

## ORIGINAL PAPER

**SEARCHING FOR NEW BREAST CANCER-ASSOCIATED GENES.  
*ABRAXAS1* GENE MUTATIONS IN THE GROUP  
OF *BRCA1*-NEGATIVE PATIENTS**

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In the present study, we analysed the association of mutations of a *BRCA1*-associated gene, *ABRAXAS1*, with the risk of development of breast cancer (BC) in *BRCA1*-negative women from North-Central Poland. A hundred women with consecutively diagnosed BC and 100 women belonging to the control group were screened for new mutations predisposing to breast cancer. The first step was a test carried out in order to find one of the three Polish founder mutations in the *BRCA1* gene. In 96 *BRCA1*-negative patients two missense variants: c.422C>T and c.1042G>A as well as two intronic variants: IVS3-34G>A, IVS3-44T>C were detected in the *ABRAXAS1* gene. The c.422C>T mutation was detected in one of 96 women diagnosed with breast cancer (1.04%); it was not associated with increased risk of disease in this group, compared to the controls ( $p = 0.49$ ), but the odds ratio was 3.314; 95% CI: 0.122-75.352. IVS3-44T>C was found more frequently in the control group (15/93) than in the tested group (1/85), OR 0.062; 95% CI: 0.008-0.480,  $p = 0.007$ , which may suggest protective properties of this variant against tumorigenicity. The data obtained from the present study suggest the necessity for further research to be conducted on the *ABRAXAS1* gene in relation to hereditary predisposition to breast cancer.

**Key words:** breast cancer, *ABRAXAS1* mutations, breast cancer predisposing genes.

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## Introduction

A family history of breast cancer is a major cancer risk factor. However, the mutations of high-penetrant genes, such as *BRCA1*, explain only 20-25% of familial breast cancer and 5% of all breast cancers [1]. The *BRCA1* gene is involved in the processes of DNA damage response (DDR), such as repair of DNA double strand breaks (DSB) and cell cycle checkpoint control. The *BRCA1* protein plays

a crucial role in DDR through its interactions with many other proteins, such as *BRCA2*, *CHEK2*, *NBS1*, and *Abraxas* [2, 3, 4]. *Abraxas* (the protein encoded by the *ABRAXAS1* gene) seems to be a central adaptor protein of *BRCA1* Complex A. This complex contains *BRCA1/BARD1* (*BRCA1*-associated RING domain protein 1) heterodimer, *Abraxas*, *RAP80* (receptor-associated protein 80), *BRCC45* (*BRCA1/BRCA2*-containing complex subunit 45, also known as *BRE*), and *BRCC36* (*BRCA1/BRCA2*-containing

complex subunit 36, also known as BRCC3) [5]. BRCA1 binds through its BRCT domain (BRCA1 C-terminal domain) to phosphorylated Abraxas, which acts as a scaffold and binds to BRCC36, BRCC45, and RAP80 [6, 7]. Although Abraxas appears to be a key player in the BRCA1-dependent DNA damage response, the mechanism of its participation in DNA repair activity, BRCA1 signalling, and tumour suppression is not entirely clear. However, similarly to *BRCA1*, mutations in *ABRAXAS1* appear to be involved in susceptibility to cancer [6, 8].

In the present report we show the results of research on association between *ABRAXAS1* mutations and the risk of BC in women originating from North-Central Poland, which is a rather homogenous population, as well as the relation of these mutations to familial history of BC [9].

## Material and methods

### Patients

A hundred women from North-Central Poland (Kujawy-Pomerania voivodeship) with consecutively diagnosed BC, treated in 2012-2016 at the Oncology Centre in Bydgoszcz, were included in the study, regardless of the histopathological type of cancer.

