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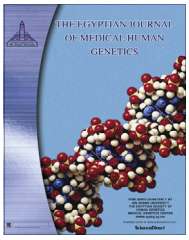

ORIGINAL ARTICLE

The population structure of Ukraine in relation to the phenylthiocarbamide sensitivity

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Abstract *Background:* The taste sensitivity to phenylthiocarbamide (PTC) is one of the classical genetic markers in human studies. PTC is of great interest from the medical point of view since a number of associations of the taster status with human diseases have been found. The aim of our study was to evaluate the population structure of Ukraine in relation to PTC sensitivity.

Methods: The study involved 533 people (78 males and 455 females) aged from 16 to 25 years. The PTC solution in the concentration of 0.13% was prepared according to the method of Harris and Kalmus. The participants of the study analyzed the taste of the filter paper impregnated with PTC. If the trial subjects tasted PTC as "bitter", "very bitter", "bitterish", the phenotype was defined as a taster. If the trial subject did not taste PTC ("no taste", "taste of paper"), he/she was referred to a non-taster.

Results: The structure of the sample of the Ukrainian population studied in relation to the phenotypic and genotypic frequency associated with the phenylthiocarbamide sensitivity has been studied. It has been shown that in the population there are 22% of those who do not feel the taste of phenylthiocarbamide. Among males there are a few more non-tasters than among females, however, the differences are not significant. The frequency of the dominant and recessive allele of the phenylthiocarbamide sensitivity gene in the sample calculated on the basis of the Hardy–Weinberg equation is generally $p_T = 0.55$ and $q_t = 0.45$, respectively.

Conclusions: Frequencies of alleles T and t obtained in the male and female population under research are very close to the frequencies of the same alleles in some populations of India. Data of this study supplement the currently available information in relation to the genetic structure of modern Ukrainian cities.

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1. Introduction

The ability to detect signals with a different taste and smell is the integral part of adaptation of biological species to the environment. To date, several hundreds of chemoreceptors in the human body have been studied. Despite the fact that taste receptors are much less described than the olfactory ones, the population polymorphism extends increasingly to the taste sensitivity, especially the sensitivity to bitter, with respect to which 25 receptors are known. As for olfactory receptors, there are more than 400, however, the expressed olfactory polymorphism has been studied only with regard to a small number of compounds, particularly, androsterone (musk flavor), isovaleric acid (cheese flavor), *cis*-3-hexen-1-ol (grass flavor) and asparagus metabolites in the urine [1].

The taste sensitivity to phenylthiocarbamide is one of the classical genetic markers in the human studies. Phenylthiocarbamide (PTC) is a synthetic compound, which by the interaction with certain human taste receptors is felt bitter in some individuals (tasters) and tasteless in others (non-tasters). In a number of studies among tasters, the supertasters, who are capable of identifying a bitter taste in extremely low concentrations, are distinguished [2]. Despite the fact that PTC is synthesized in the laboratory and is not found in nature, the ability to sense this substance is highly correlated with the ability to sense other bitter compounds of natural origin, many of which are toxic, such as strychnine, ricin and quinine [3], and some are very useful, such as grapefruit, green tea, broccoli, arugula, cauliflower, and others [4] having in their composition compounds with anticancer activity (citrus flavonoids, polyphenols of green tea and red wine, cruciferous glucosinolates and isoflavones of soy products) [5]. On the other hand, according to some estimates, about 70% of cancers arise from the consumption of certain foods or smoking [6]. At the same time, it is essential to consider that sensitivity of certain compounds with a bitter taste, for example, goitrin, does not correlate with sensitivity to PTC, while they may be contained in the same products [7]. A variety of gustatory senses in the population can significantly affect the eating behavior of a person and, therefore, the state of his/her health; moreover, associations may have a sex specificity. For instance, in one of the studies it was shown that among females, lovers of tea with a bitterish taste, there were more PTC tasters, while among males, who consumed alcohol and preferred bitter coffee and tea, there were more PTC non-tasters [8]. At the same time, the unambiguity of this issue is still missing as in some studies, particularly in Africa, no relationship between the frequency distribution of PTC tasters and non-tasters and food preferences was found [9].

