Journal of Human Genetics (2013) 58, 501–507

www.nature.com/jhg

ORIGINAL ARTICLE

Serbian high-risk families: extensive results on *BRCA* mutation spectra and frequency

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Mutations in *BRCA* genes elevate risk for breast and ovarian cancer. These mutations are population specific. As there are no data on *BRCA* mutation screening on larger number of probands in Serbia to date, aim of this study was to determine types and frequencies of *BRCA* mutations in individuals from high-risk families from Serbia, as well as to determine which *BRCA* mutations may be considered as founder for Serbian population. We analyzed 94 probands and detected 9 frameshift mutations in 12 individuals, 1 benign *BRCA2* nonsense mutation and numerous missense and synonymous mutations in both genes. Frequency of frameshift mutations is 12.77%. In addition to two novel mutations detected in our population we reported previously, we detected another novel mutation—c.7283delT in *BRCA2* exon 14. None of the detected deleterious mutations may be considered as founder mutations for Serbian population, as each of them was found in no more than two high-risk families. This mutation diversity is most probably due to high migration rate in history of this part of Europe. Interpretation of genetic testing results with missense mutations of unknown clinical importance is very challenging and should be approached with caution, using all available data sources for results' interpretation.
Journal of Human Genetics (2013) **58,** 501–507; doi:10.1038/jhg.2013.30; published online 2 May 2013

Keywords: *BRCA* genes; genetic testing; hereditary breast and ovarian cancer; mutation

INTRODUCTION

BRCA1 (breast cancer susceptibility gene 1) gene was the first gene associated with elevated breast/ovarian cancer risk.[1] Its sequence is divided into 24 exons, 22 of which are coding exons for 1863 amino-acid (aa) protein product.[2,3] BRCA1 protein has four main structural domains: RING domain at N-terminus (aa 24–64),[4] two nuclear localization signals (NLS; aa 503–508 and 606–615),[5] DNA-binding domain (aa 452–1079)[6] and two BRCA1 C-terminal domains[7] (aa 1642–1736 and 1756–1855, http://www.uniprot.org/uniprot/P38398).

The second gene associated with hereditary breast cancer, *BRCA2* (breast cancer susceptibility gene 2, also known as *FANCD1*), is divided into 27 exons, 26 of which are coding exons for 3418 aa protein product.[8,9] BRCA2 protein contains some distinctive domains: eight BRC motifs (aa 1009 and 2083),[10] single strand DNA-binding domain[11] and two NLSs in last 156 aa.[12]

After DNA damage occurs, the information about it needs to be transmitted via various molecules (signaling) in order to stop cell cycle, choose appropriate DNA repair mechanism depending on the type of DNA damage, to remodel chromatin so that proteins of repair machinery may approach DNA damage site and correct the mutation. If the damage cannot be repaired, cell activates pathways that lead to apoptosis.

BRCA1 and BRCA2 proteins are involved in almost all mentioned functions. BRCA1 has a role in signaling, DNA repair, chromatin remodeling, ubiquitination and regulation of cell cycle, apoptosis and transcription.[13] BRCA2 is involved in cell cycle M-phase checkpoint regulation, homologous recombination and DNA repair.[14] Mutations that compromise BRCA protein functions lead to decreased ability of DNA repair and regulation of cell cycle and apoptosis. This leads to accumulation of mutations (genomic instability) and uncontrolled proliferation, hallmarks of cancerous cells. That is why individuals who inherit mutation in one of *BRCA* genes are more prone to developing cancer.

Mutations in *BRCA* genes are scattered throughout numerous coding regions without clustering, complicating mutation detection. More than 1600 and 1900 mutations in *BRCA1* and *BRCA2*, respectively have been identified.[15] The majority of them are frameshift mutations (around 70%), while nonsense and missense mutations contribute with around 10% each. In addition to clinically significant mutations that impair protein function, numerous variants, especially missense ones, have been identified. These variants may have unknown impact on protein function and may low or moderately elevate cancer risk.

