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The Frequency of Alleles in the *GSTT*1 and *GSTM*1 Genes Involved in Phase II of Xenobiotic Transformation in Long-Lived People of Subcarpathia

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Abstract—Polymorphism of genes of Phase II of xenobiotic biotransformation was studied in 166 long-lived people and in 169 control subjects who live in the Ivano-Frankivsk region. The frequency of the functionally inactive allele of the *GSTT*1 gene among the long-lived people was 24.70% and in the control group it was 20.12%. The frequency of the functionally inactive allele of *GSTM*1 in the long-lived people was 46.99 and in the control group it was 54.44%. The area of residence of the subjects was divided into zones with regard to environmental factors: environmentally comfortable, under moderate environmental load, and environmentally uncomfortable. Analysis of the combinations of alleles of glutathione-*S*-transferase genes revealed an elevated frequency of the *GSTM*1+/*GSTT*1+ allele combination in the long-lived people of the uncomfortable zone in comparison to the control group of the same zone: 54.55 and 35.09%, respectively ($\chi^2 = 4.29$; *OR* = 2.22 (1.04–4.75)). The *GSTM*1–/*GSTT*1+ combination was significantly more frequent in the control group than in the long-lived people: 21.82 and 43.86%, respectively ($\chi^2 = 6.15$; *OR* = 0.36 (0.16–0.82)). Significant differences were detected between the frequencies of combinations *GSTM*1+/*GSTT*1+, *GSTM*1+/*GSTT*1–, *GSTM*1–/*GSTT*1+, and *GSTM*1–/*GSTT*1– in long-lived people of the environmentally comfortable and uncomfortable zones: $\chi^2 = 6.44$, *OR* = 0.36 (0.16–0.80); $\chi^2 = 4.86$, *OR* = 4.89 (1.28–18.72); $\chi^2 = 5.89$, *OR* = 2.82 (1.20–6.58); $\chi^2 = 3.90$; *OR* = 0.19 (0.16–6.58), respectively.

Keywords: alleles, *GSTM*1, *GSTT*1, long-lived people

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INTRODUCTION

The development of disorders depends on personal hereditary features, which determine the variation in sensitivity and responses to ambient factors. The xenobiotic detoxication system holds the key place in the response of a person to the environment. Therefore, investigation of the individual features of detoxication processes is essential for the determination of the individual risk of disorders with regard to the present-day industrial load [4, 6]. The biotransformation of alien substances, which includes their enzymatic conversion, is divided into three phases. The first phase is activation. It involves modification by attaching functional groups (–OH, –SH, or $-NH_3$) through oxidation, reduction, or hydrolysis and generates intermediate metabolites. This process is catalyzed by the microsomal cytochrome P_{450} system and several other enzymes belonging to oxidases, reductases, hydrolases, or dehydrogenases. In the second phase, neutralization, endogenous ligands are attached to the intermediate metabolites, making them even more hydrophilic, which helps their washout. Thus, the second phase is the conjugation of high-molecular-weight hydrophilic substances with various substrates and the generation of hydrophilic conjugates, which can be excreted with bile. In the third phase, the water-soluble nontoxic products are excreted from the body. This process is done by special carriers, viz., P-glycoproteins. They mediate the excretion of xenobiotics to bile or blood. Most studies are dedicated to enzymes that are involved in phase II of xenobiotic detoxication, in particular, *N*-acetyl- and glutathione-*S*-transferases [13, 16, 19]. The multigenic glutathione-*S*-transferase (GST) family detoxicates various aliphatic, aromatic, and heterocyclic compounds by conjugating them with glutathione. Cytosolic GSTs are divided into seven classes: α, μ, ω, π, σ, θ, and ζ. Numerous alleles of *GST* genes are known. Several deletions in the *GSTM*1 and *GSTT*1 genes for glutathione-*S*-transferases μ and θ, respectively, determine the absence of the activity of the corresponding enzymes. It is believed that persons who are homozygous for such deletions are predisposed to some multifactorial diseases. The roles of detoxication system genes and their alleles in aging are under investigation [5, 7, 8, 12, 14, 16, 18]. The aging of the pop-

