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EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Association of *BARD1* and *BRIP1* Gene Polymorphisms with the Risk of Uveal Melanoma

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Considering the limited information about the role of hereditary predisposition to the development of uveal melanoma, we have performed an analysis of the frequencies of *BARD1* (*rs1048108*, *rs2229571*, *rs2070094*) and *BRIP1* (*rs4986764*) gene polymorphisms in patients with uveal melanoma and benign choroidal nevus in comparison with healthy volunteers (control). It has been found that the minor alleles of *BRIP1 rs4986764* and *BARD1 rs2070094* polymorphisms, as well as the homozygosity of *T* allele at the *BARD1 rs1048108* locus are common genetic markers for the predisposition to uveal melanoma and benign choroidal nevus, while the homozygous genotype *GG* for the *BARD1 rs2229571* polymorphism is a specific marker for the predisposition to uveal melanoma and progressive choroidal nevus. We have also found that the heterozygous genotype at *BARD1 rs1048108* polymorphic locus is a specific marker for protection against uveal melanoma and progressive choroidal nevus. Thus, our results indicate the advisability of studying polymorphisms of the *BARD1* gene (*rs1048108*, *rs2229571*, and *rs2070094*) and the *BRIP1* gene (*rs4986764*) in patients with uveal melanoma and progressive choroidal nevus. The obtained findings can be used for forming risk groups, prevention of uveal melanoma, and differential diagnosis of intraocular neoplasms.

Key Words: *gene polymorphisms; uveal melanoma; BARD1; BRIP1*

Uveal melanoma (UM) is one of the most common intraocular malignancies. It comprises about 86% of all intraocular malignant tumors [1,2]. The difficulties in the clinical diagnosis of UM are attributed to the need to perform differential diagnosis with other intraocular diseases. The lack of early diagnosis makes the detection of the disease late at stages III-IV [3]. The cytogenetic abnormalities in UM are most studied. In tumor cells, they are presented by aberrations in chromosomes 1, 3, 6, and 8 [4]. In 70% of patients, monosomy of chromosome 3 has been detected, whereas in the other 30%, the allelic deletion in the short arm of chromosome 1 (1p36) was revealed, which was associated with extraocular tumor invasion [4,5]. A variety of molecular genetic alterations affecting the signaling pathways has also been identified, including the mitogen-activated protein kinase pathway (MAPK) [5].

Germinal mutations that determine the genetic predisposition to malignant neoplasms and, in particular, to UM are as interesting as the changes mentioned above. Thus, it is known that mutations in the *BARD1* (BRCA1-associated RING domain protein 1) gene located in chromosome 2 are associated with certain

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types of oncopathology [6]. *BARD1* can initiate the p53-dependent apoptosis independently of the *BRCA1* gene. Mutations in the *BARD1* gene in the absence of *BRCA1/2* mutations were found in some families with a risk of breast cancer. Therefore, it can be considered as an important marker for predisposition to UM [7]. Some polymorphisms of the *BARD1* gene, specifically *rs17489363*, *rs2229571*, *rs3738888*, *rs3768716*, *rs6435862*, *rs3768707*, *rs17487792*, *rs2229571*, and *rs1048108*, are associated with the risk of developing a number of malignant neoplasms [8].

The *BRIP1* (*BRCA1* interacting helicase 1) gene is located on chromosome 17 (17q22-q24) and encodes a helicase that interacts with *BRCA1* and is involved in DNA double-strand break repair [9]. Its mutations are used as a potential biomarker to predict the survival of patients with certain types of oncopathology, since some variants in *BRIP1* gene are related to susceptibility to some kind of malignant neoplasms [10].

Despite recent progress, the predictive diagnostics of UM remains an extremely complicated issue primarily due to inadequate knowledge of the factors that determine the genetic predisposition to the disease development. Currently, there is no effective personalized UM therapy, which leads to the disease progression and inevitable fatal outcomes [11].

The purpose of the presented work was to study the frequencies of *BARD1* gene polymorphisms (*rs1048108*, *rs2229571*, and *rs2070094*), as well as the *rs4986764* polymorphism of the *BRIP1* gene in patients with UM to analyze their possible association with the disease.

MATERIALS AND METHODS

Clinical data. The study involved 126 participants: 65 patients (17 men and 48 women) with choroidal neoplasms (mean age 49.6±15.2 years) and 61 individuals without intraocular neoplasms (15 men and 46 women, mean age of 38.72±11.13 years; control group). Informed consent was signed by all participants. Exclusion criteria were the presence of sub- or decompensated somatic diseases or other oncological diseases.

