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BRCA1 and *BRCA2* germline variants in breast cancer patients from the Republic of Macedonia

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Abstract

Purpose We aimed to establish the spectrum of *BRCA1/2* mutations among the breast cancer (BC) patients from the Republic of Macedonia.

Methods We used targeted next-generation sequencing (NGS), Sanger DNA sequencing, and multiplex ligation probe amplification analysis (MLPA) to search for point mutations and deletions/duplications involving *BRCA1* and *BRCA2*-coding regions.

Results We have analyzed a total of 313 BC patients, enriched for family history of cancer, early age of onset and bilateral and/or triple negative (TN) BC. A total of 26 pathogenic mutations were observed in 49 unrelated BC patients (49/313, 15.7%). *BRCA2* mutations (27/49, 55.1%) were more common than *BRCA1* mutations (22/49, 44.9%). We identified five novel point mutations, one in *BRCA1* (c.4352_4356delA) and four in *BRCA2* (c.151G>T, c.4707_4708delCA, c.7811_7814delTGTG, and c.9304_9305delG), as well as two novel deletions involving parts of the *BRCA1* gene (c.81−?_593+?del and c.5470−?_5530+?del). The most common mutations were c.181T>G, c.5266dupC, and c.3700_3704del5 in *BRCA1* and c.7879A>T, c.8317_8330del14 and c.5722_5723delCT in *BRCA2* gene. Thus far, *BRCA2* c.7879A>T and c.8317_8330del14 mutations have been described in several isolated cases; however, our study is the first one showing that they have a founder effect among Macedonian population. Nine recurrent mutations account for 65.3% of all of the detected mutations allowing for implementation of a fast first-step *BRCA1/2* mutational screening strategy in our country.

Conclusion This study provides a comprehensive view of known and novel *BRCA1/2* mutations in BC patients from the Republic of Macedonia and contributes to the global spectrum of *BRCA1/2* mutations in breast cancer.

Keywords Breast cancer · *BRCA1/2* mutations · Next-generation sequencing (NGS) · Macedonian population · Albanian population

Introduction

Breast cancer (BC) is the most widespread cancer in the world and the second most common cause of death from a neoplastic disease affecting women. Although lifestyle and hormonal factors play a significant role, family history is

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one of the most powerful risk factor for BC development. Patients who have close relatives with breast and/or ovarian (Br/Ov) cancer comprise a group of familial breast cancers (FBC) that represent 5–7% of all BC cases. Around 25% of FBC patients have germline mutations in two high-risk breast cancer susceptibility genes, *BRCA1* [1] and *BRCA2* [2]. The penetrance of deleterious *BRCA1/2* mutations has been variably estimated. Based on combined analysis of different reports, *BRCA1* and *BRCA2* mutations confer an average cumulative risk of 71 and 45% for developing BC [3].

Up to date, a total of 5784 germline variants in *BRCA1* and 7720 germline variants in *BRCA2* genes have been reported in the NCBI's ClinVar database. The mutations are distributed across the entire coding sequence of both genes, with more than 50% of the observed mutations being unique to particular individuals. Small insertions/deletions (in-dels) or nonsense mutations leading to premature protein truncation, translational frameshifts, and defective splice sites are the most commonly observed *BRCA* gene mutation types [4, 5]. In addition to the recognized pathogenic variants, a large number of missense mutations and in-frame deletions known as variants of unknown significance (VUS) have been reported. They have unclear pathogenic potential; therefore, the clinical interpretation of these variants in cancer patients represents a challenging task.

With an aim to determine the spectrum of *BRCA1/2* gene mutations responsible for hereditary breast cancer in the Republic of Macedonia, we have analyzed a large cohort of BC patients, enriched for family history of cancer, early age of onset, and bilateral and/or triple negative (TN) BC.

Materials and methods

Our patient group consisted of 313 individuals diagnosed with invasive breast cancer during the years 2009–2017. The patients were selected for *BRCA1* and *BRCA2* genetic testing according to several criteria, including family history of cancer, early age of onset, and bilateral and/or triple negative (TN) BC. Personal and clinical characteristics, including ethnicity, age at diagnosis, family history, and histopathological findings, are presented in Table 1. The mean age of onset of the BC was 49 years (range 24–87 years). For each patient, DNA was isolated from peripheral blood using the standard phenol–chloroform extraction method.

We used two library preparation kits for the targeted next-generation sequencing (NGS): TruSeq Custom Amplicon for targeting *BRCA1* and *BRCA2*-coding regions and TruSight Cancer sequencing panel for targeting cancer-associated genes including *BRCA1* and *BRCA2* (Illumina, San Diego, CA, USA). The sequencing was performed on the NGS MiSeq Illumina Personal Sequencer and the data analyses were performed on the Illumina Variant Studio.

All of the variants detected in one or two patients by NGS were validated using Sanger sequencing method. In addition, the entire exon 23 in *BRCA1*, not covered by TruSeq Custom Amplicon kit, was sequenced using the Sanger method. The conventional Sanger sequencing was performed with the use of BigDye terminator sequencing kit v1.1 on ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Screening for large genomic alterations was performed in a group of patients ($n = 145$) by MLPA using the SALSA MLPA Kit P002 BRCA1 and SALSA MLPA Kit P045 BRCA2 according to the instructions provided by the manufacturer (MRC-Holland, Amsterdam, The Netherlands).