All 100 patients from the study group originated from families fulfilling Hereditary Breast Cancer criteria (HBC) defined by Gronwald *et al.* [10]. Forty-seven of 100 (47%) probands were affected by breast cancer at  $\leq 40$  years old. In 53 of 100 (53%) probands breast cancer was diagnosed at over 40 years of age. In their families, at least one additional breast cancer in a close relative was detected. In 54% (54/100) of all HBC families the most frequently diagnosed additional cancers were ovary, lung, colon, and prostate.

The median age at BC diagnosis in the whole group was 41 years (range 30-67 years). The molecular investigations for the detection of pathogenic mutations in the *BRCA1* and *ABRAXAS1* genes were not carried out in any probands' relative with cancer.

The control group consisted of 100 age-matched women from North-Central Poland, without cancer at the time of molecular investigations on a questionnaire basis. These women came from families without a history of cancer.

Informed consent was obtained from all patients and control persons. The study was approved by the Ethics Committee of the *Collegium Medicum*, Nicolaus Copernicus University in Bydgoszcz, Poland.

### Molecular analysis

All the mutations were investigated in DNA from peripheral blood leukocytes extracted by standard salting-out method. The first step of analysis con-

sisted of the multiplex-PCR test for the presence of three Polish founder mutations in the *BRCA1* gene: c.181T>G, c.4035delA, and c.5266dupC [11]. This analysis was conducted in all 100 patients and 100 control persons. The next step was carried out in the *BRCA1*-negative persons (96 out of 100 patients and 100 control persons). Encoding fragments with about 50 nucleotides of introns surrounding all nine exons of *ABRAXAS1* (NG\_051599.1) gene were analysed by Sanger automated sequencing. Sequences of primers for all *ABRAXAS1* exons were established by Novak *et al.* [12]. Evaluation of pathogenicity of detected missense mutations was carried out using PROVEAN [13], PolyPhen-2 [14], and AlignGVGD [15] tools.

Statistical significance of differences in the frequency of variant alleles between tested and control groups was estimated using the chi-squared test with Yates's correction for continuity.

## Results

The *BRCA1* mutations c.181T>G, c.4035delA, and c.5266dupC are the founder mutations for hereditary BC in the Polish population [11, 16]. In the first step of analysis we looked for their presence in the *BRCA1* gene. At least one of them was found in four out of 100 women diagnosed with BC (4%); three c.5266dupC mutations and one c.181T>G. Thus, these women were excluded from the group investigated for mutations in *ABRAXAS1* gene.

Mutations in *ABRAXAS1* gene identified in this study were two missense variants: c.422C>T (p.Thr141Ile, rs150207999) and c.1042G>A (p.Ala348Thr, rs12642536) and two intronic variants: IVS3-34G>A and IVS3-44T>C (not recorded in dbSNP; Table I).

The c.422C>T variant was found in one patient from the investigated group and in no person from the control group. We evaluated its pathogenicity *in silico* with PolyPhen-2, AlignGVGD and PROVEAN tools. The prediction analysis indicated its deleterious character. The carrier of c.422C>T variant was a woman diagnosed with breast cancer at the age of 35 years (Fig. 1). Her mother's sister also had breast cancer diagnosed at a young age (BC32), but samples of her DNA as well as DNA of the patient's mother were not available.

The second missense variant c.1042G>A was found in 28 patients from the investigated group and 36 persons from the control group. The prediction analysis using PolyPhen-2, AlignGVGD, and PROVEAN tools indicated its probable deleterious character.

Two missense alterations (c.422C>T and c.1042G>A) of the *ABRAXAS1* gene we found did not show a statistically significant association with

cancer susceptibility (Table I). However, there was a high odds ratio: 3.314 for c.422C>T and 3.176 for c.1042G>A.

Two intronic *ABRAXAS1* variants were also found as a result of investigation (Table I). The difference between the frequency of one of IVS3-44T>C in the tested and control groups was statistically significant. This was found to be 15-times more frequent in the control group than in the tested group ( $p = 0.007$ ), which may suggest protective properties of this variant against tumorigenicity. The pathogenicity of IVS3-44T>C and IVS3-34G>A intron variants, evaluated by *in silico* analyses with Human Splicing Finder and NetGene2 tools, indicated that both have no impact on splicing.

