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GENETIC MARKERS OF CANCER PATHOLOGIES

Identification of high-risk populations is a research priority in cancer prevention. Genetic predisposition to cancer development is an obvious marker for risk to reduce cancer mortality in this group of patients.

Genetic factors affect the tendency to develop cancer. Predisposing mutations often influence DNA repair, cell-cycle regulation and cell-death pathways. In this article we present the results of research devoted to identifying of genetic risk factors for cervical and esophagus cancer. For the "case-control" study we have used clinical material (blood, buccal smears, biopsy materials, cervical smears) of 115 patients suffering from esophageal cancer and 207 patients with cervical cancer. In accordance to the ethnic and age data of esophagus and cervical cancer patients abnormalities of control groups of healthy individuals (100 and 160 respectively) were selected. Genes involved in the processes of xenobiotics detoxification (GSTM1 and GSTT1), DNA repair (XRCC1 and XRCC3), and cell cycle regulation and apoptosis (CCND1 and TP53), were examined as a genes candidates for esophagus and cervix cancer. Conducted research allowed to determine the panels of genetic markers of predisposition to the development: cervical cancer - deletions of GSTT1 and GSTM1 genes; XRCC1 Arg194Arg; XRCC1 Gln399Gln, XRCC3 Met241Met and TP53 Arg72Arg; esophageal cancer - deletions of GSTT1 and GSTM1 genes; XRCC3 Met241Met; TP53 Pro72Pro, CCND1 A870A. These results will be used for developing of test-kits for the defining susceptibility to esophageal and cervical cancers. The introduction of these tests to the screening programs will allow to develop large-scale preventive measures and will have an impact on reducing cancer incidence and mortality in Kazakhstan, helping to extend the qualitative longevity.

Keywords: *cervical cancer, esophageal cancer, genetic susceptibility, single nucleotide polymorphism, GSTT1 and GSTM1 deletions, DNA repair, xenobiotic detoxification.*

Introduction. Cancer is the leading cause of death worldwide. According to estimates from the International Agency for Research on Cancer (IARC), there were 12,7 million new cancer cases in 2008 worldwide. The total number of cancer continues to increase. By 2030, it is projected that there will be ~26 million new cancer cases and 17 million cancer deaths per year [1].

One of the most perspective directions in modern molecular genetics and medicine is search and identification of genetic markers of cancer development.

Cancer is a genetic disorder in which the normal control of cell growth is lost. Individuals differ in their inherited tendency to develop cancer. Major single-gene defects that cause early cancer onset have been known for many years from their inheritance patterns, and inherited defects that have weaker effects on predisposition were also suspected to exist. Recent progress in cancer genetics has identified specific loci that are involved in cancer progression, many of which have key roles in DNA repair, cell-cycle control and cell-death pathways. Those loci, which are often mutated somatically during cancer progression, sometimes also contain inherited mutations. Recent genetic studies and quantitative population-genetic analyses provide a framework for understanding the frequency of inherited mutations and the consequences of these mutations for increased predisposition to cancer [2]. Identification of high-risk populations is a research priority in cancer prevention. Genetic predisposition to cancer development is an obvious marker for risk to reduce cancer mortality in this group of patients.

Mutations of the same gene may cause the several cancer types. Thus, mutations of tumor suppressor gene TP53 were detected in the tumors of all tissues and organs. The spectrum of mutations in key genes, involved in the control of genome instability, DNA repair, cell cycle, apoptosis, and such processes as xenobiotics detoxification, may differ for different cancer types.

Genetic polymorphism spectrums depend on the geographical conditions, diet, ethnicity, etc. and are the result of natural selection. In certain circumstances, genetic polymorphisms can predispose to the development of specific diseases, or, on the contrary, to protect organism. Analysis of genomic polymorphism, which forms the basis of predictive medicine, helps to identify the individual genotypes that predispose to the development of diseases [3, 4].

Here we present the results of molecular epidemiological study of populations from Kazakhstan, representing healthy individuals and patients with esophageal and cervical cancers. The choice of these cancer types is caused by high levels of morbidity and mortality in Kazakhstan.

Esophageal cancer is one of the most aggressive forms of cancer. It is ranked on the 9th place by malignancy and on the 7th place by mortality. Esophageal cancer often diagnosed at an advanced stage, and therefore the five-year survival rate for this type of cancer is only 5-10%. This is particularly acute problem for Kazakhstan, where the incidence of esophageal cancer in the male population reaches 25,7 cases per 100 000 population.

