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Substitutions of the *NBS1* gene and clinicopathological characteristics of young breast cancer patients

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The purpose of this study was to determine characteristics potentially related to *NBS1* mutations and polymorphisms in young (≤50 years of age) breast cancer patients. Blood from 80 breast cancer patients was collected. *NBS1* mutations *c.657_661del*, *p.R215W*, *p.I171V*, and polymorphisms *c.8360G>C*, *c.30537G>C* were genotyped by the PCR-RFLP method. Two-sided Chi-square test was used for univariate analysis and logistic regression analysis was used to evaluate the odds ratio. No carriers of the *c.657_661del*, *p.R215W* and *p.I171V* mutations were found. *NBS1 c.8360G>C* logistic regression analysis showed that *GC* and *CC* genotypes compared with *GG* genotype had decreased risk of low grade tumour, 2.885-fold (OR = 2.885, 95% CI 0.173–0.735, $P = 0.005$) and 2.186-fold (OR = 2.186, 95% CI 0.188–0.888, $P = 0.024$), respectively. *8360 CC* genotype (OR = 3.034, 95% CI 0.156–0.778, $P = 0.010$) significantly increased the chances of HER2 amplification compared to *GG* genotype. *NBS1 8360 GC* genotype had a higher risk for breast cancer progression (OR = 1.673, 95% CI 0.233–0.915, $P = 0.027$). The homozygote *8360 CC* carriers had approximately a six times higher risk for the disease progress (OR = 5.946, 95% CI 0.098–0.585, $P = 0.002$). The prevalence of triple negative breast cancer type was significantly higher in individuals with *NBS1 8360 CC* genotype (OR = 2.186, 95% CI 0.188–0.888, $P = 0.024$). Regarding *c.30537G>C* polymorphism, none of the genotypes had a significant influence on pathological characteristics. *NBS1* gene *c.8360G>C* polymorphism might be associated with breast cancer aggressiveness in young breast cancer patients.

Keywords: breast cancer, mutation, polymorphism, *NBS1* gene

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INTRODUCTION

It is estimated that breast cancer (BC) comprises nearly 25% of all cancers in women and it is one of major causes of women cancer mortality worldwide (Ferlay et al., 2012). Preventive breast cancer prophylactic, new diagnostics, and treatment options have improved survival of breast cancer but there is still a need of better understanding of the disease. Realisation of molecular breast cancer mechanisms established on the basis of genetic differences in patients is important to individual medicine, therapy optimization, and cancer prognosis. Recent studies have focused on genetic factors, which increase cancer risk, but there is little data about the association of the genetic variation with prognostic aspects of the malign disease (Lima et al., 2019).

Nibrin (95 kDa), the expression product of the Nijmegen Breakage Syndrome 1 (*NBS1*) gene [OMIM 602667], – is responsible for maintaining genome stability. Hypomorphic mutations that lead to decreased functions of the *NBS1* gene are responsible for the Nijmegen Breakage Syndrome (NBS), a rare autosomal recessive syndrome characterized by microcephaly, growth retardation, immunodeficiency, and predisposition to different malignancies. Mostly lymphoid and haematological cancers are observed, however, an increased susceptibility to solid tumours for homozygotes or heterozygotes of the mutated *NBS1* was reported (Cybulski et al., 2004; Steffen et al., 2004). Recently published data has provided the information that the mutated *NBS1* gene has influence on breast cancer risk mainly in Slavic populations (Cybulski et al., 2019; Rusak et al., 2019). The highest rate of heterozygous carriers of present gene mutations is noted in Eastern and Central Europe, especially in Poland, the Czech Republic and Ukraine (Huzarski et al., 2013).

