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Different association of *gBRCA1* and *gBRCA2* variants with HER2-low status in invasive breast cancer: findings from a Ukrainian study

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HER2-low breast cancer (BC) representing about 40–55% of all BC has emerged as a targetable entity. However, little is known about the link between germline *BRCA1/2* mutations (*gBRCA1/2*) and HER2-low status in BC, especially in Ukrainian population. This study aims to elaborate on the rates of HER2-low status among patients with sporadic and *BRCA1/2*-associated hereditary BC in the Ukrainian population and investigate the relationship between *gBRCA1/2* and HER2 status. This was a retrospective multicenter cross-sectional study on 1412 cases of BC. HER2 status was assessed according to ASCO-CAP Guidelines. All patients underwent germline NGS testing to detect SNV and indel variants in *BRCA1* and *BRCA2* genes. Overall, *gBRCA1/2* genetic variants were found in 212 (15.0%) patients with BC. *gBRCA1* variants were associated mostly with TNBC molecular subtype, while *gBRCA2* mutations were linked to Luminal-like BC. The majority (343 of 436; 78.7%) of HER2-low BC was associated with luminal-like BC ($P < 0.001$). We also found significant relationships between *gBRCA1/2* and HER2 status ($P = 0.006$). There were 837 HER2-zero (59.3%), 436 HER2-low (30.9%) and 139 HER2-positive (9.8%) BC. More than 70% of patients with *gBRCA1* were HER2-negative. Alternatively, *gBRCA2* cases possessed a higher rate of HER-low BC status (37.5%) as compared to WT (31.2%) and *gBRCA1*-associated *BC (25.7%)*. In conclusion, *gBRCA1* and *gBRCA2* variants differed in their association with breast carcinoma molecular subtype and HER2-low status. *gBRCA1* variants were linked to the prevalence of TNBC type and HER2 zero status. In contrast, *gBRCA2* cases had a higher rate of HR + and HER-low breast cancer.

Keywords Breast cancer, Germline *BRCA1/2*, *HER2*-low status

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Abbreviations

ADC	Antibody-drug conjugate
ACGS	Association for clinical genomic science
ASCO/CAP	American Society of Clinical Oncology / College of American Pathologists
BC	Breast cancer
ER	Estrogen receptors
ESMO	European Society for Medical Oncology
HBOC	Hereditary breast and ovarian cancer
HR	Hormone receptors
gBRCA1/2	Germline BRCA1/2 variants
IHC	Immunohistochemistry
IQR	Interquartile range
LPV	Likely pathogenic variant
NGS	next generation sequencing
PgR	Progesterone receptors
PV	Pathogenic variant
SNV	Single nucleotide variant
TNBC	Triple-negative breast cancer

Background

Breast cancer (BC) is the most commonly diagnosed malignancy and the leading cause of cancer death in women[1,2]. The intimidating trend of continuous rise in BC incidence has been a driving force stimulating biomarker discovery and exploration of molecular landscape of BC[3,4]. Molecular classification of BC, evaluating the expression of estrogen (ER) and progesterone (PgR) receptors, human epidermal growth factor receptor 2 (HER2), and Ki-67 was recognized as a robust approach for analysis of tumor biology, guiding patient prognosis and approach to treatment[3]. According to the 2011 St. Gallen Consensus Conference, depending on the status of these biomarkers, luminal A, luminal B, HER2-positive, and triple-negative breast cancer (TNBC) entities were distinguished[5]. Among them HER2-positive BC was traditionally defined as the one with increased HER2 protein expression level (reaching IHC score 3 + or 2 + demonstrating amplification of *ERBB2* gene, coding HER2, confirmed via in situ hybridization)[6]. HER2-positive BC is associated with highly aggressive tumor behavior and poor prognosis, as well as sensitivity to anti-HER2 monoclonal antibodies, such as trastuzumab and pertuzumab[7]. Anti-HER2 agents were shown to improve the prognosis in patients with HER2-positive primary and metastatic BC[8].

Notably, only around 15–20% of BC are HER2-positive which presents a limited application of targeted anti-HER2 therapeutic agents[9,10]. This paradigm shifted when researchers uncovered the specific biological features of BC tumors with low HER2 expression[11]. As a result, American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines and the European Society for Medical Oncology (ESMO) expert consensus statements established a new subtype of HER2-low BC, defined as those with a low but still detectable level of HER2 expression (IHC score 1 + or 2 + without *ERBB2* gene amplification)[12]. Based on this definition, HER2-negative breast cancer was further divided into HER2-low and HER2-zero (tumors with IHC score 0) BC. HER2-low BC demonstrated a good response to antibody-drug conjugates (ADC)[13]. Thereafter, the statement of HER2-negative tumors being insensitive to anti-HER2 therapy was reconsidered in August 2022, when trastuzumab deruxtecan (Enhertu, T-DXd) became the first therapy approved to treat patients with metastatic HER2-low breast cancer[14].

Approximately 40–55% of all BC cases belong to HER2-low subtype[15,16]. Recent studies showed that most HER2-low BC exhibit hormone-receptor (HR) expression[13]. According to four prospective neoadjuvant clinical trials, more than 60% patients with HER2-low tumors were HR positive[16]. At the same time only 36.7% of HR positive tumors were HER2-zero ($P < 0.001$)[16]. Similarly, HR-positive BC demonstrated around two times higher prevalence of HER2-low tumors compared to TNBC[17]. Similarly, Tarantino et al. in a large cohort of BC patients, showed the prevalence of HR expression among HER2-low compared to HER2-negative BC tumors, underscoring that high ER score is associated with elevated HER2 expression[18].