Sequence variants were checked for previously published reports in four databases: Breast Cancer Information Core, Leiden Open Variation Database, Universal Mutation Database and ClinVar. In silico prediction analysis for deleteriousness of the novel mutations and those with undetermined pathogenic effects were performed using eight online available software tools: PolyPhen-2 [6], SIFT [7], PROVEAN [8], Mutation Assessor [9], Mutation Taster [10], SuSPect [11], and PANTHER [12] for missense and Human Splice Finder [13] for splicing variants. The guidelines and standards for interpretation of sequence variants recommended by the American College of Medical Genetics and Genomics (ACMG) were followed for presenting and classifying the variants [14].

Results

The mutation screening of 313 patients resulted in the discovery of 86 sequence variations, 32 in *BRCA1,* and 54 in *BRCA2* gene, while the MLPA screening for large genomic alterations in a smaller group of 145 patients revealed two deletions involving parts of the *BRCA1* gene.

Pathogenic variants

A total of 26 different pathogenic variants were detected in 49 unrelated patients. Eleven different *BRCA1* mutations were observed in 22 patients and 15 different *BRCA2* mutations in 27 patients (Table 2). The age of onset, histopathological findings, and family history of *BRCA1/2* carriers are given in Supplementary Table 1, whereas the distribution of *BRCA1/2* mutations according to patients' parameters is presented in Table 1.

The most common *BRCA1* gene mutation was c.181T>G found in five patients (5/49, 10.2%), followed by c.5266dupC found in four (4/49, 8.2%), and c.3700_3704delGTAAA found in three patients (3/49, 6.1%). *BRCA1* c.1102G>T and c.5212G>A mutations were found in two patients each (2/49, 4.1%), while all

Table 1 Personal and clinical characteristics of 313 BC patients and the distribution of *BRCA1/2* mutations according to patients' parameters

	No. of individuals	*BRCA1*		*BRCA2*		TOTAL *BRCA1/2*	
		n	(%)	*n*	(%)	*n*	(%)
Ethnicity							
Macedonian	252	18	7.14	24	9.52	42	16.67
Albanian	55	4	7.27	3	5.45	7	12.72
Other	6	0	0	0	0	0	0
Age of onset							
< 40	100	8	8.0	11	11.00	19	19.00
41–60	141	11	7.80	14	9.93	25	17.73
> 60	71	2	2.82	3	4.23	5	7.04
Bilateral breast cancer							
Yes	37	3	8.11	6	16.22	9	24.32
No	263	17	6.46	21	7.98	38	14.45
No data	13	2	15.38	1	7.69	3	23.08
Cancer family history							
Br/Ov cancer	180	16	8.89	18	10.00	24	13.33
Other cancers	40	2	5.00	3	7.50	5	12.50
No cancers	83	3	3.61	7	8.43	10	12.05
No data	10	1	10.00	1	10.00	2	20.00
Estrogen receptor (ER)							
Positive	204	8	3.92	22	10.78	30	14.71
Negative	88	12	13.64	4	4.55	16	18.18
No data	21	2	9.52	2	9.52	4	19.05
Progesterone receptor (PR)							
Positive	178	7	3.93	17	9.55	24	13.48
Negative	113	13	11.50	8	7.08	21	18.58
No data	22	2	9.09	3	13.64	5	22.73
Her2/neu receptor							
Positive	55	1	1.82	2	3.64	2	3.64
Negative	228	18	7.89	23	10.09	41	17.98
No data	30	3	10.00	3	10.00	6	20.00
Triple negative							
Yes	68	13	19.12	4	5.88	17	25.00
No	224	7	3.13	22	9.82	29	12.95
No data	21	2	9.52	2	9.52	4	19.05
Tumour Stage							
In situ	5	0	0.00	0	0.00	0	0.00
I	56	2	3.57	0	0.00	2	3.57
II	130	14	10.77	12	9.23	26	20.00
III	100	3	3.00	13	13.00	16	16.00
No data	22	3	13.64	3	13.64	6	27.27

other mutations were found in one patient each (1/49, 2%) (Fig. 1). Three *BRCA1* mutations (c.4352_4356delA, c.81−?_593+?del, and c.5470−?_5530+?del) were described for the first time among our patients. All novel *BRCA1* mutation carriers had at least one first or second degree relative with Br/Ov cancer. BC patients with c.4352_4356delA and c.5470−?_5530+?del mutations were diagnosed with TN ductal cancer, whereas no histopathological data were available for the c.81−?_593+?del carrier.