Complex clinical and instrumental examination including standard ophthalmological (viscometry, perimetry, tonometry, biomicroscopy, and ophthalmoscopy) and special (echography, optical coherence tomography including angiography) research methods were performed in all cases. According to the results of the clinical and instrumental examination, all patients were divided into two groups: experimental group (patients with UM and progressive choroidal nevus, *n*=42; 56.3±9.8 years) and a risk group (patients with benign choroidal nevus, *n*=23; 47.5±7.9 years).

In all patients of the experimental group, the presence of progressive intraocular melanocytic neoplasm was determined by ophthalmoscopy, echography, and optical coherence tomography (in case of tumor size <2 mm). Choroidal melanomas of T1-T3 stages amounted to 83.3% (*n*=35), progressive choroidal nevi were 16.7% (*n*=7). In all cases, the presence of a benign choroidal nevus without signs of growth within two years of observation was determined.

DNA extraction and genotyping. Genomic DNA was extracted from the peripheral blood samples by standard procedures using a commercially available kit (Syntol). Genotyping for polymorphisms of the *BARD1* (*rs1048108*, *rs2229571*, and *rs2070094)* and *BRIP1* (*rs4986764*) genes was carried out using a PCR with restriction fragment length polymorphism method (Table 1). DNA fragments were separated by agarose gel electrophoresis.

TABLE 1. Genotyping Conditions

Gene polymorphisms	Primer	$T_{annealing}$, °C	Restriction endonucleases	DNA fragments, bp
BARD1 rs2229571 (Arg378Ser)	F: 5'-AAGTTGGTGGTACATCAGGGCG-3' R: 5'-ATTGGGCAACAGCTTCATTGCT-3'	56	AspLEI	*GG*: 146 *GC*: 147,122,22 *CC*: 124,22
BARD1 rs1048108 (Pro24Ser)	F: 5'-CGGGACGATGCCGGATAA-3' R: 5'-CGGAAGGAGGAAACGGAAGA-3'	57	AspLEI	*CC*: 109, 76, 46, 26, 5 *CT*: 122, 109, 76, 46, 26, 5 *TT*: 122, 109, 26, 5
BARD1 rs2070094 (Val50Met)	F: 5'-ATTATTGCTCCAGCATAAGGCA-3' R: 5'-GGAAAGTAACAGCTTGACTATATGCA-3'	53	Zsp21	*GG*: 112 *GA*: 112, 90, 22 *AA*: 90, 22
BRIP1 rs4986764 (*C>T*)	F: 5'-AAGTGACCTCTTTAAAGTACAGTAGC-3' R: 5'-TTAGGACACTGTAGTTCCTGGA-3'	54	AluI	*CC*: 121 *CT*: 121, 97, 24 *TT*: 97, 24

Statistical data analysis. To compare genotype and allele frequencies between studied groups, the χ^2 test or Fisher's exact test were applied using the R-language program. The differences were considered statistically significant at $p \leqslant 0.05$. The odds ratio (OR) and 95% confidence interval (95%CI) were also calculated.

RESULTS

Analysis of the *BRIP1 rs4986764* genotype distribution revealed significant differences between patient and control groups (Table 2). In particular, *CC* homozygotes were significantly more frequent in the control group than in the experimental group (p=0.002) and the risk group (p=0.004), while the opposite situation was observed with the frequency of *CT* heterozygotes (p=0.002 and p=0.04, respectively; Table 2). It is noteworthy that minor *TT* homozygotes were not found among healthy volunteers. The *T* allele was significantly less frequent in the control group in comparison with the experimental group (p=0.003) and the risk group (p=0.002), therefore, it can be considered as a genetic marker for predisposition to ocular neoplasms (UM: OR=2.667; 95%CI=1.417-5.020; benign nevus: OR=2.780, 95%CI=1.478-5.227).

According to published data, the *BRIP1 rs4986764* polymorphism was associated with the reduced risk of cervical cancer in European and Chinese populations (p<0.05) [10]. Only one study showed that the extremely rare minor variant in the *BRIP1* gene (*rs374334794*) was associated with the risk of developing both cutaneous and uveal melanoma [12]. There are no other studies on the association of the *BRIP1* gene with UM.

