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Structure of population of Ukraine by phenylthiocarbamide sensitivity

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The structure of the sample of population of Ukraine regarding the frequency of phenotypes, genotypes and genes, 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 by the given sample size (n=255) are not statistically significant. The frequency of dominant and recessive allele of the phenylthiocarbamide sensitivity gene calculated on the basis of the Hardy-Weinberg equation amounted in the sample as a whole p_T=0.47 and q_t=0.53 respectively.

Key words: *phenylthiocarbamide, taste sensitivity, tasters/non-tasters for bitter taste, genetic marker, population of Ukraine.*

Структура населения Украины по чувствительности к фенилтиокарбамиду

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Изучена структура выборки населения Украины в отношении частоты фенотипов, генотипов и генов, связанных с чувствительностью к фенилтиокарбамиду. Показано, что в популяции присутствует 22% лиц, которые не ощущают вкуса фенилтиокарбамида. Среди мужчин несколько больше нетестеров, чем среди женщин, однако различия при данном размере выборки (n=255) носят статистически не значимый характер. Рассчитанная на основании уравнения Харди-Вайнберга частота доминантного и рецессивного аллеля гена чувствительности к фенилтиокарбамиду составила в целом в выборке p_T=0,47 и q_t=0,53 соответственно.

Ключевые слова: *фенилтиокарбамид, вкусовая чувствительность, тестеры/нетестеры горького вкуса, генетический маркёр, население Украины.*

Структура населення України за чутливістю до фенілтіокарбаміду

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Вивчена структура вибірки населення України відносно частоти фенотипів, генотипів і генів, пов'язаних з чутливістю до фенілтіокарбаміду. Показано, що в популяції присутні 22% осіб, які не відчувають смаку фенілтіокарбаміду. Серед чоловіків дещо більше нетестерів, ніж серед жінок, проте відмінності при даному розмірі вибірки (n=255) носять статистично не значущий характер. Розрахована на підставі рівняння Харді-Вайнберга частота домінантного і рецесивного алеля гена чутливості до фенілтіокарбаміду склала в цілому у вибірці p_T=0,47 і q_t=0,53 відповідно.

Ключові слова: *фенілтіокарбамід, смакова чутливість, тестери/нетестери гіркого смаку, генетичний маркер, населення України.*

Introduction

The ability to detect signals with different taste and smell is the integral part of the adaptation of species to the environment. To date, several hundred of chemoreceptors have been studied in the human body. Despite the fact that taste receptors are much less described than the olfactory, population polymorphism increasingly covers the taste sensitivity, especially the sensitivity to bitter, in relation to which 25 receptors are known. As for olfactory receptors, there are more than 400, however, the expressed olfactory polymorphism was studied only with regard to a small number of compounds, particularly, androsterone (musk flavour), isovaleric acid (cheese flavour), cis-3-hexen-1-ol (grass flavour) and asparagus metabolites in urine (Newcomb et al., 2012).

The high taste sensitivity to phenylthiocarbamide is one of the classical genetic markers of a human. Phenylthiocarbamide (PTC) is a synthetic compound, which by the interaction with certain taste receptors of a human is felt bitter in some individuals (tasters) and tasteless in other (non-tasters). In a number of studies among tasters, the supertasters, which are capable of defining a bitter taste in extremely low concentrations, are distinguished (Bartoshuk, 2000). 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 (Wooding, 2006), and some are very useful, such as grapefruit, green tea, broccoli, arugula, cauliflower, and others (Garcia-Bailo et al., 2009), having in their composition compounds with oncoprotective activity (citrus flavonoids, green tea and red wine polyphenols, cruciferous glucosinolates and isoflavones of soy products) (Drewnowski et al., 2001). On the other hand, according to some estimates, about 70% of cancers arise from the consumption of certain food or smoking (Banning, 2005). At the same time, it is essential to consider that sensitivity of certain compounds with a bitter taste, for example, goitrin, is not correlated with sensitivity to PTC, while they may be contained in the same product (Wooding et al., 2010). Variety of gustatory senses in the population can significantly affects eating behavior of a human and, therefore, the state of his health and the associations may have 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 consume alcohol and prefer bitter coffee and tea, there were more PTC non-tasters (Shivaprasad et al., 2012). At the same time, the unambiguity of this issue is still missing, as in some studies, particularly in Africa, no connection between the frequency distribution of PTC tasters and non-tasters and food preferences was defined (Campbell, et al., 2012).

Geneticists offered different types of PTC inheritance sensitivity, including both single-locus and double-locus models (Olson et al., 1989). Most family studies indicated monogenic nature of sensitivity to PTC. It was considered that the ability to sense the compound was controlled by a dominant allele, and the inability, respectively, by a recessive allele of the autosomal gene. A nature of PTC was determined later by molecular genetics methods in the description of the gene of sensitivity receptor to a bitter taste *h*TAS2R38. It was shown that this receptor was also involved in the perception of related compounds with a bitter taste, propylthiouracil, in particular (Bufe et al., 2005). Moreover, it was found that the threshold value of sensitivity to PTC and propylthiouracil were highly correlated with each other (Sung Yong Choi et al., 2010). 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*) (Behrens et al., 2013). At the moment the obvious nature of inheritance of sensitivity to PTC allows to use this feature as learning applications (Merritt et al., 2008).