Cervical cancer very often suffers women in the reproductive age. Kazakhstan is among the countries with high levels of cervical cancer cases. Among Kazakhstan women the cervical cancer is on the second place, after the breast cancer.

Identification of genetic markers for these types of cancer will help to determine strategies of prevention, early diagnosis and personalized treatment.

The choice of candidate genes for this research was based on literature data. The types of genetic markers for study: 1) deletion polymorphism of genes participating in xenobiotic detoxification - glutation-S-transferases - GSTM1 and GSTT1; 2) 2 types of single nucleotide polymorphism (SNP) of XRCC1 (Arg194Trp and Arg399Gln), responsible for the repair of double strand DNA breaks; 3) SNP of XRCC3 (Thr241Met), responsible for the repair of single strand DNA breaks; 4) SNP of gene regulating cell cycle and apoptosis - TP53 (Arg72Pro); 5) SNP of cell cycle regulating gene cyclin D1 - CCND1 (A870G).

Material and Methods. This "case-control" study was approved by the Ethics Committee of Kazakh National Medical University named after S.D. Asfendiyarov. The material was collected on the basis of Kazakh Research Institute of Oncology and Radiology (Almaty, Kazakhstan) by approbation of the patients. We used clinical material (blood, buccal smears, biopsy materials, cervical

smears) of 115 patients suffering from esophageal cancer and 207 patients with cervical cancer. In accordance to the ethnic and age data of esophagus and cervical cancer patients abnormalities of control groups of healthy individuals (100 and 160 respectively) were selected.

A detailed questionnaires and informed consents were collected when we took biosamples. The clinical diagnosis verification was carried out cytologically and histologically on biopsy material.

DNA samples were extracted by standard phenol-chloroform method with modifications in lysis buffer composition (for blood samples: 0.2 M sodium acetate and 1% sodium dodecylsulfate, pH 8.0; for tissue: 0,02 M ethylenediaminetetraacetic acid (EDTA); 0,02 M tris-HCl, pH=8,0; 0,16 M NaCl; 0,3% sodium dodecylsulfate, 1 U of protease E).

Water diluted DNA samples were used for all type of polymerase chain reaction (PCR). 20-100 nanograms of target DNA was amplified in total volume of 20 μL of PCR mixtures using amplifier "Mastercycler" (Eppendorf, Germany). Term and time conditions were selected to each gene individually taking in the account the size of primers.

The genotyping of GSTM1 and GSTT1 deletion polymorphisms was carried out by multiplex PCR using Taq-polymerase (Sigma-Aldrich, USA). The results were recorded as each gene being present or absent. The specific primers for GSTM1 and GSTT1 genes and a primer set for a β-globin fragment using as a control of amplification were used for the same amplification reaction. The 215-bp GSTM1 and the 480-bp GSTT1 fragments were analyzed using 1.4% agarose gel electrophoresis and Lambda/Hind III marker. The absence of either GSTM1 or GSTT1 fragments indicated the corresponding null genotype.

The method of site-specific PCR with following restriction of amplified fragments was used for the genotyping of XRCC1 Arg194Trp; XRCC1 Arg399Gln and XRCC3 Thr241Met single nucleotide polymorphisms. We use the enzymes produced by Fermentas (Lithuania): PvuII, Bcn1, and Nco1, respectively. Restriction products were analysed using 3% agarose MetaPhor (Lonza, USA) gel. 490 bp fragment characterizes XRCC1194Arg allele, 2 bands (294/196 bp.) - XRCC1194Trp allele, 2 fragments (89/159 bp) - XRCC1399Arg, 1 fragment 248 bp -XRCC1399Gln allele. 1 band 136 bp corresponds to XRCC3 241Trp allele and 2 bands (97/39 bp) gives the XRCC3 241Met allele.

The genotyping of TP53 Arg72Pro and CCND1 A870G polymorphisms was carried out by direct sequencing method and by TaqMan allelic discrimination method. The direct sequencing was performed using BigDye® Terminator v3.1 kit and Genetic Analysis System ABI PRISM® 3130 (Applied Biosystems). TaqMan allelic discrimination was performed using ABI PRISM® 7700 Sequence Detection System (Applied Biosystems).

The statistical analysis of the obtained data was performed using GraphPad InStat™ Software (V. 2.04. Ralf Stahlman, Purdue University) and “Case-Control Study Estimating Calculator” from TAPOTILI company (Laboratory of Molecular Diagnostics and Genomic Dactiloscopy of “GosNII Genetika” State Scientific Centre of Russian Federation; http://www.tapotili.ru). The odd ratio calculation was carried out taking into account dominant and recessive model.