Beyond molecular classification, genetic predisposition also impacts prognosis, surveillance, and management of BC patients. This involves hereditary syndromes that increase the risk of BC and influence treatment approaches. Around 10% of all BC are caused by germline mutations, mostly attributed to hereditary breast and ovarian cancer (HBOC) syndrome[19]. The most renowned and clinically impactful here are germline mutations in *BReast CAncer gene 1 and 2* (*gBRCA1/2*). Individuals with *gBRCA1/2* variants face an increased absolute risk of developing BC that exceeded 60%. The incidence of *gBRCA1/2* varies between populations and among different clinical groups. For example, patients with TNBC are reported to have *gBRCA1/2* mutation in up to 15.4% cases[20]. However, little is known about the prevalence of HER2-low BC in patients harboring pathogenic and likely pathogenic variants (PV and LPV) in *BRCA1/2*. Recent study shed light on this issue demonstrating that HER2-low status was found in 32.3% young patients with early-stage Luminal-like BC harboring *gBRCA1/2* PVs[21]. Importantly, this international multicenter study enrolled women under 40 from different countries and ethnic groups. While epidemiology of HBOC is well established in most high-income countries, the incidence and spectrum of g*BRCA1/2* variants in low- and middle-income countries including Ukraine is lesser known. Within the published studies, there is a great discrepancy in patient groups, methods and scopes of testing performed, as well as in sampling approaches, limiting the groups to specific territories of Ukraine. Most studies employ a PCR-based method detecting founder *BRCA1/2* mutations which are most common worldwide and a single next generation sequencing (NGS) based study focused on specifically western

Ukraine[22,23]. A common limitation to our understanding of Ukrainian *BRCA1/2* mutation carriers, however, lies within the scopes of these studies, reaching up to 200 patients each. Moreover, there is no data about the links between HER2 status and *gBRCA1/2* status in Ukrainian population.

This study aims to elaborate on the rates of HER2-low status among patients with sporadic and hereditary BC in the Ukrainian population and investigate the relationship between *gBRCA1/2* and HER2 status.

Results

General characteristics of BC patients

The median age of patients at the time of diagnosis was 46 (IQR 41–54). Overall, *gBRCA1/2* genetic variants were found in 212 (15.0%) out of 1412 enrolled patients with BC. The rate of *gBRCA1* variants was more than twice as high (10.5%) compared to *gBRCA2* (64; 4.5%). In this study, 843 patients (59.7%) had Luminal-like BC, 430 cases (30.45%) possessed TNBC and a small portion of cases (139 of 1412, 9.84%) had HER2-enriched invasive BC. Among the study group, 117 patients (8.3%) had grade 1 (G1) tumors, 731 (51.8%) had G2 tumors, and 564 (39.9%) were diagnosed with G3 tumors (Table 1). Most patients carrying *gBRCA1/2* pathogenic or likely pathogenic variants were diagnosed with BC of G3 (123 of 212 patients, 58.02%; $P<0.001$). Less frequently, patients with *gBRCA1/2* were diagnosed with G2 BC (79/212; 37.3%) and G1 BC (10/212, 4.72%; Fig. 1A).

The presence of *gBRCA1* variants was also associated with the highest rate of cell proliferation in BC samples assessed through Ki-67 expression assessment by IHC (50.0; IQR 32.5–80.0%; $P<0.001$) compared to tumors with *gBRCA2* mutation (35.0; IQR 21.3–60.0) and wild type (WT) tumors (30.0; 20.0–50.0) (Fig. 1B).

Spectrum of *gBRCA1/2* variants in Ukrainian patients with BC

According to our data, we have identified 8 major g*BRCA1/2* variants (7 in *BRCA1* and 1 in *BRCA2*) that were found in 53.3% of patients with BC harboring *gBRCA1/2*. *BRCA1* c.5266dup (p.Gln1756fs) genetic variant was the most common pathogenic mutation among Ukrainian patients with BC and accounted for 32.54% of patients harboring *gBRCA1/2* (69 of 212 patients). Genetic variant c.181T > G (p.Cys61Gly) of *BRCA1* gene was the second most frequent pathogenic mutation found in 8.49% of cases (18 of 212 patients). Variants *BRCA1* c.4035del (p.Glu1346fs) and *BRCA2* c.475 + 1G > T were detected in 2.35% each (5 patients out of 212). The next most frequent variants were identified in 1.88% cases each (4 patients out of 212) and included 4 pathogenic mutations of *BRCA1* gene, namely c.1510del (p.Arg504fs), c.1961del (p.Lys654fs), c.68_69del (p.Glu23fs) and c.5177_5180del (p.Arg1726fs). The rest of *BRCA1/2* variants were present in smaller subsets of patients. Four genetic variants (3 frameshift and 1 stop gain variant) were identified in 3 patients each: c.5030_5033del (p.Thr1677fs) in *BRCA1* and c.5238dup (p.Asn1747Ter), c.658_659del (p.Val220fs), c.9097dup (p.Thr3033fs) in *BRCA2*.

Characteristics	Whole cohort	*gBRCA1/2*m	*gBRCA1/2* WT	*P*-value
Age*	46 (41–54)	45 (40–51)	46 (41–55)	0.00014
Age category 40				
- Under 40	332 (22.8%)	61 (18.9%)	261 (81.1%)	0.0247
- Over 40	1090 (77.2%)	151 (13.9%)	939 (86.1%)	
Age category 50				
- Under 50	904 (64%)	156 (17.3%)	748 (82.7%)	0.0017
- Over 50	508 (36%)	56 (11.0%)	452 (89.0%)	
Age category 60				
- Under 60	1217 (86.2%)	199 (16.4%)	1018 (83.6%)	< 0.001
- Over 60	195 (13.8%	13 (6.7%)	182 (93.3%)	
Age category 65				
- Under 65	1307 (92.6%)	208 (15.9%)	1099 (84.1%)	< 0.001
- Over 65	105 (7.4%)	4 (3.81%)	101 (96.2%)	
Molecular subtype				
Luminal-like	843 (59.7%)	90 (10.7%)	753 (89.3%)	< 0.001
HER2 enriched	139 (9.8%)	9 (6.5%)	130 (93.5%)	
Triple-negative	430 (30.5%)	113 (26.3%)	317 (73.7%)	
Tumor grade				
G1	117 (8.3%)	10 (8.54%)	107 (91.45%)	< 0.001
G2	731 (51.8%)	79 (10.8%)	652 (89.2%)	
G3	564 (39.9%)	123 (21.8%)	441 (78.2%)	

