Genetic testing in Poland and Ukraine: should comprehensive germline testing of BRCA1 and BRCA2 be recommended for women with breast and ovarian cancer?

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Abstract

**Purpose.** To characterize the spectrum of BRCA1 and BRCA2 pathogenic germline variants in women from south-west Poland and west Ukraine affected with breast and ovarian cancer. Testing in women at high risk of breast and ovarian cancer in these regions is currently mainly limited to founder mutations.

**Methods.** Unrelated women affected with breast and/or ovarian cancer from Poland (n = 337) and Ukraine (n = 123) were screened by targeted sequencing. Excluded from targeted sequencing were 34 Polish women who had previously been identified as carrying a founder mutation in BRCA1. No prior testing had been conducted among the Ukrainian women. Thus, this study screened BRCA1 and BRCA2 in the germline DNA of 426 women in total.

**Results.** We identified 31 and 18 women as carriers of pathogenic/likely pathogenic (P/LP) genetic variants in BRCA1 and BRCA2, respectively. We observed five BRCA1 and eight BRCA2 P/LP variants (13/337, 3.9%) in the Polish women. Combined with the 34/337 (10.1%) founder variants identified prior to this study, the overall P/LP variant frequency in the Polish women was thus 14% (47/337). Among the Ukrainian women, 16/123 (13%) women were identified as carrying a founder mutation and 20/123 (16.3%) were found to carry non-founder P/LP variants (10 in BRCA1 and 10 in BRCA2).

**Conclusions.** These results indicate that genetic testing in women at high risk of breast and ovarian cancer in Poland and Ukraine should not be limited to founder mutations. Extended testing will enhance risk stratification and management for these women and their families.

1. Introduction

Women who carry a pathogenic mutation in BRCA1 or BRCA2 are at increased risk of developing breast and ovarian cancer. Kuchenbaecker et al. estimated the cumulative breast cancer risk to age 80 years to be 72% (95% confidence interval (CI) = 65–79%) and 69% (95% CI = 61–77%) for BRCA1 and BRCA2 pathogenic variant carriers, respectively (Kuchenbaecker et al., 2017). For ovarian cancer, the cumulative cancer risk to age 80 years is estimated to be 44% (95% CI = 36–53%) and 17% (95% CI = 11–25%) for BRCA1 and BRCA2 pathogenic variant carriers, respectively (Kuchenbaecker et al., 2017).

In many countries, genetic testing for BRCA1 and BRCA2 has shown clear clinical utility and validity. Evidence-based best practice guidelines are available to inform the clinical management of women who carry a BRCA1 or BRCA2 pathogenic variant. These guidelines support personalized risk assessment, targeted treatment regimens and informed decision-making about the use of risk-reducing medications, bilateral salpingo-oophorectomy, mammography, risk-reducing mastectomy, magnetic resonance imaging and other screening modalities.

It is well established that, as a consequence of a founder effect, different ethnic and geographical regions can have different BRCA1 and BRCA2 mutation spectra and prevalence rates.
Cybulski et al. recently reported on the mutation spectrum in BRCA1, BRCA2 and other genes associated, or putatively associated, with increased risk of breast cancer in 1018 probands from multiple-case breast cancer families from Poland. In their study, three founder mutations were identified with high prevalence: BRCA1:c.5266dup (20%, 204/1018), BRCA1:c.181T>G (8.3%, 84/1018) and BRCA1:c.4035del (1.5%, 15/1018). Other mutations reported at lower prevalence (≤1.0%) in the 1108 familial breast cancer cases included BRCA1:c.3700–3704del (1.0% 10/1018), BRCA1:c.68_69del (0.9%, 9/1018), BRCA1:c.5251C>T (0.6%, 6/1018) and BRCA1:c.5346G>A (0.5%, 5/1108) (Cybulski et al., 2019). Recurrent mutations were also reported in BRCA2 by Cybulski et al., all at a prevalence of 0.5% or below in the Polish women with familial breast cancer (Cybulski et al., 2019).