Results. Association of genomic polymorphism with development of esophageal cancer

All of 115 persons representing esophageal cancer case group were suffering from squamous- cell carcinoma. Among these patients there are 8 cases of high differentiated carcinoma, 48 cases of moderately differentiated carcinoma and 59 cases of low-grade differentiated carcinoma.

The control cohort representing healthy people without any noticeable pathologies was matched to case cohort by the age, sex, and ethnicity and smoking habit (table 1).

Table 1. The correspondence of the esophageal cancer case and control cohorts by age, ethnicity, sex and smoking habit.

Cohort	Years of birth	Nationality, persons (%)		Sex, persons (%)		Smoking habit, persons (%)	Total, persons
		Kazakh	Russian	Male	Female		
Case	1920-1977	102 (88.69)	13 (11.31)	62 (53.91)	53 (46.09)	30 (26,08)	115
Control	1921-1976	89 (89.00)	11 (11.00)	54 (54.00)	46 (46.00)	26 (26,00)	100

For the genotyping we used DNA samples extracted from frozen (-80^{0}C) EDTA-containing blood samples preferably. The buccal smears used only in the case of blood sample absence. Genotyping of candidate genes polymorphism (deletions GSTM1 and GSTT1, XRCC1 Arg194Trp and Arg399Gln, XRCC3 Thr241Met, TP53 Arg72Pro and CCND1 A870G) was performed by the different methods (see Materials and Methods).

All candidate genes genotyping results show no contradictions with the Hardy-Weinberg equilibrium.

The genotyping of genes participating in xenobiotic detoxification - GSTM1 and GSTT1 was performed in multiplex mode. A differences in the allele distributions in control and case cohorts were found for the both genes. The frequency of GSTT1 deletion in case cohort was significantly higher than in control cohort: 0,822 versus 0,635, respectively. Also the frequency of GSTM1 deletion (0,796) in case cohort is more higher then in control population (0,490).

Genotyping of 2 types of DNA repair genes was done separately using site-specific PCR with following restriction of amplified fragments. The distribution of allele variants for the 2 SNP polymorphisms of gene, responsible for the repair of double strand DNA breaks - XRCC1 Arg194Trp and Arg399Gln is opposite to each other. The frequencies of rare allele variants XRCC1 194Trp in control and case cohorts are the same (0,145 and 0,148, respectively). But the frequencies of rare allele XRCC1 399Gln are significantly differ in control and case cohort (0,275 and 0,343, respectively). For the polymorphism of another DNA repair gene - XRCC3 Thr241Met, there are no significant differences in the allele variants distribution between control (0,815 - for allele XRCC3 241Thr and 0,185 - for allele XRCC3 241Met) and case (0,822 - for allele XRCC3 241Thr and 0,178 - for allele XRCC3 241Met) populations.

Cell cycle regulating gene cyclin D1 polymorphism (CCND1 A870G) and Arg72Pro polymorphism of TP53, key gene regulating genome instability, cell cycle and apoptosis, were defined using direct sequencing and TaqMan allelic discrimination methods. Genotyping results for the TP53 Arg72Pro polymorphism show differences between control and esophageal cancer case cohort. The frequency of rare allele variant TP53 72Pro in control population is 0,240 and in case population – 0,343. The frequencies of CCND1 allele variants also differ in control and case cohorts. Control cohort show the prevalence of CCND1 870A allele (0,550). And the CCND1 870G allele (0,596) predominates in case cohort.

We performed the statistical analysis of association between genetic polymorphism and development of esophageal cancer (table 2).

Table 2 – Association between genetic polymorphism and development of esophageal cancer