## Discussion

The genetic basis of breast cancer is very complex, and it is suggested that many factors could play a role in disease development. Researchers are looking for biomarkers and cancer susceptibility genes to diagnose the disease at an early stage and quickly implement the treatment. A biomarker is defined as an indicator of objective measurement that can be used to detect various diseases or to evaluate treatment risks or effectiveness. For example, a clinical trial in 71 breast cancer patients proved that topoisomerase II expression can be considered a proliferation marker and a prognostic factor in oestrogen receptor (ER)-positive human epidermal growth factor type-2 (HER2)-negative breast cancer [17]. Our study

shows the results of the first studies on *ABRAXAS1* gene mutations in the Polish population and one of the first in the world. Previous studies on the function of *ABRAXAS1* by Castillo *et al.*, Wang *et al.*, and Solyom *et al.* indicated its important role in the maintenance of genome stability, and the necessity of its co-operation with BRCA1 for efficient DNA damage response [5, 6, 7]. Novak *et al.* studied the *ABRAXAS1* gene as a potential BC risk factor in BRCA1 mutation-negative patients with hereditary breast cancer and described new pathogenic variants that may play role in cancer development [12]. Similarly, Solyom *et al.* found new deleterious *ABRAXAS1* variants, which is why *ABRAXAS1* was suggested by the authors as a new breast cancer susceptibility gene [6]. In our investigation on quite a small group of breast cancer patients, one probable pathogenic *ABRAXAS1* exon variant (c.422C>T) was found with frequency of 1.04% (1/96) in the tested group and not detected in the control group. The difference in frequency of mutation between these two groups was not statistically significant ( $p = 0.49$ ) but the odds ratio was 3.314; 95% CI: 0.122-75.352. The results of our study confirm the results obtained by Laure-Renault *et al.*, who analysed 1318 BC cases with diagnosed cancer below the age of 45 years, of Caucasian, Latino, East Asian, or African-American ancestry. Cases were matched with 1115 controls within each centre according to the age at recruitment ( $\pm 10$  years from the age at diagnosis) and race/ethnicity. They found the same c.422C>T mutation frequency (0.01%) in

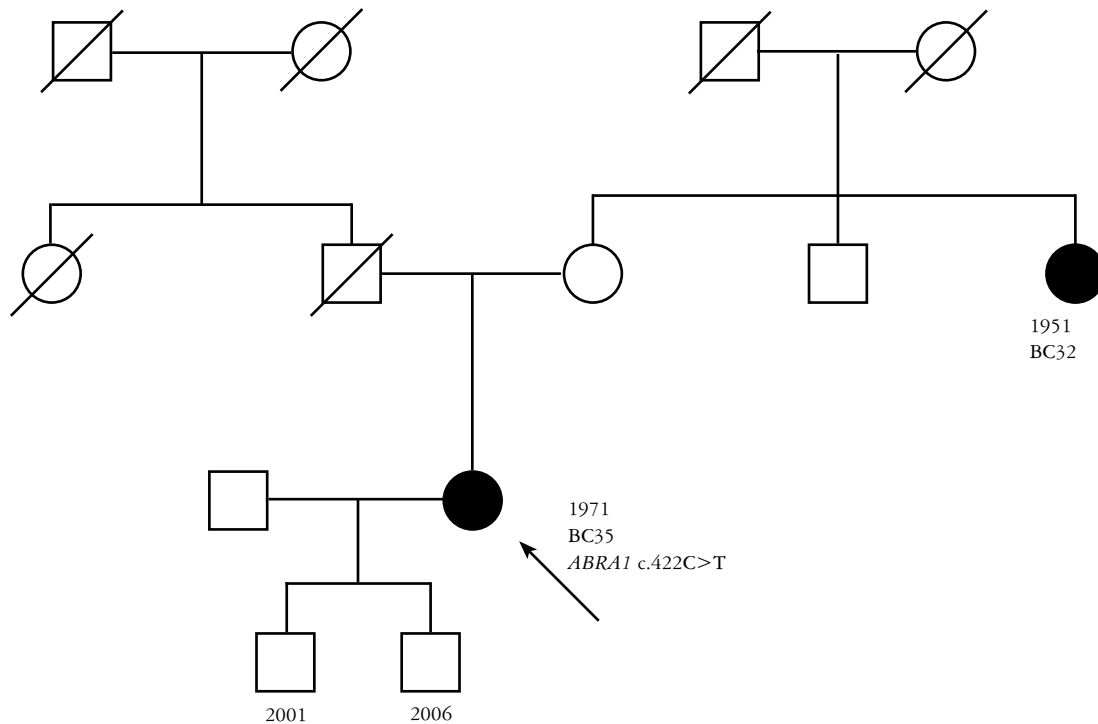
**Table I.** Statistical analysis of the presence of *ABRAXAS1* gene variants in the investigated and control groups