The BRCA1 mutations c.5266dupC and c.4035delA have been confirmed by haplotype analysis as founder mutations in Eastern Europe (Hamel et al., 2011; Janavicius et al., 2013). BRCA1: c.181T>G mutation carriers of Polish and Jewish ancestry have been shown to present the same haplotype (Kaufman et al., 2009). Haplotype analysis of BRCA1:c.68_69delAG showed a common haplotype among Ashkenazi Jews (Laitman et al., 2013). Currently, genetic testing in the Polish population mainly relies on testing of the founder mutations. Comprehensive BRCA1 and BRCA2 genetic testing could, however, identify more women with pathogenic variants, thus leading to improved cancer prevention for women at high risk of breast and ovarian cancer.

Participants in this study were unrelated women affected with breast or ovarian cancer from south-west Poland and west Ukraine. The Polish participants had previously been genotyped for BRCA1:c.5266dup, BRCA1:c.181T>G, BRCA1:c.4035del, BRCA1:c.68_69del and BRCA2:c.5946del. Thirty-four women identified as carrying one of these mutations were excluded from further testing in this study. Participants recruited in Ukraine had not undergone prior genetic testing and have thus all been included in the genetic testing reported in this study.

### 2. Materials and methods

#### 2.1. Study participants

The women participating in this study were unrelated women diagnosed with breast and/or ovarian cancer recruited after or during oncological treatment from Wroclaw Medical University, Lower Silesia, Poland, between 2004 and 2008, or Livit State Oncology Regional Treatment and Diagnostic Center, Lviv, Ukraine, between 2004 and 2010, as described previously (Myszka et al., 2018). The Polish cohort consisted of 238 women affected with breast cancer, 95 women affected with ovarian cancer and 4 women affected with breast and ovarian cancer. Of the 242 women with breast cancer, 95 had hereditary breast cancer, 18 had familial breast cancer and 125 were sporadic cases, according to the criteria described by Berliner et al. (2006). Of the 95 Polish women with ovarian cancer, 28 had hereditary ovarian cancer, 10 had familial ovarian cancer and 57 were sporadic ovarian cancer cases. All four women with breast and ovarian cancers met the criteria for hereditary disease. The Polish cohort thus consisted of 337 women, all of whom had previously been genotyped for four mutations in BRCA1 (c.5266dup, c.181T>G, c.4035del, c.68_69del) (Table 1) and one mutation in BRCA2 (c.5946delT). Thirty-four women were identified as carriers of one of these BRCA1 founder mutations and were thus not included in the targeted sequence screening described in this study. No BRCA2 c.5946delT carrier was observed.

The Ukrainian cohort consisted of 112 women with breast cancer, 10 women with ovarian cancer and 1 woman with breast and ovarian cancer. Seventy-three women affected with breast cancer met the hereditary cancer criteria and 38 women met the familial cancer criteria. For one Ukrainian participant with breast cancer, insufficient information was available to classify her cancer. Of the women with ovarian cancer, six had hereditary ovarian cancer and four had familial ovarian cancer. The woman with breast and ovarian cancer met the hereditary cancer criteria. There had been no previous testing for mutations in BRCA1 and BRCA2 conducted in the Ukrainian participants.

All participants provided informed consent for participation in this research programme, which was approved by the Commission of Bioethics of the Institute of Hereditary Pathology of the National Academy of Medical Sciences of Ukraine, the Ethics Committee of Wroclaw Medical University (Poland), the Ethics Committee of the University of Rzeszow (Poland) and the University of Melbourne Human Research Ethics Committee (Australia).

