High frequency of pathogenic non-founder germline mutations in BRCA1 and BRCA2 in families with breast and ovarian cancer in a founder population

J. Maksimenko*, A. Irmejs, G. Trofimovičs, D. Bērziņa, E. Skuja, G. Purkalne, E. Miklaševičs and J. Gardovskis

Abstract

Background: Pathogenic BRCA1 founder mutations (c.4035delA, c.5266dupC) contribute to 3.77% of all consecutive primary breast cancers and 9.9% of all consecutive primary ovarian cancers. Identifying germline pathogenic gene variants in patients with primary breast and ovarian cancer could significantly impact the medical management of patients. The aim of the study was to evaluate the rate of pathogenic mutations in the 26 breast and ovarian cancer susceptibility genes in patients who meet the criteria for BRCA1/2 testing and to compare the accuracy of different selection criteria for second-line testing in a founder population.

Methods: Fifteen female probands and 1 male proband that met National Comprehensive Cancer Network (NCCN) criteria for BRCA1/2 testing were included in the study and underwent 26-gene panel testing. Fourteen probands had breast cancer, one proband had ovarian cancer, and one proband had both breast and ovarian cancer. In a 26-gene panel, the following breast and/or ovarian cancer susceptibility genes were included: ATM, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, and XRCC2. All patients previously tested negative for BRCA1 founder mutations.

Results: In 44% (7 out of 16) of tested probands, pathogenic mutations were identified. Six probands carried pathogenic mutations in BRCA1, and one proband carried pathogenic mutations in BRCA2. In patients, a variant of uncertain significance was found in BRCA2, RAD50, MRE11A and CDH1. The Manchester scoring system showed a high accuracy (87.5%), high sensitivity (85.7%) and high specificity (88.9%) for the prediction of pathogenic non-founder BRCA1/2 mutations.

Conclusion: A relatively high incidence of pathogenic non-founder BRCA1/2 mutations was observed in a founder population. The Manchester scoring system predicted the probability of non-founder pathogenic mutations with high accuracy.

Keywords: Pathogenic non-founder BRCA1/2 mutations, Triple-negative breast cancer, Familial breast cancer

* Correspondence: Jelena.Maksimenko@rsu.lv
Institute of Oncology, Riga Stradins University, Dzirciema iela 16, Riga LV1007, Latvia

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Background
Hereditary breast cancers account for approximately 10% of all breast cancers, and approximately 23% of all ovarian cancers are considered hereditary [1, 2]. According to Plakhins et al., BRCA1 pathogenic founder mutations (c.4035delA, c.5266dupC) contribute to 3.77% of all consecutive primary breast cancers and 9.9% of all consecutive primary ovarian cancers [3]. BRCA1 and BRCA2 pathogenic founder mutation analysis is a relatively straightforward and cost-effective screening strategy to identify mutation carriers [4]. In Latvia, all consecutive breast and ovarian cancer cases are eligible for BRCA1 pathogenic founder mutations (c.181 T > G, c.4035delA, c.5266dupC) screening [5], and the costs of the test are covered by the public health care system. However, according to recent studies, non-founder BRCA1 and BRCA2 pathogenic mutations account for up to 21.6% of all BRCA1 and BRCA2 pathogenic mutations in the Ashkenazi Jewish population [6, 7]. There is little information about pathogenic BRCA1/2 non-founder mutations in Latvia. In a study published by Berzina et al., pathogenic non-founder mutations in BRCA1 and BRCA2 were identified in 4 out of 30 high-risk breast/ovarian cancer families from the Latvian population [8]. In another study published by Tihomirova et al., non-founder pathogenic mutations in BRCA1 and BRCA2 were detected in 9 out of 160 patients with breast and ovarian cancer [5]. These findings suggest that the proportion of pathogenic BRCA1/2 non-founder mutations is small and that family cancer history alone is of limited value to find subgroups of individuals, where expensive complete BRCA1/2 testing is indicated.

The remaining hereditary breast and ovarian cancer cases are associated with mutations in other breast and ovarian cancer susceptibility genes, such as BRCA1/2, TP53, PTEN, CDH1, STK11, MLH1, MSH2, MSH6, PMS2, PALB2, CHEK2, ATM, RAD51C, RAD51D, BRIP1 and other [9]. Patients and their relatives harbouring mutations in hereditary cancer predisposing genes could benefit from prevention and screening strategies or novel therapeutic approaches [10, 11]. Advances in next-generation sequencing allowed the implementation of low-cost multi-gene panel testing in clinical practice to detect pathogenic mutations in hereditary cancer predisposing genes [12].

Therefore, knowledge of the frequency and phenotypic features of pathogenic mutations beyond BRCA1 pathogenic founder mutations in breast and ovarian cancer susceptibility genes is essential for determining the role of second-line testing with multi-gene panels in counselling unsolved high-risk breast and ovarian cancer patients.