The most frequent *BRCA2* mutation was c.7879A>T, observed in six patients (6/49, 12.2%), followed by c.8317_8330del14 (5/49, 10.2%), c.5722_5723delCT (3/49, 6.1%), and c.5851_5854deAGTT (2/49, 4.1%). The other 11 *BRCA2* mutations were found in one patient each (1/49, 2%) (Fig. 1). Three frameshift *BRCA2*

Table 2 Pathogenic variants in *BRCA1* and *BRCA2* genes identified in our study

	Exon	HGVS nomenclature	AA change	Variant ID	Mutation type	Functional domain	Times observed in patients	Times observed in patients' family members
	BRCA 1							
1	03–08	c.81−?_593+?del	–	–	Large deletion	Zinc finger	1	0
2	5	c.181T>G	p.Cys61Gly	rs28897672	Missense	Zinc finger	5	4
3	11	c.1102G>T	p.Glu368Ter	rs80357139	Nonsense	Serine rich domain	2	0
4	11	c.1612C>T	p.Gln538Ter	rs80356893	Nonsense	–	1	0
5	11	c.1687C>T	p.Gln563Ter	rs80356898	Nonsense	–	1	1
6	11	c.2933_2934insA	p.Tyr978 fs	rs878853292	Frameshift	–	1	0
7	11	c.3700_3704del5	p.Val1234 fs	rs80357609	Frameshift	–	3	0
8	13	c.4352_4356delA	p.Ala1453 fs	–	Frameshift	–	1	0
9	20	c.5212G>A	p.Gly1738Arg	rs80356937	Missense	BRCT2 domain	2	5
10	20	c.5266dupC	p.Gln1756 fs	rs80357906	Frameshift	BRCT2 domain	4	1
11	23	c.5470−?_5530+?del	–	–	Large deletion	BRCT2 domain	1	0
	BRCA 2							
1	3	c.151G>T	p.Glu51Ter	–	Nonsense	–	1	2
2	9	c.775A>T	p.Arg259Ter	rs397507937	Nonsense	–	1	0
3	9	c.1599_1600delTG	p.Glu534Serfs	rs80359293	Frameshift	–	1	0
4	11	c.2808_2811delACAA	p.Ala938Profs	rs80359351	Frameshift	–	1	0
5	11	c.3189_3192delGTCA	p.Ser1064Leufs	–	Frameshift	–	1	0
6	11	c.4707_4708delCA	p.Tyr1569Terfs	–	Frameshift	–	1	2
7	11	c.5351dupA	p.Asn1784Lysfs	rs80359508	Frameshift	–	1	0
8	11	c.5722_5723delCT	p.Leu1908Argfs	rs80359531	Frameshift	–	3	1
9	11	c.5851_5854delAGTT	p.Ser1951Trpfs	rs80359543	Frameshift	–	2	0
10	17	c.7811_7814delTGTG	p.Cys2605Thrfs	–	Frameshift	Helical domain	1	0
11	17	c.7879A>T	p.Ile2627Phe	rs80359014	Missense	Helical domain	6	2
12	18	c.8168A>G	p.Asp2723Gly	rs41293513	Missense	Oligosaccharide binding domain 1	1	2
13	18	c.8317_8330del14	p.Ser2773Aspfs	rs397507976	Frameshift	Oligosaccharide binding domain 1	5	1
14	25	c.9304_9305delG	p.Ala3102Glnfs	–	Frameshift	Oligosaccharide binding domain 3	1	0
15	25	c.9352_9353delAT	p.Met3118Valfs	rs786203318	Frameshift	Oligosaccharide binding domain 3	1	0

mutations (c.4707_4708delCA, c.7811_7814delTGTG, and c.9304_9305delG) and one nonsense variant (c.151G>T) were novel. *BRCA2* c.151G>T, c.4707_4708delCA, and c.9304_9305delG mutations were found in very young patients, while the c.7811_7814delTGTG mutation was present in a patient with bilateral BC diagnosed at the age of 73.

Variants with uncertain significance (VUS)

We detected a total of nine VUS; eight missense (c.4031A>G in *BRCA1* and c.3938A>C, c.6613G>A, c.7081C>A, c.7417T>C, c.7916C>T, c.7975A>G, and c.9863C>T in *BRCA2*) and one in-frame *BRCA2* deletion (c.4410_4412delAAG). The *BRCA2* c.7081C>A and c.4410_4412delAAG variants have not been reported previously. The histopathological findings and family history of the VUS carriers, along with the variant descriptions, are given in Table 3.

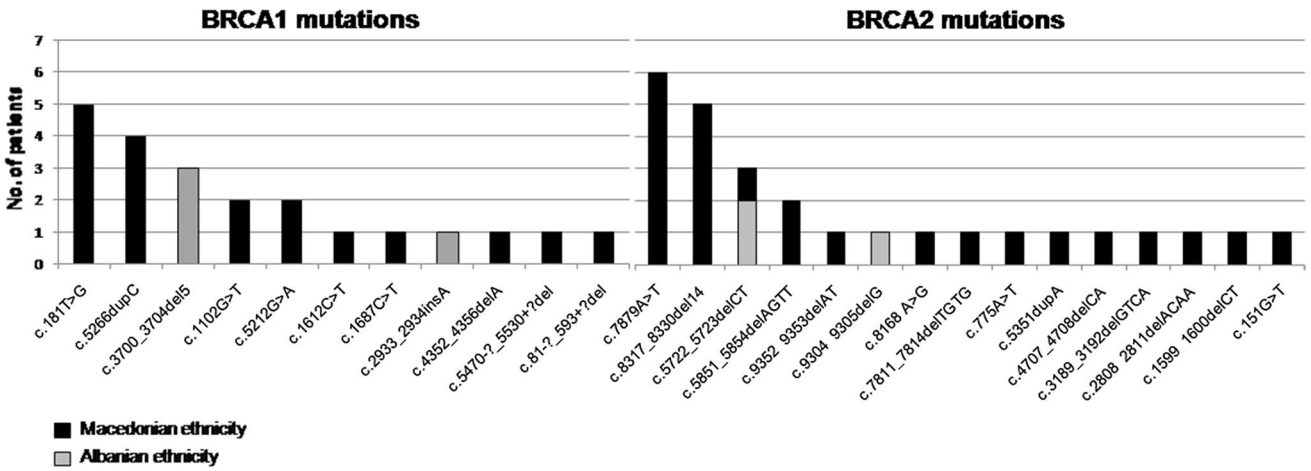

Fig. 1 Distribution of the *BRCA1* and *BRCA2* mutations among patients with Macedonian and Albanian ethnicity