#### 2.2. Mutation screening

Amplicon-based massively parallel sequencing of the protein-coding regions and proximal intron–exon junctions of BRCA1 (NM_0007294.3) and BRCA2 (NM_000059.3) was performed using lymphocyte-derived germline DNA and the Hi-Plex protocol (Nguyen-Dumont et al., 2015). All oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA, USA). Gene-specific primers and adapter primers were purified to standard desalting and high-performance liquid chromatography grade, respectively. All oligonucleotide sequences are available upon request. Massively parallel sequencing (150 bp paired-end) was performed on the MiSeq system (Illumina, San Diego, CA, USA). Mapping to the human reference build GRCh37 was performed using bwa-mem 0.7.17 (Li & Durbin, 2009).
Table 2. Pathogenic and likely pathogenic\(^a\) BRCA1 and BRCA2 mutations carriers identified in 460 women affected with breast or ovarian cancer in south-west Poland and west Ukraine.

| Gene       | Variant type      | HGVS\(_c^{b}\)          | HGVS\(_p^{b}\)          | Classification\(^c\) | Number of carriers | Total | Pol\(^d\) | Ukr\(^d\) | BC\(^e\) | OC\(^e\) |
|------------|-------------------|-------------------------|-------------------------|----------------------|-------------------|-------|----------|----------|---------|---------|
| **BRCA1**  | Nonsense          | c.5251C>T              | p.Arg1751Ter            | Pathogenic           | 1                 | 1     | 1        | 0        | 1       | 0       |
|            |                   | c.5346G>A              | p.Thr1782Ter            | Pathogenic           | 1                 | 1     | 0        | 1        | 0       | 0       |
|            | Frameshift        | c.68_69del\(^f\)       | p.Glu23ValfsTer17       | Pathogenic           | 3                 | 1     | 2        | 2        | 1       | 1       |
|            |                   | c.374dup               | p.Gln126ProfsTer16      | No data              | 1                 | 1     | 0        | 0        | 1       | 1       |
|            |                   | c.843_846del           | p.Ser282TyrfsTer15      | Pathogenic           | 1                 | 1     | 0        | 1        | 0       | 0       |
|            |                   | c.844_850dup           | p.Gln284LeufsTer5       | Pathogenic           | 1                 | 0     | 1        | 0        | 0       | 1       |
|            |                   | c.1510del              | p.Arg504ValfsTer28      | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.1612_1616del         | p.Gln538GlyfsTer11      | Pathogenic           | 1                 | 1     | 0        | 0        | 1       | 1       |
|            |                   | c.2217dup\(^g\)       | p.Val740SerfsTer3       | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.2291_2292del         | p.Val764GlyfsTer3       | No data              | 1                 | 0     | 0        | 1        | 1       | 0       |
|            |                   | c.4035del\(^h\)       | p.Glu1346LysfsTer20     | Pathogenic           | 3                 | 2     | 2        | 0        | 0       | 2       |
|            |                   | c.5030_5033del         | p.Thr1677IlefsTer2      | Pathogenic           | 3                 | 0     | 3        | 2        | 1       | 1       |
|            |                   | c.5177_5180del         | p.Arg1726LysfsTer3      | Pathogenic           | 2                 | 0     | 2        | 2        | 0       | 0       |
|            |                   | c.5266dup\(^h\)       | p.Gln1756ProfsTer74     | Pathogenic           | 35                | 24    | 11       | 25       | 12      | 12      |
|            | Splice donor      | c.4357+1G>C            | –                       | No data              | 1                 | 0     | 1        | 1        | 0       | 0       |
|            | Missense          | c.181T>G\(^g\)        | p.Cys61Gly              | Pathogenic           | 10                | 7     | 4        | 3        | 6       | 6       |
| **BRCA2**  | Nonsense          | c.3075_3076delinsTT    | p.Lys1025_Lys1026delinsAsnTer | Pathogenic         | 3                 | 3     | 0        | 0        | 3       | 3       |
|            |                   | c.5857G>T              | p.Glu1953Ter            | Pathogenic           | 1                 | 1     | 0        | 1        | 0       | 0       |
|            |                   | c.7721G>A              | p.Thr2574Ter            | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.8623G>T              | p.Glu2875Ter            | No data              | 1                 | 1     | 0        | 1        | 0       | 0       |
|            | Frameshift        | c.2945del              | p.Ile982AsnfsTer9       | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.5205_5208del         | p.Gln1736IlefsTer4      | Pathogenic           | 1                 | 1     | 0        | 1        | 0       | 0       |
|            |                   | c.6315_6318del         | p.Pro2107ValfsTer11     | No data              | 1                 | 1     | 0        | 1        | 0       | 0       |
|            |                   | c.6405_6409del         | p.Asn2135LysfsTer3      | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.6408_6414del         | p.Asn2137LysfsTer29     | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.7069_7070del         | p.Leu2357ValfsTer2      | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.9097dup              | p.Thr3033AsnfsTer11     | Pathogenic           | 1                 | 0     | 1        | 1        | 0       | 0       |
|            |                   | c.9253dup              | p.Thr3085AsnfsTer26     | Pathogenic           | 1                 | 1     | 0        | 1        | 0       | 0       |
|            |                   | c.10095delinsGAATTATATCT| p.Ser3366AsnfsTer4      | Not yet reviewed     | 1                 | 0     | 1        | 1        | 0       | 0       |
|            | Splice donor      | c.475+1G>T             | –                       | No data              | 3                 | 0     | 3        | 3        | 0       | 0       |