BRCA mutation spectra are population specific. Some mutation(s) may be frequent in one population and rare in the other. This is usually due to so-called founder effect. In populations that are geographically and/or reproductively isolated, or derived from limited number of individuals (founders), whole gene pool of that population

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Received 21 December 2012; revised 18 March 2013; accepted 3 April 2013; published online 2 May 2013

is based on the founders' gene pool. The consequence is high frequency of mutations that may be rather rare in other populations.[16] Such are Ashkenazi Jew and Polish populations, which are characterized by three founder *BRCA* mutations each.[17,18] Testing for founder mutations covers majority of *BRCA* mutation carriers in these populations, making genetic testing faster, cheaper and easier. At the same time, large portion of *BRCA* mutations have been detected only once, and they are considered as family-specific mutations.

Apart from our previous results on new *BRCA* mutations detected in two families in Serbia[19] and some earlier reports on mutation testing of small number of probands from Serbia,[20,21] to date there are no data on more extensive *BRCA* mutation screening in Serbia.

Aim of this study was to determine types and frequencies of *BRCA* mutations in larger number of probands from Serbia that meet criteria for *BRCA* testing, as well as to determine which *BRCA* mutations are the most frequent in Serbian high-risk families and may be considered as founder for Serbian population.

MATERIALS AND METHODS

We analyzed 94 (87 female and 7 male) probands from 71 families. The criteria for proband selection were:

- More than one breast cancer case on the same side of pedigree,
- Ovarian cancer with positive family history for breast or ovarian cancer,
- Breast and ovarian cancer in same individual,
- Bilateral breast cancer,
- Male breast cancer, with or without positive family history,
- Early breast cancer (before age 35), with or without family history,
- Relative who is *BRCA* mutation carrier.

Of 94 probands, 52 had breast cancer (including early, bilateral and male breast cancer cases), 4 had ovarian cancer and 6 had both breast and ovarian cancer. Details on probands' personal history are listed in Table 1.

Of 94 probands tested, 54 had family history of breast cancer (including early, bilateral and male breast cancer cases), 20 had family history of ovarian cancer or both breast and ovarian cancer, while 18 did not have positive family history for breast or ovarian cancer. Details on family histories of tested individuals are listed in Table 2.

Blood samples were collected from various parts of Serbia. All probands signed informed consent form approved by the Ethics Committee of Institute for Oncology and Radiology of Serbia.

DNA was isolated from blood samples on ABI Prism 6100 Nucleic Acid PrepStation using BloodPrep Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. *BRCA1* and *BRCA2* coding regions were amplified and dye-labeled using PCR and cycle sequencing conditions described previously.[19] Samples were sequenced on ABI PRISM 310 or 3130 Genetic Analyzer. Obtained sequences were examined for the presence of mutations by alignment with wild-type complementary DNA sequence (GenBank U14680 for *BRCA1*; GenBank U43746 for *BRCA2*) using BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/). Observed mutations were then analyzed for their clinical significance and effect on protein product in Breast Cancer Information Core database (http://www.research.nhgri.nih.gov/bic/) and Leiden Open Variation Database (http://www.lovd.nl/2.0/), as well as using PolyPhen software (http://genetics.bwh.harvard.edu/pph/).

RESULTS

We tested 94 individuals. Genes to be tested were determined according to the mutation carrier probability using BRCAPRO software for each individual. Twenty-four individuals were tested for the presence of family mutations only.

We detected 9 frameshift mutations (5 in *BRCA1* and 4 in *BRCA2* gene) in 12 individuals (Table 3). None of the detected frameshift mutations may be considered as founder mutations for Serbian population, as each of them was found in no more than two high-risk families.

Frequency of frameshift mutations is 12.77% (12/94) (Figure 1).

We detected one nonsense mutation in *BRCA2* gene (10204A>T, K3326X, c.9976A>T, p.Lys3326*) in one individual.

We also detected numerous missense mutations, classified by PolyPhen software as 'benign', 'possibly' or 'probably damaging' (Table 4), as well as numerous synonymous mutations in *BRCA1/2* genes (data not shown).

Table 1 Personal history of individuals in our test group

Personal history	*Number of individuals*
Breast cancer[a]	23
Early breast cancer (before age 35)	17
Multiple cancers (including bilateral breast cancer)[b]	9
Male breast cancer	4
Ovarian cancer and fallopian tube cancer[c]	5
Breast cancer and ovarian cancer	5
Renal cancer (with relative who is *BRCA* mutation carrier)	1
Healthy	30

[a]Including two *in situ* breast cancer cases.
[b]Including bilateral breast cancer, first of which was early breast cancer (one individual), bilateral breast cancer and colon cancer (two individuals), bilateral breast cancer and lung cancer (one individual), early breast cancer, ovarian cancer, colon cancer and lung cancer (one individual).
[c]Four individuals with ovarian cancer and one individual with fallopian tube cancer.

