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Original Research Article

BRCA1 gene-related hereditary susceptibility to breast and ovarian cancer in Latvia

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ABSTRACT

Purpose: In this report, we summarise data on *BRCA1* gene analysis in Latvia to characterise criteria of genetic testing for breast and ovarian cancer susceptibility.
Material/methods: Analysis by SSCP/HD, MALDI-TOF mass spectrometry or DNA sequencing was used for mutation detection. Mutations identified were confirmed by direct DNA sequencing.
Results: Out of 1068 breast and 231 ovarian cancer patients from different families: 58 carried the c.5266dupC and 43 carried the c.4035delA mutations. Every 4th patient in our study did not report cancer in the family. The breast cancer was diagnosed earlier in carriers of the c.5266dupC than in carriers of the c.4035delA ($p = 0.003$). The incidence of breast or ovarian cancer does not differ among the 2 mutation carriers in our patient group. The nature of the c.5266dupC mutation might be more deleterious.
Conclusions: We recommend the screening of 4 founder *BRCA1* mutations in all breast and ovarian cancer patients in Latvia at diagnosis of disease regardless of family history or age. The *BRCA1* screening can be carried out efficiently using the MALDI-TOF mass spectrometry mutation detection method developed in the Biomedical Research and Study Centre (Riga, Latvia).

1. Introduction

After the linkage of *BRCA* gene dysfunction to hereditary breast and ovarian cancer, genetic testing for mutations in these genes has become an essential procedure carried out in different countries to facilitate decisions about the treatment and follow up of patients, to identify the risk to individuals among healthy family members of mutation carriers and to offer timely and appropriate preventive activities for these carriers [1,2].

The qualification of breast and ovarian cancer patients for genetic testing to identify *BRCA1/2* mutation carriers is usually based on the data of family history of cancer and early age of disease onset in a patient or a family member. This approach is considered the most efficient for the identification of mutation carriers in large mixed populations, but in populations with a high frequency of founder mutations the family history may not be so informative. Furthermore, in several countries (Poland, Norway, Belgium) it was shown, that a significant proportion of *BRCA* gene mutation carriers have no history of cancer in their family [3–5]. As a result, the mutation carrier frequencies can be underestimated in some populations.

The criteria for genetic testing are usually rather strong, first of all because of the large size of *BRCA* genes and the work involved with the genetic testing procedure. Criteria may differ between countries and should be based on the characterisation of gene variations in the population [6]. In most countries, therefore, genetic testing is offered to women from families with several breast or ovarian cancer cases in first- or second-degree relatives, often taking into account histological and immunological features of the tumour [7,8]. The biotechnology company Myriad Genetics (Salt Lake City, UT, USA) recently suggested to increase genetic testing for *BRCA1/2* gene mutations by the age of 65 to identify virtually all mutation carriers [9].

Genetic testing is facilitated in populations characterised by a high prevalence of specific mutations. Several prevalent founder

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mutations have been detected in many populations [10]. The Ashkenazi Jewish population and eastern European region are characterised by a high prevalence of a small number of *BRCA1* founder mutations. Nonetheless, other non-founder mutations can be associated with cancer predisposition in some families, and the impact of these mutations in disease predisposition would be useful to estimate in every founder population.

The *BRCA1* mutation spectrum has been characterised in most European and eastern European populations. The most frequently detected mutation in this region is the c.5266dupC (traditionally known as 5382insC), found from the Baltic Sea to the Pacific Ocean and from Europe to Siberia [11,12]. The c.4035delA (4154delA) mutation is the second most prevalent mutation in the Baltic states [13–15]. The prevalence of the c.181T>G in Latvia and Russia is not high [14,16]. The c.68_69delAG, known as Ashkenazim founder mutation, was detected previously in Latvia only once [14].