\(^a\) Genetic variants in BRCA1 and BRCA2 that are classified as pathogenic by the expert panel Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) (Spurdle et al., 2012) as reported on the BRCA Exchange portal (http://brcaexchange.org), unreported truncating variants and variants occurring in consensus splice sites.

\(^b\) Variant nomenclature based on +1 as A of ATG start codon, according to the Human Genome Variation Society (HGVS), HGVS\(_c\) for coding DNA and HGVS\(_p\) for protein variants.

\(^c\) Classification according to the ENIGMA expert panel (Spurdle et al., 2012), available from the BRCA Exchange portal (http://brcaexchange.org).

\(^d\) Pol = Polish, Ukr = Ukrainian.

\(^e\) BC = breast cancer; OC = ovarian cancer. Some women were diagnosed with both BC and OC.

\(^f\) Transcript sequences are BRCA1: NM_007294.3 and BRCA2: NM_00059.3.

\(^g\) Founder mutation - identified via targeted sequencing (this study) or via Sanger sequencing (prior testing) (Table 1).

\(^h\) No DNA was available for validation by Sanger sequencing.
Variant calling was performed using the Java version of VarDict in single-sample, amplicon mode (Lai et al., 2016).

### 2.3. Annotation and classification of variants

Variants were annotated using VEP (v.90) and loaded into GEMINI, according to the authors’ recommendations (Paila et al., 2013; McLaren et al., 2016). Classification of genetic variants in BRCA1 and BRCA2 was then performed in accordance with the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium’s recommendations (Spurdle et al., 2012). Pathogenicity calls were retrieved from the BRCA Exchange portal (http://brcaexchange.org; accessed 17 June 2019). When no information was available (i.e., variants reported as ‘no data’, ‘not available’ or ‘not yet reviewed’), variants were classified in accordance with the ENIGMA criteria. Specifically, truncating variants (i.e., nonsense, frameshift insertions or deletions and variants occurring in consensus splice sites) were classified as pathogenic/likely pathogenic (P/LP) by the expert panel Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) (Spurdle et al., 2012) as reported on the BRCA Exchange portal (http://brcaexchange.org), unreported truncating variants and variants occurring in consensus splice sites.