Likely benign and benign variants

Fifty four variants were classified according to ClinVar database as likely benign and benign (22 in *BRCA1* and 32 in *BRCA2*) (Supplementary Table 2). Three were novel variants, while all others have already been reported with minor allele frequencies (MAF) varying from 9.9e−05 to 0.363 in the European population. Comparable MAF was observed in our study (0.002–0.328).

Discussion

Genetic testing of *BRCA1* and *BRCA2* genes in patients with positive family history of Br/Ov cancer has important implications for the mutation carrier and for the family members, since new targeted therapies are constantly arising and well-established management protocols are proven to be life-saving. In most of the studied populations, particular mutations demonstrate a founder effect.

The contribution of *BRCA1* and *BRCA2* mutations to hereditary breast cancer (BC) in women from the Republic of Macedonia has been largely unknown. The only published study included a screening for six *BRCA1* and four *BRCA2* common mutations among 100 BC patients and identified five *BRCA1* and one *BRCA2* mutations [15]. In the current study of 313 BC patients, enriched for family history of cancer, early age of onset and bilateral and/or TN BC, an overall mutation prevalence of 15.7% (49/313) was observed. The *BRCA2* was more common (27/49, 55.1%) than *BRCA1* mutations (22/49, 44.9%) among our BC patients. The tumors of *BRCA1* carriers were more commonly TN (59.1%, $p = 0.001$) in comparison with *BRCA2* carriers (14.8%).

The frequencies of the *BRCA1* and *BRCA2* gene mutations detected in our study in comparison with the neighboring [16–19] and some other European populations [20–28] is given in Table 4. The most prevalent *BRCA1* mutation among our BC patients was the Slavic founder c.181T>G mutation (10.2%). The worldwide most frequent *BRCA1* c.5266dupC mutation was observed with a frequency of 8.16%. Comparable frequencies were reported in studies from Serbia (8.33%) [16], Slovenia (8.93%) [22], and Croatia (10%) [20].

The most common *BRCA2* gene mutation in our study was c.7879A>T; p.Ile2627Phe (12.3%). It was found in six patients of Macedonian ethnic origin, with a mean age of onset of the BC at 41 years. All of these patients had positive family history for breast, prostate, uterus, or gastric cancer, with at least one first or second degree affected family member. The variant has been previously known as 8107A>T and has been reported as a variant of unknown clinical significance in isolated breast and ovarian cancer families in Slovenia [29], Poland [25] and in Germany [27]. This variant has not been reported in ExAC and Ensembl.

The second most common *BRCA2* c.8317_8330del14 mutation was observed in five carriers (10.2%), three of whom had lobular tumor type. Different cancers, including breast, ovarian, pancreatic, lung, colon, and gastric cancers, were present in the family members of the c.8317_8330del14 carriers. To date, this mutation has been reported only once in a family from The Netherlands [30]. Although both *BRCA2* c.7879A>T and c.8317_8330del14 mutations have been described in several isolated cases, our study is the first one, showing that they have a founder effect among Macedonians.

Nine recurrent mutations account for 65.3% of all of the detected mutations in BC patients from the Republic of Macedonia. This observation supports the implementation of a two-step *BRCA1/2* genetic testing strategy in our country. Every woman with BC may undergo the cost-effective

Table 3 Description of the variant with unknown clinical significance and patients' histopathological data and family history

	Exon	HGVS nomenclature	AA change	Variant ID	Functional domain	ExAC European (Non-Finnish)	In silico prediction tools (damaging/total)	Patient ID	Found with BRCA pathogenic mutation	BC type	ER,PR/HER2	Family history data	
												No. of relatives	Cancer type in family
	BRCA 1												
1	10	c.4031A>G	p.Asp1344Gly	rs55639854	–	NA	2/8	BC-283	Yes (c.7879A>T BRCA2)	Ductal	+/−	1	PC
	BRCA 2												
1	11	c.3938A>C	p.Tyr1313Ser	rs80358639	–	NA	1/8	BC-220	No	Ductal	TN	2	BC
2	11	c.4410_4412delAAG	p.Arg1471del	–	–	NA	1/1	BC-516	No	Ductal	TN	No data available	
								BC-789	No	Ductal	+/−	2	BC
3	11	c.6613G>A	p.Val2205Met	rs80358889	–	6,03E − 02	1/8	BC-260	No	Ductal	+/−	No family history	
4	14	c.7081C>A	p.His2361Asn	–	Interaction with FANCD2	NA	0/8	BC-360	No	Ductal	+/−	1	BC
5	15	c.7417T>C	p.Cys2473Arg	rs786202720	Interaction with FANCD2	NA	0/8	BC-540	No	Ductal in situ	n.d.	4	BC, OC, LC
6	17	c.7916C>T	p.Pro2639Leu	rs774723315	OB fold 1, helical domain	NA	5/8	BC-282	No	Ductal	TN	1	BC
7	17	c.7975A>G	p.Arg2659Gly	rs80359026	OB fold 1, helical domain	NA	6/8	BC-557	No	Lobular	+/−	2	BC,LC
8	27	c.9863C>T	p.Thr3288Ile	rs754588394	OB fold 3	NA	4/8	BC-370	No	Ductal	TN	No family history	
								BC-1014	No	Ductal	+/−	3	BC, PC