It is estimated that approximately 0.5% of the Eastern European population are heterozygous for 5 bp deletion *657_661 delACAAA* (*c.657_661del*) in *NBS1* (Varon et al., 2000). This truncating germ line mutation that reside in 6th *NBS1* gene exon leads to formation of two alternative forms of nibrin with a lower molecular weight, of approximately 26 and 70 kDa (di Masi, Antoccia, 2008). Therefore majority of studies in this restricted European region analysed the *NBS1 c.657_661del* mutation association with breast cancer predisposition. Although an elevated risk of breast cancer has been observed among carriers of *c.657_661del*, it was not confirmed to be associated with the course of the disease. According to *c.657_661del* mutation status, Huzarski et al. (2013) identified that mutation carriers and non-carriers presented with similar clinicopathological breast tumour characteristics and survival rate.

Since the identification of the *NBS1* gene, many other mutations have been discovered. Recently, missense substitutions *p.R215W* and *p.I171V* have been suggested to be breast cancer susceptibility factors (Cybulski et al., 2004; Bogdanova et al., 2008a; Roznowki et al., 2008). In the study by Roznowski et al. (2008), the association between clinicopathological tumour features and mutation status was analysed. Although no statistically significant association was reported, it was suggested that *p.I171V* mutation could be a negative prognostic factor.

It has been suggested that *NBS1* polymorphisms contribute to the aetiology of malignancies. One of the most commonly studied *NBS1* polymorphism is *c.8360G>C* (rs1805794), which has been determined to increase the risk of specific cancer in relation to person's ethnicity (Lu et al., 2009; He et al., 2014). Lu et al. (2006) findings suggest that *NBS1 c.8360G>C* polymorphism and haplotypes may contribute to the aetiology of sporadic breast cancer in young non-Hispanic white woman. According to Sun et al. (2007), *CC* genotype of this polymorphism is associated with increased neutropenic fever in breast cancer patients. Also, these studies assessed the contribution of *c.30537G>C* (rs1805787) polymorphism to increased breast cancer risk, but the results were inconclusive (Lu et al., 2006; Sun et al., 2007).

In the present study, we performed the analysis for *NBS1* gene mutations (*c.657_661del*, *p.I171V*, *p.R215W*) and polymorphisms (*c.8369G>C*, *c.30537G>C*) for young women with breast cancer diagnosis. The majority of the studies analyse the relative risk of breast cancer in the case of current gene substitutions (Lu et al., 2006; Steffen et al., 2006; Bogdanova et al., 2008b; di Masi, Antoccia, 2008; Wang et al., 2010). However, the aim of this research was to determine the possible association between *NBS1* and clinicopathological features together with BC progression.

MATERIALS AND METHODS

Study population

Biological material was obtained from 80 breast cancer patients. In genetic analysis, young premenopausal women (≤50 years of age) with I or II stage breast cancer were involved. All samples were analysed at the Oncology Research Laboratory of the Oncology Institute of the Lithuanian University of Health Sciences. The study was approved by the local Biomedical Research Ethical Committee (protocol number BE-2-13). Patients provided written informed consent for the participation in the study.

The data on tumour clinicopathological characteristics was obtained from medical records.

Tumour oestrogen, progesterone receptors, and HER2 (human epidermal growth factor receptor 2) status were determined with immunohistochemistry. In the case of HER2, it was considered positive when immunohistochemically detected HER2 (3+) or (2+) amplification was confirmed by positive Fluorescence *in situ* hybridization. Testing for HER2 status gave as a score of 0 to 3+ that measured the amount of HER2 proteins on the surface of the cells from breast tumour biopsy. Score from 0 to 1+ was considered as HER2-negative. A score of 2+ and 3+ was considered HER2-positive.