Table 1. Characteristics of patients with invasive breast carcinoma. *Data presented as Me (IQR; Q_I – Q_{III}). The categorical variables are presented as n (%). The Kruskal-Wallis test was applied to compare age among groups. To compare frequencies χ^2 test was applied. The molecular subtypes of breast cancer were defined by IHC and included Luminal-like (HR+, HER2-); HER2-enriched (any HR; HER2+); triple-negative (HR-, HER2-); wild type was defined as a case with no *gBRCA1/2*.

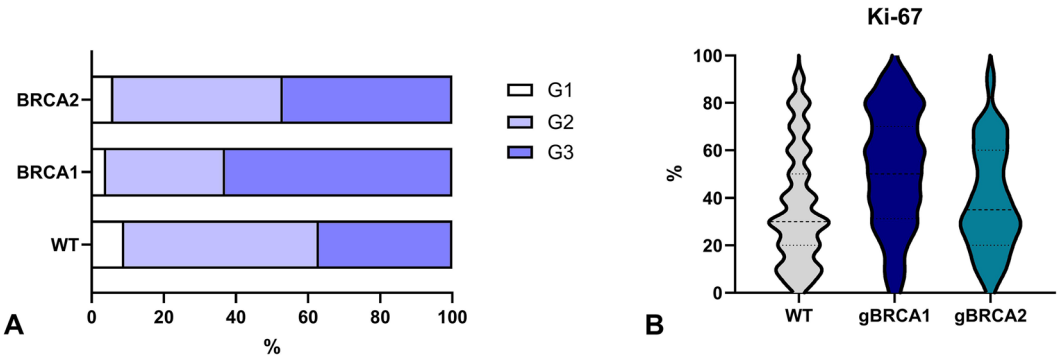

Fig. 1. The relationship between the presence of *gBRCA1/2* variants and tumor biology. **A** — demonstrates the link between presence *gBRCA1/2* genetic variants and poor differentiation of cancer cells. **B** — shows differences in Ki-67 expression in nuclei of tumor cells, highlighting the elevated cell proliferation in BC with *gBRCA1* variants.

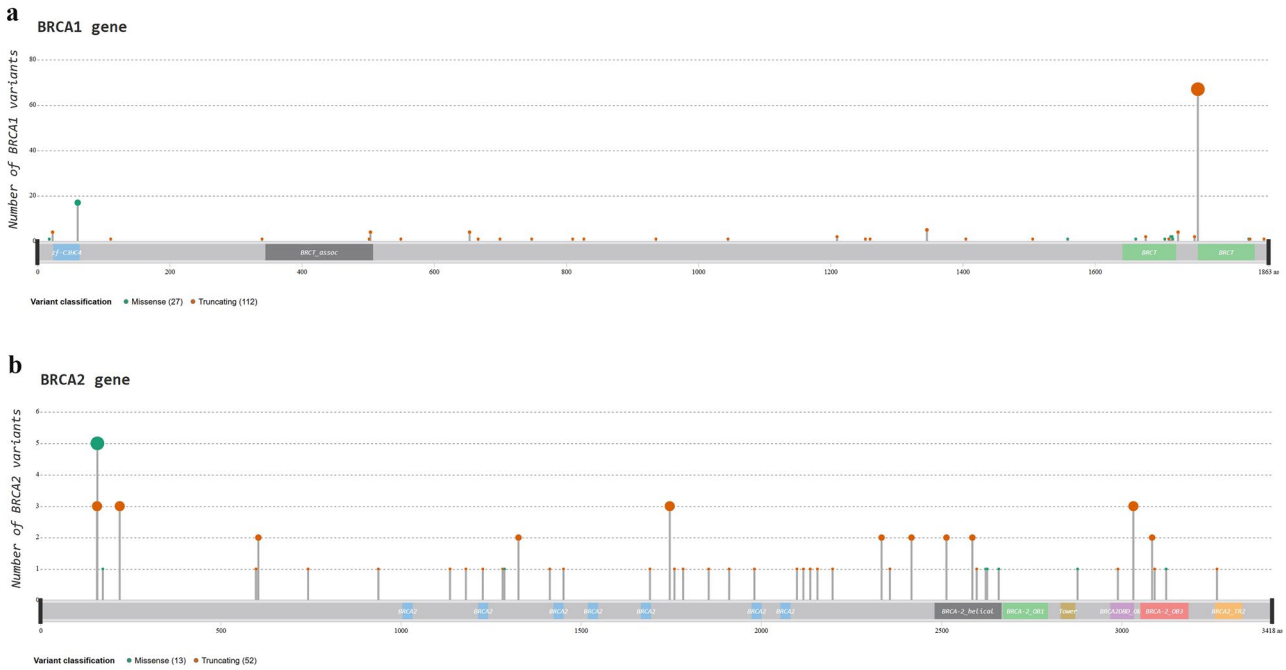

Fig. 2. The spectrum of *gBRCA1* and *gBRCA2* genetic variants. The frequency of various g*BRCA1/2* variants is represented along the vertical axis.

