentiation between the continental populations samples was low (Fst=0.056) in comparison with estimate based on other genes. These results combine to suggest that balancing natural selection has acted to maintain "taster" and "non-taster" alleles at the PTC locus in humans at different populations.

Table (4) Allele of PTC and genetic breeding coefficients (Fis and Fst)

	Allele frequency		Heterozygosity%		Fis*	Fst*
	T (p)	t (q)	Observed	expected		
Whole population	0.427	0.573	41.20	48.74	0.161	0.0003
Males	0.365	0.635	16.00	31.12	0.488	
Females	0.410	0.590	66.00	66.46	0.007	

***Fis calculated as described by Weir and Cockerium (1984)**

PTC as quantitative trait

When PTC measured as a quantitative trait, it is evident that not all of the phenotypic variance in PTC taste perception is heritable (table, 5). Genetic portion was more than 70% (heritability=0.739) the rest (less than 30%) was environmental factor. PTC scored show positive genetic and additive value (breeding value) with low dominant effect as dominant deviation of Tt genotype was 0.18. PTC scored also revealed high additive variance (35.684), low dominant variance (0.032). Morton, et al. (1981) reported heritability value of 55% for taste threshold for PTC. The modifiers of the genotype–phenotype relationship such as gender, age or smoking may partially account for the non-heritable fraction of the phenotypic variance (Wooding, 2006). The PTC polymorphism, because it combines both bimodal and continuous variation, is an appealing model to develop methods and strategies for complex traits. Also, since the phenotyping is relatively stable, inexpensive, can be measured accurately, and

the gene frequency is high, it is an ideal complex trait to test out current gene mapping methodology.

Table (5) Genetic and breeding value, dominance deviation, additive variance (σ^2A), dominance variation (σ^2D) and phenotypic variance (σ^2p) of PTC locus

Genotype	PTC mean score	Genetic value	Breeding value	Dominance deviation
TT	11.553	5.526	6.395	-0.242
Tt	6.395	0.368	1.819	0.180
tt	2.282	-3.745	-4.765	-0.134

α (average gene substitution) is equal to 5.580, σ^2A=35.684, σ^2D= 0.032, σ^2p=48.273 and Heritability ($h^2=\sigma^2A/\sigma^2p$)=0.739

Association of PTC and BMI

Phenotypic and genetic correlation values (Table, 6) between PTC, body weight and BMI were negative. Negative correlation means an increase in one variable associated with decrease of other variable value. BMI showed negatively significant phenotypic correlation (-0.40) with PTC threshold. PTC threshold directed to high values as BMI going toward lower values. Genetic correlation between PTC and body weight was medium, whereas, that of PTC and BMI was very low. Gene responsible for PTC has reverse relationship with those genes responsible for body weight.

Regression of body weight, BMI and PTC on genotype are shown in table (7). There is negative and significant regression between BMI and genotype. PTC genotype determined around 14.8% of body weight variation, whereas, it determined 87.6% of PTC threshold.

Table (6) Phenotypic and genetic correlation between PTC, body weight, height and body mass index (BMI)

	Body weight	height	BMI	PTC
Body weight		0.593**	0.868**	-0.211
height	0.593		0.124	0.203
BMI	0.503	0.452		-0.400**
PTC	-0.211	0.203	-0.031	

Figures above and below diagonal are phenotypic and genetic correlations respectively.** Significant at 1%.

Table (7) Regression of body weight, BMI and PTC on genotypes

	Body weight	BMI	PTC
Regression coefficient	-4.065	-2.367**	4.524
R^2%	4.4	14.8	87.6

**** Significant at 1%**

Analysis of variance revealed significant differences between body weight and BMI of different genotypes (table, 8). The genotypes TT and Tt showed lower (P<0.05) BMI than that of tt genotype. Body weight of the Tt genotype was the lowest (P<0.05) among other genotypes and that of TT genotype came in the middle. The mechanism linking PTC status to body weight is not known, but one suggestion is that it may influence the discriminability (Hayes & Duffy, 2007) and palatability (Hayes & Duffy, 2008) of dietary fat.

Table (8) mean of body weight, height and body mass index of different genotypes

Traits	Genotypes		
	TT	Tt	tt
Body weight (kg)	b65.36±15.39	c57.74±13.02	a72.21±9.88
Height (cm)	165.27±8.20	161.00±7.90	160.93±9.53
BMI (kg/m2)	b23.62±3.43	b22.13±4.05	a27.93±3.57

***means with different letter within each row indicate a presence of significant differences (P<0.05)**

Because non-tasters have fewer taste buds receiving trigeminal input, they have decreased ability to discriminate textural cues from food (Essick et al, 2003). Tepper and Nurse (1997) reported that non-tasters had decreased abilities to discriminate differences in fat content between high-(40%) and low- (10%) fat salad dressings. It is possible that non-tasters need higher levels of fat in foods to not only detect it, but also to achieve the same level of satisfaction that a taster would receive with a lower fat level. Several other studies have found that non-taster adults like higher fat foods such as ice cream, doughnuts, bacon, mayonnaise, and cheeses (Tepper, 2008), and non-taster children report higher liking of cheese and in females, only whole fat milk (Keller et al, 2002). Still, other studies do not support the above relationships (Drewnowski et al, 2007), and the topic remains controversial.

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