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### **The study of association between candidate gene polymorphisms and age-dependent diseases in population from Kazakhstan**

**Abstract.** Here, we show that several aging related genes may serve as the genetic risk factors for cervical, esophagus and colorectal cancers. We examined the genes involved in the processes of xenobiotics detoxification (*GSTM1* and *GSTT1*), DNA repair (*XRCC1*, *XRCC3*, *hMLH1*), cell cycle regulation and apoptosis (*CCND1*, *TP53*, *DCC*). The study results will lead to development of screening for detection of individuals susceptible to esophageal, cervical and colorectal cancers. Introduction of the screening programs will allow the early and effective preventive measures that will reduce cancer incidence and mortality in Kazakhstan.

**Key words:** age-dependent disease, genetic susceptibility, genetic polymorphisms, Kazakhstan population.

#### **Introduction**

Aging is a complex biological process determined by genetic and environmental factors. Aging processes are associated with the accumulation of toxic metabolites, damages of biologically important molecules and increased predisposition to the development of a number of pathological conditions. Age-related pathologies include cancer, cardiovascular, neurodegenerative, autoimmune diseases, diabetes, obesity and other. The development of age-related diseases or the ability to take an active longevity is greatly influenced by ethnicity and many individual genetic characteristics. The important areas of aging medicine are the elucidation of genetic and molecular mechanisms of aging including the role of genetic and epigenetic factors in the etiology and pathogenesis of various age-related pathologies. The determination of genetic features of centenarians and genetic status of key genes involved in the pathogenesis of age-related pathologies are the main approaches to define the key components of active aging and longevity.

Candidate genes participating in aging can be classified as following: 1) genes involved in tissue homeostasis (apoptosis and telomerase); 2) genes

controlling integrity of genome and DNA repair; 3) genes involved in stress resistance (heat shock and oxidation). Cancer is primarily a disease of older people where incidence rates increased substantially with age for most cancers. The genetic components of cancer overlays the range of candidate genes controlling aging.

At present the cancer incidence in Kazakhstan is higher than in the European region and one of the highest among the Central Asian countries. The most common are tumors arising from permanently renewing tissues such as epithelium. The frequencies of some cancer types (esophageal, colorectal and cervical) grow each year in Kazakhstan. These cancer types have been selected in our study because of their high morbidity and mortality in Kazakhstan.

Esophageal cancer (EC) is one of the most aggressive forms of cancer. It is ranked on the 9<sup>th</sup> place by malignancy and on the 7<sup>th</sup> 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 in a range of 5 to 10%. The incidence of esophageal cancer in males reaches 25.7 cases in population of 100 000.

Cervical cancer (CC) in women is diagnosed in the reproductive age. Kazakhstan is among the

countries with high levels of cervical cancer and its incidence is on the second place following the breast cancer.

Colorectal cancer (CRC) is the third most commonly diagnosed type of cancer in men and women worldwide. CRC is age related, colonic cancer more so than rectal. CRC continues to be one of the most common fatal types of cancer. Among CIS countries, Kazakhstan is in the seventh place regarding to the colorectal cancer incidence. Most cases are diagnosed at the late stages of cancer progression (III-IV st.). About 75% are the sporadic cases and about 25% are familial. In recent years, worldwide there has been considerable increase of CRC morbidity and a significant rejuvenation of this type of cancer. The possible reason is lifestyle change, which implies the reducing of physical activity, many hours sitting at a computer, «fast food»-nutrition, smoking and alcohol consumption habits among young people.

Here we present the results of case-control study of populations from Kazakhstan, representing healthy individuals and patients with esophageal (EC), cervical (CC) and colorectal (CRC) cancers.

### Material and methods

Biological samples were collected from the patients (100 – EC, 217 – CC, and 249 – CRC) of cancer clinics of Almaty city. The control groups of healthy individuals were selected in accordance to the ethnic and age data of cancer patients (115 – EC, 160 – CC, and 245 – CRC). Detailed questionnaires and informed consents were filled prior collection of samples. The clinical diagnosis of cancer patients was verified by the cytological or histological methods using biopsy materials.

Genomic DNA was isolated from peripheral blood leukocytes using the standard phenol-chloroform method with modifications in the composition of the lysis buffer; 0.2 M sodium acetate and 1% sodium dodecyl sulfate, pH 8.0 [1]. The DNA was dissolved in distilled water and the quantity and quality of the dissolved DNA samples were evaluated by spectrophotometric analysis (Eppendorf BioPhotometer plus). The dissolved DNA samples were stored at – 20 °C until further use.