Table 2 Number of individuals with positive family history for various cancers

Family history	*Number of individuals*
Breast cancer (including early and bilateral breast cancers)	48
Various cancers[a]	16
Male breast cancer[b]	4
Ovarian cancer	3
Breast cancer and ovarian cancer[c]	3
Colon cancer	2
No family history	18

[a]Including family history of breast cancer, early breast cancer and colon cancer (one individual), early breast cancer and renal cancer (one individual), ovarian cancer and prostate cancer (one individual), ovarian cancer, prostate cancer and fallopian tube cancer (six individuals), breast cancer, ovarian cancer and renal cancer (seven individuals).
[b]Including three individuals with family history of breast cancer and male breast cancer.
[c]Including one individual with family history of early breast cancer and ovarian cancer.

DISCUSSION

Key event in tumorigenesis is losing control over genome stability and proliferation. To ensure that they do not proliferate after DNA damage, cells have developed crosslinked mechanisms of cell cycle checkpoints and DNA repair.[22] If cell acquires damage in one of the genes involved in DNA repair and cell cycle control (such as *BRCA* genes), mutations that occur afterward remain unrepaired, leading to mutation accumulation and genome instability, uncontrolled proliferation and tumorigenesis. Therefore, individuals who inherit mutations in one of *BRCA* genes are more prone to develop cancers. Depending on position and type of mutation, protein function may be more or less impaired, more or less elevating cancer risk. Frameshift mutations impair protein function severely, it loses some of domains essential for its function, significantly elevating

Table 3 Frequency of *BRCA* genes

		Mutation		*Protein effect*		
Gene	*Exon*	*BIC classification*	*HGVS classification*	*BIC classification*	*HGVS classification*	*Number of individuals*
BRCA1	11	962delCTCA	c.843_846delCTCA	STOP 297	p.Ser282Tyrfs*15	1
	11	969ins7	c.844_850dupTCATTAC	STOP 288	p.Gln284Leufs*5	2
	11	2138delA	c.2019delA	STOP 700	p.Glu673Aspfs*28	2
	15	4765del20	c.4646_4665del	STOP 1566	p.Val1549Alafs*18	1
	20	5382insC	c.5266dupC	STOP 1829	p.Gln1756Profs*74	1
BRCA2	10	1991del4	c.1763_1766delATAA	STOP 612	p.Asn588Serfs*25	1
	11	4366insTT	c.4139_4140dupTT	STOP 1388	p.Lys1381Leufs*8	2
	11	5215delGTCA	c.4987_4990delGTCA	STOP 1668	p.Val1163Leufs*6	1
	14	7511delT	c.7283delT	STOP 2468	p.Leu2428Trpfs*41	1

Abbreviation: BIC, Breast Cancer Information Core database.

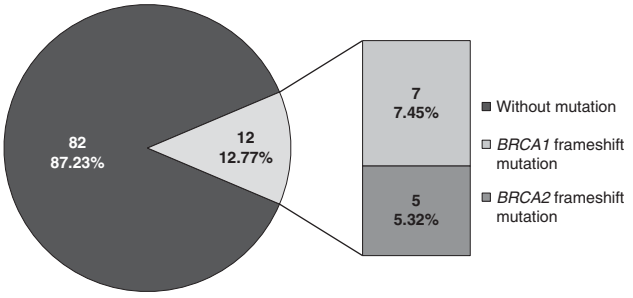

Figure 1 Frequency of *BRCA* frameshift mutations. *BRCA1* mutations were detected in seven individuals, while *BRCA2* mutations were detected in five individuals. Frequency of frameshift mutations is 12.77% (12/94).

cancer risk. Missense mutations may have different impact on protein function depending on the position of altered aa: the more important role of altered amino acid in protein function, the more severe impact of the amino-acid alteration is.