Type of polymorphysm	Genotype	Esophageal cancer, persons (%)	Control, persons (%)	Odds ratio (OR)	Confidence interval (CI), (95%)	χ^2	P
GSTT1	+/+	5 (4,35)	13 (13,00)	0,30	0,10-0,89	18,66	9.0e-5
	+/-	31 (26,96)	47 (47,00)	0,42	0,24-0,74		
	-/-	79 (68,69)	40 (40,00)	3,29	1,88-5,77		
GSTM1	+/+	4 (3,48)	26 (26,00)	0,10	0,03-0,31	40,64	2.0e-9
	+/-	39 (33,91)	50 (50,00)	0,51	0,30-0,89		
	-/-	72 (62,61)	24 (24,00)	5,30	2,93-9,61		
XRCC1 Arg194Trp	Arg/Arg	85 (73,91)	72 (72,00)	1,108	0,60-2,01	1,86	0,39
	Arg/Trp	26 (22,61)	27 (27,00)	0,79	0,42-1,47		
	Trp/Trp	4 (3,48)	1 (1,00)	3,57	0,39-32,46		
XRCC1 Arg399Gln	Arg/Arg	47 (40,87)	49 (49,00)	0,72	0,42 – 1,23	3,24	0,2
	Arg/Gln	57 (49,57)	47 (47,00)	1,11	0,65 – 1,90		
	Gln/Gln	11 (9,56)	4 (4,00)	2,54	0,78 – 8,24		
XRCC3 Thr 241Met	Trp/Trp	82 (71,30)	64 (64,00)	1,40	0,79 – 2,48	8,32	0,02
	Trp/Met	25 (21,74)	35 (35,00)	0,52	0,28 – 0,94		
	Met/Met	8 (6,96)	1 (1,0)	7,40	0,91 –60,25		
TP53 Arg72Pro	Arg/Arg	51 (44,38)	57 (57,00)	0,60	0,35-1,03	5,71	0,06
	Arg/Pro	49 (42,61)	38 (38,00)	1,21	0,70-2,09		
	Pro/Pro	15 (13,04)	5 (5,00)	2,85	1,00-8,15		
CCND1 A870G	G/G	22 (19,13)	28 (28,00)	0,61	0,32-1,15	10,87	0,004
	G/A	49 (42,61)	54 (54,00)	0,63	0,37-1,08		
	A/A	44 (38,26)	18 (18,00)	2,82	1,50-5,32		

The presented data show significant association of “null” GST-genotypes (-/-) with susceptibility to esophageal cancer both for GSTM1 gene (OR=5,30) and for GSTT1 gene (OR=3,29). These findings are also confirmed by the dominant and recessive models of OR calculation. Thus, according to the dominant model the risk of esophageal cancer development was significantly higher for the following combinations of genotypes: GSTM1 (+/- and -/-) (OR = 9,75; p=2,0 e-9); and GSTT1 (+/- and -/-) (OR=3,29; p=0,02). The recessive model corresponds to the results of general model of inheritance: for the GSTM1 -/- (OR=5,30; p=1,0e-8) and GSTT1 -/- (OR=3,29; p=2,0e-5). The presence in genotype the functional allele variants GSTM1 and GSTT1 genes in homozygous states shows strong protective effect (for GSTM1 +/+ genotype - OR=0,10 and for GSTT1 +/+ genotype – OR=0,30).

The XRCC1 Trp194Trp homozygote show not statistically reliable association with esophageal cancer (OR=3,57; p=0,39). Using the dominant and recessive model of OR calculation did not reveal any significant risk. The same situation we have noticed regarding another type of XRCC1 polymorphism (Arg399Gln). Homozygous genotype XRCC1 Gln241Gln shows statically not significant OR. According to total model of inheritance – OR=2,54; p=0,2. According to the dominant model - OR=1,39; p=0,23. Strong association with risk of esophageal cancer development shows the homozygote genotype XRCC3 Met241Met (OR=7,40; p=0,02). This is confirmed by using dominant model.

CCND1 G870A polymorphism statistically reliable associates with susceptibility to easophageal cancer. The risk is revealed for the CCND1 A870A genotype (OR=2,82; p=0,004). Combination with CCND1 G870A genotype reduces the risk (OR=1,64; p=0,12).

The same risk shows the TP53 Pro72Pro genotype: by total model of inheritance - OR=2,85; p=0,06; and by dominant model (in combination with TP53 Arg72Pro genotype) - OR=1,66; p=0,06.

Association of genomic polymorphism with development of cervical cancer

All 217 women representing cervical cancer case cohort were cytologicaly or histologically examined for cancer type. Squamous-cell carcinoma (cancer in situ) is the predominant histotype in selected cohort. The I stage of cervical cancer was defined at 15 women, the II stage – at 167 women, the III stage (invasive) – at 26 patients, and IV stage (invasive, metastatic) – at 9 women.

The control cohort was matched to cases. The age, sex, and ethnicity smoking habit data of case and control groups represent in table 3.