Geneticists offered different types of inheritance for the PTC sensitivity, including both single-locus and double-locus models [10]. Most family studies indicated the monogenic nature of the sensitivity to PTC. It was considered that the ability to sense the compound was controlled by a dominant allele, and the inability by a recessive allele of the autosomal gene, respectively. The molecular genetic nature of PTC was determined later when describing the gene of the sensitivity receptor to a bitter taste *h*TAS2R38 [11]. Moreover, it was found that the threshold values of the sensitivity to PTC and a related compound with a bitter taste propylthiouracil are highly correlated with each other [12]. Molecular studies of the mutant gene variants *h*TAS2R38 revealed the presence of three basic single-nucleotide substitutions that encode three different amino acids (*C145G*/*P49A*, *C785T*/*A262V* and *A886G*/*I296V*) [13]. At the moment the obvious nature of inheritance of the sensitivity to PTC allows to use this trait as learning applications. One can show students in real life examples the mechanism of the PTC sensitivity inheritance and to solve genetic problems during the classes in genetics [14].

The ability to sense PTC is not unique for *Homo sapiens* species. The experiments conducted on animals kept in the zoo environment showed that among chimpanzees, orangutans, gibbons and gorillas PTC tasters and non-tasters were also found [3]. PTC, which is close to rodenticide strychnine by the acute toxicity in mice, often acts as an object of various studies of eating behavior [15]. In animal models studied the compounds close to PTC, such as propylthiouracil, which, however, did not show the relationship with the sensitivity to ethanol in mice were also studied [16].

PTC is of great interest from the medical point of view since a number of associations of the taster status with human diseases have been found. In this connection, the sensitivity to PTC detected in the young age may be a predictor of a number of pathological conditions, including genetically determined (multifactorial) ones, which development in individuals in the risk group can be prevented by optimally selected environmental factors. Cheapness and availability of this type of testing appear to be attractive diagnostically. The following examples illustrate the diversity of associations of the sensitivity to PTC. In particular, in early studies the inhibitory activity of PTC in relation to the thyroid gland was found [17], and subsequently the toxic effect of the compound on the thyroid gland and a higher frequency of its pathologies, including goiter in non-tasters were observed. In particular, among non-tasters there were more individuals with pathology of the thyroid gland (68%) compared to tasters (32%) [8]. In one of the studies it was shown that the frequency of PTC non-tasters among individuals with idiopathic and symptomatic epilepsy was higher (35.5% and 32.5%, respectively) than in the control (20%) [18]. In another study non-tasters were more frequently found among patients with schizophrenia and their first degree relatives [19–20] although, according to other authors, this relationship was not confirmed [21]. A higher threshold of the sensitivity of secondary school students to PTC and sucrose was associated with a higher risk of dental caries [22]. Among adult non-tasters an increased inclination to obesity was reported, in particular, the odds ratio among PTC non-tasters to have BMI $\geqslant 25\ kg/m^2$ was 2.51 times more than among PTC tasters [23]. Among children with obesity there were 72% of non-tasters, while among children with the normal weight – only 28% [24]. At the same time non-tasters compared to tasters were less susceptible to malaria [25], and they had less high level of anxiety [26]. The relationship of the sensitivity to PTC and rheumatoid arthritis was studied, but it was not detected [27].

In Ukraine similar studies were carried out restrictedly in the western region of the country among the secondary school students of the Khmelnitsky region (Kamenetz-Podolsk) [26]. Data obtained in this population may not reflect the pattern of distribution of the sensitivity to PTC in Ukraine because different populations of Ukraine differ significantly in the genetic and demographic structure as it has been shown many times in our previous studies [28–32]. Actually, the frequencies

of the PTC taster and non-taster phenotypes obtained within the same country can vary by tens of times. Thus, in the populations of India, which are characterized by a significant structuredness, multiethnicity and differences in frequencies of tasters/non-tasters [33], non-tasters were found from 1.7% to 66.7% according to different data [34]. In this regard, it is interesting to perform research in other regions of Ukraine and henceforth to expand the study on the search of possible associations of the sensitivity to PTC with the human somatic and behavioral characteristics (and their deviant forms) in the local population. The aim of this study was to analyze distribution of the sensitivity to PTC in the sample of the population of Ukraine presented by residents of Kharkov and some other populations of Ukraine.