The majority of breast tumours were not larger than 2 cm (63.8%). Most of selected young breast cancer patients (85%) had negative tumour stromal lymphocyte infiltration. Approximately half of the cases (48.8%) were positive for lymph node involvement. The majority of the tumours (68.8%) were well to moderate differentiated (grade 1 (G1) or grade 2 (G2)). Low grade (G1) is well differentiated breast cancer. Cancer cells look like normal breast cells and grow (spread) slowly. Intermediate grade (G2) is moderately differentiated breast cancer. Cancer cells look slightly different to normal breast cells. High grade (G3) is poorly differentiated breast cancer. Cancer cells look very different to normal breast cells. In the case of tumour receptor expression, almost half patients were positive for estrogen (51.2%) and progesterone (46.3%) receptors, HER2 amplification was determined in 22.5% of tumours. Most of breast cancer patients had Luminal A tumour subtype (47.5%). Luminal B, HER2 and triple negative (tumours that does not have any of the receptors that are found in breast cancer, e.g. oestrogen, progesterone receptors, and HER2) breast cancer subtypes were determined in 12.5%, 10.0%, and 30.0% of selected cases, respectively. During the follow-up period (of at least two years), 27.5% patients were documented with a local or systemic disease progression.

Genetic analysis

DNA was isolated from peripheral blood leukocytes using commercially available GeneJET genomic DNA purification kit, according to manufacturer's recommendations (Thermo Fisher Scientific Baltics, Lithuania). Polymerase chain reaction (PCR) primer sequences and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) conditions for genotyping are listed in Table 1. All restriction enzymes for PCR-RFLP were from Thermo Fisher Scientific Baltics, Lithuania. Genetic testing for current gene mutation or polymorphism was done as described above, with some modifications when necessary (Resnick et al., 2003; Lu et al., 2006; Bogdanova et al., 2008a; Bogdanova et al., 2008b).

Table 1. **Primer sequences, PCR-RFLP conditions for the analysis of *NBS1* mutations and polymorphisms**

Substitution	Primer sequence	Annealing temperature (°C)	Restriction enzyme	DNA fragment size (bp)	Reference
NBS1 c.657_661del	F: 5'-ACCCACCTCTT-GATGAACCA-3' R: 5'-CTGTTTG-GCATTCAAAAA-3'	55	–	WT 112bp Mutant 107bp	Resnick et al., 2003
NBS1 p.R215W	F: 5'-GGAAGTA-AAAATGTTGATCT-GTCAGTA-3' R: 5'-TGAAATACGT-TAACAACTACTG-3'	60	*Rsa*I	WT 153+25bp Mutant 178bp	Bogdanova et al., 2008a
NBS1 p.I171V	F: 5'-TCCT-GAAAGCAGTT-GAGTCC-3' R: 5'-ACAAGCAT-TAAAGAGGGAGT-TAAC-3'	59	*Tsp*509I	WT 127+43bp Mutant 170bp	Bogdanova et al., 2008b
NBS1 c.8360G>C	F: 5'- CGTCCAATTG-TAAAGCCAGAA-3' R: 5'-TCCTGAAA-CAAGCATTA-AAGAGG-3'	54	*Hinf*I	*GG* genotype 125+49bp *CC* genotype 174bp *GC* genotype 174+125+49 bp	Lu et al., 2006
NBS1 c.30537G>C	F: 5'-TTGCTTGAAT-GACAGCCTGA-3' R: 5'-TCAGCCAGATG-GCAGACTC-3'	61	*Ear*I	*GG* genotype 197bp *CC* genotype 172+25bp *GC* genotype 197+172+25 bp	Lu et al., 2006

* WT – wild type.

Statistical analysis

Statistical analysis was done with SPSS 20.0 for Windows (SPSS, Inc., Chicago, IL, USA). In the case of qualitative variables, comparison was made by the 2-sided Pearson's Chi-square or 2-sided Monte Carlo exact test (in the cases where >25% of the expected value were <5). In order to calculate the crude and adjusted odds ratio (OR) and their 95% confidence intervals (CIs) (with and without adjustment for cancer diagnosis age), logistic regression analysis was used. Results were statistically significant at *P* value lower than 0.05.

