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Gene Polymorphism of DNA Excision Repair in Pathogenesis of Malignancy

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***Abstract* – The interaction towards a risk of breast cancer in the ethnically homogeneous group of the Chechens and the polymorphism of DNA repair genes was studied: XPC (rs2228000, rs2228001) XPA (rs 1800975) ERCC2 (rs13181; rs1799793). DNA samples taken from venous blood of 241 women diagnosed with breast cancer and of other 300 representatives of a control group were used. According to the study, no significant connection was found between the alleles of the studied polymorphisms of DNA repair and breast cancer genes; the exception is in a minor allele of ERCC2 rs13181 gene (OR = 1.33 with 95 % CI 1.06 – 1.79). However, regression analysis revealed some significant links (p <0.05) between XPC rs2228000 polymorphism and a risk in developing breast cancer OR = 4.80 (95 % CI 1.56 – 14.78) and OR = 8.95 (95 % CI 2.92 – 27.44), respectively. Also, reliable connections were noted for recessive model AA / CA + CC of the XPC gene (rs2228001): OR = 0.63 (95 % CI 0.40 – 0.98). Likewise, some significant associations (p <0.05) were observed in TT / TG + GG recessive models of polymorphic variants of ERCC2 rs13181 OR = 2.39 95 % CI 1.30 – 4.39 and AA / AG + GG rs1799793 (OR = 2.26 95 % CI 1.33 – 3.83). The experiment results showed that recessive GG homozygote raises a possibility towards breast tumor formations (OR = 2.39 with 95 % CI 1.30 – 4.39) and can be used in a diagnostic panel to determine a risk rate in breast cancer in the Chechen population.**

Keywords – gene polymorphism, repair genes, XPC, XPA , ERCC2, breas cancer, Chechen population.

I. INTRODUCTION

Breast cancer (BC) is one of the most common malignant tumors among women. One of the ways to solve the problem of the annual increase in a number of cases is early diagnosis and prevention. The problem of breast cancer pathologies is complicated by multiple factors underlying carcinogenesis: genetic, environmental, reproductive, and others. At present, it is proven that breast cancer carcinogenesis is based on induction of DNA damage by endogenous and exogenous agents [8].

The research studies to identify the role of DNA repair impairment in cancer pathology continue throughout the world. It has been shown that single nucleotide polymorphisms (SNPs) of DNA repair genes can alter protein functions and simulate DNA repair mechanisms, which, in turn, can provoke breast cancer [7]. The failure in a reparation program plays the leading role in cancer progression [9]. Population studies show inconsistent results in finding associations between DNA repair genes and a risk in developing breast cancer. Many authors believe that the reason for this is in ethnic and geographical diversity. In this vein, in the present work we made an attempt to study the interaction between the risk in developing breast cancer in an ethnically homogeneous group of the Chechens and DNA repair gene polymorphism: XPC (rs2228000, rs2228001) XPA (rs 1800975) ERCC2 (rs13181; rs1799793).

II. METHODS AND MATERIALS

In the study we used the DNA samples taken from the venous blood of women diagnosed histologically breast cancer (n = 241). As control comparing, we used the genomic DNA of women who, at the time of the blood sampling, had no deviations in health (n = 300) and had no family history of cancers. The average age of the patients was 49.19 ± 9.84 years, healthy (without deviations in health) – 48.37 ± 11.30 years. The minimum age at which breast cancer was diagnosed was 16 years; the maximum was 76 years. All representatives gave the consent to participate in the study. The data on age, menopause, concomitant diseases, a family history of breast cancer or other forms of cancer, and ethnicity was obtained from each participant. In addition, we made available for the patients to know the date of tumor detection, a type of tumor, a stage of tumor, and a receptor status.

All representatives of the experiment belonged to one ethnic group – Chechens.

DNA extraction was conducted with DiatomTM DNA Prep200 (Izogen LLC, Moscow) in line with a manufacturer's instructions. The reagents' action is based on lysing cells with guanidine thiocyanate, dissolving cell debris, destroying nucleases, active DNA sorption on Nucleos S sorbent.

Genotyping was performed with a set of lyophilized ready-made reaction mixtures, including DNA polymerase inhibited for 'hot start' of Taq, dNTP and electrophoresis paint, GenPak® PCR Core (12x8) (Izogen, Moscow) with tetrapray PCR method followed by electrophoretic detection (horizontal electrophoresis) of amplification results in a 2 % agarose gel, by adding ethidium bromide. The amplification program was chosen empirically, taking into account the temperature minima for primers selected with Primer 3.0 program. Visualization was performed in transmitted ultraviolet light with the help of a transilluminator.

The distribution of genotypes in the experimental and control groups was tested for consistency with Hardy-Weinberg equilibrium (HW) using $\chi 2$ criteria. The potential risk factors were determined by risk ratio (OR) at a 95 % confidence interval using WinPepi (2011, version 11.15). A comparative analysis of age in two groups was performed with Student T test program. For all types of statistical analysis, WinSTAT for Excel was used. (2009, R. Fitch Software).