The aim of the study was to evaluate the rate of pathogenic mutations in the 26 breast and ovarian cancer susceptibility genes in patients who meet the criteria for BRCA1/2 testing and to compare the accuracy of different selection criteria for second-line testing in a founder population.

Methods
Patient group
Sixteen sequential patients with primary breast and/or ovarian cancer who met all inclusion criteria were included in the study between October 2016 and August 2017. The inclusion criteria were as follows: 1) fulfil at least one of the National Comprehensive Cancer network (NCCN) BRCA1/2 testing criteria (Table 1) (www.nccn.org); 2) previously tested negative for BRCA1 pathogenic founder mutations (c.181 T > G, c.4035delA, c.5266dupC); 3) able to cover the cost of the 26 multi-gene tests.

The following clinical information was obtained: age at testing, personal cancer history, age at cancer diagnosis, breast and/or ovarian cancer pathology, BRCA1/2 testing history, a family cancer history that covers a 3-generation pedigree according to probands information. The median patient age was 45.6 years (33–63 years). Fifteen out of 16 (93.75%) patients were females, and 1 out of 16 (6.25%) patients was male. Thirteen patients had unilateral breast cancer, 1 patient had bilateral breast cancer, 1 patient had ovarian cancer, and in 1 patient had both breast and ovarian cancer. Four out of 16 (25%) breast cancers were luminal-like HER2 negative, 2 out of 16 (12.5%) breast cancers were luminal B HER2 positive, 8 out of 16 (50%) breast cancers were triple-negative, and 1 out of 16 (6.25%) breast cancers was HER2 positive. The patient characteristics are summarized in Table 2.

DNA testing
Informed consent for genetic testing was obtained for all patients. All patients underwent DNA testing with a 26-gene panel (myBRCA HiRisk Hereditary Breast and

Table 1 NCCN selection criteria for screening of mutations in BRCA1 and BRCA2

| At least one of the following criteria has to be met: |
|-----------------------------------------------------|
| 1. Personal history of breast cancer diagnosed < age 45 years |
| 2. Personal history of breast cancer diagnosed < age 50 years and at least one case of breast cancer at any age in close blood relative |
| 3. Personal history of triple negative breast cancer diagnosed < age 60 years |
| 4. Personal history of breast cancer diagnosed at any age and at least two cases of breast cancer diagnosed at any age or at least one close blood relative with breast cancer diagnosed ≤50 years or at least one blood relative with ovarian carcinoma or a close male blood relative with breast cancer |
| 5. Personal history of ovarian cancer |
| 6. Personal history of male breast cancer |
Ovarian Cancer screening Test, VeritasGenetics, USA) that is a targeted next-generation sequencing assay for the detection of mutations in 26 breast and ovarian cancer susceptibility genes. The genes included high-penetrance breast-ovarian genes (BRCA1, BRCA2, PTEN, TP53, CDH1, STK11, PALB2), moderate-penetrance breast and/or ovarian genes (CHEK2, BRIP1, ATM), and additional genes (BARD1, BLM, EPCAM, RAD50, RAD51C, RAD51D, MEND, MRE11A, MSH2, MLH1, NBN, MSH6, PMS2, FAM175A, XRCC2). In all patients, the test was performed using saliva. The specificity and sensitivity of the assay are 99.9% for point mutations and small insertions/deletions in the 24 sequenced genes and 99.9% for structural variations in BRCA1 and BRCA2.

**Statistical analysis**

The specificity, sensitivity and accuracy of the NCCN criteria, Manchester scoring system and Swedish Breast cancer group criteria for the prediction of pathogenic non-founder mutations were evaluated. The Manchester score of 15 points threshold was used to assess the likelihood of BRCA1/2 pathogenic mutation [13]. The specificity, sensitivity and accuracy of different selection criteria for BRCA1/2 testing in our cohort were calculated using MedCalc Statistical Software version 17.9.

**Results**

In seven out of sixteen (44%) patients included, pathogenic non-founder BRCA1/2 mutations were identified. Six patients carried pathogenic variants of BRCA1 and one of BRCA2. In four patients, variants of uncertain significance of BRCA2, RAD50, MRE11A and CDH1 were found. Detailed results are shown in Table 3. The NCCN criteria showed a high sensitivity (100%) with low specificity (50%) for the prediction of non-founder pathogenic BRCA1/2 mutations. The Swedish Breast cancer group criteria showed a low sensitivity (57.1%) with three false negative results. The Manchester scoring system showed a high accuracy (87.5%) for the prediction of pathogenic non-founder BRCA1/2 mutations with high sensitivity (85.7%) and specificity (88.9%). The specificity, sensitivity and accuracy of different criteria/scoring systems for the detection of probability of BRCA1/2 pathogenic mutations in our cohort are compared in Table 4.