Table 1. Distribution of subjects from the long-lived and control groups over environmental living conditions

Environmental zone	Number of long-lived people	Control
Environmentally comfortable (zone 1)	50	35
Moderate environmental load (zone 2)	61	77
Environmentally uncomfortable (zone 3)	55	57

ulation stimulates studies of primary aging mechanisms and factors that affect longevity. Therefore, top priority is given to works that concern patterns in the relationships among exogenous and endogenous factors, longevity, and molecular mechanisms. These efforts are aimed at the prediction and stimulation of active aging.

The goal of the study is to investigate the relationships of the frequencies of alleles of the *GSTM*1 and *GSTT*1 genes with longevity in long-lived people who inhabit various environmental zones of Subcarpathia.

MATERIALS AND METHODS

Experiments were performed with DNA samples taken from 166 long-lived people. The samples were genotyped for the *GSTM*1 and *GSTT*1 genes by multiplex PCR with amplificate resolution in 1.5% agarose gel. Primer sequences for detecting alleles of *GSTM*1 and *GSTT*1 and amplification regimes were chosen according to [10]. The results were compared with the control group. It included 169 persons of various ages who had no long-lived people in their pedigrees according to clinical and genealogical records and lived under the same environmental conditions as the long-lived group.

The subjects were divided into three groups according to living conditions (Table 1). The division of the region into environmental zones followed the ecological certificate of the Ivano-Frankivsk oblast and the results of environmental studies in Ukraine [9].

Statistical evaluation of the results was performed by the Chi-square method (Statistica 10.0) and odds ratio (OR).

RESULTS AND DISCUSSION

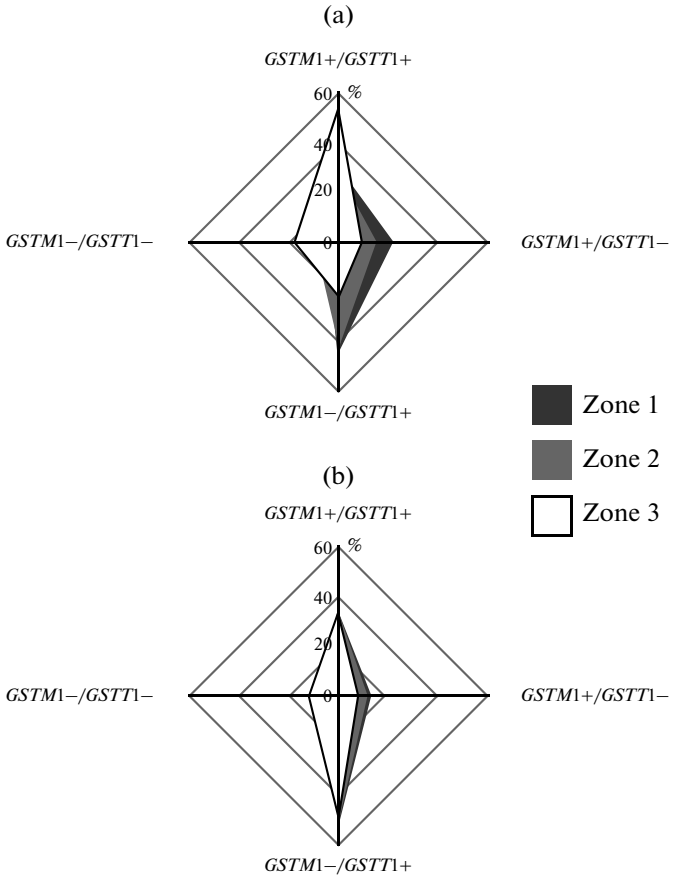
In our study, the frequency of the nonfunctional allele of *GSTT*1 in long-lived people of the Ivano-Frankivsk oblast was 24.70% vs. 20.12% in the control group. The frequencies of the nonfunctional allele of *GSTM*1 in long-lived people and control subjects were 46.99 and 54.44%, respectively, with the difference being insignificant (Table 2). The frequencies of the combinations *GSTM*1+/*GSTT*1+, *GSTM*1+/*GSTT*1−, *GSTM*1−/*GSTT*1+, *GSTM*1−/*GSTT*1− revealed no significant differences between long-lived people and control subjects either (Table 2).