| Table 3. Clinical characteristics of pathogenic and likely pathogenic BRCA1 and BRCA2 variant carriers, identified from 460 women affected with breast or ovarian cancer in south-west Poland and west Ukraine. |
|---|
| Cohort | Cancer type | Cancer classification | Studied cases | Carriers of a founder mutation | Carriers of a non-founder P/LP variant | Carriers of any P/LP variant |
|---|---|---|---|---|---|---|
| Polish | Breast | Hereditary | 95 | 11 (11.6%) | 4 (4.2%) | 15 (15.8%) |
| | | Familial | 18 | 1 (5.5%) | 1 (5.5%) | 2 (11.1%) |
| | | Sporadic | 125 | 1 (0.8%) | 2 (1.6%) | 3 (2.4%) |
| | | Total | 238 | 13 (5.5%) | 7 (2.5%) | 20 (8.4%) |
| | Ovarian | Hereditary | 28 | 17 (60.7%) | 2 (7.1%) | 19 (67.8%) |
| | | Familial | 10 | 0 | 3 (30%) | 3 (30%) |
| | | Sporadic | 57 | 1 (1.7%) | 0 | 1 (1.7%) |
| | | Total | 95 | 18 (18.9%) | 5 (5.3%) | 23 (24.2%) |
| | Breast and ovarian | Hereditary | 4 | 3 (75%) | 1 (25%) | 4 (100%) |
| | | Total | 337 | 34 (10.1%) | 13 (3.9%) | 47 (13.9%) |
| Ukrainian | Breast | Hereditary | 73 | 12 (16.4%) | 14 (19.2%) | 26 (35.1%) |
| | | Familial | 38 | 2 (5.3%) | 4 (10.5%) | 6 (15.8%) |
| | | Sporadic | 0 | – | – | – |
| | | Unknown | 1 | 0 | 1 | 1 |
| | | Total | 112 | 14 (12.5%) | 19 (15.4%) | 33 (29.5%) |
| | Ovarian | Hereditary | 6 | 1 (16.7%) | 1 (16.7%) | 2 (33.3%) |
| | | Familial | 4 | 0 | 0 | 0 |
| | | Sporadic | 0 | – | – | – |
| | | Total | 10 | 1 (0.9%) | 1 (0.9%) | 2 (18.2%) |
| | Breast and ovarian | Hereditary | 1 | 1 | 0 | 1 (100%) |
| | | Total | 123 | 16 (13%) | 20 (16.3%) | 36 (29.3%) |
| Polish and Ukrainian | Breast | Hereditary | 168 | 23 (13.7%) | 18 (10.7%) | 41 (24.4%) |
| | | Familial | 56 | 3 (5.4%) | 5 (8.9%) | 8 (14.3%) |
| | | Sporadic | 125 | 1 (0.8%) | 2 (1.6%) | 3 (2.4%) |
| | | Unknown | 1 | 0 | 1 | 1 |
| | | Total | 350 | 27 (7.7%) | 26 (7.4%) | 53 (15.1%) |
| | Ovarian | Hereditary | 34 | 18 (52.9%) | 3 (8.8%) | 21 (61.8%) |
| | | Familial | 14 | 0 | 3 (21.4%) | 3 (21.4%) |
| | | Sporadic | 57 | 1 (1.8%) | 0 | 1 (1.8%) |
| | | Total | 105 | 19 (18.1%) | 6 (5.7%) | 25 (23.8%) |
| | Breast and ovarian | Hereditary | 5 | 4 (80%) | 1 (20%) | 5 (100%) |
| | | Total | 460 | 50 (10.9%) | 33 (7.2%) | 83 (18.0%) |

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*Genetic variants in BRCA1 and BRCA2 that are classified as pathogenic/likely pathogenic (P/LP) by the expert panel Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) (Spurdle et al., 2012) as reported on the BRCA Exchange portal (http://brcaexchange.org), unreported truncating variants and variants occurring in consensus splice sites.

*Classification as per Berliner et al. (2007).

*Founder mutations in Table 1.
junctions) were classified as pathogenic/likely pathogenic (P/LP). Missense substitutions that had not been reviewed by ENIGMA were classified as variants of unknown significance (VUS).