Table 3. The correspondence of the cervical cancer case and control cohorts by age, ethnicity and smoking habit

Cohort	Years of birth	Nationality, persons (%)		Smoking habit, persons (%)	Total, persons
		Kazakh	Russian		

Case	1945-1990	176 (81,11)	41 (18,89)	10 (4,61)	217
Control	1942-1987	128 (80,00)	32 (20,00)	8 (5,00)	160

All DNA samples, extracted from frozen blood, buccal and pap smears, were genotyped for candidate genes polymorphism: deletions GSTM1 and GSTT1, XRCC1 Arg194Trp and Arg399Gln, XRCC3 Thr241Met, TP53 Arg72Pro and CCND1 A870G). The genotyping results revealed that in all cases the distribution of genotypes follows to Hardy-Weinberg equilibrium.

The genotyping of GST-polymorphism in cohorts of healthy women and women suffering to cervical cancer revealed, that the frequency of GSTT1 deletions in case cohort (0,770) is more higher, than in control cohort (0,456). Also case cohort shows the prevalence of GSTM1 deletions: 0,323 versus 0,150 in control cohort.

The frequency of rare allele of DNA repair gene XRCC1, coding Trp in 194 codon higher in control population (0,219), than in case population (0,138). Genotyping results of polymorphism 399 codon of XRCC1 gene shows that the frequency of rare 399Gln allele variant is higher in case cohort: 0,366 versus 0,306. There are differences in allele distributions between control and case cohort regarding polymorphism of another DNA repair participating gene - XRCC3 Thr241Met. The frequency of rare allele variant XRCC3 241Met in case cohort (0,224) is more higher than in control (0,125).

Genotyping results for the TP53 Arg72Pro polymorphism show differences between control and cervical cancer case cohort. The frequency of allele variant TP53 72Pro in control population is higher than in case population: 0,428 versus 0,353, respectively. But between control and case populations there are no strong differences regarding distributions of allele variants CCND1 A870G polymorphism: in control cohort – 0,500 (870A) and 0,500 (870G); in case cohort – 0,491 (870A) and 0,509 (870G).

The data of statistical analysis of associations between investigated gene polymorphism and development of cervical cancer is presented in table 4.

Table 4. Association between genetic polymorphism and development of cervical cancer

Type of polymorphysm	Genotype	Cervical cancer, persons (%)	Control, persons (%)	Odds ratio (OR)	Confidence interval (CI), (95%)	χ^2	P
GSTT1	+/+	12 (5,53)	57 (35,62)	0,11	0,05-0,21	67,15	0,00
	+/-	76 (35,02)	60 (37,50)	0,90	0,59-1,37		
	-/-	129 (59,45)	43 (26,88)	3,99	2,56-6,21		
GSTM1	+/+	108 (49,77)	116 (72,50)	0,38	0,24 – 0,58	25,31	3,0e-6
	+/-	78 (35,94)	40 (25,00)	1,68	1,07 – 2,65		
	-/-	31 (14,29)	4 (2,5)	6,5	2,25 – 18,81		
XRCC1 Arg194Trp	Arg/Arg	163 (75,12)	105 (65,63)	1,58	1,01-2,48	8,72	0,01
	Arg/Trp	48 (22,12)	40 (25,00)	0,85	0,53 – 1,38		
	Trp/Trp	6 (2,76)	15 (9,37)	0,27	0,10-0,73		
XRCC1 Arg399Gln	Arg/Arg	78 (35,94)	66 (41,25)	0,80	0,53 – 1,22	7,24	0,03
	Arg/Gln	119 (54,84)	90 (56,25)	0,94	0,63 – 1,42		
	Gln/Gln	20 (9,22)	4 (2,50)	3,96	1,33 – 11,82		
XRCC3 Trp241Met	Trp/Trp	140 (64,51)	124 (77,50)	0,53	0,33 – 0,84	10,28	0,006
	Trp/Met	57 (26,27)	32 (20,00)	1,43	0,87 – 2,33		
	Met/Met	20 (9,22)	4 (2,50)	3,96	1,33 – 11,82		
TP53 Arg72Pro	Arg/Arg	85 (39,17)	49 (30,63)	1,46	0,95 – 2,25	5,15	0,08
	Arg/Pro	111 (51,15)	85 (53,12)	0,92	0,61 – 1,39		
	Pro/Pro	21 (9,68)	26 (16,25)	0,55	0,30 – 1,02		
CCND1 A870G	G/G	54 (25,12)	41 (25,62)	0,97	0,61 – 1,56	0,09	0,96
	G/A	103 (47,91)	78 (48,75)	0,97	0,64 – 1,46		
	A/A	58 (26,97)	41 (25,63)	1,07	0,67 – 1,71		