BC breast cancer, *OC* ovarian cancer, *PC* prostate cancer, *LC* lung cancer, *NA* not available, *TN* triple negative receptor status

Table 4 Frequencies of the BRCA1 and BRCA2 mutations detected in our study among BRCA carriers from the neighbouring and some other European populations

Citations		Cohord type	n-carriers/ n-total (%) in studied cohord	BRCA1										BRCA2														Other mutations
				c.81-?_593+?del	c.181T>G	c.1102G>T	c.1612C>T	c.1687C>T	c.2933_2934insA	c.3700_3704del5	c.4352_4356delA	c.5212G>A	c.5266dupC	c.5470-?_5530+?del	c.151G>T	c.775A>T	c.1599_1600delTG	c.2808_2811delACAA	c.3189_3192delGTCA	c.4707_4708delCA	c.5351dupA	c.5722_5723delCT	c.5851_5854delAGTT	c.7811_7814delTGTG	c.7879A>T	c.8168A>G	c.8317_8330del14	c.9304_9305delG	c.9352_9353delAT	
Serbia, 2013	[16]	High-risk families	12/62 (19,3)	0	0	0	0	0	0	0	0	0	8,33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91,67
Bulgaria, 2015	[17]	NCCN Guidelines	39/200 (19,5)	0	5,13	0	0	0	0	0	0	0	56,41	0	0	0	0	0	0	0	0	2,56	5,13	0	0	0	0	0	0	30,77
Greece,2008	[18]	enriched for a family history for cancer	59/287 (20,5)	0	1,69	0	0	0	0	1,69	0	15,25	30,51	0	0	0	0	1,69	0	0	0	1,69	0	0	0	0	0	0	0	47,46
Greece,2014	[19]	enriched for a family history for cancer	153/473 (32)	0	3,92	0	0	0,65	0	4,58	0	11,11	16,99	0	0	0	0	0,70	0	0	0	0,70	0	0	0	0	0	0	0	61,35
Turkey, 2014	[28]	young onset and high-risk families	16/117 (14)	0	0	0	0	0	0	0	0	0,00	25,00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75,00
Croatia, 2012	[20]	enriched for a family history for cancer	10/145 (6,89)	0	10,00	0	0	10,00	0	0	0	0	10,00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70,00
Slovenia, 2008	[21]	High-risk families	56/145 (38,6)	0	12,50	0	0	17,86	0	0	0	0	8,93	0	0	0	0	0	0	0	1,79	0	0	0	0	0	0	0	0	58,93
*Slovenia, 2011	[22]	enriched for a family history for cancer	97**/322 (29,8)	0	15,46	0	0	13,40	0	0	0	0	8,25	0	0	1,03	0	0	0	0	0	1,03	0	0	0	1,03	0	0	0	0	59,79
Poland, 2000	[23]	High-risk families	35/66 (53)	0	20,00	0	0	0	0	0	0	0	51,00	0	Not analyzed															29,00
Poland, 2004	[24]	High-risk families	129/200 (64,5)	0	24,03	0	0	0	0	2,33	0	0	52,71	0	Not analyzed															20,93
*Poland, 2015	[25]	enriched for a family history for cancer	52/512 (10,1)	0	0	0	1,92	15,38	0	0	0	0	0	0	0	0	0	1,92	0	0	0	0	0	0	1,92	0	0	0	0	78,85
Czech Repub., 2008	[26]	enriched for a family history for cancer	294/1010 (29,1)	0	6,80	0	0	2,04	0	9,86	0	0	25,51	0	0	0	0	1,70	0	0	0	0,34	0	0	0	0	0	0	0	53,74
*Germany, 2000	[27]	enriched for a family history for cancer	10**/120 (8,3)	0	20	0	0	0	0	0	0	0,00	10,00	0	0	0	0	10	0	0	0	0	0	0	10	0	0	0	0	50,00
Our study, 2017		enriched for a family history for cancer	49/313 (15,6)	2,04	10,20	4,08	2,04	2,04	2,04	6,12	2,04	4,08	8,16	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	6,12	4,08	2,04	12,24	2,04	10,20	2,04	2,04	0,00

* Article describing c.7879A>T mutation

** Number includes BRCA2 c.7879A>T which was reported as VUS

first-step screening for the most common nine mutations, while the comprehensive NGS analysis using panel of cancer genes might be reserved for those with negative first-step genetic *BRCA1/2* result and a high risk of having a mutation in the BC susceptibility genes.