For the esophageal and cervical cancer we studied the following genetic markers:

1) Deletion polymorphism of genes participating in xenobiotic detoxification – glutathione-S-transferases – *GSTM1* and *GSTT1*; single nucleotide polymorphism (SNP) of *GSTP1* Ile105Val;

2) 2 types of SNP of *XRCC1* (Arg194Trp and Arg399Gln), responsible for the repair of double strand DNA breaks;

3) SNP of *XRCC3* (Trp241Met), 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).

For the sporadic cases of colorectal cancer we have studied the following genetic markers:

1) SNP of tumor suppressor gene – *DCC* G32008376A (c.985+67534A>G, rs 714);

2) SNP of tumor suppressor gene regulating cell cycle and apoptosis – *TP53* (Arg72Pro) (rs 1042552);

3) SNP of DNA mismatch repair gene – *hMLH1* A-93G (rs 1800734);

4) Deletion polymorphism of genes participating in xenobiotic detoxification – glutathione-S-transferases – *GSTM1* and *GSTT1*.

The genotyping of *GSTM1* and *GSTT1* deletion polymorphisms was carried out by multiplex PCR. The PCR with following restriction was used for the genotyping of *XRCC1*-Arg194Trp; *XRCC1*-Arg399Gln, *XRCC3*-Thr241Met, *DCC*-A32008376G, *MLH1*-G93A, and *TP53*-Arg72Pro SNPs. The genotyping of *CCND1*-A870G was detected by direct sequencing. The PCR and sequencing details were described previously [1,2].

### Results and their discussion

The case-control study of sporadic cases of esophageal, cervical and colorectal cancers allowed determine the panels of genetic markers of predisposition to the development of:

1) esophageal cancer – deletions of *GSTT1* (OR=3.45; p=0.00013) and *GSTM1* (OR=8.08; p=0.000001) genes; *XRCC3*-Met241Met (OR=7.40; p=0.006); *XRCC1*-Gln399Gln (OR=16.33; p=0.006); *TP53*-Pro72Pro (OR=3.34; p=0.039), *CCND1*-A870A (OR=2.92; p=0.009);

2) cervical cancer – deletions of *GSTT1* (OR=3.99; p=0.0001) and *GSTM1* (OR=6.50; p=0.0001) genes; *XRCC1*-Arg194Arg (OR=1.58; p=0.08); *XRCC1*-Gln399Gln (OR=3.83; p=0.06), *XRCC3*-Met241Met (OR=2.84; p=0.05) and *TP53*-Arg72Arg (OR=3.96; p=0.06);

3) colorectal cancer – *DCC*-32008376 G/G and G/A vs. A/A (OR=3.45; p<=0.0002), *MLH1*-G93G (OR=1.45; p=0.04), *TP53* homozygous (*Pro72Pro*, OR=3.80, p<0.0001), *GSTT1* (deletions and hetero-

zygous vs. normal homozygous –  $OR=1.43$ ,  $p=0.05$ ) and *GSTM1* deletions ( $OR=1.83$ ,  $p=0.001$ ).

Identified associations between candidate genes polymorphism and esophageal, cervical and colorectal cancer are not surprising.

Glutathione S-transferases (*GSTs*), a multi-gene family of phase II metabolic enzymes, are active in the detoxification of a wide variety of potentially toxic and carcinogenic substances by conjugating them to glutathione. Deletions of *GST*-genes are associated with susceptibility to many cancer types. The previous study [3; 4; 5; 6] reported that deletions of *GSTT1* and *GSTM1* genes play a significant role in development of esophageal or cervical cancers. Most of these studies were carried on Chinese and Caucasian populations. Association of *GSTT1* and *GSTM1* deletions with esophageal and cervical cancer susceptibility is supported by data obtained by studying populations from India, Korea, Turkey, Great Britain, Italy, USA and other countries [7; 8; 9]. Several studies have shown that deletions of *GSTM1*, rather than *GSTT1*, are associated with CRC susceptibility in the Caucasian [10; 11], Japanese [12], and mixed American populations [13; 14]. Our results demonstrate that *GSTT* and *GSTM* «null» genotypes are strongly associated with susceptibility to esophageal, cervical and colorectal cancers in population from Kazakhstan.