Deletion of GSTT1 in homozygous state (-/-) shows the significant association with susceptibility to cervical cancer (OR=3,99; p=0,0). This “null” genotype with combination with heterozygous genotype (-/- and +/-) significantly increases the risk: in accordance with dominant model of OR calculation - OR=9,45; p=0,0. The GSTM1 “null” genotype also show strong association with development of cervical cancer (OR=6,50; p=3,0e-6). But the GSTM1 functional allele presence in genotype reduces the risk: in accordance with dominant model to the combination of genotypes GSTM1 (+/- and -/-) - OR=2,66; p=9,0e-6. The presence in genotype the functional allele variants GSTM1 and GSTT1 genes in homozygous states shows strong protective effect (for GSTM1 +/+ genotype - OR=0,38 and for GSTT1 +/+ genotype – OR=0,11).

The XRCC1 194Arg allele variant associates with susceptibility to cervical cancer in homozygous (Arg194Arg) and heterozygous (Arg194Trp) states. According to total model of inheritance for Arg194Arg genotype – OR=1,58; p=0,01. According to the dominant

model for combinations of genotypes (Arg194Arg and Arg194Trp) - OR=3,64; p=0,006. The homozygotes XRCC1 Gln399Gln show the association with susceptibility to cervical cancer: OR=3,96; p=0,03. But in combination with heterozygotes (Arg399Gln and Gln399Gln) the risk not statistically reliably reduces (in accordance with dominant model – OR=1,25; p=0,90). Another DNA repair gene XRCC3 also shows the associations between Trp399Met polymorphism and susceptibility to cervical cancer. The risk expressed to homozygotes Met399Met: OR=3,96; p=0,006.

TP53 Arg72Pro polymorphism show that 72Arg allele in homozygous state can increase risk of cervical cancer development (OR=1,46; p=0,08). And TP53 Pro72Pro genotype has strong protective effect (OR=0,55; p=0,08). However, the CCND1 G870A polymorphism does not show any associations with susceptibility to cervical cancer.

Discussion. Comparing the frequencies of allele variants of genes-candidates obtained on control cohorts of Almaty residents with data presented in NCBI SNP database and literature, it could be noticed that frequencies of most of investigated allele variants more close to Asian and mixed populations, than European. Thus, the frequencies of GSTT1 deletion in our control populations (control for esophageal cancer (CEC) – 0,635; control for cervical cancer (CCC) – 0,456) are more similar to Asian (0,480-0,540 [5, 6] than to European populations (according to NIH NCBI. SNP. Database - 0,160-0,385).

Our control populations are mixed by ethnicity (see tables 1 and 3), but about 80% of persons represented by Kazakh and about 20% represented by Russian. Should be noted that the studied control populations differ in the frequencies of defined allelic variants, showing the similarity to Asian and European populations data both.

The GSTM1 deletions are widely distributed among Asian and European peoples with similar rates [5, 6]: 0,490-0,540 for Asian populations and 0,420-0,540 for European populations. But our control cohorts show the significant differences in frequencies of GSTM1 deletions: CEC – 0,490; CCC – 0,150. It is very likely that not only the mixed ethnic composition of the studied populations plays a role here, and also the fact that deletion of this gene has a strong association with the development of cervical cancer.

The obtained frequencies of rare allele variants XRCC1 194Trp (CEC – 0,145; CCC – 0,219) are more close to Asian populations (0,239-0,289) than European (0,092-0,093). The frequency of rare XRCC1 399Gln allele in CEC (0,275) is more close to Asian populations data (0,274-0,279), but XRCC1 399Gln allele rate in CCC (0,306) is more similar to European people (0,303). Reverse observations we have noted regarding distribution of rare XRCC3 241Met allele: CEC (0,185) is more close to European data (0,000-0,417) and CCC (0,125) show the rate of this allele comparing with Asian populations (0,000-0,148).

The rates of TP53 72Pro (0,760) and CCND1 870G (0,550) in CEC populations are more close to the distributions of these allele variants in European (0,233 and 0,475-0,483, respectively). And the frequencies of this alleles (TP53 72Pro - 0,428; CCND1 870G - 0,500) in CCC population are more similar to Asian populations (0,409-0,511 and 0,456-0,656, respectively).

The possible explanation of the fact may be distinction of Kazakh population from other Asian populations (Chinese, Japanese, Malaysian, etc.) by genotype, and affinity to European populations.