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 there were also found PTC tasters and non-tasters (Wooding, 2006). PTC, which is close in mice to rodenticid strychnine by the acute toxicity, often acts as an object of various studies of eating behavior (Johna et al., 2005). In animal models there were also studied the compounds close to PTC, such as propylthiouracil, which, however, didn't display the connection with sensitivity to ethanol in mice (White et al., 2007).

PTC is of great interest from the medical point of view, since a number of associations of taster status with human diseases is determined. In this connection, sensitivity to PTC defined in the young age may be a predictor of a number of pathological conditions, including genetically determined (multifactorial), 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 attractive in the way of diagnostic. The following examples illustrate the diversity of the associations of sensitivity to PTC. In particular, early studies ascertained the inhibitory activity of PTC for tyrosinase (Bernheim, Bernheim, 1942), and subsequently there has been studied the toxic effect of the compound on the thyroid gland and a higher frequency of its pathologies, including goiter in non-tasters. In particular, among non-tasters there were more individuals with pathology of the thyroid gland (68%) as compared with tasters (32%) (Shivaprasad et al., 2012). 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%) (Pal et al., 2004). In another study non-tasters were more frequently found among patients with schizophrenia and their

first-degree relatives (Moberg et al., 2005, 2007), although according to other authors, this relationship was not confirmed (Compton et al., 2007). A higher threshold of sensitivity both to PTC and sucrose in pupils was associated with a higher risk of dental caries (Sourav Sen et al., 2012). Among adult non-tasters there was indicated an increased inclination to obesity, in particular, the odds ratio among PTC non-tasters to have BMI ≥ 25 kg/m^2 was 2.51 times more than among PTC tasters (Gandhi et al., 2012). Among children with obesity there were 72% of non-tasters, while among children with normal weight – only 28% (Saraswathi et al., 2011). At the same time non-tasters as compared with tasters were less susceptible to malaria (Igbeneghu et al., 2014) and they had less high level of anxiety (Pavlovich, Bezrukov, 2007). The connection between sensitivity to PTC and rheumatoid arthritis was studied, but was not detected (Yagur, 2008).

Populations of the world have been well studied by this monogenic factor. Thus, in some samples of the healthy population the frequency of PTC non-tasters was: 18.6% – in Pakistan (Iqbal et al., 2006), 20% – in India (Pal et al., 2004) and 9.4% – in Japan (Sato et al., 1997). The review (Guo, Reed, 2001) provides the data on many other populations and it has been indicated, for example, that the frequency of PTC non-tasters of 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. (Guo, Reed, 2001). It has been shown that the distribution of PTC tasters and non-tasters in the populations is bimodal (Jaiswal, 2012).

In Ukraine, the similar studies were carried out restrictedly in the western region of the country among the pupils of Khmelnitsky region (Kamenetz-Podolsk) (Pavlovich, Bezrukov, 2007). The data obtained in this population may not reflect the pattern of distribution of sensitivity to PTC in Ukraine. Indeed, the frequencies of PTC tasters and non-tasters 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, multi-ethnicity and differences in frequencies of tasters/non-tasters (Yumnam Luxmi, Kapoor, 2011), non-tasters were found from 1.7 to 66.7% by the different data (Guo, Reed, 2001). In this regard, it is interesting to perform a research in other regions of Ukraine and henceforth to expand the study on the search of possible associations of sensitivity to PTC with somatic and behavioral characteristics of a human (and their deviant forms) in the local population. The purpose of this study was to analyze the distribution of sensitivity to PTC in the sample of the population of Ukraine, presented by residents of Kharkiv and some other populations of Ukraine.

Objects and methods of the study

Participants of the study

The study involved 255 people (47 males and 208 females) aged from 16 to 20 years. All participants of the study completed specially designed questionnaires, which included a list of issues of demographic and medical kind, as well as issues related to food preferences. This study included the analysis of only the main characteristics of the surveyed participants, which were needed to calculate the structure of the population by the studied genetic marker. The surveyed persons were not relatives and they largely represented the general population sample regarding 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.

Preparations and probation of PTC testing-system

PTC solution in concentration of 0.13% was prepared according to the method of Harris and Kalmus (1949). To prepare 0.13% PTC solution, 130 mg of dry powder were dissolved in 100 ml of water. A filter paper was immersed into a freshly prepared solution, after which it was dried and cut into strips of 1 × 4 cm. The strips of clean filter paper were used as a control system. The surveyed were first offered to taste the ordinary strip of filter paper, keeping it in the mouth and chewing for a few seconds, and record their feelings on paper. After that the participants of the study analyzed the taste of filter paper impregnated with PTC in a similar way. If the surveyed tasted PTC as "bitter", "very bitter", "bitterish", the phenotype was defined as a taster. If the surveyed didn't taste PTC ("no taste", "taste of paper"), he was referred to a non-taster.