Fourteen *BRCA1/2* variants (7 in *BRCA1* and 7 in *BRCA2)* were found in two patients each. Among them 9 were frameshift variants, 3 – stop gain, 1 – splice site and 1 – missense. Most of the less common g*BRCA1/2* variants were well annotated and presented in public databases with clear evidence of clinical significance. There were also singular variants found in patients: 23 in *BRCA1* and 36 in *BRCA2*, of which 30 are frameshift variants, 16 – stop gain, 10 – missense, 2 – splice site, and 1 – inframe deletion (Fig. 2).

Among these findings, however, we identified rare single nucleotide variants (SNV) and insertions-deletions (indels) that had not yet been described previously in literature or public databases. These were found in singular patients and classified as Pathogenic/Likely pathogenic using the Association for Clinical Genomics Science (ACGS) 2020 guidelines[24]. They included *BRCA2* c.9795 C > A (p.Cys3265Ter), *BRCA2* c.5564del (p.Ser1855fs), *BRCA2* c.3410dup (p.Leu1137fs), *BRCA1* c.1647dup (p.Asn550Ter), *BRCA1* c.2099_2108del (p.Leu700fs), *BRCA2* c.6593del (p.Thr2199fs), *BRCA1* c.1505T > A (p.Leu502Ter), *BRCA2* c.2228dup (p.His743fs), *BRCA2* c.4240del (p.Thr1414fs), *BRCA2* c.7538_7547del (p.Lys2514fs), *BRCA1* c.4190_4191insGGATACCATGCAACATAACCTGA (p.Ile1405fs). Further studies are necessary to elaborate on the functional effects of these discovered variants.

Correlation between age of onset and *gBRCA1/2* mutation incidence in women with BC

The incidence of *gBRCA1/2* mutation was high in young women diagnosed with BC and decreases progressively with age. Among the observed patients there were 322 diagnosed with BC under 40 years old, with 61 cases carrying *gBRCA1/2* (18.9%). Naturally the incidence of *gBRCA1/2* was lower among women over 40 (13.9%; $P = 0.0247$). The frequency of *gBRCA1/2* variants among women under 50 (904; 64%) reached 17.3% that was significantly higher than in women over 50 at the time of diagnosis (11.0%; $P = 0.0017$). Comparing other age categories, 195 (13.8%) patients with BC were older than 60 at the time of diagnosis, and only 105 women (7.4%) in the observed cohort were over 65 years old (Table 1). The incidences of *gBRCA1/2* variants among cases under 60 and 65 were 16.4% and 15.9% ($P < 0.001$) respectively. Thus, early onset of BC suggests a higher probability that the woman may harbor *gBRCA1/2* variants[25]. However, we did not find the association between age and HER2-status.

Importantly, early-onset breast cancer was associated with specific germline variants in *BRCA1* and *BRCA2*. Among the 61 cases with *gBRCA1/2* variants found in patients under 40, c.5266dup (p.Gln1756fs) was the most prevalent (17 of 61 patients; 27.9%), however this number represented just 24.6% of all patients with this variant. Next, c.181T > G (p.Cys61Gly) was detected in 7 of 61 patients (11.5%), corresponding to 38.9% of this all the carriers of this variant. The rest (more than half) of young patients had rare variants. Among them c.3756_3759del (p.Ser1253fs) and c.5251 C > T (p.Arg1751Ter) of *BRCA1* and c.468dup (p.Lys157Ter) of *BRCA2* were found in 2 cases each being exclusively related to age under 40. Many other variants of *BRCA1* and *BRCA2* were found in young age women with BC (Table 2).

The similar analysis of *gBRCA1/2* variants incidence among women under and over 50 years old demonstrated that 72.5% and 83.3% of c.5266dup and c.181T > G BRCA1 variants respectively were associated with BC development in age before 50. Similarly, the association with early onset BC was found for c.1510del, c.1961del, c.5177_5180del, c.68_69del, and c.5030_5033del, c.3627dup, c.3756_3759del, c.5251 C > T variants in *BRCA1* and c.658_659del, c.1813dup, c.3975_3978dup, c.468dup, c.5152 + 1G > T, c.6998dup, c.7251_7252del, c.7758G > A variants in *BRCA2*. In contrast, there were some variants detected in women with BC onset after the age of 50 (Suppl. Table 1).

The relationship between *gBRCA1/2* and HR status of BC

The presence of *gBRCA1/2* variants was closely related to the BC molecular subtype and HR expression ($P < 0.001$; Suppl. Table 2). *gBRCA1* variants were associated mostly with TNBC molecular subtype, while *gBRCA2* variants were linked to Luminal-like BC subtypes (Fig. 3, Suppl. Table 3). The rate of *gBRCA1/2m* in TNBC was high, reaching 26.3%. Most of these patients (98; 22.8%) had g*BRCA1* and a smaller fraction harbored g*BRCA2* variant (15; 3.5%). The rate of *gBRCA1/2* variants was lower among women with Luminal-like BC subtypes (90 of 843 patients had g*BRCA1/2* variants, 10.7%), while HER2-enriched subtype was associated with the lowest rate of *gBRCA1/2* mutation (6.5%). We found specific *BRCA1/2* genetic variants associated with different molecular subtypes (see Suppl. Table 2).

Naturally, these distinctions between genetic findings and tumor biology were closely related to the different association of *gBRCA1/2* variants on HR status. Among patients with *gBRCA2m* allele 75% (48 of 64) were associated with HR-positive status, while women with *gBRCA1* variants demonstrated only 31.1% of HR positive BC (Fig. 4).

There was also an association between HR and HER2 status in BC ($P < 0.001$). HR-positive status was found in 920 of 1412 BC cases (65.2%). Among them 77 (8.4%) tumors were HER2-positive, 343 (37.3%) – HER2-low and 500 (54.3%) – HER2-zero. The rates of HER2 expression differed in HR-negative BC ($n = 492$ of 1412; 34.8%). Although the rate of HER2-positive BC was found in 12.5% (62 of 492 HR-negative BC), only 93 cases of this subgroup (18.9%) were HER2-low, and the rest 337 (68.5%) demonstrated HER2-zero status.