3. Results
Targeted sequencing identified a total of 31 and 18 women as carriers of a P/LP variant in BRCA1 and BRCA2, respectively (Tables 2 & 3). The clinical characteristics of these women are available in Supplementary Table S1.

The 49 P/LP variants with a variant allele fraction >0.2 and total depth ≥10× were verified by Sanger sequencing, except for the case that was identified as carrying the BRCA1 variant NM_007294.3: c.4357+1G>C, as there was insufficient DNA available. That position was covered by 1302 reads, and the variant allele fraction was 0.53 and thus highly unlikely to be a sequencing artefact.

### Table 4. Variants of unknown significance in BRCA1 and BRCA2 identified by Hi-Plex targeted sequencing, in 426 women affected with breast or ovarian cancer in south-west Poland and west Ukraine.

| Gene   | HGVS_c | HGVS_p | MAF ExAC | CADD PHRED score | REVEL score | Pol | Ukr | BC | OC |
|--------|--------|--------|----------|------------------|-------------|-----|-----|----|----|
| BRCA1  | c.5047G>C | p.Glu1683Gln | 23.5 | 0.525 | 1 | 0 | 0 | 1 |
|        | c.5005G>T | p.Ala1669Ser | 5.53E-05 | 25.5 | 0.704 | 1 | 0 | 1 | 0 |
|        | c.4730C>A | p.Ser1577Tyr | 3.349 | 0.574 | 1 | 0 | 1 | 0 |
|        | c.4036G>A | p.Glu1346Lys | 7.36E-05 | 25.3 | 0.546 | 0 | 1 | 1 | 0 |
|        | c.3092T>G | p.Ile1031Ser | 10.5 | 0.551 | 1 | 0 | 0 | 1 |
|        | c.2686A>T | p.Ser938Cys | 22.4 | 0.44 | 1 | 0 | 0 | 1 |
|        | c.1441C>G | p.Leu481Val | 18.7 | 0.668 | 2 | 1 | 0 | 3 |
|        | c.429A>C | p.Glu143Asp | 1.84E-05 | 22.5 | 0.57 | 1 | 0 | 1 | 0 |
|        | c.358G>A | p.Aspl20AASN | 23.4 | 0.384 | 1 | 0 | 1 | 0 |
|        | c.116G>A | p.Cys39Tyr | 37 | 0.932 | 1 | 0 | 1 | 0 |
| BRCA2  | c.353G>A | p.Arg118His | 9.59E-05 | 9.169 | 0.334 | 2 | 0 | 2 | 0 |
|        | c.955A>G | p.Asn319Asp | 2.463 | 0.216 | 1 | 0 | 1 | 0 |
|        | c.1040A>C | p.Gln347Pro | 7.396 | 0.203 | 0 | 1 | 1 | 0 |
|        | c.1292C>T | p.Thr431Ile | 0.115 | 0.26 | 1 | 0 | 1 | 0 |
|        | c.1514T>C | p.Ile505Thr | 0.00108 | 5.755 | 0.216 | 1 | 0 | 1 | 0 |
|        | c.1556G>C | p.Ser513Thr | 1.52 | 0.244 | 1 | 0 | 1 | 0 |
|        | c.1645A>G | p.Lys549Glu | 4.923 | 0.133 | 1 | 0 | 1 | 0 |
|        | c.1792A>G | p.Thr598Ala | 0.00371 | 6.88 | 0.236 | 0 | 1 | 1 | 0 |
|        | c.2153A>C | p.Glu718Ala | 8.074 | 0.219 | 0 | 1 | 1 | 0 |
|        | c.2803G>A | p.Asp935Asn | 0.000832 | 1.58 | 0.092 | 3 | 1 | 4 | 0 |
|        | c.3515C>G | p.Ser1172Thr | 13.93 | 0.195 | 1 | 0 | 0 | 1 |
|        | c.4696A>G | p.Thr1566Ala | 0.683 | 0.229 | 1 | 0 | 0 | 1 |
|        | c.5479A>G | p.Ile1827Val | 0.356 | 0.175 | 1 | 0 | 1 | 0 |
|        | c.5737T>C | p.Cys1913Arg | 1.84E-05 | 0.993 | 0.287 | 1 | 0 | 1 | 0 |
|        | c.6317T>C | p.Leu2106Pro | 0.00013 | 15.92 | 0.076 | 1 | 0 | 1 | 0 |
|        | c.6455C>A | p.Glu2152Tyr | 0.000468 | 14.74 | 0.512 | 1 | 0 | 0 | 1 |
|        | c.7994A>G | p.Asp2665Gly | 0.000168 | 32 | 0.801 | 1 | 0 | 1 | 0 |
|        | c.8182G>A | p.Val2728Ile | 0.00326 | 1.787 | 0.462 | 2 | 1 | 2 | 1 |
|        | c.9038C>T | p.Thr3013Ile | 0.000353 | 10.91 | 0.368 | 0 | 1 | 1 | 0 |
|        | c.9371A>T | p.Asn3124Ile | 1.84E-05 | 28.2 | 0.828 | 0 | 1 | 0 | 1 |