Different *BRCA1/2* mutations were present among BC patients of Macedonian and Albanian ethnic origin (Fig. 1). Only one mutation (*BRCA2* c. 5722_5723decCT) was present in both ethnic groups. The mutations c.3700_3704del5 and c.2933_2934insA in *BRCA1* and c.9304_9305delG in *BRCA2* gene were found only in Albanian patients. The *BRCA1* c.3700_3704del5 variant was detected in three patients of the 55 screened Albanian BC patients (5.45%) and it represents 42.9% (3/7) of the *BRCA1/2* carriers of Albanian ancestry, suggesting it as a founder mutation in the Albanian population. There are no other data available in the literature for the contribution of the *BRCA1/2* mutations among Albanian BC patients.

We report nine VUS (one in *BRCA1* and eight in *BRCA2*). The two missense *BRCA2* VUS c.7916C>T and c.7975A>G were predicted to be damaging with 5 and 6 in silico predictor tools, respectively. The c.7916C>T variant is located in the first oligonucleotide binding fold in the DNA-binding domain at the C-terminal region. This region is considered as the most conserved region in *BRCA2* gene [31]. The *BRCA2* c.7975A>G, p.Arg2659Gly variant, is a missense variant located in the region that has a role in the interaction with the *SEM1* gene. It was suggested that the AGA-to-GGA codon change alters the splice donor site, thus creating a low amount of an alternative transcript with an in-frame deletion of exon 17 [32], [33]. It was also shown that it affects the homologous repair activity [34]. Only one VUS was in-frame variant (c.4410_4412delAAG), not reported in the literature and the databases thus far. It causes a removal of arginine at position 1471; however, this amino acid position is not conserved. The detection of this mutation in two of our BC patients may suggest that it may have a pathogenic effect or it represents a common variant in our population. Further analyses are warranted to clarify the role of *BRCA2* c.4410_4412delAAG mutation.

The nonsense *BRCA2* c.9976A>T (p.Lys3326Ter, rs11571833) variant, which leads to the loss of the final 93 amino acids of BRCA2 protein is a common variant (0.9% among European population in ExAC) and is classified as a benign variant in ClinVar. Large genome-wide association studies identified it as a low-risk variant for breast cancer (OR 1.26, 95% CI 1.14–1.39) [35]. In our study, p.Lys3326Ter was found in two patients diagnosed with ductal cancer with estrogen positive receptor status without co-occurrence of other pathogenic mutation.

In conclusion, this study provides a comprehensive view of known and novel *BRCA1/2* gene mutations in BC patients from the Republic of Macedonia and contributes to the global spectrum of BRCA1/2 mutations in breast cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the insti-