Thus, *gBRCA1* and *gBRCA2* variants differ in their effects on BC biology and molecular subtypes. While *gBRCA1* variants were associated with HR expression loss and TNBC subtype, *gBRCA2* variants were linked to HR-positive status and Luminal-like BC subtype.

gBRCA1 and *gBRCA2* genetic variants differently related to HER2-status

Overall, there were 837 HER2-negative (59.3%), 436 HER2-low (30.9%) and 139 HER2-positive (9.8%) BC among the study sample. The HER2-low status was twice as high in Luminal-like BC as compared to TNBC ($P < 0.001$). HER2-low status was found in 343 (40.7%) out of 843 Luminal-like BC while only 93 (21.6%) of 430 TNBC demonstrated the same level of HER2 expression at IHC (Fig. 5). Thus, the Luminal-like BC had proportionately more HER2-low expression, while TNBC were mainly HER2-zero.

When comparing *BRCA1/2* variants-associated tumors with WT BC it seemed that carrying *gBRCA1/2* was linked to HER2 negativity and lower rate of HER-low and HER2+ status ($P = 0.006$). There were 141 (66.5%) HER2 negative cases among 212 patients with *gBRCA1/2*, 62 (29.2%) demonstrated HER2-low status and only 9 (4.2%) were HER2-positive ($P < 0.001$). However, *gBRCA1* and *gBRCA2* differently impacted tumor biology. HER2-zero status was mostly linked to *gBRCA1* variants ($P = 0.006$; Table 3), as more than 70% of *gBRCA1* cases were HER2-zero. In patients with *gBRCA2* and WT BC the rate of HER-zero status was lower comprising 56.2% and 58% respectively. Among WT tumors the proportion of HER2-positive status was the highest reaching 10.8%, while in *gBRCA2m* BC it accounted for 6.2% and in *gBRCA1m* BC was the lowest (3.6%). Notably, HER-low status rate was detected in 37.5% of patients with *gBRCA2* carriers, that was higher than in WT-breast carcinomas (31.2%) and patients with *gBRCA1* variants (25.7%). Thus, *gBRCA1* cases demonstrated the highest rate of HER2-negative BC (70.9%), while *gBRCA2m BC* possessed HER2-low status in more than one third of cases (Suppl. Table 4).

At the next step we assessed the relationship between various HER2 status and different *BRCA1/2* variants (Table 3). HER2-positive BC was related to the limited number of variants. The following variants demonstrated

Gene	*gBRCA1/2* variants	Under 40 years	Over 40years	Number of carriers
BRCA1	c.5266dup (p.Gln1756fs)	17 (24.6%)	52 (75.4%)	69 (4.9%)
BRCA1	c.181T > G (p.Cys61Gly)	7 (38.9%)	11 (61.1%)	18 (1.3%)
BRCA1	c.4035del (p.Glu1346fs)	0	5 (100%)	5 (0.4%)
BRCA2	c.475 + 1G > T	0	5 (100%)	5 (0.4%)
BRCA1	c.1510del (p.Arg504fs)	1 (25%)	3 (75.0%)	4 (0.3%)
BRCA1	c.1961del (p.Lys654fs)	0	4 (100.0%)	4 (0.3%)
BRCA1	c.5177_5180del (p.Arg1726fs)	0	4 (100.0%)	4 (0.3%)
BRCA1	c.68_69del (p.Glu23fs)	1 (25%)	3 (75%)	4 (0.3%)
BRCA1	c.5030_5033del (p.Thr1677fs)	1 (33.3%)	2 (66.7%)	3 (0.2%)
BRCA2	c.5238dup (p.Asn1747Ter)	0	3 (100%)	3 (0.2%)
BRCA2	c.658_659del (p.Val220fs)	1 (33.3%)	2 (66.7%)	3 (0.2%)
BRCA2	c.9097dup (p.Thr3033fs)	1 (33.3%)	2 (66.7%)	3 (0.2%)
BRCA1	c.1016dup (p.Val340fs)	0	2 (100.0%)	2 (0.1%)
BRCA2	c.1813dup (p.Ile605fs)	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA1	c.3627dup (p.Glu1210fs)	0	2 (100.0%)	2 (0.1%)
BRCA1	c.3700_3704del (p.Val1234fs)	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA1	c.3756_3759del (p.Ser1253fs)	2 (100%)	0	2 (0.1%)
BRCA2	c.3975_3978dup (p.Ala1327fs)	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.468dup (p.Lys157Ter)	2 (100.0%)	0	2 (0.1%)
BRCA1	c.5145 C > G (p.Ser1715Arg)	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA1	c.5152 + 1G > T	0	2 (100.0%)	2 (0.1%)
BRCA1	c.5251 C > T (p.Arg1751Ter)	2 (100.0%)	0	2 (0.1%)
BRCA2	c.6998dup (p.Pro2334fs)	0	2 (100.0%)	2 (0.1%)
BRCA2	c.7251_7252del (p.His2417fs)	0	2 (100.0%)	2 (0.1%)
BRCA2	c.7758G > A (p.Trp2586Ter)	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.9253dup (p.Thr3085fs)	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA1	c.1505T > A (p.Leu502Ter)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.1647dup (p.Asn550Ter)	0	2 (100.0%)	1 (0.07%)
BRCA2	c.1794_1798del (p.Ser599fs)	0	3 (100.0%)	1 (0.07%)
BRCA2	c.1796_1800del (p.Thr598_Ser599insTer)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.1999 C > T (p.Gln667Ter)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2099_2108del (p.Leu700fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.220 C > T (p.Gln74Ter)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.2228dup (p.His743fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2241dup (p.Lys748fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2429del (p.Asn810fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2479_2480del (p.Glu827fs)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.2806_2809del (p.Asp936fs)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.2808_2811del (p.Ala938Profs)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.3132del (p.Asn1045fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.329dup (p.Glu111fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3410dup (p.Leu1137fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3545_3546del (p.Gln1181_Phe1182insTer)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3682_3685del (p.Asn1228fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.3779del (p.Leu1260fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3847_3848del (p.Val1283fs)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.3861TAA[1] (p.Asn1288del)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.4190_4191insGGATACCATGCAACATAACCTGA (p.Ile1405fs)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.4240del (p.Thr1414fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.4354 C > T (p.Gln1452Ter)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.4516del (p.Asp1506fs)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.4675G > A (p.Glu1559Lys)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.470_474del (p.Lys157fs)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.4986 + 3G > C	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5073dup (p.Trp1692fs)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.5117G > A (p.Gly1706Glu)	1 (100.0%)	0	1 (0.07%)
Continued				