In addition to *BRCA1* mutation c.4646_4665del we previously reported and whose possible consequences to protein functions we described,[19] we detected another four *BRCA1* frameshift mutations (Table 3). All these mutations lead to synthesis of truncated protein products that lack BRCA1 C-terminal domains, disrupting interaction with other proteins, disturbing essential BRCA1 functions. In the presence of c.843_846delCTCA, c.844_850dupTCATTAC and c.2019delA mutations, BRCA1 protein is severely truncated and has only 296, 287 and 699 aa, respectively. These proteins lack numerous phosphorilation sites that activate *BRCA1* to stop cell cycle in various cell cycle phases after DNA damage. These truncated proteins have only RING domain and one of the NLS signals, and it is highly unlikely that such short proteins have any function preserved. Cell with such truncated BRCA1 protein continues with cell cycle even after DNA damage, accumulating mutations, leading to genome instability and tumorigenesis.

BRCA2 mutation c.4139_4140dupTT was first reported by us[19] and later by a group from Croatia.[23] In addition, we detected another three frameshift *BRCA2* mutations (Table 3). One of them is novel mutation that has not been reported previously in Breast Cancer Information Core or Leiden Open Variation databases: c.7283delT *BRCA2* exon 14. Protein truncated by this mutation has intact BRC domains and is able to bind RAD51. However, each of frameshift mutations found in *BRCA2* gene lead to syntheses of truncated proteins that lack NLSs at BRCA2 C-terminus. This leaves both BRCA2 and RAD51 proteins in cytoplasm, unable to have their roles in DNA repair in nucleus, leading to genomic instability and tumorigenesis. Findings that truncated BRCA2 proteins that lack NLSs are located in cytoplasm with majority of RAD51 protein support this conclusion.[12,24]

BRCA2 nonsense mutation p.Lys3326* leads to truncated BRCA2 protein that lacks last 93 amino acids on C-terminus. In some studies this mutation has been found together with deleterious mutations,[25,26] whereas other studies showed no difference in frequency of this mutation in patients and in control group,[27] so its neutral effect is assumed, as this mutation is classified in Breast Cancer Information Core and Leiden Open Variation databases. Functional analysis gave results similar to those for wild-type BRCA2.[28] However, it was found that this nonsense mutation is five times more frequent in families with hereditary pancreatic cancer comparing with control group.[29] In our study, *BRCA2* mutation p.Lys3326* has been found in one healthy individual with positive family history for breast cancer (her sister, mother, maternal aunt and maternal grandmother developed breast cancers). It is possible that healthy proband did not inherit family deleterious mutation. As samples from cancer patients from this family were not available for testing, it was not possible to determine potential presence of family deleterious *BRCA* mutation that would eliminate suspicions about possible effect of detected nonsense mutation on cancer risk in this family.

Missense mutations that do not lead to complete disruption of protein function may slightly change structure of domains important for protein function. These mutations are marked as unclassified variants owing to their unknown clinical impact. The effect of these mutations is estimated according to their position and type of altered amino acid using computer softwares, and is described by probability of disrupting protein function and therefore elevating disease risk. PolyPhen software terms missense mutations as 'benign', 'probably' or 'possibly damaging'. Missense mutation may increase cancer risk by altering gene penetrability in deleterious mutation carriers. In noncarriers, presence of more than one unclassified variants may have cumulative effect and increase cancer risk. Classification of missense mutations may change in time with acquiring more information on their impact, and some mutation first classified as possibly damaging may turn out to be benign, and *vice versa*.

On the basis of comparative analyses of BRCA1 protein sequences in different species, it was shown that majority of *BRCA1* missense mutations found in our group are neutral or with small clinical significance.[30] By combining several methods it was confirmed that p.Ser1512Ile, p.Met1652Ile and p.Ser1040Asn are neutral.[31,32,33] Earlier studies showed that mutations p.Gln356Arg and p.Pro871Leu

