

## Research Paper

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# 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 or 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.

**Table 1.** Reported prevalence of *BRCA1* founder mutations in the Polish population.

| HGVS_cDNA <sup>a</sup> | HGVS_p                | BIC <sup>b</sup> | MAF ExAC <sup>c</sup> | MAF Polish population                        | References   |
|------------------------|-----------------------|------------------|-----------------------|--|--|
| c.5266dup              | p.(Gln1756ProfsTer74) | 5382insC         | 0.00016               | $1.7 \times 10^{-3}$ to $3.5 \times 10^{-3}$ | Gorski <i>et al.</i> (2005); Brozek <i>et al.</i> (2011) |
| c.181T>G               | p.(Cys61Gly)          | 300T>G           | 5.64e-05              | $0.5 \times 10^{-3}$ to $0.8 \times 10^{-3}$ | Gorski <i>et al.</i> (2005); Brozek <i>et al.</i> (2011) |
| c.4035del              | p.(Glu1346LysfsTer20) | 4153delA         | 3.68e-05              | $0.2 \times 10^{-3}$                         | Gorski <i>et al.</i> (2005)                              |
| c.68_69del             | p.(Glu23ValfsTer17)   | 185delAG         | 0.000406              | $<0.1 \times 10^{-3}$                        | Brozek <i>et al.</i> (2011)                              |

<sup>a</sup> Variant nomenclature according to the Human Genome Variation Society (HGVS). Transcript sequences is *BRCA1*: NM\_007294.3.

<sup>b</sup> Variant nomenclature according to the Breast Cancer Information Core (BIC) (<https://research.nhgri.nih.gov/bic>).

<sup>c</sup> Minor allele frequency (MAF) in the ExAC database, in the non-Finnish European population minus The Cancer Genome Atlas (Lek *et al.*, 2016).

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 ( $\leq 1.0\%$ ) 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 more 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 Lviv 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.* (2007). 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\_007294.3) 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,