Associations of alleles of various gene groups, including genes of the xenobiotic detoxication system, with longevity have been considered by other scientists as well. For example, the polymorphism of genes for cytochromes P450 *CYP1A1*, *CYP1B1* and glutathione-*S*-transferases *GSTM*1, *GSTT*1, *GSTP*1 was analyzed in 205 Germans of ages of 80 years and older in comparison with 294 younger Germans [18]. The allele of *GSTT*1 most clearly associated with longevity contained a deletion within the gene. It was found in 14% of the long-lived people and in 21% of the control subjects [18].

The localities we studied were zoned with regard to environmental conditions: environmental comfort

Table 2. Frequencies of genotypes for *GSTM*1 and *GSTT*1 in Subcarpathian long-lived people and in the control group

Gene	Genotype	Subcarpathian long-lived people, $n = 166$		Control group, $n = 169$		χ^2	*OR*	95% CI	*p*
		abs. number	%	abs. number	%				
*GSTM*1	*GSTM*1−	78	46.99	92	54.44	1.86	0.74	0.48–1.14	>0.05
	*GSTM*1+	88	53.01	77	45.56	1.86	1.35	0.88–2.07	>0.05
*GSTT*1	*GSTT*1−	41	24.70	34	20.12	1.01	1.30	0.78–2.08	>0.05
	*GSTT*1+	125	75.30	135	79.88	1.01	0.74	0.46–1.29	>0.05
*GSTM*1+/*GSTT*1+		65	39.16	57	33.72	1.07	1.26	0.81–1.98	>0.05
*GSTM*1+/*GSTT*1−		23	13.86	20	11.84	0.31	1.20	0.63–2.28	>0.05
*GSTM*1−/*GSTT*1+		60	36.14	78	46.16	3.46	0.66	0.43–1.02	>0.05
*GSTM*1−/*GSTT*1−		18	10.84	14	8.28	0.63	1.35	0.65–2.80	>0.05



The distribution of frequencies of genotype combinations for *GSTM*1 and *GSTT*1 in (a) Subcarpathian long-lived people and (b) control subjects living in various environmental zones.

(zone 1), moderate environmental load (zone 2), and environmentally uncomfortable (zone 3). The study concerned frequencies of *GSTM*1 and *GSTT*1 alleles and genotypes *GSTM*1+/*GSTT*1+, *GSTM*1+/*GSTT*1−, *GSTM*1−/*GSTT*1+, and *GSTM*1−/*GSTT*1−. Analysis of combinations of genotypes for GST enzymes in persons living for long periods in zone 3 showed that the *GSTM*1+/*GSTT*1+ combination occurred significantly more frequently in long-lived people than in the controls: 54.55 and 35.09%, respectively ($\chi^2 = 4.29$, $OR = 2.22$ (1.04–4.75), see figure). There were no other significant differences.

Comparison of frequencies of *GSTM*1/*GSTT*1 allele combinations in long-lived groups living in various environmental zones revealed significant differences between long-lived people of the environmentally comfortable and uncomfortable zones in the frequencies of the allele combinations *GSTM*1+/*GSTT*1+, *GSTM*1+/*GSTT*1−, *GSTM*1−/*GSTT*1+, and *GSTM*1−/*GSTT*1−. The *GSTM*1+/*GSTT*1+ combination was found in 30% of the long-lived people who had lived for long periods in zone 1 and in 54.55% of long-lived people of zone 3 ($\chi^2 = 6.44$, $OR = 0.36$ (0.16–0.80)). The combinations *GSTM*1+/*GSTT*1− and *GSTM*1−/*GSTT*1+ were significantly more frequent among long-lived people of zone 1: $\chi^2 = 4.86$, $OR = 4.89$ (1.28–18.72), 44.00 and 21.82%; $\chi^2 = 5.89$; $OR = 2.82$ (1.20–6.58), respectively. The *GSTM*1−/*GSTT*1− combination was significantly more frequent in long-lived people of zone 3 than in zone 1: 18.18 and 4.00%, respectively: ($\chi^2 = 3.90$, $OR = 0.19$ (0.16–6.58)) (Tables 3 and 4).