BRCA1 gene mutations are the major cause of hereditary breast and ovarian cancer in Latvian women. The testing of 44 Latvian patients with the onset of breast or ovarian cancer before the age of 48 for mutations in the *BRCA2* gene by Sinicka [17] in the laboratories of the Department of Oncology and Surgical Sciences at the Busonera University Hospital in Padua (Italy) by DHPLC method using Transgenomic WAVE 3500 System under the guidance of Drs. E. D'Andrea, M. Montagna and S. Agata, resulted in finding only polymorphic variants of BRCA2 gene. No pathogenic mutations were found. However, pathogenic *BRCA2* gene mutations (possibly 2 founder mutations) were detected recently by Berzina et al. [18] through the analysis (real time PCR/HRM or RFLP) of *BRCA2* gene in more patients from Latvia. The analysis of *BRCA2* gene might be important for the identification of risk individuals in Latvia; however, further studies would be useful.

The aim of the present study was to characterise *BRCA1*-associated hereditary breast and ovarian cancer in Latvian patients in terms of genetic testing criteria, the *BRCA1* gene mutation spectrum and to relate the carrier status of founder mutations with the age at diagnosis and family history of cancer.

2. Material and methods

2.1. Subjects

The present study included 1299 female patients regardless of their ethnicity diagnosed with breast or ovarian cancer and 12 healthy relatives. In all cancer cases, the diagnosis was confirmed histologically. The number of patients is summarised in Table 1. The Oncology Clinic of the city of Liepaja (the most south-western region of Latvia) provided blood samples of 214 breast cancer patients, collected between 2004 and 2008, and 63 ovarian cancer patients, collected between 2004 and 2012. The Latvian Oncology Centre (Riga, Latvia) (LOC) provided blood samples of 286 patients with breast cancer, collected between 1996 and 2007, and 168 (80 + 88) with ovarian cancer, collected between 1999 and 2004.

Table 1
The origin of study subjects.

Source of blood samples	Number of samples
Oncology clinic, Liepaja	
Breast cancer patients	214
Ovarian cancer patients	63
Healthy relatives (saliva samples)	12
Latvian Oncology Centre, Riga	
Breast cancer patients	286
Ovarian cancer patients	80
The Latvian Genome Database	
Breast cancer patient DNA samples	568
Ovarian cancer patient DNA samples	88
Total	1311

DNA samples of 568 breast cancer patients from LOC were provided by the Latvian Genome Database (LGDB) in the Biomedical Research and Study Centre (collected between 2003 and 2012), and 348 of them (collected between 2009 and 2012) were analysed for mutation detection by mass spectrometry.

In this study, we included data obtained from the analysis of patient DNA samples for prevalent mutations and entire *BRCA1* gene analysis (SSCP/HD), partly during our previous studies [14,19] and sequencing of the entire *BRCA1* gene in the current study. Only data of the patients from different families were used in this study. Duplication of DNA samples was excluded specifically.

Patients were invited to fill in a personal and family history questionnaire. The family history data of 101 mutation carriers reported by patients (58 with the c.5266dupC and 43 with the c.4035delA) identified in the Biomedical Research and Study Centre (BMC, Riga, Latvia) were analysed. Unfortunately, it was impossible to verify the information concerning the family history data before the 1980 presented by the patients in Latvia, however, there was no reason to assume that carriers of one mutation would be better informed than carriers of other mutation.

The study conforms to The Code of Ethics of the World Medical Association. Written informed consent was obtained from all participants and the study protocol was approved by the Central Medical Ethics Committee of Latvia.

2.2. DNA isolation

The DNA of study participants was isolated from peripheral EDTA blood samples using standard phenol-chloroform extraction.

The DNA of mutation carrier family members was isolated from saliva samples collected using the saliva self-collection system (DNA Genotek Inc., Ottawa, ON, Canada) and extracted according to manufacturer's instructions.