Identified associations between candidate genes polymorphism and esophageal and cervical cancer are not surprising facts. Deletions of GST-genes associates with susceptibility to many cancer types. Some literature data [7, 8, 9, 10] confirms that deletions of GSTT1 and GSTM1 genes can play significant role in development of esophageal or cervical cancer. Most of these research works were done on Chinese populations. But some data obtained on different populations are not in accordance with our results [11, 12]. This fact can be explained either by insufficient knowledge about influence of GST-deletions on development of esophageal and cervical cancer, or ethnic characteristics of genotypes, or accounting related factors, such as smoking, chemotherapy or radiation therapy.

There are many opinions about influence of XRCC1 (X-ray repair complementing defective repair in Chinese hamster cells 1) and XRCC3 (X-ray repair complementing defective repair in Chinese hamster cells 3) genes on different cancer types. These genes participate in excision repair of bases and repair of single and double strand breaks. Some authors point to the relation of XRCC1 (Arg399Gln, Arg194Trp) and XRCC3 Trp241Met polymorphisms with colorectal cancer, skin cancer, lung cancer and others. There are data confirming the participation of XRCC-genes polymorphism to cervical cancer [13] (Barbisan G. et al., 2011). Interestingly, that in this article there is a conclutions, that Arg194Trp polymorphism may be associated with cervical cancer risk, Arg399Gln polymorphism might be a low-penetrent risk factor for cervical cancer only in Asians. One article [14] shows the strong association between XRCC1 Gln399Gln genotype and squamous-cell carcinoma of esophagus, and the smoking people have 4,2 fold increased risk in comparison with not smoking persons.

Mutations and polymorphisms of cell cycle regulating genes (CCND1 and TP53) can play the main role in cancer induction process, also in esophageal cancer development [15, 16]. In our study we show that CCND1 G870A polymorphism associates with susceptibility to esophageal cancer, but not to cervical cancer.

Polymorphism of TP53 Arg72Pro can play dual role in cancer development [17]. On the one side, protein product of 72Arg allele more effectively induces apoptosis [18]. On the other side, 72Pro allele variant provide longevity of being in cell cycle G1-phase in which DNA repair processes are active [19]. Also it was established, that oncoprotein E6 coding by viruses HPV-18 and HPV-16, can interact with p53 protein inducing its degradation. And 72Arg allele more faster depredates E6 than 72 Pro [20, 21]. Further investigations show contradictive results. Thus, women from Taiwan, Thailand, Korea, Japan, China and Hong-Kong show no association between TP53 72Arg/Pro polymorphism and HPV-associated and HPV-nonassociated cervical cancer [22, 23, 24]. The study of women from India, Brazil, Chili, Peru and women from Africa show this association [25, 26, 27]. Study of women in Greece, Holland and Hungary revealed this positive association [28, 29]. And also there are evidence of influence of TP53 Arg72Pro on development of esophageal cancer [30, 31]. We find out that TP53 72Pro allele associates with susceptibility to cervical cancer and 72Arg allele shows strong association with esophageal cancer development.

Conducted research allowed to determine the panels of genetic markers of predisposition to the development:

1) esophageal cancer - deletions of GSTT1 (OR=3,29) and GSTM1 (OR=5,30) genes; XRCC3 Met241Met (OR=7,40); TP53 Pro72Pro (OR=2,85), CCND1 A870A (OR=2,82).

2) cervical cancer - deletions of GSTT1 (OR=3,99) and GSTM1 (OR=6,50) genes; XRCC1 Arg194Arg (OR=1,58); XRCC1 Gln399Gln (OR=3,83), XRCC3 Met241Met (OR=2,84) and TP53 Arg72Arg (OR=3,96).

These results statistically reliable and will be used for developing of test-kits for the defining susceptibility to esophageal and cervical cancers. The introduction of these tests to the screening programs will allow to develop large-scale preventive measures and will have an impact on reducing cancer incidence and mortality in Kazakhstan, helping to extend the qualitative longevity.

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ГЕНЕТИЧЕСКИЕ МАРКЕРЫ РАКА

Резюме: Выявление популяционных групп с высоким риском развития онкологических заболеваний является одним из важных научно-исследовательских приоритетов в профилактике рака. Определение генетической предрасположенности к развитию рака может привести к снижению уровня смертности в этих группах.