Gene	*gBRCA1/2* variants	Under 40 years	Over 40years	Number of carriers
BRCA1	c.5136G > A (p.Trp1712Ter)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.5154G > T (p.Trp1718Cys)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.517G > T (p.Gly173Cys)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5279 C > G (p.Ser1760Ter)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5351dup (p.Asn1784fs)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.53T > C (p.Met18Thr)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.5497G > A (p.Val1833Met)	1 (100.0%)	0	1 (0.07%)
BRCA1	c.5503 C > T (p.Arg1835Ter)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5564del (p.Ser1855fs)	0	1 (100.0%)	1 (0.07%)
BRCA1	c.5566 C > T (p.Arg1856Ter)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5734G > T (p.Glu1912Ter)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5946_5949del (p.Ser1982fs)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.6298 C > T (p.Gln2100Ter)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.6352_6353del (p.Val2118fs)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.6408_6414del (p.Asn2137fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.6468_6469del (p.Gln2157fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.6593del (p.Thr2199fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7069_7070del (p.Leu2357fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7538_7547del (p.Lys2514fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7540_7549del (p.Lys2514fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7792G > T (p.Glu2598Ter)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7868 A > G (p.His2623Arg)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7879 A > T (p.Ile2627Phe)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.7975 A > G (p.Arg2659Gly)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.8633-1G > A	0	1 (100.0%)	1 (0.07%)
BRCA2	c.8969G > A (p.Trp2990Ter)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.9275_9278del (p.Tyr3092fs)	0	1 (100.0%)	1 (0.07%)
BRCA2	c.9371 A > T (p.Asn3124Ile)	1 (100.0%)	0	1 (0.07%)
BRCA2	c.9795 C > A (p.Cys3265Ter)	0	1 (100.0%)	1 (0.07%)

Table 2. The rate of *gBRCA1* and *gBRCA2* variants linked to early breast cancer development (under 40).

the high incidence of association with HER2-positive status: c.181T > G and c.4035del in *BRCA1* and 4 different variants in *BRCA2* (c.658_659del, c.517G > T, c.7975 A > G, and c.9253dup). Only 2 of 69 (2.89%) patients with common c.5266dup variant were linked to HER2-positive BC. Notably, several *gBRCA2* variants demonstrated links to HER2-low status: c.475 + 1G > T (3/5, 60%), c.5238dup (2/3; 66.7%), c.9097dup (2/3; 66.7%). At the same time there were several *gBRCA1* variants that were related to HER2-low expression (Table 3). Notably, the most common variants of g*BRCA1* demonstrated a lower incidence of HER-2-low status: 15 of 69 c.5266dup cases (21.7%) and 2 of 18 patients with c.181T > G (22.2%) were HER2-low.

To sum up, the most common variants of g*BRCA1* were associated with HER2-zero status of BC, while g*BRCA2* demonstrated a higher rate of HER2 up-regulation defining HER2-low status. Several variants of g*BRCA1/2* were linked to low or even high HER2 expression.

Discussion

This large-scale Ukrainian study defined the rate and the spectrum of *gBRCA1/2* variants in large sample of Ukrainian population, although there were the previous studies describing *BRCA1/2* variants in Ukraine based on limited cohorts up to 123 Ukrainian patients with breast and ovarian cancers[22]. Moreover, some other studies relied on PCR testing focusing on the most common variants[23]. In this research we provided the results of NGS testing in 1412 women with histologically confirmed invasive BC and revealed that 212 of them (15%) had *gBRCA1/2* variants. This relatively high rate of *gBRCA1/2* could be related to medical indications for genetic testing and prevalence of young age patients in the study population with the average age 46 (41–54), though in previous study the rate of patients with *gBRCA1/2* was even higher (16.3%)[22].

The most common variants of *BRCA1* were c.5266dup (p.Gln1756fs) and c.181T > G (p.Cys61Gly). In Ukrainian cohort they accounted for 32.54% and 8.5% among *gBRCA1/2* cases. These data align with other reports demonstrating that c.5266dup (p.Gln1756fs) and c.181T > G (p.Cys61Gly) *BRCA1* variants are common European founder mutations and are also prevalent in Poland and in the Czech Republic[22,26]. Using NGS technology we were able to define the whole spectrum of *gBRCA1/2* variants most of which were previously annotated, including c.4035del (p.Glu1346fs), c.5030_5033del (p.Thr1677fs), c.1510del (p.Arg504fs), c.1961del (p.Lys654fs), c.68_69del (p.Glu23fs) and c.5177_5180del (p.Arg1726fs) variants in *BRCA1*. The rate of *gBRCA2* variants in Ukrainian patients with BC was lower as compared to *gBRCA1* comprising 30.1% of all *BRCA1/2*-