Statistical analysis

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

The connection between the features was determined by means of tetrachoric index of connection K and criterion χ^2. The significance of differences was determined at the level of $p \leq 0.05$.

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

Results and discussion

In relation to the mentioned demographic indicators, 104 people of all surveyed were natives of Kharkiv and Kharkiv region, 144 people were natives of other regions of Ukraine and 4 people were born outside Ukraine. 198 people identified themselves as Ukrainians, 38 – as Russians and 13 – as representatives of other nationalities. 168 of the surveyed had Ukrainian father, 35 had Russian father and 13 had fathers of other nationalities. 195 of the surveyed had Ukrainian mother, 41 had Russian mother and 9 had mothers of other nationalities. All surveyed were students of National University of Pharmacy (Kharkiv).

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 there may be mentioned the population-based study on the estimate of the frequency of PTC tasters in Pakistan (students of medical college, 600 people) (Iqbal et al., 2006), Japan (students of dental faculty, 915 people) (Sato et al., 1997), and the study on the search of associations of PTC with smoking (medical students, 80 people) (Krut et al., 1961) and with diabetes mellitus type 2 at relatives with the first degree of relationship (students of medical college, 41 people) (Joseph, 2013).

In the study of phenotype frequencies of PTC tasters and non-tasters there were obtained the data, which indicated that among all the studied population 22% of PTC non-tasters were found. There is a tendency of increase of the frequency of non-tasters among males as compared with females (29.8% and 20.2% respectively), however, the connection between gender and taster/non-taster status was not detected (p=0.15). At the same time we cannot exclude the fact that with the expansion of the sample followed by the increase of power, the connection may appear to be statistically significant (table 1).

Table 1.

The distribution of phenotypes of PTC tasters and non-tasters in the studied sample

	Males		Females		Sum total	
	n	% (of all males)	n	% (of all females)	n	% (of all the sample)
Tasters	33	70.2	166	79.8	199	78.0
Non-tasters	14	29.8	42	20.2	56	22.0
Sum total	47	100	208	100	255	100
Statistics	χ^2=2.059, v=1, K=-0.17, p=0.15					

Notes: χ^2 – Pearson criterion, v – the number of degrees of freedom, K – the index of connection, p – the significance level.

For discussion and for comparative purposes it should be noted that despite the autosomal nature of sensitivity to PTC, in the studied populations of the world there is a trend to that some more tasters are found among females than among males (Fareed et al., 2012), although in one of the studies it was shown that the average threshold of sensitivity to PTC was slightly higher among females as compared with males (the differences were not statistically significant) (Hussain et al., 2013). It can be assumed that males, phenotypic non-tasters, have an 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 PTC sensitivity is higher among smokers, although for the sensitivity to the other three species of the studied taste sensitivities (sweet, salty and sour) there were not observed any significant differences as compared with non-smokers (Krut et al., 1961). In another study there were presented the 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, which could affect the age-related changes of the threshold of sensitivity to PTC (Padmavathi, 2013). At the same time, in other studies this trend of the sexual specificity of sensitivity to PTC it 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 (Iqbal et al., 2006).

With the assumption that sensitivity to PTC is not a feature by which there exists an assortative mating (i.e., population is panmictic), taking into account the monogenic nature of a feature, separately among males and females there were calculated the frequencies of the gene responsible for the presence of (*T*) and the absence (*t*) of sensitivity to this compound (table 2). Also on the basis of the Hardy-Weinberg equilibrium the frequencies of three possible genotypes (dominant homozygotes *TT*, heterozygotes *Tt* and recessive homozygotes *tt*) (table 3) were calculated.

The derived frequencies of alleles *T* and *t* in the male and female parts of the surveyed population are very close to the frequencies of the same alleles in one of the populations of India (*Gujjar* and *Bakarwal*, *T* and *t* are 0.44 and 0.56 respectively among males, and *T* and *t* are 0.55 and 0.45 respectively among females) (Fareed et al., 2012).

Table 2.

The frequencies of alleles of sensitivity to PTC genes

	Alleles	
	T	*t*
Males	0.45	0.55
Females	0.55	0.45
Sample in tote	0.47	0.53

Table 3.

The genotype frequencies by the locus of sensitivity to PTC

	Genotypes		
	TT	*Tt*	*tt*
Males	0.2025	0.495	0.3025
Females	0.3025	0.495	0.2025
Sample in total	0.2209	0.4982	0.2809

The data of this study supplement the currently available information regarding the genetic structure of modern Ukrainian cities.

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

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