Table 4 Missense mutation detected in *BRCA* genes

Gene	*Exon*	*BIC classification*	*HGVS classification*	*Mutation effect BIC database*	*Mutation effect Polyphen*	*Number of individuals*
BRCA1	11	1186A>G Q356R	c.1067A>G p.Gln356Arg	Polymorphism	Possibly damaging	14
	11	1563A>T I482F	c.1444A>T p.Ile482Phe	Not reported	Benign	1
	11	2196G>A D693N	c.2077G>A p.Asp693Asn	Polymorphism	Benign	7
	11	2640C>T R841W	c.2521C>T p.Arg841Trp	Unclassified variant	Possibly damaging	1
	11	2731C>T P871L	c.2612C>T p.Pro871Leu	Polymorphism	Benign	31
	11	3232A>G E1038G	c.3113A>G p.Glu1038Gly	Polymorphism	Benign	36
	11	3238G>A S1040N	c.3119G>A p.Ser1040Asn	Unclassified variant	Benign	5
	11	3667A>G K1183R	c.3548A>G p.Lys1183Arg	Polymorphism	Benign	35
	15	4654G>T S1512I	c.4535G>T p.Ser1512Ile	Polymorphism	Probably damaging	1
	16	4956A>G S1613G	c.4837A>G p.Ser1613Gly	Polymorphism	Benign	37
	16	5075G>A M1652I	c.4956G>A p.Met1652Ile	Unclassified variant	Probably damaging	6
BRCA2	9	962G>A R245K	c.734G>A p.Arg245Lys	Not reported	Benign	1
	10	1075A>G I283V	c.847A>G p.Ile283Val	Unclassified variant	Benign	1
	10	1093A>C N289H	c.865A>C p.Asn289His	Polymorphism	Possibly damaging	1
	10	1342C>A H372N	c.1114C>A p.His372Asn	Polymorphism	Benign	5
	10	2024T>C S599F	c.1796T>C p.Ser599Phe	Not reported	Possibly damaging	22
	11	3007A>G M927V	c.2779A>G p.Met927Val	Not reported	Probably damaging	2
	11	3199A>G N991D	c.2971A>G p.Asn991Asp	Polymorphism	Benign	1
	11	3941T>C V1238A	c.3713T>C p.Val1238Ala	Not reported	Benign	2
	11	5972C>T T1915M	c.5744C>T p.Thr1915Met	Unclassified variant	Possibly damaging	1
	11	6328C>T R2034C	c.6100C>T p.Arg2034Cys	Unclassified variant	Benign	1
	15	7834T>C 7836T>C S2536P	c.7606T>C c.7608T>C p.Ser2536Pro	Not reported	Possibly damaging	12

Abbreviation: BIC, Breast Cancer Information Core database.

are associated with elevated ovarian cancer risk.[34] However, analyzing larger number of probands it was shown that these mutations are not associated with elevated breast[35,36] or ovarian cancer risk.[37] Risk contribution of *BRCA1* missense mutations was estimated based on segregation with disease, absence in ethnically matched controls, amino-acid conservancies, and it was shown that majority of mutations we found are probably neutral.[38,39] *BRCA1* mutation p.Arg841Trp, which was found together with known deleterious mutation, is considered to be neutral.[40] However, possibility that this mutation moderately elevates cancer risk cannot be excluded,[40] which is supported by PolyPhen analysis that classifies this mutation as 'possibly damaging'. By combining various methods, several studies associated this mutation with elevated cancer risk.[34,41,42,43] Some data suggest that mutations p.Gln356Arg and p.Ser1512Ile found together elevate cancer risk.[44] Among our probands, these two *BRCA1* mutations were found together in one female with *in situ* breast cancer, which may suggest the confirmation of mentioned literature data.

Functional analysis of p.His372Asn *BRCA2* mutation gave results similar to those for wild-type *BRCA2* gene, so it is possible that this mutation is benign.[28] However, it has been suggested that this mutation in homozygous His/His form elevates breast and ovarian cancer risk (relative risk = 1.3–1.5).[45,46] Elevated breast cancer risk associated with this mutation was found only in a group of *BRCA1/2*-negative high-risk women.[36] However, in large study that included 15 000 patients and 15 000 controls, this association has not been confirmed: for His/His homozygotes relative risk = 1.12, whereas for heterozygotes relative risk = 1.05.[47] This mutation changes amino acid in region of BRCA2 protein (aa 290–453) that interacts with histone acetiltransferase P/CAF, modulating transcription by modifying chromatin.[48] It is possible that this amino-acid change at position 372 has impact on this interaction, affecting chromatin remodeling. In our tested group, all three individuals with His/His genotype developed disease: first one developed early female breast cancer, second one ovarian cancer and third male breast cancer. These results may confirm that this mutation in His/His homozygous form contributes to cancer risk.