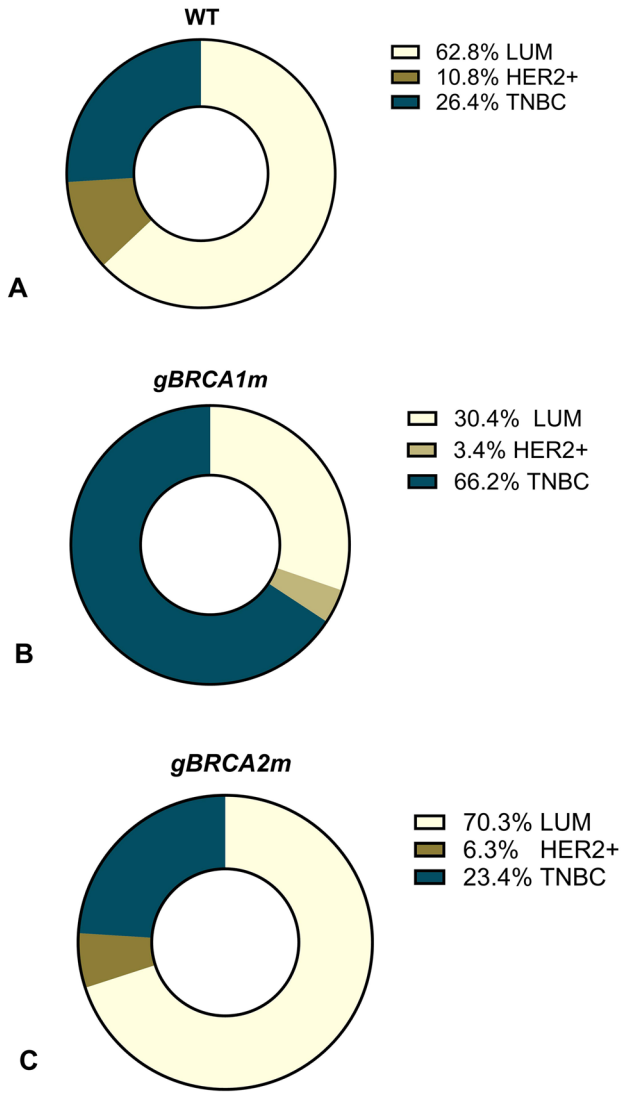

Fig. 3. *gBRCA1* variants are associated with TNBC, while *gBRCA2* variants are linked to Luminal-like breast cancer. **A —** demonstrates the distribution of various molecular subtypes in patients with no *gBRCA1/2* (WT). **B —** demonstrates the prevalence of TNBC in patients with *gBRCA1*. C - shows prevalence of Luminal-like BC in patients with *gBRCA2* variants.

associated BC. The most common *gBRCA2* variant was c.475 + 1G > T. Other *gBRCA2* variants included c.5238dup (p.Asn1747Ter), c.658_659del (p.Val220fs), c.6998dup (p.Pro2334fs), c.7251_7252del (p.His2417fs), c.7758G > A (p.Trp2586Ter), c.9097dup (p.Thr3033fs) and c.9253dup (p.Thr3085fs).

Most of the rarer *BRCA1/2* variants were well-annotated and listed in public databases with clear evidence of clinical significance. There were also singular variants found in patients: 23 in *BRCA1* and 36 in *BRCA2*, of which 30 are frameshift variants, 16 – stop gain, 10 – missense, 2 – splice site, and 1 – inframe deletion (Suppl. Table 2). Among our findings, however, we identified rare SNV and indels that had not yet been described in literature or public databases. These were found in singular patients and classified as Pathogenic/Likely pathogenic using ACGS 2020 guidelines[24]. It is worth noting, in this study included the data on *BRCA1/2* testing without PALB2 and other Homologous recombination repair (HRR) genes recommended by European Molecular Genetics Quality Network the (EMQN) best practices for hereditary breast and ovarian cancer (HBOC)[27].

Carrying *gBRCA1/2m* was associated with the younger age of BC development. We also demonstrated that in Ukrainian population more than half cases of early onset of BC are linked to rare variants of *gBRCA1* and *gBRCA2*. Our findings highlight the need in NGS testing covering the coding sequence of *BRCA1* and *BRCA2* for identifying rare variants predisposing to early BC. This finding highlights the importance of *BRCA1/2* sequencing instead of common variants testing, for detecting a variety of germline variants, predisposing to early onset of BC. Besides, the incidence of *gBRCA1/2* variants differed significantly between women under and over 60–65 years old. Considering the impact of *gBRCA1/2* variants on breast cancer patients' surveillance, surgery, and treatment, it is essential to broaden the indications for *BRCA1/2* testing in patients under 65 years, as recommended by ASCO[28]. ASCO guidelines recommend g*BRCA1/2* testing to all newly diagnosed people

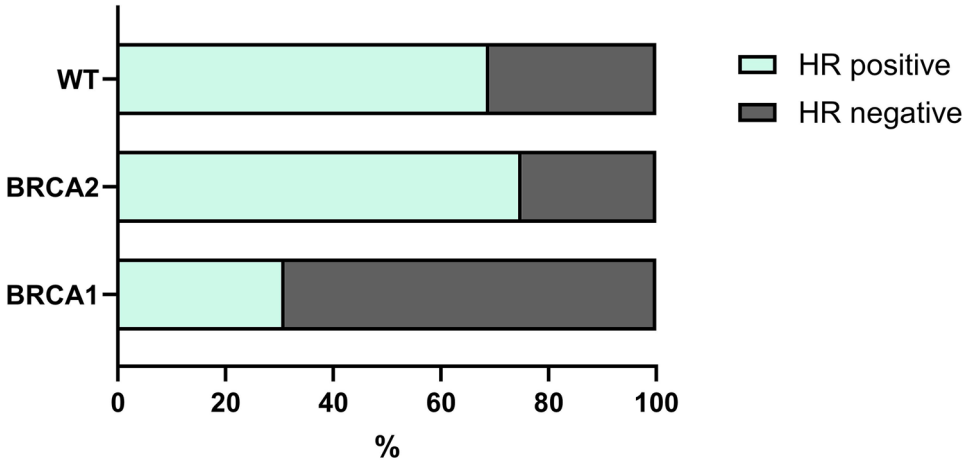

Fig. 4. The relationship between *gBRCA1* and *gBRCA2* genetic variants and HR expression. *gBRCA1* variants were associated with loss of HR expression. In contrast, *gBRCA2* variants were associated with HR positive BC status.