2.3. Genetic testing

All DNA samples of patients were tested for the c.5266dupC and c.4035delA mutations, and all patients from the Oncology Clinic of Liepaja (Liepaja, Latvia) and most of the patients from LOC were tested for two other mutations i.e. c.181T>G and c.68_69delAG by SSCP/HD analysis as described in the BIC database [20] and direct DNA sequencing of mutant DNA samples detected. The SSCP/HD method is not suitable to identify every variation in the DNA structure, therefore, positive controls with DNA from known mutation carriers were always used to identify carriers of the 4 aforementioned founder mutations and, nonetheless, several other mutations were detected using this method.

Suspected DNA fragments were amplified using the same primers for mutation screening and for DNA sequencing. Primers [20] and PCR conditions were as described [14]. Analysis of 348 DNA samples. provided by LGDB, was carried out by minisequencing for the 4 mutations, and the resulting products were analysed on a MALDI-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) as described by Ignatovica et al. [21].

The DNA of 12 patients from the Oncology Clinic in Liepaja (all possibly suspected of hereditary cancer because of early onset, recurrence of disease or cancer history in first-degree relatives) were tested for mutations within the entire *BRCA1* gene by DNA sequencing, including all exons and exon/intron boundaries using the same primers as for mutation detection.

2.4. Sequencing

Sequencing reactions were performed using fluorescent BigDye Terminator vs.3.1 Cycle Sequencing protocol (Applied Biosystems, Foster City, CA, USA). The sequencing reactions and product

purification were as described by Tarasova et al. [22]. The analysis of DNA sequencing products was carried out using the ABI Prism Genetic analyser model 3130xl (Applied Biosystems). Chromatograms were inspected using DNA Sequencing Analysis software v.5.2 (Applied Biosystems).

2.5. Genotyping

DNA (348 samples) obtained from the LGDB was distributed into 96-well PCR plates (28 ng per well) using the Freedom Evo robotic workstation (Tecan, Mannedorf, Switzerland). PCR primers for amplification of genomic DNA fragments containing the BRCA1 mutations of interest (the c.5266dupC, c.4035delA, c.181T>G and 68_69delAG) were designed by the modified Primer3 program [23] and synthesised by Operon Biotechnology GmbH, Cologne, Germany. The primer sequences are available on request (ilona@biomed.lu.lv). Amplification was carried out by multiplex PCR.

For minisequencing, the CalcDalton program [24] was used to design special primers that contained a biotin cap at the 5′-end and a photolinker cleavage site (synthesised by BioTeZ Berlin-Buch GmbH, Germany). The primer sequences and mass of cleaved products are available on request (ilona@biomed.lu.lv). The minisequencing reaction conditions and MALDI-TOF mass spectrometry analysis were performed as previously described by Ignatovica et al. [21].

2.6. Statistical analysis

Results are presented as means with standard deviation and 95% confidence interval where appropriate. Statistical analysis was done using MedCalc 12.6.1.0, (MedCalc.org), *t*-test was used to compare means, Chi-square test to compare proportions. Agresti-Coull interval approximation was used for confidence intervals of binomial proportion [25]. *p* Values less than 0.05 were deemed significant.

3. Results

Because many patients in our study did not meet any accepted criteria to be selected for genetic testing, we summarised all our data to find criteria for genetic testing of breast and ovarian cancer patients in Latvia. Altogether we have analysed 1299 patients (Table 1) and identified 115 deleterious BRCA1 mutation carriers.

At the beginning of our study, we tested mainly women before 45 years of age at the onset of disease. In later stages, we invited unselected women, however, we cannot exclude that women with an earlier age of cancer diagnosis may be more interested to participate in genetic testing for possible inheritance of cancer. All patients from Liepaja and LOC were analysed for the c.5266dupC and c.4035delA and all patients from Liepaja and most of patients from LOC for two more *BRCA1* gene mutations – the c.181T>G and c.68_69delAG.

Data in Table 2 show that in total 19 of the *BRCA1* c.5266dupC mutation carriers can be found among breast and ovarian cancer patients diagnosed before 40 years of age. A proportion of the c.5266dupC carriers identified among breast cancer patients diagnosed between 40 and 49 years of age was lower, however, more carriers of the c.5266dupC in this age group were detected among ovarian cancer patients. Patients between 40 and 49 years of age were also a group with the highest proportion of the c.4035delA mutation.