Генетические факторы влияют на тенденцию в развитии рака. В сигнальном пути предрасполагающие к раку мутации часто влияют на процессы репарации ДНК, регуляции клеточного цикла и клеточной смерти. В этой статье приведены результаты исследования, посвященного выявлению генетических факторов риска развития рака шейки матки и рака пищевода. Для исследования "случай-контроль" мы использовали клинические материалы (кровь, буккальные мазки, биопсийные материалы, соскобы эпителия шейки матки) 115 пациентов с диагнозом рак пищевода и 207 пациентов с диагнозом рак шейки матки. В соответствии с этническими и возрастными данными пациентов с раком пищевода и шейки матки были сформулированы контрольные группы людей без этих патологий (100 и 160 человек, соответственно). Гены, участвующие в процессах детоксикации ксенобиотиков (GSTM1 и GSTT1), репарации ДНК (XRCC1 и XRCC3), регуляции клеточного цикла и апоптоза (CCND1 и TP53), были рассмотрены в качестве генов кандидатов рака пищевода и шейки матки. Проведенные исследования позволили определить панели генетических маркеров предрасположенности к развитию: рака шейки матки – делецииGSTT1 и GSTM1 генов; XRCC1 Arg194Arg; XRCC1 Gln399Gln, XRCC3 Met241Met и TP53 Arg72Arg, рака пищевода - делеции GSTM1 и GSTT1 генов; XRCC3 Met241Met, TP53 Pro72Pro, CCND1 A870A.Эти результаты будут использованы для разработки тест-систем для определения предрасположенности к раку пищевода и шейки матки.

Ключевые слова: рак шейки матки, рак пищевода, генетическая предрасположенность, однонуклеотидный полиморфизм, делеции GSTT1 и GSTM1, репарация ДНК, детоксикация ксенобиотиков.

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ІСІКТІҢ ГЕНЕТИКАЛЫҚ МАРКЕРЛЕРІ

Түйін: Онкологиялық аурулардың дамуының жоғарғы қауіпі бар популяциялық топтарды анықтау, ісік профилактикасында маңызды бір ғылыми зерттеудің артықшылығы болып табылады. Ісік дамуының генетикалық бейімділігін анықтау, бұл топтардың өлімөжітім деңгейін төмендетуге әкелуі мүмкін.

Генетикалық факторлар ісіктің даму тенденциясына әсер етеді. Белгі берілу жолында ісікке беуімділігі бар мутациялар ДНҚ репарациясына, клеткалық цикл мен клеткалық өлімінің реттелуіне жиі әсер етеді. Бұл жұмыста жатыр мойын және өңеш ісіктерінің даму қауіпінің генетикалық факторларын анықтауға бағытталған зерттеу жұмысының нәтижелері көрсетілген. Біз «кездейсоқ-бақылау» әдісімен зерттеу үшін өңеш ісігі диагнозы бар 115 емделушілердің және жатыр мойын ісігі диагнозы бар 207 емделушілердің клиникалық материалдарын қолдандық. Емделушілердің өңеш және жатыр мойыны ісігі бар, сәйкесінше, этникалық құрамы мен жас шамасына байланысты бұл ақаулары жоқ адамдардың бақылау топтары құрылды (сәйкесінше, 100 және 600 адам). Ксенобиотиктердің детоксикация үрдістеріне (GSTM1 и GSTT1), ДНҚ репарациясына (XRCC1 и XRCC3), клеткалық цикл мен апоптозға (CCND1 и TP53) қатысатын гендер, өңеш және жатыр мойын ісігінің ген-кандидаттары ретінде қарастырылды. Жүргізілген зерттеу жұмыстар ісіктің дамуына бейімділіктің генетикалық маркерлер панелін анықтауға мүмкіндік берді: жатыр мойын ісігі - GSTT1 и GSTM1 гендерінде делеция; XRCC1 Arg194Arg; XRCC1 Gln399Gln, XRCC3 Met241Met және TP53 Arg72Arg, өңеш ісіri - GSTM1 и GSTT1 гендерінде делеция; XRCC3 Met241Met, TP53

Pro72Pro, CCND1 A870A. Бұл нәтижелер өңеш және жатыр мойын ісіктеріне бейімділігін анықтау үшін тест-жүйелерді өңдеуге қажет болады.

Түйінді сөздер: жатыр мойын ісігі,өңеш ісігі,генетикалық бейімділік, бір нуклеотидтік полиморфизм, GSTT1 и GSTM1 делециялары, ДНК репарация, ксенобиотиктер детоксикациясы.