III. Results

A comparative analysis of clinical and demographic data in both groups is given in Table 1.

TABLE I. CLINICAL AND DEMOGRAPHIC CHARACTERISTICS IN GROUPS

Parameter	**Experimental (n=239)**	**Control (n=300)**
Age	p=0,000	
	49,2±9,85	48,4±11,30
BMI (Body Mass Index)	32.5 ± 6,9	29,7 ± 5,2
Age at menarche	p=0,038	
younger 14 years	34,73 % (83)	26,33 %(79)
older 14 years	65,27 % (156)	73,67 % (221)
Number of children	p=0.600	
no	13,4 % (32)	11,67 % (35)
One or more	86,6 % (207)	88,33 % (265)
Family history	p=0,158	
	6,28 % (15)	10 % (30)

The significant differences between two groups were noted in the age parameter and in the age menarche parameter ($p = 0.000$; $p = 0.038$). In other cases the differences were slight ($p = 0.600$; $p = 0.512$; $p = 0.158$).

The results in genotyping of XPC genetic loci (rs2228000, rs2228001) XPA (rs 1800975) ERCC2 (rs13181; rs1799793) are presented in table 2.

No significant connection was established between the alleles of the studied polymorphisms of DNA repair genes and malignant tumors among the Chechen women, with the exception of a minor allele G of the polymorphic variant of ERCC2 rs13181 gene, the frequency of which in the experimental group was 35.3 % versus 28.3 % that was a figure for the control group. The associative analysis showed an increased risk of breast cancer (OR = 1.33 with 95 % CI 1.06–1.79).

Some interesting results were revealed with the multivariate logistic regression analysis: significant connections ($p<0.05$) were found between XPC rs2228000 polymorphism and breast cancer development in codominant and recessive models: OR was 4.80 (95 % CI 1.56 – 14.78) and 8,95 (95 % CI 2.92 – 27.44), respectively.

Similarly, reliable relations were noted for a recessive model AA / CA + CC of XPC gene (rs2228001): OR = 0.63 with 95 % CI 0.40-0.98. It is likely to observe here the protective role of homozygote along the recessive type CC in relation to carcinogenesis.

The significant associations ($p <0.05$) were noted for TT / TG + GG recessive models of polymorphic variants of ERCC2 rs13181 OR = 2.39 95 % CI 1.30–4.39 and AA / AG + GG rs1799793 (OR = 2.26 95 % CI 1.33–3.83).

IV. Conclusion

Polymorphisms in DNA repair genes can cause a decrease in ability to restore DNA due to a disorder in the functional characteristics of the encoded proteins. In turn, the accumulation of errors leads to instability of the genome and to carcinogenesis development [2]. However, there are no definitive conclusions about the role of polymorphism of DNA repair genes in relation to malignancy development; or they have not been found. The majority part of scholars agree that the ethnicity of the respondents plays an important role in the existing contradictions. It is clear that the currently existing population groups of women are the result of a long natural selection of certain gene variants that increase the adaptive potential of populations in specific environmental conditions. Thus, the population studies despite many present day solutions in the search for specific genetic risk factors towards development of MN, such as GWAS, continue to be relevant and remain in a big demand.

XPC repair gene polymorphisms studied in this study (rs2228000, rs2228001) XPA (rs 1800975) ERCC2 (rs13181; rs1799793) are involved in NER pathway, restoring single-stranded DNA breaks and the effects of oxidative damage. ERCC2 is localized in the long arm of chromosome 19 (locus 19q13.3). It possesses both single-stranded DNA-dependent ATPase and 5'-3 'DNA helicase activity [5]. Changes in the activity of the helicase will affect the unwinding of double-stranded DNA, thereby blocking the DNA repair process [6].

A number of molecular epidemiological studies have shown that polymorphisms of ERCC1 and ERCC2 genes can affect susceptibility towards colorectal cancer [6], lung cancer, larynx cancer [10], ovarian cancer [14], glioma [12]. Sileng A. and et al. (2016) observed an risk-increase in pancreatic cancer for those who carry minor alleles of the polymorphic variant of ERCC2 rs13181 gene [5]. The variants of ERCC2 gene have been found to be associated with susceptibility towards breast cancer [4]. Whereas, Brewster and et al. could not find a significant connection between rs13181 genotypes and a risk towards breast cancer [3]. In the study conducted by A.Özgöz and co-authors (2017), a connection between rs13181 and a risk of breast cancer could not be detected. The distribution of the alleles of the polymorphic variants of ERCC2 rs1799793 gene and XPC rs2228000, rs2228001 gene did not reveal a connection with histopathological characteristics and a risk of breast cancer [2]. Recently, various studies have focused on the relations between polymorphism in XPA gene and carcinogenesis [1].