In patients over 60 years of age and diagnosed with cancer, only two carriers of the c.5266dupC (2/397; 0.5%; CI 95% = 0–1.2%) were detected (61 and 63 years), as compared to 11; 2.8%; CI 95% = 1.2–4.4% carriers of the c.4035delA (5 of them detected over the age of 65). However, more patients from different age groups should be tested to confirm the difference ($p = 0.024$). The total *BRCA1* founder mutation rate in this older patient group was only 3.3%, mainly due to carriers of the c.4035delA. Majority of the c.4035delA carriers were ethnic Latvians.

The analyses of family history data, reported by patients in questionnaires (Table 3), show that every 4th prevalent mutation carrier detected in our study did not report cancer in family members (25/101; $p < 0.0001$). This proportion could probably vary if more patients from different settings were tested. In 348 patients involved from LOC between 2009 and 2011 (mean age 56.9 years, range 21–87, this cohort may be considered as unselected), 50% of patients did not report cancer in the family. However, out of 14 mutation carriers among these patients, only two carriers of the c.5266dupC did not have cancer in family, but 6 mutation carriers reported other cancer types.

The characterisation of detected mutation carriers by family history of cancer is shown in Table 3. More families with multiple cancer cases were reported by carriers of the c.5266dupC. The difference among carriers of the two mutations may be accidental and more carriers should be analysed to confirm this.

The differences in the mean age at onset of disease in carriers of the two mutations are shown in Table 4. These data confirm a statistically significant difference between the mean ages at disease onset in breast cancer patients carrying the two different *BRCA1* gene mutations (c.5266dupC or c.4035delA). The number of mutation carriers (23 and 17) detected among ovarian cancer patients tested (231) was not large enough to statistically confirm the difference between ages at onset of disease in carriers of the two mutations.

Table 2
Analysed patients and detected prevalent mutation carriers.

Individuals tested		BRCA1 mutations detected				
Patient group	*n*	*n* (%)	c.5266dupC		c.4035delA	
			BC[a]	OC[b]	BC	OC
Mean age at cancer diagnosis	56.8					
Personal cancer history	1299	101				
Breast cancer	1068	61 (5.7)				
Ovarian cancer	231	40 (17.3)				
Patients diagnosed						
Cancer under the age of 40	165	23 (13.9)	16	3	4	0
Cancer between the age of 40 and 49	394	48 (12.2)	15	14	11	8
Cancer between the age of 50 and 59	343	17 (4.9)	3	5	3	6
Cancer over the age of 60 (60–87)	397	13 (3.3)	1	1	8	3

[a] BC – breast cancer.
[b] OC – ovarian cancer.

Table 3
All deleterious BRCA1 mutation carriers detected and their family history of cancer.

BRCA1 gene mutation	*n* detected *n* (%)	Carriers detected		Family history of cancer in carriers						Recurrence of cancer		
		BC[a] (%)	OC[b] *n* (%)	No cancer *n* (%)	Other type of cancer *n* (%)	BC *n* (%)	OC *n* (%)	Gyn. ca[c] ± *n* (%)	FCA[d] *n* (%)	In patient		In relative *n* (%)
										BC first *n* (%)	OC first *n* (%)	
c.5266dupC	58 (4.4)	35 (60)	23 (40)	16 (27.6)	10 (17.2)	12 (20.6)	4 (6.9)	8 (14)	7 (12)	6 (10.3)	1 (1.7)	4 (6.9)
c.4035delA	43 (3.3)	26 (60)	17 (40)	9 (20.9)	7 (16.3)	14 (32.5)	6 (13.9)	4 (9)	3 (7)	6 (14)	2 (4.7)	0 (0)
c.181T>G	7	5	2	2	3	2				+		1
c.68_69delAG	2	2	0	1	1							
c.843_846delCTCA	1	1			2+[e]					1		
c.3531delT	1	1		1								
c.4357+1G>A	1	1				1						
4675G>A	1	+	1		+		+	+			1	
c.3756delGTCT	1	+	1		3+			2+	+		1	
Total families	115	71	44	29	24	31	11	13	11	14	5	5