2. Subjects and methods of the study

2.1. Participants of the study

The study involved 533 people (78 males and 455 females) aged from 16 to 25 years. All participants of the study filled in specially designed questionnaires, which included a list of issues of the demographic and medical nature, as well as the issues related to food preferences. This study included the analysis of only the main characteristics of the respondents required to calculate the structure of the population by the genetic marker studied. The respondents were not relatives, and they largely represented the general population sample in relation to the sensitivity to PTC. The collection of information was conducted taking into account the ethical principles when dealing with a person in accordance with the Declaration of Helsinki (*World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects*). All participants of the study gave the written consent to participate in the study.

2.2. Preparation and the experimental trial of PTC testing-system

The PTC solution in the concentration of 0.13% was prepared according to the method of Harris and Kalmus [35]. To prepare 0.13% PTC solution 130 mg of a dry powder of the substance was dissolved in 100 ml of water. A filter paper was immersed into a freshly prepared solution, after that it was dried and cut into strips of 1×4 cm. The strips of a clean filter paper were used as a control system. The trial subjects were first offered to taste the ordinary strip of a filter paper, keeping it in the mouth and chewing for a few seconds, and record their sensations in writing. After that the participants of the study analyzed the taste of the filter paper impregnated with PTC in a similar way. If the trial subjects tasted PTC as ''bitter'', ''very bitter'', ''bitterish'', the phenotype was defined as a taster. If the trial subjects did not taste PTC (''no taste'', ''taste of paper''), he/she was referred to a non-taster.

2.3. Statistical analysis

The design of the experiment corresponded to the blind cohort cross-sectional study.

The relationship between the traits was determined by means of Pearson criterion χ^2. The significance of differences was determined at the level of $p \leqslant 0.05$.

The database was formed in the Microsoft Excel program. Calculations were made in the Statistica 6.0 program.

3. Results and discussion

In relation to the demographic indicators mentioned of all trial subjects 159 people were natives of the Kharkov and Kharkov region, 348 people were natives of other regions of Ukraine and 9 people were born outside Ukraine. 466 people identified themselves as Ukrainians, 45 – as Russians and 16 – as representatives of other nationalities. 385 of the respondents had the Ukrainian father, 80 had the Russian father and 23 had fathers of other nationalities. 432 of the trial subjects had the Ukrainian mother, 60 had the Russian mother and 20 had mothers of other nationalities. All trial subjects were students of the National University of Pharmacy (Kharkov).

Based on the analysis of the works of other authors it may be noted that samples, including school and college students, are used quite often in these studies. As examples one can mention the population-based study on the estimate of the frequency of PTC tasters in Pakistan (students of Medical college, 600 people) [36], Japan (students of Dental Faculty, 915 people) [37], the study on the search of associations of PTC with smoking (medical students, 80 people) [38] and with diabetes mellitus type 2 in the first degree relatives (students of Medical college, 41 people) [39].

When studying the phenotype frequencies of PTC tasters and non-tasters the data indicating that among all the populations studied 20% of PTC non-tasters were obtained. There is a tendency of increase of the frequency of non-tasters among males compared to females (24.4% and 19.1%, respectively), however, the relationship between sex and taster/non-taster status has not been found (Table 1).