a Missense substitutions in BRCA1 and BRCA2 that are present at less than 1% in ExAC, that have not been reviewed yet or are classified as variants of unknown significance on BRCA Exchange by the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) expert panel (Spurdle et al., 2012).

b Variant nomenclature based on +1 as A of ATG start codon, according to the Human Genome Variation Society (HGVS), HGVS_c for coding DNA and HGVS_p for protein variants.

c ExAC non-Finnish European population minus The Cancer Genome Atlas (Lek et al., 2016).

d CADD (Kircher et al., 2014); REVEL (Ioannidis et al., 2016).

e Pol = Polish; Ukr = Ukrainian.

f BC = breast cancer; OC = ovarian cancer.

g Transcript sequences are BRCA1: NM_007294.3 and BRCA2: NM_00059.3.

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The Polish women participating in this study were previously genotyped for mutations in BRCA1 and BRCA2. The prevalence of BRCA1 founder mutations (c.1566dup, c.181T>G, c.405del, c.68_69del) identified prior to this study was 10.1% (34/337) (Tables 2 & 3). Targeted sequencing did not identify any additional carrier of the mutations previously tested. We detected five carriers of a non-founder P/LP BRCA1 variant and eight carriers of a P/LP BRCA2 variant (3.9% combined prevalence of non-founder mutations) (Table 3).

Among the Ukrainian women, the prevalence of founder mutations was 13% (16/123) (Table 3). We identified 11 carriers of BRCA1:c.5266dup, 3 carriers of BRCA1:c.181T>G and 2 carriers of BRCA1:c.68_69del. We did not observe BRCA1:c.405del in the Ukrainian cohort. There were 10/123 carriers of a P/LP variant in BRCA1 and 10/123 carriers of a P/LP variant in BRCA2 (16.3% combined prevalence of non-founder P/LP variants) (Table 3). BRCA1:c.5030_5033del was observed in three unrelated women with breast cancer from Ukraine, all of whom having a family history of cancer (one had a sister with breast cancer and two had mothers with ovarian cancer) (Supplementary Table S1).

Targeted sequencing also identified 11 and 27 rare missense substitutions in BRCA1 and BRCA2, respectively, which are currently classified as VUS (Table 4).

4. Discussion

The overall prevalence of P/LP BRCA1 and BRCA2 variants in the Polish women in this study was 13.9% (47/337) (Table 3). Of these, over a quarter were non-founder mutations (27.7%, 13/47). Our findings are consistent with a recent report by Kowalik et al., who screened for BRCA1 and BRCA2 in Polish women who qualified for genetic testing and identified 161 P/LP variants, 64% (103/161) of which were founder mutations and 24.8% (40/161) of which were non-founder P/LP variants (Kowalik et al., 2018).