tutional research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266(5182):66–71
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378(6559):789–792. https://doi.org/10.1038/378789a0
3. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tulinius H, Thorlacius S, Eerola H, Nevanlinna H, Syrjakoski K, Kallioniemi OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 72(5):1117–1130. https://doi.org/10.1086/375033
4. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE (1994) Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 343(8899):692–695
5. Easton DF, Ford D, Bishop DT (1995) Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet 56(1):265–271
6. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010) A method and server for predicting damaging missense mutations. Nat Methods 7(4):248–249. https://doi.org/10.1038/nmeth0410-248
7. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4(7):1073–1081. https://doi.org/10.1038/nprot.2009.86
8. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP (2012) Predicting the functional effect of amino acid substitutions and indels. PLoS ONE 7(10):e46688. https://doi.org/10.1371/journal.pone.0046688
9. Reva B, Antipin Y, Sander C (2011) Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res 39(17):e118. https://doi.org/10.1093/nar/gkr407
10. Schwarz JM, Cooper DN, Schuelke M, Seelow D (2014) MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 11(4):361–362
11. Yates CM, Filippis I, Kelley LA, Sternberg MJ (2014) SuSPect: enhanced prediction of single amino acid variant (SAV) phenotype using network features. J Mol Biol 426(14):2692–2701
12. Mi H, Poudel S, Muruganujan A, Casagrande JT, Thomas PD (2016) PANTHER version 10: expanded protein families and functions, and analysis tools. Nucleic Acids Res 44(D1):17
13. Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C (2009) Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 37(9):e67. https://doi.org/10.1093/nar/gkp215
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424. https://doi.org/10.1038/gim.2015.30
15. Maleva I, Madjunkova S, Bozhinovski G, Smickova E, Kondov G, Spiroski Z, Arsovski A, Plaseska-Karanfilska D (2012) Genetic variation of the brca1 and brca2 genes in macedonian patients. Balkan J Med Genet 15(Suppl):81–85. https://doi.org/10.2478/v10034-012-0025-8
16. Dobricic J, Krivokuca A, Brotto K, Malisic E, Radulovic S, Brankovic-Magic M (2013) Serbian high-risk families: extensive results on BRCA mutation spectra and frequency. J Hum Genet 58(8):501–507
17. Dodova RI, Mitkova AV, Dacheva DR, Hadjo LB, Vlahova AI, Hadjieva MST, Valev SS, Caulevska MM, Popova SD, Popov IE, Dikov TI, Sedloev TA, Ionkov AS, Timcheva KV, Christova SL, Kremensky IM, Mitev VI, Kaneva RP (2015) Spectrum and frequencies of BRCA1/2 mutations in Bulgarian high risk breast cancer patients. BMC Cancer 15(523):015–1516
18. Konstantopoulou I, Rampias T, Ladopoulou A, Koutsodontis G, Armaou S, Anagnostopoulos T, Nikolopoulos G, Kamakari S, Nounesis G, Stylianakis A, Karanikiotis C, Razis E, Gogas H, Keramopoulos A, Gaki V, Markopoulos C, Skarlos D, Pandis N, Bei T, Arzimanoglou I, Fountzilas G, Yannoukakos D (2008) Greek BRCA1 and BRCA2 mutation spectrum: two BRCA1 mutations account for half the carriers found among high-risk breast/ovarian cancer patients. Breast Cancer Res Treat 107(3):431–441
19. Konstantopoulou I, Tsitlaidou M, Fostira F, Pertesi M, Stavropoulou AV, Triantafyllidou O, Tsotra E, Tsiftsoglou AP, Tsionou C, Droufakou S, Dimitrakakis C, Fountzilas G, Yannoukakos D (2014) High prevalence of BRCA1 founder mutations in Greek breast/ovarian families. Clin Genet 85(1):36–42
20. Levanat S, Musani V, Cvok ML, Susac I, Sabol M, Ozretic P, Car D, Eljuga D, Eljuga L (2012) Three novel BRCA1/BRCA2 mutations in breast/ovarian cancer families in Croatia. Gene 498(2):169–176
21. Krajc M, Teugels E, Zgajnar J, Goelen G, Besic N, Novakovic S, Hocevar M, De Greve J (2008) Five recurrent BRCA1/2 mutations are responsible for cancer predisposition in the majority of Slovenian breast cancer families. BMC Med Genet 9(83):1471–2350
22. Stegel V, Krajc M, Zgajnar J, Teugels E, De Greve J, Hocevar M, Novakovic S (2011) The occurrence of germline BRCA1 and BRCA2 sequence alterations in Slovenian population. BMC Med Genet 12:9. https://doi.org/10.1186/1471-2350-12-9
23. Gorski B, Byrski T, Huzarski T, Jakubowska A, Menkiszak J, Gronwald J, Pluzanska A, Bebenek M, Fischer-Maliszewska L, Grzybowska E, Narod SA, Lubinski J (2000) Founder mutations in the BRCA1 gene in polish families with breast-ovarian cancer. Am J Hum Genet 66(6):1963–1968
24. Gorski B, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Grzybowska E, Mackiewicz A, Stawicka M, Bebenek M, Sorokin D, Fiszer-Maliszewska L, Haus O, Janiszewska H, Niepsuj S, Gozdz S, Zaremba L, Posmyk M, Pluzanska M, Kilar E, Czudowska D, Wasko B, Miturski R, Kowalczyk JR, Urbanski K, Szwiec M, Koc J, Debniak B, Rozmiarek A, Debniak T, Cybulski C, Kowalska E, Toloczko-Grabarek A, Zajaczek S, Menkiszak J, Medrek K, Masojc B, Mierzejewski M, Narod SA, Lubinski J (2004) A high proportion of founder BRCA1 mutations in Polish breast cancer families. Int J Cancer 110(5):683–686
25. Kluska A, Balabas A, Paziewska A, Kulecka M, Nowakowska D, Mikula M, Ostrowski J (2015) New recurrent BRCA1/2 mutations in Polish patients with familial breast/ovarian cancer detected by next generation sequencing. BMC Med Genom 8(19):015–0092
26. Machackova E, Foretova L, Lukesova M, Vasickova P, Navratilova M, Coene I, Pavlu H, Kosinova V, Kuklova J, Claes K (2008)