BRCA2 missense mutations p.Ser599Phe and p.Ser2536Pro have not been described previously. PolyPhen software classifies these mutations as 'possibly damaging', so their potential impact on BRCA2 protein function is suggested. However, unclassified variant p.Ser599Phe has been detected in 22 probands in our laboratory, almost all (20/22) as homozygous. Mutation p.Ser2536Pro is found in 12 probands so far, and all of them are homozygous. Although these are unclassified variants with potential impact on disease development, the fact that both of these mutations are common in our population, as well as the fact that they are most often found as homozygous, decreases the possibility that these mutations may be considered as deleterious or with high impact on cancer risk.

BRCA2 missense mutation p.Met927Val is detected in two individuals from family with breast cancer cases. This mutation is not described in literature, and PolyPhen analysis classifies this mutation as 'probably damaging', assuming that this mutation may increase cancer risk. The fact that both affected members of this family are mutation carriers suggests that this assumption may be correct.

Possible clinical significance of missense mutations changes in time, with acquiring more data regarding association of these mutations with increased cancer risk. Therefore, interpretation of results with missense mutations of unknown clinical impact is very challenging and should be approached with caution, using all available data sources (softwares, literature data, personal experience, and so on).

Overview of mutation carriers in our group, type of cancer, age of onset and number of cancers in their families are given in Table 5. In all individuals affected with ovarian cancer or in those who had family

Table 5 Overview of diseases and family history of mutation carriers

Gene	Frameshift mutation	Cancer type (age of onset)	Number of affected relatives: Breast cancer	Number of affected relatives: Ovarian cancer	Other cancer types (number of relatives)
BRCA1	c.843_846delCTCA	Ovarian cancer (47)	2		
	c.844_850dupTCATTAC	Breast cancer (34)	1[a]	1[a]	Renal cancer (1)
		Breast cancer (70)	1 (1 early)		Renal cancer (1)
		Ovarian cancer (70)			
	c.2019delA	Fallopian tube cancer (55)			Prostate cancer (1)
		Not affected		2	Fallopian tube cancer (1)
					Prostate cancer (1)
	c.4646_4665del	Breast cancer (55)		2	
		Ovarian cancer (59)			
	c.5266dupC	Breast cancer (49)	3 (1 bilateral, 1 early)	2	
BRCA2	c.1763_1766delATAA	Breast cancer (34)	2 (1 male breast cancer)		
	c.4139_4140dupTT	Breast cancer (bilateral) (54)	1		
		Not affected	2 (1 bilateral)		
	c.4987_4990delGTCA	Not affected	1 (early)		
	c.7283delT	Breast cancer (37)	1		

[a]Breast and ovarian cancer in same individual.

member(s) affected with ovarian cancer, *BRCA1* mutation was detected. Among *BRCA2* mutation carriers, there are neither ovarian cancer patients nor individuals with relative(s) affected with ovarian cancer. These findings are in accordance with literature data that ovarian cancer is more often associated with *BRCA1* than with *BRCA2* mutations.[49] *BRCA1* mutations were also found in individuals affected with both breast and ovarian cancer or in those who had family member affected with both types of cancer. This is in accordance with data found in literature that *BRCA1* mutation carriers affected with breast cancer are at an increased risk for ovarian cancer.[50] Among *BRCA2* mutation carriers, there is one individual with one male relative affected with breast cancer, which is in accordance with the fact that male breast cancers are more often associated with *BRCA2* mutations.[51] Among 12 *BRCA1/2* mutation carriers, three of them were not affected with cancer. One of them was *BRCA1* mutation carrier (c.2019delA), age 33, from the family with ovarian cancer cases only. As *BRCA1*-related ovarian cancer is not associated with earlier onset of disease, it is possible that this mutation carrier will develop ovarian cancer later in life, as she did not opt for prophylactic surgery. In remaining two healthy mutation carriers *BRCA2* frameshift mutations were detected. One of them was 43 (c.4139_4140dupTT), and the other was 35 years old (c.4987_4990delGTCA), both were from site-specific breast cancer families. Both of them opted for follow-up, refusing prophylactic surgery. As *BRCA2* mutations are more often associated with later onset of breast cancer,[52] these two individuals still may develop disease later in life. Furthermore, it is possible that not all of healthy mutation carriers will develop disease because of incomplete penetrability of *BRCA* genes.