**Table 2.** Pathogenic and likely pathogenic<sup>a</sup> *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 <sup>b</sup>     | HGVS_p <sup>b</sup>           | Classification <sup>c</sup> | Number of carriers |                  |                  |                 |                 |
|---------------------------|--------------------------|-------------------------|-------------------------------|-----------------------------|--------------------|------------------|------------------|-----------------|-----------------|
|                           |                          |                         |                               |                             | Total              | Pol <sup>d</sup> | Ukr <sup>d</sup> | BC <sup>e</sup> | OC <sup>e</sup> |
| <i>BRCA1</i> <sup>f</sup> | Nonsense                 | c.5251C>T               | p.Arg1751Ter                  | Pathogenic                  | 1                  | 1                | 0                | 1               | 0               |
|                           |                          | c.5346G>A               | p.Trp1782Ter                  | Pathogenic                  | 1                  | 1                | 0                | 1               | 0               |
|                           | Frameshift               | c.68_69del <sup>g</sup> | p.Glu23ValfsTer17             | Pathogenic                  | 3                  | 1                | 2                | 2               | 1               |
|                           |                          | c.374dup                | p.Gln126ProfsTer16            | No data                     | 1                  | 1                | 0                | 0               | 1               |
|                           |                          | c.843_846del            | p.Ser282TyrfstTer15           | Pathogenic                  | 1                  | 1                | 0                | 1               | 0               |
|                           | c.844_850dup             | p.Gln284LeufsTer5       | Pathogenic                    | 1                           | 0                  | 1                | 0                | 1               |                 |
|                           | c.1510del                | p.Arg504ValfsTer28      | Pathogenic                    | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.1612_1616del           | p.Gln538GlyfsTer11      | Pathogenic                    | 1                           | 1                  | 0                | 0                | 1               |                 |
|                           | c.2217dup <sup>h</sup>   | p.Val740SerfsTer3       | Pathogenic                    | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.2291_2292del           | p.Val764GlyfsTer3       | No data                       | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.4035delA <sup>g</sup>  | p.Glu1346LysfsTer20     | Pathogenic                    | 2                           | 2                  | 0                | 0                | 2               |                 |
|                           | c.5030_5033del           | p.Thr1677IlefsTer2      | Pathogenic                    | 3                           | 0                  | 3                | 2                | 1               |                 |
|                           | c.5177_5180del           | p.Arg1726LysfsTer3      | Pathogenic                    | 2                           | 0                  | 2                | 2                | 0               |                 |
|                           | c.5266dup <sup>g</sup>   | p.Gln1756ProfsTer74     | Pathogenic                    | 35                          | 24                 | 11               | 25               | 12              |                 |
|                           | Splice donor             | c.4357+1G>C             | -                             | No data                     | 1                  | 0                | 1                | 1               | 0               |
| Missense                  | c.181T>G <sup>g</sup>    | p.Cys61Gly              | Pathogenic                    | 10                          | 7                  | 3                | 4                | 6               |                 |
| <i>BRCA2</i> <sup>f</sup> | Nonsense                 | c.3075_3076delinsTT     | p.Lys1025_Lys1026delinsAsnTer | Pathogenic                  | 3                  | 3                | 0                | 0               | 3               |
|                           |                          | c.5857G>T               | p.Glu1953Ter                  | Pathogenic                  | 1                  | 1                | 0                | 1               | 0               |
|                           |                          | c.7721G>A               | p.Trp2574Ter                  | Pathogenic                  | 1                  | 0                | 1                | 1               | 0               |
|                           | c.8623G>T                | p.Glu2875Ter            | No data                       | 1                           | 1                  | 0                | 1                | 0               |                 |
|                           | Frameshift               | c.2945del               | p.Ile982AsnfsTer9             | Pathogenic                  | 1                  | 0                | 1                | 1               | 0               |
|                           |                          | c.5205_5208del          | p.Gln1736IlefsTer4            | Pathogenic                  | 1                  | 1                | 0                | 1               | 0               |
|                           |                          | c.6315_6318del          | p.Pro2107ValfsTer11           | No data                     | 1                  | 1                | 0                | 1               | 0               |
|                           | c.6405_6409del           | p.Asn2135LysfsTer3      | Pathogenic                    | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.6408_6414del           | p.Asn2137LysfsTer29     | Pathogenic                    | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.7069_7070del           | p.Leu2357ValfsTer2      | Pathogenic                    | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.9097dup                | p.Thr3033AsnfsTer11     | Pathogenic                    | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | c.9253dup                | p.Thr3085AsnfsTer26     | Pathogenic                    | 1                           | 1                  | 0                | 1                | 0               |                 |
|                           | c.10095delinsGAATTATATCT | p.Ser3366AsnfsTer4      | Not yet reviewed              | 1                           | 0                  | 1                | 1                | 0               |                 |
|                           | Splice donor             | c.475+1G>T              | -                             | No data                     | 3                  | 0                | 3                | 3               | 0               |

<sup>a</sup> 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.

<sup>b</sup> 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.

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

<sup>d</sup> Pol = Polish; Ukr = Ukrainian.

<sup>e</sup> BC = breast cancer; OC = ovarian cancer. Some women were diagnosed with both BC and OC.

<sup>f</sup> Transcript sequences are *BRCA1*: NM\_007294.3 and *BRCA2*: NM\_000593.3.

<sup>g</sup> Founder mutation – identified via targeted sequencing (this study) or via Sanger sequencing (prior testing) (Table 1).

<sup>h</sup> No DNA was available for validation by Sanger sequencing.