EXON	VARIANT	AMINO ACID CHANGE	TEST GROUP		CONTROL GROUP		OR	95% CI	P		
			TOTAL	%	TOTAL	%					
INTRON	3	IVS3-34G>A	-	G/G=21/85	24.71	G/G=29/93	31.18	-	-	-	
				G/A=35/85	41.17	G/A=42/93	45.16	0.850	0.469-1.540	0.592	
				A/A=29/85	34.12	A/A=22/93	23.66	1.671	0.867-3.21	0.124	
	IVS3-44T>C	-	T/T=84/85	98.82	T/T=78/93	83.87	-	-	-		
			T/C=1/85	1.18	T/C=15/93	16.13	0.062	0.008-0.480	0.007*		
			C/C=0/85	0	C/C=0/93	0	-	-	-		
5	c.422C>T	p.Thr141Ile	C/C=95/96	98.96	C/C=96/96	100	-	-	-		
			C/T=1/96	1.04	C/T=0/96	0	3.314	0.122-75.352	0.499		
			T/T=0/96	0	T/T=0/96	0	-	-	-		
9	c.1042G>A	p.Ala348Thr	G/	64.77	G/G=54/91	59.34	-	-	-		
			G=57/88		G/A=28/88	31.82	G/A=36/91	39.56	0.713	0.386-1.318	0.280
			A/A=3/88	3.41	A/A=1/91	1.1	3.176	0.324-31.134	0.321		

Informative results were obtained in the tested group in 96 and 88 patients for exons 5 and 9, respectively, and in 85 for intron 3. Informative results for control group consisting of 100 persons were obtained in 96 and 91 patients for exon 5 and 9, respectively, and in 93 patients for intron 3.

OR – odds ratio, CI – confidence interval

\* statistically significant



**Fig. 1.** Pedigree of the family with c.422C>T mutation (BC35 – breast cancer and year of diagnosis, black symbol – affected family member, white symbol – non-affected family member)

the tested and control groups (17/1318 and 13/1115, respectively) [18].

The prediction analysis using PolyPhen-2, AlignGVGD, and PROVEAN tools indicated deleterious character of c.422C>T mutation, but in a previous investigation performed by Osorio *et al.* this mutation was described as a benign amino acid change [19]. The second missense variant c.1042G>A was described in previous investigations performed by Novak *et al.* and Osorio *et al.* as a non-pathogenic change [12, 19]. An *in silico* analysis performed by us showed a probable deleterious character of this mutation.

Further investigations on the role of *ABRAXAS1* in BC development on a larger group of patients are needed, as well as further research including hitherto undescribed intron variant (IVS3-44C>T), which may play a protective role in cancer development.

Our study shows that the *ABRAXAS1* gene should be investigated more extensively because its mutations/variants may constitute new important hereditary breast cancer risk factors.

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*The authors declare no conflict of interest.*

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