According to literature data, in families with breast and/or ovarian cancers frequency of *BRCA1* and *BRCA2* mutations are 0.7–29% and 1.5–25%, respectively, and about 20% in total for both genes.[53] In our sample this frequency is 12.77%, which is in accordance with data from other authors.

In early breast cancer cases, independent on family history, *BRCA1* and *BRCA2* mutations are found in 0.7–10% and 1–6%, respectively.[53] In our test group, 19 individuals had early breast cancer (including one person who, in addition to early breast cancer, later developed contralateral breast cancer, and one individual who developed early breast cancer, ovarian cancer, colon cancer and lung cancer). Among these 19 early breast cancer patients, mutations were found in two individuals (10.53%). One of the mutations was found in *BRCA1* gene, which is in accordance with data that associated *BRCA1* mutations with early breast cancer.[52] This mutation was found in individual whose mother (also a mutation carrier) first developed breast cancer and later ovarian cancer, which is supported by data that *BRCA1* mutation carriers who developed breast cancer are at increased risk for developing ovarian cancer.[50] Second mutation in early breast cancer group is found in *BRCA2* gene, in individual who after early breast cancer developed contralateral breast cancer. *BRCA2* mutations are rarely associated with early or bilateral breast cancers (more frequently they are associated with *BRCA1* mutations), but this result is understandable when proband's family history of male breast cancer is taken into account. In individuals who were affected with early breast cancer only ($n=17$), one *BRCA1* mutation has been found (5.88%).

In eight probands who developed bilateral breast cancer, one mutation in *BRCA2* gene was found. This is not in accordance with literature data that indicate *BRCA1* mutations as more frequent in individuals with bilateral breast cancer,[54] but this finding becomes understandable in the light of proband's family history of male breast cancer. Mutation frequency in bilateral breast cancer group is 12.50%.

Literature data show that *BRCA2* mutation frequency in male breast cancer cases, independently of family history and the age of onset, is 7–14%.[53] So far, we tested four males affected with breast cancer and none of them were found to be *BRCA2* mutation carriers. Among female individuals with family history of male breast cancer ($n=4$), who also developed breast cancer, we detected one *BRCA2* mutation. In total, taking into account all individuals with male breast cancers in their families (eight individuals from seven families), one *BRCA2* mutation was detected, frequency being 12.50%, which is in accordance with the given literature data.

In four probands with ovarian cancer, one *BRCA1* mutation was found, frequency being 25%. In five probands with both breast and ovarian cancer (one of them developed early breast cancer, ovarian, colon and lung cancer), two *BRCA1* mutations were found

(40%; 2/5). This is in accordance with data in literature, suggesting that *BRCA1* mutation carriers are at increased ovarian cancer risk after developing breast cancer.[50] In addition, *BRCA1* mutations increase risk for developing Fallopian tube cancer, which is supported by our finding that one proband with this type of cancer is *BRCA1* mutation carrier. Individual who developed early breast cancer, ovarian, colon and lung cancer is not *BRCA* mutation carrier, although high probability of *BRCA* mutation presence may be supposed due to numerous various types of cancer this individual developed.

In conclusion, we detected nine deleterious *BRCA* mutations in 12 individuals. Frequency of *BRCA* mutations in our group is 12.77% (12/94). In addition to two novel mutations detected in our population and reported previously,[19] we detected another novel mutation that was not previously reported in Breast Cancer Information Core or Leiden Open Variation databases by others—c.7283delT in *BRCA2* exon 14. We did not identify *BRCA* mutation that would be characteristic for Serbian population, most probably because of high rate of migrations in history of this part of Europe. Possible clinical significance of missense mutations changes in time, with acquiring more data regarding association of these mutations with increased cancer risk. Therefore, interpretation of results showing missense mutations of unknown clinical importance is very challenging and should be approached with caution, using all available data sources.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a grant of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant number 41026). We thank Professor Dr Zvonko Magic and his co-workers on their permanent support.

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