**Discussion**

Our study is the first report on the use of a 26 gene panel in to examine breast and ovarian cancer susceptibility genes in patients in Latvia. We demonstrated a high frequency of pathogenic non-founder germline mutations in BRCA1 and BRCA2 genes. In seven out of sixteen (44%) primary breast and ovarian cancer patients...
matching the criteria for BRCA1/2 testing pathogenic non-founder BRCA1/2 mutations were identified. All 7 pathogenic mutations, including 2 large deletions, are novel in populations of Latvia [5, 8]. These results may suggest that the present practice of testing only the 3 most frequent BRCA1 pathogenic founder mutations is insufficient and fails to detect a considerable number of pathogenic mutations in BRCA1/2. However, our study comprises a relatively small cohort of selected patients. In a study published by Frank et al., 21.6% of patients with Ashkenazi ancestry pathogenic non-founder BRCA1 and BRCA2 mutations were identified [6]. In contrast, in the Finnish population of high-risk individuals tested negative for 28 BRCA1/2 pathogenic founder mutations, additional pathogenic mutations in BRCA1 and BRCA2 accounted for just 1.2% [12]. Much larger numbers are necessary to assess the real proportion of pathogenic non-founder mutations in the population of Latvia.

Despite the drawbacks of such a small study group, the initial results raised some observations.

### Table 3 Results

| Nr. | Mutation | Clinical significance of mutation | NCCN inclusion criteria | Manchester score [13] | Swedish Breast cancer group criteria for screening of mutation in BRCA1 and BRCA2 |
|-----|----------|----------------------------------|-------------------------|-----------------------|-------------------------------------------------------------------------------------|
| 1   | RAD50c.980G > A | VUS | NCCN4 | 17 | One case of male breast cancer |
| 2   | BRCA1c.5075-T_5152 +1del | PAT | NCCN2 | 29 | One case of triple-negative breast cancer | Sage 40 |
| 3   | BRCA1c.1752+1del | PAT | NCCN3 | 20 | One case of breast cancer | Sage 35 |
| 4   | BRCA2c.6998dupT | PAT | NCCN4 | 19 | Breast cancer and ovarian cancer in one individual. |
| 5   | BRCA1c.5117G > A | PAT | NCCN5 | 15 | Do not match |
| 6   | RAD50c.251 T > A | VUS | NCCN4 | 6 | NA |
| 7   | BRCA1c.1961delA | PAT | NCCN3 | 14 | Do not match |
| 8   | BRCA2c.280C > T | VUS | NCCN4 | 14 | Do not match |
| 9   | BRCA1c.5117G > A | PAT | NCCN4 | 16 | Do not match |
| 10  | BRCA1c.4996_4997dupTA | PAT | NCCN4 | 20 | One case of triple-negative breast cancer | Sage 40 |
| 11  | Negative | Negative | NCCN4 | 2 | Do not match |
| 12  | Negative | Negative | NCCN1 | 2 | Do not match |
| 13  | Negative | Negative | NCCN3 | 8 | Do not match |
| 14  | Negative | Negative | NCCN1 | 8 | Do not match |
| 15  | CDH1 c.808 T > G | VUS | NCCN4 | 8 | Do not match |
| 16  | Negative | Negative | NCCN1 | 0 | Do not match |

PAT, pathological; VUS, variant of uncertain significance; *Triple-negative breast cancer was defined as ER-0%; PR-0%; HER2- negative; 

Interestingly, probands that carried a pathogenic non-founder mutation had some common features. All six breast cancer patients in our study with proven pathogenic non-founder BRCA1/2 mutations had a triple-negative phenotype. It is well established that approximately 80% of all BRCA1/2– related tumours have a triple-negative phenotype [14–18]. The prevalence of pathogenic germline BRCA1/2 mutations in the selected triple-negative breast cancer patients ranged from 9.2 to 34.4% [19–22]. Additio nal analyses of cDNA microarray data from van’t Veer showed that BRCA1–related tumours have a sporadic basal-like breast cancer gene expression profile [23]. Additionally, according to Richardson et al., loss of BRCA1 function could play a role in the development of basal-like breast cancers [24]. Couch et al. identified BRCA1/2 pathogenic mutations in 11.2% of triple-negative breast cancer patients and other breast-ovarian cancer predisposing gene mutations in 3.7% of triple-negative breast cancer patients [25].

In our study we used the NCCN criteria for screening pathogenic mutations in BRCA1 and BRCA2, where triple-negative breast cancer is used as a criterion together with an age limit < 60. Only one out of six breast cancer patients in our study who carried a pathogenic BRCA1/2 non-founder mutation was older than 60 years of age, but in this case, family cancer history was positive in the study published by Couch et al., 3.1% of triple-negative breast cancer patients older than 60 years and only 1.4% with no family history of breast or ovarian
cancer were diagnosed with BRCA1/2 pathogenic mutation [25]. Therefore, our study results support the current NCCN guidelines for screening all triple-negative breast cancer patients younger than 60 years of age.