RESULTS

Among the 80 patients, no carriers of the *NBS1 c.657_661del*, *p.R215W*, and *p.I171V* mutations were found. *NBS1 c.8360G>C* genotyping identified 11 patients with *GG* (13.75%), 38 with *GC* (47.5%), and 31 with *CC* (38.75%). Regarding *c.30537G>C* polymorphism, 46 patients were *GG* (57.5%), 29 *GC* (36.25%), and five with *CC* genotype (6.25%). The genotypes of *NBS1 c.8360G>C* and *c.30537G>C* were under Hardy-Weinberg Equilibrium. The *NBS1* polymorphism genotype distribution between pathological characteristics

is summarized in Table 2. Our descriptive data indicate that *c.8360G>C* and *c.30537G>C* variants do not statistically contribute to breast cancer characteristics. Dominant and recessive

Table 2. **The distribution of tumour clinicopathological characteristics by *NBS1* genotype**

Variable	*NBS1 c.8360*			*P* value	*NBS1 c.30537*			*P* value
	GG	*GC*	*CC*		*GG*	*GC*	*CC*	
	N	N	N		N	N	N	
	%	%	%		%	%	%	
Tumour size				0.822				0.118
<2 cm	7	26	19		34	16	2	
	63.6	68.4	61.3		73.9	55.2	40.0	
≥2 cm	4	12	12		12	13	3	
	36.4	31.6	38.7		26.1	44.8	60.0	
Histological grade				0.194				0.403
Grade 1+2	5	28	22		34	17	4	
	45.5	73.7	71.0		73.9	58.6	80.0	
Grade 3	6	10	9		12	12	1	
	54.5	26.3	29.0		26.1	41.4	20.0	
Stroma lymphocytic infiltration				0.843				0.457
Positive	1	6	5		6	6	0	
	9.1	15.8	16.1		13.0	20.7	0.0	
Negative	10	32	26		40	23	5	
	90.9	84.2%	83.9		87.0	79.3	100	
Lymph nodes				0.084				0.549
Positive	8	20	11		25	12	2	
	72.7	52.6	35.5		54.3	41.4	40.0	
Negative	3	18	20		21	17	3	
	27.3	47.4	64.5		45.7	58.6	60.0	
Oestrogen receptor				0.670				0.487
Positive	7	19	15		26	12	3	
	63.6	50.0	48.4		56.5	41.4	60.0	
Negative	4	19	16		20	17	2	
	36.4	50.0	51.6		43.5	58.6	40.0	
Progesterone receptor				0.662				0.418
Positive	4	17	16		21	15	1	
	36.4	44.7	51.6		45.7	51.7	20.0	
Negative	7	21	15		25	14	4	
	63.6	55.3	48.4		54.3	48.3	80.0	
HER2 amplification				0.704				0.316
Amplified	3	7	8		13	4	1	
	27.3	18.4	25.8		28.3	13.8	20.0	
Non-amplified	8	31	23		33	25	4	
	72.7	81.6	74.2		71.7	86.2	80.0	

Table 2. **(continued)**

Variable	*NBS1 c.8360*			*P* value	*NBS1 c.30537*			*P* value
	GG	*GC*	*CC*		*GG*	*GC*	*CC*	
	N	N	N		N	N	N	
	%	%	%		%	%	%	
Molecular breast cancer type								
Luminal A				0.866				0.718
Positive	6	18	14		20	15	3	
	54.5	47.4	45.2		43.5	51.7	60.0	
Negative	5	20	17		26	14	2	
	45.5	52.6	54.8		56.5	48.3	40.0	
Luminal B				0.896				0.314
Positive	2	4	4		8	2	0	
	18.2	10.5	12.9		17.4	6.9	0.0	
Negative	9	34	27		38	27	5	
	81.8	89.5	87.1		82.6	93.1	100.0	
HER2 type				0.876				0.729
Positive	1	3	4		5	2	1	
	9.1	7.9	12.9		10.9	6.9	20.0	
Negative	10	35	27		41	27	4	
	90.9	92.1	87.1		89.1	93.1	80.0	
Triple negative				0.587				0.737
Positive	2	13	9		13	10	1	
	18.2	34.2	29.0		28.3	34.5	20.0	
Negative	9	25	22		33	19	4	
	81.8	65.8	71.0		71.7	65.5	80.0	
Disease progress				0.410				0.238
Present	4	12	6		16	5	1	
	36.4	31.6	19.4		34.8	17.2	20.0	
Absent	7	26	25		30	24	4	
	63.6	68.4	80.6		65.2	82.8	80.0	

models showed no significant associations with studied characteristics in our study (Table 3).