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. There are data confirming the participation of *XRCC1*-genes polymorphism to cervical cancer [15]. The one study [16] shows the strong association between *XRCC1* Gln399Gln genotype and squamous-cell carcinoma of esophagus. In our study *XRCC1* Trp194Trp genotype was associated with susceptibility to esophageal cancer, and *XRCC1* Arg194Arg genotype – with cervical cancer. Regarding the *XRCC1* Arg399Gln polymorphism our results show the evidence of associations between *XRCC1* Gln399Gln genotype carriers and increased risk of cervical and esophageal cancer development, which is confirmed by other studies [16]. Also, our data demonstrate the strong association between *XRCC3* Met241Met genotype and expressed risk of susceptibility to both cervical and esophageal cancer in Kazakhstan populations.

Mutations and polymorphisms of cell cycle regulating genes (*CCND1* and *TP53*) can play the main role in many types of cancer. Proto-oncogene cyclin D1 is an activator of CDK kinases, whose activity is required for cell cycle G1/S transition. This protein has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb. One meta-analysis [17] exhibited the statistically significant association between *CCND1* G870A polymorphism and a risk for cancers of the digestive tract, including esophageal cancer. There are no substantial data confirming the correlation of this type of polymorphism with cervical cancer. In our study we have shown that *CCND1* A870A genotype associates with susceptibility to esophageal cancer, but not to cervical cancer.

Polymorphism of *TP53* Arg72Pro can play dual role in cancer development. 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 faster degrades E6 than 72 Pro [20]. Further investigations show contradictory 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 [21; 22; 23]. The study of women from India, Brazil, Chili, Peru and women from Africa show this association [24; 25]. Study of women in Greece, Holland and Hungary revealed this positive association [26; 27]. And also there are evidences of influence of *TP53* Arg72Pro on development of esophageal cancer [28; 29]. The role of the *TP53* Arg72Pro polymorphism in CRC susceptibility has been examined in several studies [30; 31], with an overall controversial outcome. We find out that *TP53* 72Pro allele associates with susceptibility to esophageal and colorectal cancers and 72Arg allele shows strong association with cervical cancer development.

The human MutL homolog 1 (*hMLH1*) gene is one of the major genes in the MMR (DNA mismatch repair) pathway, and it plays an important role not only in recognition and repair of mismatched DNA base pairs, but also in other vital cellular processes including cell cycle arrest, oxidative stress and apoptosis [32].

*MLH1*-93G>A (rs1800734) is a single-nucleotide polymorphism located in the promoter region

which regulates the activity of the promoter and the rate of gene transcription [33]. Many studies have evaluated the relationship of *MLH1* -93G>A polymorphism with the risk of CRC [34; 35]. Our results show that -93 G/G genotype strongly associated with increased CRC risk.

*DCC* (deleted in colorectal cancer) gene encodes the netrin 1 receptor, a transmembrane protein that is a member of the immunoglobulin superfamily of cell adhesion molecules. Investigations on the role of the *DCC* g.32008376A>G (rs714) polymorphism closely associated with LOH in CRC started only recently [36].

A study on the Romanian population [37] showed that G allele is associated with protection for CRC (OR=0.34), while the AA genotype (OR=2.97) and A allele (OR=2.87) are associated with increased risk for CRC. Our results defined the statistically significant association of increased CRC risk with G allele carriers in Almaty (for G/G genotype – OR=1.23; for G/A genotype – OR=1.22), while the AA genotype demonstrates a strongly protective effect (OR=0.29).

Studies investigating the combined effect of *GST*-deletions, *XRCC1* (Arg194Trp and Arg399Gln), *XRCC3* (Thr241Met), *TP53* (Arg72Pro), *CCND1* (A870G), *hMLH1* A-93G, *DCC* G32008376A will be very important for further evaluate the role of these polymorphism in different cancers. Data of association between these genetic polymorphism types and 3 types of age related cancers obtained on unstudied populations from Kazakhstan can be substantial input for meta-analysis. It is required for understanding the role of studied polymorphisms in the development of age-related pathologies in populations from Eurasia. Also, these results are statistically reliable and will be used for developing of test-kits for the defining susceptibility to esophageal, cervical, and colorectal cancer types.

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