[a] Breast cancer.
[b] Ovarian cancer.
[c] Gynaecological cancers in the first degree relative, ± other type of cancer in family.
[d] FCA – family cancer aggregation – multiple (at least 4) cases of cancer in family.
[e] "+" cancer type in family besides cancer in patient.

Table 4
The mean age at onset of disease in carriers of the two prevalent BRCA1 mutations.

Prevalent mutation carriers	c.5266dupC			c.4035delA			*p*-Value	95% CI	
	n	Mean age ± sd[a]	Age range	*n*	Mean age ± sd[a]	Age range		L95	U95
BC[b]	35	42.0 ± 7.8	33–63	26	49.9 ± 12.1	33–62	0.003	2.79	13.01
OC[c]	23	46.4 ± 7.8	32–61	17	52.0 ± 19.9	40–77	0.053	0	11.26

[a] Standard deviation.
[b] Breast cancer.
[c] Ovarian cancer.

Our data indicate that most of the *BRCA1* c.5266dupC mutation carriers could be found among patients diagnosed with cancer before 50 years of age.

The number of cancer patients in families of the two prevalent mutation carriers could be compared: there were on average 1.41 relatives with cancer in carriers of the c.5266dupC and a similar number (1.35; difference for averages $p = 0.73$) of relatives with cancer in carriers of the c.4035delA, detected in our study. This should be confirmed by analysing more data.

The earlier age at onset of disease and more relatives with multiple cancer cases in families of carriers of the c.5266dupC mutations might indicate a more deleterious nature of the c.5266dupC mutation in comparison with the c.4035delA.

The proportion of breast cancer among carriers of the c.5266dupC mutation was the same as for the carriers of the c.4035delA mutation (60.3 and 60.4%, respectively). The same proportion was observed for ovarian cancer cases among the two prevalent mutation carriers (39.6 and 39.5%). But there was a significant difference in allele frequencies between breast and ovarian cancer patients for both mutations (Table 5). Both mutation carriers had significantly higher and similar odds to have ovarian or breast cancer.

All nine deleterious mutations of the *BRCA1* gene detected during this study are shown in Table 3. The entire *BRCA1* gene was analysed in 160 patients by SSCP/HD analysis, and all variants detected were confirmed by DNA sequencing. Using this method we identified three other deleterious mutations – two deletions (c.3531delT and c843_846delCTCA) and one missense mutation (c.4357+1G>A). The c.3531delT is registered in the BIC database only once (from Latvia), and the other two mutations were found repeatedly in different populations (17 and 23 times, respectively).

Two other mutations (the c.4675G>A and c.3756delGTCT) were found by DNA sequencing of the entire *BRCA1* gene (all exons and exon/intron boundaries) in 12 patients from Liepaja with two cancers: ovarian cancer followed by breast cancer, both before 50 years of age (Table 3). The mutation c.3756delGTCT has been detected before in Russia [26,27] and suspected to be a Russian founder mutation, however, additional testing of more Russian patients did not confirm this [28]. At present, c.3756delGTCT is registered in the BIC database 123 times, and in most cases it is of western European origin. The c.4675G>A has been registered in the BIC database only twice by Myriad Genetics Laboratory and classified as clinically significant mutation [20].

The screening of more patients for five other mutations detected in our patients confirmed the same mutation spectrum in Latvia [14]: not one of the mutations detected by analysing the entire *BRCA1* gene was found in additional patients tested. Regardless of the high frequency of occurrence of the c.181T>G mutation in Poland [29], only 7 carriers were detected so far in Latvia. Problematically high frequency of occurrence of the

Table 5
The difference of two prevalent BRCA1 mutation frequencies in ovarian and breast cancer patients.