The association between *NBS1* polymorphisms and clinicopathological characteristics was also assessed by the odds ratio with its corresponding 95% confidence interval. The logistic regression analysis showed no significant differences between *NBS1* polymorphism genotype and pathological features when the model was adjusted for the diagnosis age. However, there was a significant association between *c.8360G>C* polymorphism and clinicopathological characteristics in the model without adjustment for the age of breast cancer diagnosis (Table 4). It was determined that heterozygous carriers of *NBS1 8360 GC* genotype had a 2.885-fold decreased risk of low grade (well-differentiated) (OR = 2.885, 95% CI 0.173–0.735, $P = 0.005$) tumour compared with those with *GG* genotype. The homozygote *CC* genotype of this polymorphism carriers had a 2.186-fold (OR = 2.186, 95% CI 0.188–0.888, $P = 0.024$) decreased risk of low grade (G1+2) tumour differentiation. *8360 CC* genotype (OR = 3.034, 95% CI

Table 3. ***NBS1* polymorphisms genotypes models association with clinicopathological breast cancer characteristics**

Variable	NBS1c.8360 Recessive model*		*P* value	NBS1c.8360 Dominant model*		*P* value	NBS1 c.30537 Recessive model*		*P* value	NBS1 c.30537 Dominant model*		*P* value
	GG+GC	CC		GG	GC+CC		GG+GC	CC		GG	GC+CC	
	N	N		N	N		N	N		N	N	
	%	%		%	%		%	%		%	%	
Tumour size			0.468			0.727			0.337			0.052
<2 cm	34	18		9	43		50	2		18	34	
	68.0	60.0		69.2	64.2		66.7	40.0		52.9	73.9	
≥2 cm	16	12		4	24		25	3		16	12	
	32.0	40.0		30.8	35.8		33.3	60.0		47.1	26.1	
Histological grade			0.852			0.205			0.673			0.247
Grade 1+2	34	21		6	48		51	4		21	34	
	68.0	70.0		46.2	71.6		68.0	80.0		61.8	73.9	
Grade 3	16	9		7	19		24	1		13	12	
	32.0	30.0		53.8	28.4		32.0	20.0		38.2	26.1	
Stroma lymphocytic infiltration			0.746			0.420			0.593			0.569
Positive	7	5		1	11		12	0		6	6	
	14.0	16.7		7.7	16.4		16.0	0.0		17.6	13.0	
Negative	43	25		12	56		63	5		28	40	
	86.0	83.3		92.3	83.6		84.0	100		82.4	87.0	
Lymph nodes			0.094			0.108			>0.999			0.224
Positive	28	11		9	30		37	2		14	25	
	56.0	36.7		69.2	44.8		49.3	40.0		41.2	54.3	
Negative	22	19		4	37		38	3		20	21	
	44.0	63.3		30.8	55.2		50.7	60.0		58.8	45.7	
Oestrogen receptor			0.525			0.156			>0.999			0.273
Positive	27	14		9	32		38	3		15	26	
	54.0	46.7		69.2	47.8		50.7	60.0		44.1	56.5	
Negative	23	16		4	35		37	2		19	20	
	46.0	53.3		30.8	52.2		49.3	40.0		55.9%	43.5	
Progesterone receptor			0.602			0.221			0.366			0.901
Positive	22	15		4	33		36	1		16	21	
	44.0	50.0		30.8	49.3		48.0	20.0		47.1	45.7	
Negative	28	15		9	34		39	4		18	25	
	56.0	50.0		69.2	50.7		52.0	80.0		52.9	54.3	
HER2 amplification			0.890			0.957			>0.999			0.151