Populations of the world have been well studied by this monogenic factor. Thus, in some samples of healthy populations the frequency of PTC non-tasters was: 18.6% – in Pakistan [36], 20% – in India [18] and 9.4% – in Japan [37]. The review [34] provides the data concerning many other populations and it has been indicated, for example, that the frequency of PTC non-tasters of the Eskimos of Greenland equaled 53.5%, among the natives of Australia it was 50.0%, in China – 5.1–23.0%, in different populations in Africa – 2.3–34.8%, in Philippines – 2.0%, in Russia – from 5.8% to 40.0%, etc. [34]. It has been shown that distribution

Table 1 Distribution of phenotypes of PTC tasters and non-tasters in the sample studied.

	Male *n* (%)	Female *n* (%)	Total *n* (%)
Taster	59 (75.6)	368 (80.9)	427 (80.1)
Non-taster	19 (24.4)	87 (19.1)	106 (19.9)
Total	78 (100)	455 (100)	533 (100)
Statistics	$\chi^2 = 1.147$, df = 1, $p > 0.05$		

Notes: χ^2 = Pearson criterion, df = degrees of freedom, and p represents the level of significance.

of PTC tasters and non-tasters in the populations is bimodal [40].

As for discussions and for comparative purposes it should be noted that despite the autosomal nature of the sensitivity to PTC there is a trend to the fact that some more tasters are found among females than among males in the world populations studied [41]; although in one of the studies it was shown that the average threshold of the sensitivity to PTC was slightly higher among females compared to males (the differences were not statistically significant) [42]. It can be assumed that males, phenotypic non-tasters, have adaptation to the substances with a bitter taste, with which they traditionally have more frequent contact (alcohol, tobacco) than females. In particular, it was shown that the threshold of the PTC sensitivity is higher among smokers although by the sensitivity to the other three species of the taste sensitivities studied (sweet, salty and sour) any significant differences compared to non-smokers were not observed [38]. In another study there were data that among males of one of the Indian populations the habit of chewing bidis (a type of cigarettes) was observed in 85% of males and only 4% of females, and it could affect the age-related changes of the threshold of the sensitivity to PTC [43]. At the same time in the other studies this trend in the sexual specificity of the sensitivity to PTC is not confirmed, or, on the contrary, it is reversed. Thus, the sexual differences in the frequency of PTC tasters and non-tasters were found in Pakistan where there were 23% of non-tasters among males and 14% among females [36].

Assuming that the sensitivity to PTC is not a trait by which an assortative mating exists (i.e., the population is panmictic), taking into account the monogenic nature of the trait there the frequencies of the gene responsible for the presence of (*T*) and the absence of (*t*) sensitivity to this compound were calculated separately among males and females (Table 2). Based on the Hardy–Weinberg equation the frequencies of three possible genotypes (dominant homozygotes *TT*, heterozygotes *Tt* and recessive homozygotes *tt*) (Table 3) were calculated. The levels of homozygosity and heterozygosity are presented in Table 4.

The frequencies of alleles *T* and *t* obtained in the male and female population under research are very close to the frequencies of the same alleles in two populations of India (*Khan*, *T* and *t* are 0.52 and 0.48, respectively among males, and *Mir*, *T* and *t* are 0.55 and 0.45, respectively among females) [41].

Data of this study supplement the currently available information in relation to the genetic structure of modern Ukrainian cities.

In our further studies it is planned to expand the sample and study the possible associations of the sensitivity to PTC with the indices in the local population, which are significant for the state of somatic and mental health.

Table 2 The frequencies of alleles of the sensitivity gene to PTC.

	Alleles	
	T	*t*
Male	0.51	0.49
Female	0.56	0.44
Total	0.55	0.45

Table 3 The genotype frequencies by the locus of the sensitivity to PTC.

	Genotypes		
	TT	*Tt*	*tt*
Male	0.2601	0.4998	0.2401
Female	0.3136	0.4928	0.1936
Total	0.3025	0.495	0.2025

Table 4 Heterozygosity and homozygosity for PTC tasting.

	H_t	H_o
Male	0.4998	0.5002
Female	0.4928	0.5072
Total	0.4950	0.505

Notes: H_t and H_o represent heterozygosity and homozygosity, respectively.

Conflict of interest

No conflict of interests.

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