Currently, there are many publications about the *BARD1* gene. An association of the *BARD1 rs1048108* and *rs2229571* gene polymorphisms with breast cancer and neuroblastoma was revealed [7,8,13]. According to our analysis of the *BARD1 rs1048108* genotype distribution, the incidence of the minor *TT* genotype is significantly higher among patients with UM (p=0.0001) and with benign nevus (p=0.012) in comparison with the control group, while the heterozygous genotype is more frequent in the control group (Table 2). The low frequency of heterozygotes in the group of patients (7.1%) is worthy of note. Thus, the *TT* genotype can be used as a marker for predisposition to intraocular neoplasms, particularly to UM (OR=8.536; 95%CI=3.159-23.069), whereas the *CT* genotype is a specific marker for protection against UM and progressive choroidal nevus (OR=0.064, 95%CI=0.027-0.152). The allele frequencies in the studied groups did not significantly

TABLE 2. Genotype and Allele Frequencies (%) for the *BARD1* Gene Polymorphisms *rs1048108*, *rs2229571*, and *rs2070094*, and for the *BRIP1* Gene Polymorphism *rs4986764* in the Studied Groups

Polymorphism	Genotypes and alleles	Control group (n=61)	Risk group (n=23)	Experimental group (n=42)
BRIP1 rs4986764 (*C>T*)	*CC*	59.0	26.1*	28.6*
	CT	41.0	65.2*	61.9*
	TT	0.0	8.7*	9.5*
	C	79.5	58.7	59.6
	T	20.5	41.3*	40.4*
BARD1 rs1048108 (Pro24Ser)	*CC*	41.0	47.8	61.9
	CT	54.1	30.4*	7.1*
	TT	4.9	21.7*	31.0*
	C	68.1	63.0	65.5
	T	31.9	37.0	34.5
BARD1 rs2229571 (Arg378Ser)	*CC*	34.4	30.4	38.1
	CG	63.9	56.5	40.5*
	GG	1.6	13.0	21.4*
	C	66.4	58.8	58.4
	G	33.6	41.2	41.6
BARD1 rs2070094 (Val50Met)	*GG*	83.6	39.1*	31.0*
	GA	16.4	52.5*	61.9*
	AA	0.0	8.7	7.1
	G	91.8	65.4	62.0
	A	8.2	34.6*	38.0*

Note. *$p \leq 0.05$ in comparison with the control.

differ. The relationship of circulating tumor DNA (ctDNA) (*GNAQ*/*GNA11* oncogenes) and the *CC* genotype of the *ABCB1* gene with the risk of initial choroidal melanoma and choroidal nevi has already been reported [14]. Our results complement the possible differential diagnostic panel for such patients.

Our analysis of the *BARD1 rs2229571* genotype distribution revealed a significantly higher frequency of *GG* homozygotes in the experimental group in comparison with the control one (p=0.0001). In the risk group, the frequency of *GG* genotype was also increased, but the differences did not reach the level of statistical significance (p=0.091). Among healthy volunteers, heterozygotes prevailed (Table 2). The obtained results indicate an association between the homozygous *GG* genotype and the development of UM and progressive choroidal nevus (OR=13.025; 95%CI=2.964-57.242). The allele frequencies in the studied groups did not differ.

The study of the *BARD1 rs2070094* genotype and allele frequencies showed that *GG* homozygotes were more frequent in the control group in the absence of minor *AA* homozygotes (Table 2). The allele frequencies in the patient groups also differed significantly from the control (p=0.01 and p=0.032, respectively). Therefore, it can be assumed that the *A* allele in the genotype is a marker for predisposition to intraocular neoplasms (UM: OR=7.048; 95%CI=3.081-16.126; benign nevus: OR=6.192; 95%CI=2.697-14.217).

Despite the lack of published data on the association between the studied *BARD1* gene polymorphisms and the risk of developing UM, our results are consistent with the finding of research [15]. The authors have showed that *BAP1*-associated protein complexes (*FOXK2*, *ASXL1*, *BARD1*, and *BRCA1*) are directly related to the pathogenesis of UM.

Thus, we found that minor alleles of the *BRIP1 rs4986764* and *BARD1 rs2070094* polymorphic loci as well as the homozygosity for *BARD1 rs1048108 T* allele are common genetic markers of predisposition to UM, progressive and benign choroidal nevi, whereas the homozygous *GG* genotype for the *BARD1 rs2229571* polymorphism is a specific marker for predisposition to UM and progressive choroidal nevus. We have also found that the heterozygosity for the *BARD1 rs1048108* polymorphic locus is a specific marker for protection against UM and progressive choroidal nevus.

Thus, the results of our study indicate the advisability of studying polymorphisms *rs1048108*, *rs2229571*, and *rs2070094* for the *BARD1* gene and *rs4986764* for the *BRIP1* gene in patients with UM and progressive choroidal nevus. The obtained data can be used for identification of the group at risk and prevention of UM as well as for the differential diagnosis of intraocular neoplasms.

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