Table 3. **(continued)**

Variable	NBS1c.8360 Recessive model*		*P* value	NBS1c.8360 Dominant model*		*P* value	NBS1 c.30537 Recessive model*		*P* value	NBS1 c.30537 Dominant model*		*P* value
	GG+GC	CC		GG	GC+CC		GG+GC	CC		GG	GC+CC	
	N	N		N	N		N	N		N	N	
	%	%		%	%		%	%		%	%	
Amplified	11	7		3	15		17	1		5	13	
	22.0	23.3		23.1	22.4		22.7%	20.0		14.7	28.3	
Non-amplified	39	23		10	52		58	4		29	33	
	78.0	76.7		76.9	77.6		77.3%	80.0		85.3	71.7	
Molecular breast cancer type												
Luminal A			0.908			0.268			0.664			0.402
Positive	24	14		8	30		35	3		18	20	
	48.0	46.7		61.5	44.8		46.7	60.0		52.9	43.5	
Negative	26	16		5	37		40	2		16	26	
	52.0	53.3		38.5	55.2		53.3	40.0		47.1	56.5	
Luminal B			0.600			0.731			0.619			0.124
Positive	7	3		2	8		10	0		2	8	
	14.0	10.0		15.4	11.9		13.3	0.0		5.9	17.4	
Negative	43	27		11	59		65	5		32	38	
	86.0	90.0		84.6	88.1		86.7	100		94.1	82.6	
HER2 type			0.441			0.762			>0.999			>0.999
Positive	4	4		1	7		7	1		3	5	
	8.0	13.3		7.7	10.4		9.3	20.0		8.8	10.9	
Negative	46	26		12	60		68	4		31	41	
	92.0	86.7		92.3	89.6		90.7	80.0		91.2	89.1	
Triple negative			>0.999			0.209			>0.999			0.693
Positive	15	9		2	22		23	1		11	13	
	30.0	30.0		15.4	32.8		30.7	20.0		32.4	28.3	
Negative	35	21		11	45		52	4		23	33	
	70.0	70.0		84.6	67.2		69.3	80.0		67.6	71.7	
Disease progress			0.245			0.478			>0.999			0.090
Present	16	6		5	17		21	1		6	16	
	32.0	20.0		38.5	24.4		28.0	20.0		17.6	34.8	
Absent	34	24		8	50		54	4		28	30	
	68.0	80.0		61.5	74.6		72.0	80.0		82.4	65.2	

* Dominance of the alleles can be assumed by combining the heterozygote and one of the homozygotes as a single group. In c.8360G>C polymorphism (same with c.30537G>C variant), C is the "risk" allele. In the recessive model, two copies of allele C required to affect breast cancer prognosis, i.e., GG+GC vs CC are used in the analysis. The dominant effect of the "risk" C allele is described by comparing GC+CC vs GG.

Table 4. **Odds ratio for the associations of *NBS1 c.8360G>C* polymorphism with clinicopathological features of breast cancer**

Genotype	Odds ratio without adjustment for the age of breast cancer diagnosis (95% confidence interval)*	*P* value
Odds of tumour size		
NBS1 8360 GC vs GG	0.462 (0.233–0.915)	0.27
Odds of histological grade		
NBS1 8360 GC versus GG	0.357 (0.173–0.375)	0.005
NBS1 8360 CC versus GG	0.409 (0.188–0.888)	0.024
Odds of HER2 amplification		
NBS1 8360 GC versus GG	0.226 (0.099–0.513)	<0.001
NBS1 8360 CC versus GG	0.348 (0.156–0.778)	0.010
Odds of luminal B molecular BC type		
NBS1 8360 GC versus GG	0.118 (0.042–0.332)	<0.001
NBS1 8360 CC versus GG	0.148 (0.052–0.423)	<0.001
Odds of triple negative molecular BC subtype		
NBS1 8360 CC versus GG	0.409 (0.188–0.888)	0.024
Odds of disease progress		
NBS1 8360 GC versus GG	0.462 (0.233–0.915)	0.027
NBS1 8360 CC versus GG	0.240 (0.098–0.585)	0.002

* Only statistically significant associations are shown.