To the best of our knowledge, our study is the first to report the mutation screening of the complete coding regions of BRCA1 and BRCA2 in Ukrainian women affected with breast and/or ovarian cancer. In the Ukrainian women, the overall prevalence of P/LP BRCA1 and BRCA2 variants was 29.3% (36/123) (Table 3). There was no difference in the proportion of founder and non-founder mutations (44.4%, 16/36, and 55.5%, 20/36, respectively).

The higher overall prevalence of P/LP variants observed in the Ukrainian participants (29.3% versus 13.9% in the Polish participants) is likely due to differences in selection criteria. Among the Ukrainian women, 99% (122/123) of participants met the criteria for hereditary (65%), 80/123) or familial cancer (34%, 42/123), whereas the Polish cohort included a majority of sporadic cancers (54%, 182/337) (Berliner et al., 2007). Hereditary and familial cancers in the Polish cohort accounted for only 38% (127/337) and 8% (28/337) of all participants, respectively (Table 3).

Overall, non-founder variants represented 43.9% (18/41), 62.5% (5/8) and 66.7% (2/3) of all P/LP variants observed in women affected with hereditary, familial and sporadic breast cancer, respectively (Table 3). In women affected with ovarian cancer, non-founder variants represented 14.3% (3/21) and 100% (3/3) of all P/LP variants observed in hereditary and familial cancer, respectively. Expanding genetic testing beyond genotyping for founder variants has thus enabled us to identify 33 women carrying a clinically actionable variant who will be able to receive personalized clinical advice for themselves and their family. These results support the utility of comprehensive gene testing of BRCA1 and BRCA2 in Polish and Ukrainian patients, especially in women with hereditary and familial cancers.

In addition to P/LP variants, our study identified 38/427 carriers (7.7%) of rare missense variants of unknown clinical significance. Missense substitutions may result in variant proteins with functions ranging from normal to severely altered. Therefore, this group of variants is highly likely to be made up of variants with differing levels of associated risks (including none). There are substantial ongoing efforts by ENIGMA to classify VUS in BRCA1 and BRCA2 (Vallee et al., 2012). However, methods such as calibrated functional assays are essential for enabling variant classification, and we currently lack the evidence base from which to interpret and report most missense substitutions.

Our study applied an amplicon-based targeted sequencing methodology that is not designed to detect copy number variations (CNVs). The inherent nature of targeted sequencing poses substantial challenges for the detection of these variants. A number of software tools for CNV detection from targeted sequencing data have recently emerged to try to address this gap (Li et al., 2012; Ellingford et al., 2017; Kerkhof et al., 2017). However, they are developed for probe-based enrichment rather than amplicon-based methodologies and, to date, multiplex ligation-dependent probe amplification remains the gold standard method for the clinical identification of such events.

5. Conclusions

Our results show that performing comprehensive genetic testing of BRCA1 and BRCA2 instead of testing for founder mutations only will be highly valuable in Poland and Ukraine. Massively parallel sequencing is an effective way of performing comprehensive genetic testing of BRCA1 and BRCA2 that will increase the detection rate of clinically actionable variants and thus enhance risk assessment and management for these women and their families.

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Author contributions. TN-D contributed to the study design, performed the sequencing data analyses and drafted the manuscript. PK, MMS, RS HA, AgS, KP and NK collected the study sample and provided clinical information. JAS and DT contributed to the processing of the sequencing data. FH contributed to the genetic testing, DJP and BIP designed the Hi-Plex technology for the panel testing. PK, AJS and HT prepared the DNA samples. AM performed the genetic testing and validations. MCS and AM were responsible for the overall study design and contributed to the data analysis and drafting of the manuscript.

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Conflicts of interest. The authors declare that they have no conflicts of interest.
Availability of data and material. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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