The role of molecular markers associated with the rates of aging processes has been considered in a number of papers [1–3, 5, 11, 15, 17, 19, 20]. In particular, the oxidative-stress theory is proposed to be replaced by the more general *green theory of aging*. The latter implies that aging is a result of macromolecular lesions caused by various endogenous and exogenous substances and toxic metabolites, including oxidative stress and free radicals. Longevity is determined by the rate of elimination of toxic substances from the body and the efficiency of lesion repair.

CONCLUSIONS

The frequency of the nonfunctional allele of *GSTT*1 in all long-lived people of the Ivano-Frankivsk oblast was 24.70% and in the control group it was 20.12%. The frequency of the nonfunctional allele of

Table 3. Frequencies of genotype combinations for *GSTM*1 and *GSTT*1 in Subcarpathian long-lived people living in the environmentally comfortable zone and in the zone of a moderate environmental load

Genotype combination	Comfortable zone, $n = 50$		Moderate environmental load, $n = 61$		χ^2	*OR*	95% CI	*p*
	abs. number	%	abs. number	%				
*GSTM*1+/*GSTT*1+	15	30.00	20	32.79	0.10	0.88	0.39–1.97	>0.05
*GSTM*1+/*GSTT*1–	11	22.00	9	14.75	0.98	1.63	0.62–4.32	>0.05
*GSTM*1–/*GSTT*1+	22	44.00	26	42.62	0.02	1.06	0.50–2.25	>0.05
*GSTM*1–/*GSTT*1–	2	4.00	6	9.84	0.66	0.38	0.07–1.98	>0.05

Table 4. Frequencies of genotype combinations for *GSTM*1 and *GSTT*1 in Subcarpathian long-lived people living in the environmentally comfortable and uncomfortable zones

Genotype combination	Comfortable zone, $n = 50$		Uncomfortable zone, $n = 55$		χ^2	*OR*	95% CI	*p*
	abs. number	%	abs. number	%				
*GSTM*1+/*GSTT*1+	15	30.00	30	54.55	6.44	0.36	0.16–0.80	<0.05
*GSTM*1+/*GSTT*1–	11	22.00	3	5.45	4.86	4.89	1.28–18.72	<0.05
*GSTM*1–/*GSTT*1+	22	44.00	12	21.82	5.89	2.82	1.20–6.58	<0.05
*GSTM*1–/*GSTT*1–	2	4.00	10	18.18	3.90	0.19	0.04–0.90	<0.05

*GSTM*1 in all long-lived people of the Ivano-Frankivsk oblast was 46.99% and in the control group it was 54.44%. The differences were insignificant.

In subjects who had lived for long periods in the environmentally uncomfortable zone, the allelic combination *GSTM*1+/*GSTT*1+ was significantly more frequent among the long-lived people than in the control group: 54.55 and 35.09%, respectively.

The combination *GSTM*1–/*GSTT*1– was significantly more frequent in the control group than among the long-lived people: 21.82 and 43.86%, respectively.

A significant difference was detected between long-lived people of the comfortable and uncomfortable zones in the frequencies of the genotypes *GSTM*1+/*GSTT*1+, *GSTM*1+/*GSTT*1–, *GSTM*1–/*GSTT*1+, and *GSTM*1–/*GSTT*1–. The genotypes *GSTM*1+/*GSTT*1+ and *GSTM*1–/*GSTT*1– were significantly more frequent among long-lived people of the environmentally uncomfortable zone.

The combinations *GSTM*1+/*GSTT*1– and *GSTM*1–/*GSTT*1+ were more frequent among long-lived people who had lived for long periods in the environmentally comfortable zone.

Our results allow comparison with not only environmental but also other living conditions, which will add to the understanding of aging mechanisms.

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