**Table 3.** Clinical characteristics of pathogenic and likely pathogenic<sup>a</sup> *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 <sup>b</sup> | Studied cases     | Carriers of a founder mutation <sup>c</sup> | Carriers of a non-founder P/LP variant <sup>a</sup> | Carriers of any P/LP variant <sup>a</sup> |
|----------------------------|-------------------------|------------------------------------|-------------------|---|---|---|
| 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%)                                  |
|                            |                         | <b>Total</b>                       | <b>238</b>        | <b>13 (5.5%)</b>                            | <b>7 (2.5%)</b>                                     | <b>20 (8.4%)</b>                          |
|                            | 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%)                                  |
|                            |                         | <b>Total</b>                       | <b>95</b>         | <b>18 (18.9%)</b>                           | <b>5 (5.3%)</b>                                     | <b>23 (24.2%)</b>                         |
|                            | Breast and ovarian      | Hereditary                         | 4                 | 3 (75%)                                     | 1 (25%)   | 4 (100%)                                  |
|                            | <b>All Polish cases</b> |                                    |                   | <b>337</b>                                  | <b>34 (10.1%)</b>                                   | <b>13 (3.9%)</b>                          |
| 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   |
|                            | <b>Total</b>            | <b>112</b>                         | <b>14 (12.5%)</b> | <b>19 (15.4%)</b>                           | <b>33 (29.5%)</b>                                   |   |
|                            | Ovarian                 | Hereditary                         | 6                 | 1 (16.7%)                                   | 1 (16.7%)   | 2 (33.3%)                                 |
|                            |                         | Familial                           | 4                 | 0   | 0   | 0   |
|                            |                         | Sporadic                           | 0                 | –   | –   | –   |
|                            |                         | <b>Total</b>                       | <b>10</b>         | <b>1 (0.9%)</b>                             | <b>1 (0.9%)</b>                                     | <b>2 (18.2%)</b>                          |
|                            | Breast and ovarian      | Hereditary                         | 1                 | 1   | 0   | 1 (100%)                                  |
| <b>All Ukrainian cases</b> |                         |                                    | <b>123</b>        | <b>16 (13%)</b>                             | <b>20 (16.3%)</b>                                   | <b>36 (29.3%)</b>                         |
| 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   |
|                            | <b>Total</b>            | <b>350</b>                         | <b>27 (7.7%)</b>  | <b>26 (7.4%)</b>                            | <b>53 (15.1%)</b>                                   |   |
|                            | 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%)                                  |
|                            |                         | <b>Total</b>                       | <b>105</b>        | <b>19 (18.1%)</b>                           | <b>6 (5.7%)</b>                                     | <b>25 (23.8%)</b>                         |
|                            | Breast and ovarian      | Hereditary                         | 5                 | 4 (80%)                                     | 1 (20%)   | 5 (100%)                                  |
| <b>Total cases</b>         |                         |                                    | <b>460</b>        | <b>50 (10.9%)</b>                           | <b>33 (7.2%)</b>                                    | <b>83 (18.0%)</b>                         |

<sup>a</sup> 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.

<sup>b</sup> Classification as per Berliner *et al.* (2007).

<sup>c</sup> Founder mutations in Table 1.

2009). 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

**Table 4.** Variants of unknown significance<sup>a</sup> 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 <sup>b</sup> | HGVS_p <sup>b</sup> | MAF ExAC <sup>c</sup> | CADD <sup>d</sup> PHRED score | REVEL <sup>d</sup> score | Number of carriers |                  |                 |                 |
|---------------------------|---------------------|---------------------|-----------------------|-------------------------------|--------------------------|--------------------|------------------|-----------------|-----------------|
|                           |                     |                     |                       |                               |                          | Pol <sup>e</sup>   | Ukr <sup>e</sup> | BC <sup>f</sup> | OC <sup>f</sup> |
| <i>BRCA1</i> <sup>g</sup> | 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        | 1.85E-05              | 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.Ser896Cys         |                       | 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.Asp120Asn         |                       | 23.4                          | 0.384                    | 1                  | 0                | 1               | 0               |
|                           | c.116G>A            | p.Cys39Tyr          |                       | 37                            | 0.932                    | 1                  | 0                | 1               | 0               |
| <i>BRCA2</i> <sup>g</sup> | 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.Ser519Thr         |                       | 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.Ser1172Trp        |                       | 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.Ser2152Tyr        | 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               |

<sup>a</sup> 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).

<sup>b</sup> 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.

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

<sup>d</sup> CADD (Kircher *et al.*, 2014); REVEL (Ioannidis *et al.*, 2016).

<sup>e</sup> Pol = Polish; Ukr = Ukrainian.

<sup>f</sup> BC = breast cancer; OC = ovarian cancer.

<sup>g</sup> Transcript sequences are *BRCA1*: NM\_007294.3 and *BRCA2*: NM\_00059.3.

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  $\geq 10\times$  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.

The Polish women participating in this study were previously genotyped for mutations in *BRCA1* and *BRCA2*. The prevalence of *BRCA1* founder mutations (c.5266dup, c.181T>G, c.4035del, 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.4035del 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.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0016672320000075>

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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 BJP designed the Hi-Plex technology for the panel testing. PK, AIS 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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