TABLE II. GENOTYPING OF XPC REPAIR GENE POLYMORPHISMS (RS2228000, RS2228001) XPA (RS 1800975) ERCC2 (RS13181; RS1799793 IN SAMPLES UNDER STUDY

Polymorphisms	**Experimental (n=241)**	**Control (n=300)**	**OR**	**95 %DI**	**p**
rs2228000					
CC	118 (49.4 %)	151 (50.33 %)			
CT	106 (44.4 %)	145 (48.33 %)	0.94	0.66 – 1.32	0.724
TT	15 (6.3 %)	4 (1.33 %)	4.80	1.56 – 14.78	0.004*
CC/CT+TT	124 (51.45 %)	296 (98.7 %)	8.95	2.92 – 27.44	0.000*
CC+CT/TT	121 (50.2 %)	149 (49.7 %)	1.04	0.74 – 1.46	0.862
HWE	1,908	0.094			
C	342 (71.55 %)	447 (74.5 %)			
T	136 (28.45 %)	153 (25.5 %)	1.16	0.89 –1.52	0.298
rs2228001					
AA	76(31.5 %)	91(30.3 %)			
CA	130(53.9 %)	145(48.3 %)	1.07	0.73 – 1.58	0.768
CC	35(14.5 %)	64(21.3 %)	0.65	0.39 –1.09	0.123
AA/CA + CC	206 (85.5 %)	236(78.7 %)	0.63	0.40 – 0.98	0.044*
AA+CA/CC	165 (68.5 %)	209 (69.7 %)	0.95	0.66 – 1.36	0.779
HWE:	2.969	0.194			
A	282(58.5 %)	327(54.5 %)			
C	200(41.5 %)	273(44.5 %)	0.85	0.67 – 1.08	0.196
rs 1800975					
AA	50 (20.7 %)	69 (23.0 %)			
GA	119 (49.6 %)	141 (47.0 %)	1.16	0.75 – 1.80	0.506
GG	72 (29.9 %)	90 (30.0 %)	1.10	0.69 – 1.78	0.716
AA/GA+GG	169 (70.1 %)	210 (70.0 %)	0.99	0.69 – 1.44	1.000
AA+GA/GG	191 (79.25 %)	231 (77.0 %)	0.88	0.58 – 1.32	0.602
HWE	0.004	0.920			
A	219 (45.4 %)	279(46.5 %)			
G	263 (54.6 %)	321(53.5 %)	0.96	0.75 – 1.22	0.759
rs13181					
TT	100(42.70 %)	148 (49.3 %)			
TG	103(44.00 %)	134 (44.70 %)	1.14	0.79 – 1.63	0.520
GG	31 (13.20 %)	18 (6.00 %)	2.55	1.36 – 4.79	0.004*
TT/TG+GG	203 (86.75 %)	282 (94.0 %)	2.39	1.30 – 4.39	0.006*
TT+TG/GG	134 (57.3 %)	152 (50.7 %)	0.77	0.54 – 1.08	0.138
HWE	0.300	2.992			
T	303(64.7 %)	430(71.7 %)			
G	165(35.3 %)	170(28.3 %)	1.38	1.06 – 1.79	0.017*
rs1799793					
AA	91 (41.2 %)	150 (50.0 %)			
AG	91 (41.2 %)	124 (41.3 %)	1.21	0.83 – 1.76	0.339
GG	39 (17.6 %)	26 (8.7 %)	2.47	1.41 – 4.32	0.002*
AA/AG+GG	182 (83.5 %)	274 (24.7 %)	2.26	1.33 – 3.83	0.003*
AA+AG/GG	130 (58.8 %)	150 (50.0 %)	0.70	0.49 – 0.99	0.051
HWE	3.632	0.03			
A	183(64.9 %)	424 (70.7 %)			
G	99 (35.1 %)	176 (29.3 %)	1.30	0.96 – 1.76	0.087

However, we found that the recessive GG homozygote increases the possibility towards a breast tumor formation (OR = 2.39 with 95 % CI 1.30–4.39) and can be used in the diagnostic panel to determine the risk rate of breast cancer in the Chechen population.

The group A pigment xeroderma gene (XPA) encodes a DNA-binding zinc finger that plays a crucial role in nucleotide excision repair (NER) along with qXPC, TFIIH, XPG, ERCC1. Polymorphic variants of XPA gene can reduce the ability to restore DNA, provoking cancer development. Separate paired comparisons in different genetic models showed that AG genotype carriers according to the genomic model (AG versus AA) are more susceptible to lung cancer. However, in the present study, no association was found between XPA gene polymorphism (rs 1800975) and breast cancer. Some studies show that the genotyping of polymorphic variants of DNA repair genes can be a useful tool in evaluating the prognosis towards cancer possibility and its treatment.

Concluding the study results we can state that there are the significant associations between distribution of the genotypes of DNA repair genes and a risk towards breast cancer. It is worth saying that our study has limitations such as a relatively small sampling size. For these reasons, our results must be verified and confirmed by studies with a larger number of cases and a well-selected control group that will include many genes-candidates and polymorphisms.

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