In contrast, the application of the upper age limit for triple-negative breast cancer patients of 40 years (Swedish Breast cancer group criteria for screening for mutations in BRCA1 and BRCA2) would miss several BRCA-positive cases in our cohort [26].

Our small study showed the high accuracy of the Manchester scoring system for the prediction of pathogenic non-founder BRCA1/2 mutations in founder mutation-negative patients. Our finding is supported by several other studies performed on the validation of the Manchester scoring system in populations of UK, Germany and South East Asia [13, 27, 28]. However, larger numbers of cases are needed for comprehensive validation of these criteria in the population of Latvia.

Additionally, three out of eight patients tested negative for 26 breast and ovarian cancer susceptibility genes were HER2 positive. According to a recently published study, only 9% of BRCA1-related breast tumours and 13% of BRCA2-related breast tumours were HER2 positive [29]. HER2 positivity is also included in the Manchester scoring system as a BRCA1/2 probability decreasing factor [13].

Ovarian cancer in a personal or family history was documented in three out of seven patients who carried a pathogenic BRCA1/2 non-founder mutation. Additionally, in one case, unknown gynaecological cancer was reported in a paternal aunt. According to recent studies, the presence of ovarian cancer in personal or family history of pathogenic BRCA1 founder-negative breast cancer patients increases the possibility of carrying previously undetected pathogenic BRCA1/2 non-founder mutations [30, 31]. Recently, in a study published by Couch et al., ovarian cancer in family history was documented only in 1 of 54 pathogenic non-BRCA1/2 mutation carriers with triple-negative breast cancer [25].

In our study, no pathogenic mutations were detected in another 24 genes included in the panel. Some previously published studies demonstrated that the rate of pathogenic mutations in non-BRCA1/2 genes ranged from 2.9 to 9.3% [32–35].

Four of the 16 (25%) patients were identified to have a variant of unknown significance (VUS) in BRCA2, RAD50, CDH1 and MRE11. Unfortunately, due to an insufficient sample size in our study, we cannot elaborate upon those results.

Conclusion
A relatively high incidence of pathogenic non-founder BRCA1/2 mutations was observed among patients with triple-negative familial breast cancer in a founder population. The Manchester scoring system predicted the probability of non-founder pathogenic mutations with high accuracy.

Abbreviations
ATM: Ataxia-telangiectasia mutated; BARD1: BRCA1 (Breast Cancer 1) Associated RING Domain 1 gene; BLM: Bloom’s syndrome gene; BRCA1: Breast cancer susceptibility gene 1; BRCA2: Breast cancer susceptibility gene 2; BRIP1: BRCA1-interacting protein 1 gene; CDH: Cadherin-1 gene; cDNA: Complementary Deoxyribonucleic Acid; CHEK2: Checkpoint kinase 2 gene; DNA: Deoxyribonucleic Acid; EPAC1: Epac1 gene; ER: Estrogen receptor; FAM175A: Family with sequence similarity 175A gene; G2: Moderately differentiated; G3: Well differentiated; HER2: Human epidermal growth factor receptor 2; MEN1: multiple endocrine neoplasia type 1; MLH: Mismatch repair 1 gene; MRE11A: MRE11 homolog 11 gene; MXH: Mismatch repair 6 gene; MUTYH: MutY DNA glycosylase; NA: Not applicable; NBN: Nibrin gene; NCCN: National Comprehensive Cancer Network; PALB2: Partner and localizer of BRCA2 gene; PAT: Pathological; PMS2: postmeiotic segregation increased 2; PR: Progesterone receptor; Pten: Phosphatase and tensin homolog gene; RAD50: Human homolog of S. cerevisiae RAD50 gene; RAD51C: RAD51 homolog C; RAD51L: RAD51 homolog L; STK11: serine/threonine kinase 11 gene; TP53: tumor protein p53 gene; USA: United States of America; VUS: Variant of uncertain significance; XRC2C: X-ray repair cross-complementing protein 2

Funding
This work was supported by State Research Program "Biomedicine for the public health (BIOMEDICINE)" project 5 "Personalised cancer diagnostics and treatment effectiveness evaluation".

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
JM, AI and GT analyzed and interpreted the patient data regarding the disease. ES and GP analyzed and interpreted patient data regarding chemotherapy. EM and DB analyzed and interpreted genetic screening results. JM, AI, EM and JG were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by a Medical Ethics Committee of Latvia. Written consent was obtained.

Competing interests
The authors declare that they have no competing interests.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 23 December 2017 Accepted: 23 May 2018
Published online: 05 June 2018

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