0.156–0.778, $P = 0.010$) significantly increased the chances of HER2 amplification compared to *GG* genotype. The data indicated that patients with *8360 GC* genotype had a higher risk for breast cancer progression (OR = 1.673, 95% CI 0.233–0.915, $P = 0.027$). The carriers of homozygote *8360 CC* had approximately six times higher risk for the disease progress (OR = 5.946, 95% CI 0.098–0.585, $P = 0.002$) when compared to *GG* genotype. The prevalence of triple negative breast cancer subtype was significantly higher in individuals with *NBS1 8360 CC* genotype (OR = 2.186, 95% CI 0.188–0.888, $P = 0.024$). Regarding *c.30537G>C*, none of the genotypes had any significant influence on pathological characteristics.

DISCUSSION

Well-designed analysis of mutations or polymorphisms of the *NBS1* gene or other DNA repair genes could potentially identify prognostic and predictive factors for breast cancer. *c.657_661del* mutation in the *NBS1* gene clearly has association with an increased breast cancer risk, missense substitutions, e.g., *p.I171V* and *p.R215W*, may represent cancer susceptibility alleles with lower penetrance (Bogdanova et al., 2008a).

Several studies have investigated not only the risk of breast cancer but also clinical characteristics of the disease in the case of *NBS1* mutation. Previous report about the influence of *NBS1* gene *p.I171V* mutation on histological and clinical breast cancer features suggested accumulation of the negative prognostic factors (Roznowski et al., 2008). In the study by Roznowski and colleagues (2008), the carriers of *p.I171V* showed a trend of a more aggressive cancer phenotype: higher histological grade, HER2 overexpression, lower oestrogen receptor expression, and familial history of malign disease. According to Nowak et al. (2008), *NBS1 p.I171V* mutation may be a general susceptibility factor to solid tumours. The study by Huzarski et al.

(2013) showed that *NBS1 c.657_661del* did not affect breast cancer prognosis. In this case-control analysis, tumour size, grade, receptors, and lymph node status were also included. No statistically significant differences between mutation carriers and non-carriers were observed. Moreover, several studies have investigated the relation between *NBS1* gene *c.8360G>C* and *c.30537G>C* polymorphisms and cancer risk, but the results were inconclusive (He et al., 2014). Sun et al. (2007) revealed that *NBS1 c.8360G>C* polymorphism *CC* genotype may be one of the predictive neutropenic fever factors for breast cancer patients. All mentioned clinicopathological breast cancer characteristics and *NBS1* gene status association analysis included case-control groups, but there is still a need of prospective studies with a larger sample size and with broader ethnic diversity.

In the present study, we investigated the possible association between mutations and polymorphisms in the *NBS1* gene and clinicopathological characteristics of breast cancer. Compared with other studies (Roznowski et al., 2007; Huzarski et al., 2013; Rusak et al., 2019), our study is rather unique. We included young (≤50 years of age) premenopausal women with I or II stage breast cancer diagnosis. The stage of breast cancer is a strong prognostic factor; in order to avoid its influence, we chose women with an early stage of breast cancer. To reveal the relation between *NBS1* gene substitutions and clinicopathological characteristics, our analysis involved features that represent breast cancer phenotype and disease progress. No carriers of *c.657_661del*, *p.I171V* and *p.R215W* mutations were found. It might be due to the relatively young breast cancer patients' age, as it is known that mutations in the *NBS1* gene more often influence malign disease in elderly patients. In the current study, some statistically significant associations were determined. *NBS1 c.8360G>C* polymorphism *GC* and *CC* genotypes had effect on the histological tumour grade. It was observed that heterozygote *GC* and homozygote *CC* genotype holders had an increased risk for a higher histological grade. Furthermore, we revealed that *CC* genotype of *c.8360G>C* increased the odds of HER2 overexpression, which is a strong prognostic breast cancer factor. It was determined that current polymorphism heterozygous *GC* and homozygous *CC* genotypes increased the chances of breast cancer progression if compared with *GG* genotype. According to our results, *NBS1 c.8360G>C* polymorphism *CC* genotype increased the odds of triple negative breast cancer. In the case of *c.30537G>C* polymorphism, no significant associations with breast cancer characteristics were found.