Mutation	Test	Mutation frequency	OR	CI 95%	*p*
c.5266dupC	OC[a] vs. BC[b]	10.0% vs. 3.3%	3.04	1.76–5.24	0.0001
c.4035delA	OC vs. BC	7.4% vs. 2.4%	3.02	1.61–5.66	0.0006

[a] OC – ovarian cancer.
[b] BC – breast cancer.

c.181T>G mutation was identified in Belarusian breast cancer patients, but not in ovarian cancer patients [30]. Certainly, its occurrence is not high in our population. However, using MS MALDI-TOF we detected 3 carriers of this mutation among 348 breast cancer patients from LOC (all were confirmed by direct DNA sequencing of the corresponding PCR fragment from other DNA samples). The frequency of occurrence of this mutation in our population should be tested in more patients from different age groups. This could be easily performed by mass spectrometry. However, it remains important that in cases when the hereditary breast or ovarian cancer is suspected, analysis of the entire *BRCA1* gene sequence may be recommended, essentially depending on the age of patient and family cancer history.

All mutations detected were screened in a larger panel of patients who were negative for the c.5266dupC and c.4035delA. The c.181T>G was screened overall in 690 breast and 116 ovarian cancer patients and 7 mutation carriers were identified. Only 2 carriers of the c.68_69delAG were identified out of 855 patients with breast cancer and 148 patients with ovarian cancer; its frequency of occurrence, probably, is not high in Latvian patients. The other four mutations were screened in 238 breast and 29 ovarian cancer patients. Only the c.4675G>A mutation was tested by DNA sequencing of exon 15 of the *BRCA1* gene in 135 breast and 12 ovarian cancer patients, because in our experiments it could not be detected on the SSCP gel even in the presence of a positive control. No other carriers of these five mutations were detected.

This screening allowed us to conclude that the prevalence of other *BRCA1* mutations detected in our laboratory, did not likely exceed 0.5% (CI 95% 0.1–0.9%) among breast cancer patients; however, the prevalence of other mutations among ovarian cancer patients calculated from our current data could be a little higher, nevertheless, below 1% (CI 95% 0–2.3%).

4. Discussion

This paper summarises the results of *BRCA1* gene mutation analysis in the Biomedical Research and Study Centre (Riga, Latvia) between 1996 and 2011. With the accumulation of data, we were able to define the criteria for genetic testing of breast and ovarian cancer patients in Latvia more accurately as to age at onset of disease and family history of cancer. This issue becomes more important due to the extensive migration of people in the modern world. The data on the clinical manifestation of disease in two founder mutation (the c.5266dupC and c.4035delA) carriers were analysed.

The testing of all breast and ovarian cancer patients regardless of the age at onset of disease and family history of cancer for *BRCA1* founder mutations may be the best way to identify most mutation carrier families in Latvia. The mutation detection method, developed in the BMC for mass spectrometry can be easily used for screening cancer patients for the two prevalent mutations (the c.5266dupC and c.4035delA) and two mutations detected in Latvia more than once (the c.181T>G and c.68_69delAG).

However, carrier families with other rare mutations will remain unidentified, although the proportion of these families, probably, will not be high, and the analysis of the entire *BRCA1* gene could be left to patients' own decision after genetic counselling according to accepted criteria [6] and explaining the significance of information about carrier status, taking into account available data.

We analysed the data of mutation carriers (women) with diagnosed breast or ovarian cancer from different families. In total, we identified 101 carriers of the two prevalent founder mutations: 58 with the c.5266dupC and 43 with the c.4035delA. We did not consider it possible to estimate the exact proportion of all mutation carriers among breast or ovarian cancer patients since our patient group was hospital based and the patients tested did not adequately represent all age groups. Among the 1068 breast cancer study patients tested, 5.7% (CI 95% 4.3–7.1%) were carriers of the two prevalent mutations. Among 231 ovarian cancer study patients, the carrier frequency of the two mutations detected was higher (17.3%, CI 95% 12.4–18.2%; $p > 0.0001$). Our data could be biased because of the mode of involvement of patients. Obviously, the true proportion of founder mutation carriers in Latvia could be estimated if all breast and ovarian cancer patients were tested for these mutations.