Spectrum and characterisation of BRCA1 and BRCA2 deleterious mutations in high-risk Czech patients with breast and/or ovarian cancer. BMC Cancer 8(140):1471–2407
27. Spitzer E, Abbaszadegan MR, Schmidt F, Hauser A, Buwitt U, Lauter FR, Potschick K, Krocker J, Elling D, Grosse R (2000) Detection of BRCA1 and BRCA2 mutations in breast cancer families by a comprehensive two-stage screening procedure. Int J Cancer 85(4):474–481
28. Cecener G, Egeli U, Tunca B, Erturk E, Ak S, Gokgoz S, Tasdelen I, Tezcan G, Demirdogen E, Bayram N, Avci N, Evrensel T (2014) BRCA1/2 germline mutations and their clinical importance in Turkish breast cancer patients. Cancer Investig 32(8):375–387
29. Stegel V, Krajc M, Zgajnar J, Teugels E, De Greve J, Hocevar M, Novakovic S (2011) The occurrence of germline BRCA1 and BRCA2 sequence alterations in Slovenian population. BMC Med Genet 12(9):1471–2350
30. van der Hout AH, van den Ouweland AM, van der Luijt RB, Gille HJ, Bodmer D, Bruggenwirth H, Mulder IM, van der Vlies P, Elfferich P, Huisman MT, ten Berge AM, Kromosoeto J, Jansen RP, van Zon PH, Vriesman T, Arts N, Lange MB, Oosterwijk JC, Meijers-Heijboer H, Ausems MG, Hoogerbrugge N, Verhoef S, Halley DJ, Vos YJ, Hogervorst F, Ligtenberg M, Hofstra RM (2006) A DGGE system for comprehensive mutation screening of BRCA1 and BRCA2: application in a Dutch cancer clinic setting. Hum Mutat 27(7):654–666
31. Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP (2002) BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science 297(5588):1837–1848
32. Caux-Moncoutier V, Castera L, Tirapo C, Michaux D, Remon MA, Lauge A, Rouleau E, De Pauw A, Buecher B, Gauthier-Villars M, Viovy JL, Stoppa-Lyonnet D, Houdayer C (2011) EMMA, a cost- and time-effective diagnostic method for simultaneous detection of point mutations and large-scale genomic rearrangements: application to BRCA1 and BRCA2 in 1,525 patients. Hum Mutat 32(3):325–334
33. Houdayer C, Caux-Moncoutier V, Krieger S, Barrois M, Bonnet F, Bourdon V, Bronner M, Buisson M, Coulet F, Gaildrat P, Lefol C, Leone M, Mazoyer S, Muller D, Remenieras A, Revillion F, Rouleau E, Sokolowska J, Vert JP, Lidereau R, Soubrier F, Sobol H, Sevenet N, Bressac-de Paillerets B, Hardouin A, Tosi M, Sinilnikova OM, Stoppa-Lyonnet D (2012) Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants. Hum Mutat 33(8):1228–1238
34. Guidugli L, Pankratz VS, Singh N, Thompson J, Erding CA, Engel C, Schmutzler R, Domchek S, Nathanson K, Radice P, Singer C, Tonin PN, Lindor NM, Goldgar DE, Couch FJ (2013) A classification model for BRCA2 DNA binding domain missense variants based on homology-directed repair activity. Cancer Res 73(1):265–275
35. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, Schmidt MK, Chang-Claude J, Bojesen SE, Bolla MK, Wang Q, Dicks E, Lee A, Turnbull C, Rahman N, Fletcher O, Peto J, Gibson L, Dos Santos Silva I, Nevanlinna H, Muranen TA, Aittomaki K, Blomqvist C, Czene K, Irwanto A, Liu J, Waisfisz Q, Meijers-Heijboer H, Adank M, van der Luijt RB, Hein R, Dahmen N, Beckman L, Meindl A, Schmutzler RK, Muller-Myhsok B, Lichtner P, Hopper JL, Southey MC, Makalic E, Schmidt DF, Uitterlinden AG, Hofman A, Hunter DJ, Chanock SJ, Vincent D, Bacot F, Tessier DC, Canisius S, Wessels LF, Haiman CA, Shah M, Luben R, Brown J, Luccarini C, Schoof N, Humphreys K, Li J, Nordestgaard BG, Nielsen SF, Flyger H, Couch FJ, Wang X, Vachon C, Stevens KN, Lambrechts D, Moisse M, Paridaens R, Christiaens MR, Rudolph A, Nickels S, Flesch-Janys D, Johnson N, Aitken Z, Aaltonen K, Heikkinen T, Broeks A, Veer LJ, van der Schoot CE, Guenel P, Truong T, Laurent-Puig P, Menegaux F, Marme F, Schneeweiss A, Sohn C, Burwinkel B, Zamora MP, Perez JI, Pita G, Alonso MR, Cox A, Brock IW, Cross SS, Reed MW, Sawyer EJ, Tomlinson I, Kerin MJ, Miller N, Henderson BE, Schumacher F, Le Marchand L, Andrulis IL, Knight JA, Glendon G, Mulligan AM, Lindblom A, Margolin S, Hooning MJ, Hollestelle A, van den Ouweland AM, Jager A, Bui QM, Stone J, Dite GS, Apicella C, Tsimiklis H, Giles GG, Severi G, Baglietto L, Fasching PA, Haeberle L, Ekici AB, Beckmann MW, Brenner H, Muller H, Arndt V, Stegmaier C, Swerdlow A, Ashworth A, Orr N, Jones M, Figueroa J, Lissowska J, Brinton L, Goldberg MS, Labreche F, Dumont M, Winqvist R, Pylkas K, Jukkola-Vuorinen A, Grip M, Brauch H, Hamann U, Bruning T, Radice P, Peterlongo P, Manoukian S, Bonanni B, Devilee P, Tollenaar RA, Seynaeve C, van Asperen CJ, Jakubowska A, Lubinski J, Jaworska K, Durda K, Mannermaa A, Kataja V, Kosma VM, Hartikainen JM, Bogdanova NV, Antonenkova NN, Dork T, Kristensen VN, Anton-Culver H, Slager S, Toland AE, Edge S, Fostira F, Kang D, Yoo KY, Noh DY, Matsuo K, Ito H, Iwata H, Sueta A, Wu AH, Tseng CC, Van Den Berg D, Stram DO, Shu XO, Lu W, Gao YT, Cai H, Teo SH, Yip CH, Phuah SY, Cornes BK, Hartman M, Miao H, Lim WY, Sng JH, Muir K, Lophatananon A, Stewart-Brown S, Siriwanarangsan P, Shen CY, Hsiung CN, Wu PE, Ding SL, Sangrajrang S, Gaborieau V, Brennan P, McKay J, Blot WJ, Signorello LB, Cai Q, Zheng W, Deming-Halverson S, Shrubsole M, Long J, Simard J, Garcia-Closas M, Pharoah PD, Chenevix-Trench G, Dunning AM, Benitez J, Easton DF (2013) Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 45(4):353–361