Unfortunately, there are some difficulties concerning the conclusion about the *NBS1* gene status and breast cancer clinicopathological characteristics association because of small group of analysed patients compared with studies conducted by other researchers. We have to admit that the second limitation of our study is a short follow-up period of our patients.

CONCLUSIONS

Our data suggest that *NBS1* gene *c.8360G>C* polymorphism may be associated with breast cancer aggressiveness in young breast cancer patients. Further work is warranted to investigate the effect of the *NBS1* status on breast cancer prognosis and malignancies risk.

COMPETING INTEREST

The authors declare that they have no competing interests.

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NBS1 GENO POKYČIŲ IR KLINIKINIŲ PATOLOGINIŲ CHARAKTERISTIKŲ RYŠYS KRŪTIES VĖŽIU SERGANČIŲ JAUNŲ PACIENČIŲ GRUPĖJE

Santrauka

Šios bandomosios studijos tikslas – nustatyti klinikinius požymius, kurie yra potencialiai susiję su *NBS1* geno mutacijomis ir polimorfizmu krūties vėžiu sergančių jaunų (≤50 metų) pacienčių grupėje. Kraujo ėminiai paimti iš 80 pacienčių. Polimerazės grandininės reakcijos-restrikcijos fragmentų ilgio polimorfizmo metodu ištirta *c. 657_661del*, *p. R215W* ir *p. I171V* mutacijos bei *c. 8360G > C*, *c. 30537G > C* polimorfizmas. Statistinėje analizėje taikytas dvipusis Chi-kvadrato testas, o galimybių santykis įvertintas pasitelkus logistinę regresiją. Tiriamojoje grupėje pacienčių su *c. 657_661del*, *p. R215W* ir *p. I171V* mutacijomis neaptikta. *NBS1 c. 8360G > C* logistinė regresija rodo, kad *GC* (ŠS = 2,885, 95 % PI 0,173–0,735, *P* = 0,005) ir *CC* (ŠS = 2,186, 95 % PI 0,188–0,888, *P* = 0,024) genotipų nešiotojoms, palyginti su *GG* genotipu, būdinga mažesnė blogai diferencijuoto naviko rizika. *8360 CC* (ŠS = 3,034, 95 % PI 0,156–0,778, *P* = 0,010) genotipas reikšmingai padidino HER2 amplifikacijos galimybę, palyginti su *GG* genotipu. Progresuojančio krūties vėžio rizika reikšmingai didesnė *NBS1 8360 GC* (ŠS = 1,673, 95 % PI 0,233–0,915, *P* = 0,027) genotipo atveju. Homozigotinio *8360 CC* genotipo nešiotojos turėjo beveik šešis kartus didesnę progresuojančios ligos riziką (ŠS = 5,946, 95 % PI 0,098–0,585, *P* = 0,002). Trigubai neigiamo krūties vėžio tipo atvejai reikšmingai dažniau pasitaikė asmenims su *NBS1 8360 CC* genotipu (ŠS = 2,186, 95 % PI 0,188–0,888, *P* = 0,024). *c. 30537G > C* polimorfizmo atveju reikšminga įtaka patologinėms krūties vėžio charakteristikoms nenustatyta. *NBS1* geno *c. 8360G > C* polimorfizmas gali būti susijęs su krūties vėžio agresyvumu jaunų pacienčių grupėje.

Raktažodžiai: krūties vėžys, mutacija, polimorfizmas, *NBS1* genas