It is impossible to strictly control the selection process of patients for analysis, and different unselected patient groups can differ by age, family history, mutation status and the method used for mutation detection. The proportion of breast cancer among carriers of the c.5266dupC mutation is the same as for the carriers of the c.4035delA mutation. The selectivity towards ovarian versus breast cancer predisposition was not observed as well in carriers of the c.4035delA detected among patients from Russia [31]. The occurrence of breast cancer in many carriers of the c.4035delA mutation could be explained by the localisation of the c.4035delA at the very end of the region of *BRCA1* gene, which is defined as the region of elevated ovarian cancer probability (nucleotides 2401–4190) [32]. It may also be due to the specificity of our patient group, which did not correspond to widely accepted criteria for genetic testing. Our results may be biased because we tested many patients over the age of 60 and in the absence of cancer in the family history.

As to the families with multiple cancer cases, more patients were found among carriers of the c.5266dupC than among carriers of the c.4035delA mutation. Furthermore, 5 out of 7 families with multiple cancer cases among carriers of the c. 5266dupC were reported among ovarian cancer patients. Possibly some familial or genetic risk factors of ovarian cancer could be found in these families.

The observation of less c.5266dupC carriers in the group of patients over 60 years as well as more patients with multiple cancers in family might be suggestive of the more deleterious nature of the c.5266dupC mutation in our population compared to the c.4035delA. In accordance with our data, the less deleterious nature of the c.4035delA mutation was previously observed in Poland [33].

Our data confirm the earlier affirmation of high prevalence of the two *BRCA1* founder mutations (c.5266dupC and c.4035delA) in patients from Latvia (14). Several other mutations could be detected if the entire *BRCA1* gene sequence was analysed in patients negative for the two prevalent *BRCA1* gene mutations.

No other prevalent founder mutations in the *BRCA1* gene were detected. A total of nine deleterious mutations were detected in the *BRCA1* gene in patients from Latvia: seven of them using the SSCP/HD method and two by DNA sequencing of the entire *BRCA1* gene. Geographic differences [34] in the prevalence of *BRCA1* mutations in patients from Latvia were not detected in our study among the central and south-western regions. Out of the 277 patients tested from the Oncology Clinic in Liepaja, we detected 13 *BRCA1* gene mutations (including nine prevalent mutations, the c.5266dupC and the c.4035delA).

5. Conclusions

We detected a significant proportion of mutation carriers without a family history of cancer. Therefore, we recommend genetic testing to all breast and ovarian cancer patients from Latvia at least for the prevalent founder mutations at the time of breast or ovarian cancer diagnosis, regardless of the family cancer history or patients' age. This would result in the identification of most families in Latvia predisposed to hereditary breast or ovarian cancer during several years. Earlier age at onset of disease and more carriers of the c.5266dupC with multiple cancer cases in the family indicate a more deleterious nature of this mutation in

comparison to the c.4035delA. The characterisation of *BRCA1* status is important for the treatment and follow-up of cancer patients and their relatives. The testing can be realised easily using the MS MALDI-TOF mutation detection method developed in the BMC. We conclude that the *BRCA1* non-founder mutations make up the minority (<0.5%) of *BRCA1* gene mutations in Latvian patients. Mutation carriers should be encouraged to share the information with their family members so that they can consider genetic counselling and testing. The testing of women without prevalent mutations could be left to their own decision after genetic counselling, explaining available information on the clinical significance of mutation carrier status. All mutation carriers should be informed about available possibilities of genetic testing and preventive procedures.

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Conflict of interests

The authors declare no conflict of interests.

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