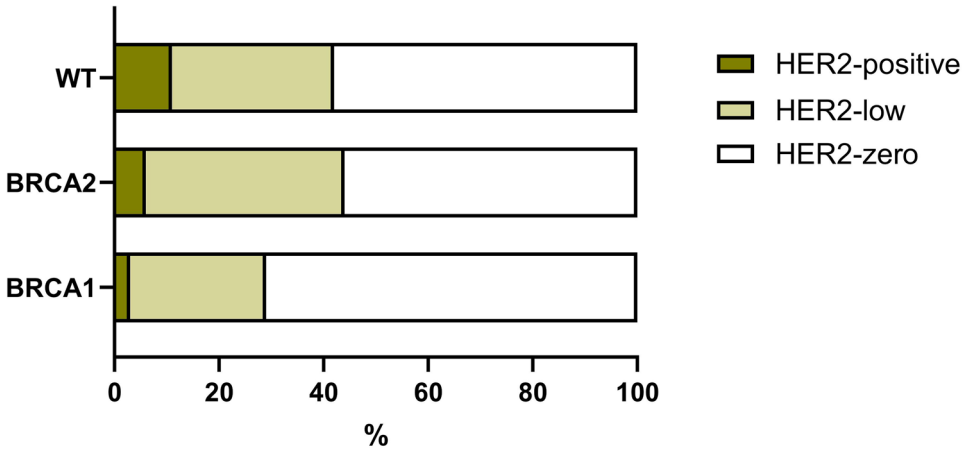

Fig. 5. The relationship between *gBRCA1/2* and HER2 status. *gBRCA2* variants were associated with a higher rate of HER2-low status, while patients with *gBRCA1* variants possessed mostly HER2-negative status.

with BC ≤ 65 years and selected patients over 65 who have personal or family history ancestry or eligible for poly(ADP-ribose) polymerase (PARP) inhibitor therapy[28]. Besides *BRCA1/2* testing should be considered in case of the second primary cancer or recurrent BC if patients are candidates for PARPi therapy regardless of family history[28].

Although HER2-positive BC is uncommonly associated with *BRCA1/2* pathogenic variants, we found the association between *gBRCA2* and HER2-low status and HR expression maintenance. Recent study also demonstrated the links between HER2-low BC and defects in homologous recombination repair genes (HRR). HER2-low BC were found to harbor frequent mutations in HRR genes that define a higher homology recombination deficiency (HRD) score and the increased level of genomic instability. These alterations can affect patient's prognosis as elevated HRD score in HER2-low tumors correlated with poorer progression-free survival, especially in hormone receptor–positive cases[29]. On the other hand, the association with DNA repair defects in HER2-low tumors opens up opportunities for innovative therapeutic approaches[30,31]. Tumors with *BRCA1/2* mutations are sensitive to PARP inhibitors and may exhibit an altered immune microenvironment, which might enhance responsiveness to immunotherapy[32]. These relationships highlight the possibility of exploiting synthetic lethality strategies and immune checkpoint inhibitors (ICI) in a subset of patients with HER2-low BC in patients with *gBRCA1/2*.

Our findings revealed that gBRCA2-associated breast cancers were more frequently HR-positive than those associated with gBRCA1 mutations, which align with results from other investigations[21,33]. For instance, large multicenter study that included 3547 women aged ≤ 40 years with newly diagnosed early-stage HER2-zero and HER2-low BC with germline *BRCA1/2* PVs demonstrated that HER2-low BC was mostly associated with HR-positive and significantly less frequently with TNBC[21]. Other studies also reported that high proportion of HER2-low tumors demonstrated Luminal-like type gene expression with maintained ER and related pathways

Gene	*gBRCA1/2* variants	HER2+	HER2low	HER2neg	Number of carriers*
BRCA1	c.5266dup (p.Gln1756fs)	2 (2.9%)	15 (21.7%)	52 (75.4%)	69 (4.9%)
BRCA1	c.181T > G (p.Cys61Gly)	2 (11.1%)	4 (22.2%)	12 (66.7%)	18 (1.3%)
BRCA1	c.4035del (p.Glu1346fs)	1 (20.0%)	1 (20.0%)	3 (60.0%)	5 (0.4%)
BRCA2	c.475 + 1G > T	0	3 (60.0%)	2 (40.0%)	5 (0.4%)
BRCA1	c.1510del (p.Arg504fs)	0	1 (25.0%)	3 (75.0%)	4 (0.3%)
BRCA1	c.1961del (p.Lys654fs)	0	1 (25.0%)	3 (75.0%)	4 (0.3%)
BRCA1	c.5177_5180del (p.Arg1726fs)	0	1 (25.0%)	3 (75.0%)	4 (0.3%)
BRCA1	c.68_69del (p.Glu23fs)	0	1 (25.0%)	3 (75.0%)	4 (0.3%)
BRCA1	c.5030_5033del (p.Thr1677fs)	0	1 (33.3%)	2 (66.7%)	3 (0.2%)
BRCA2	c.5238dup (p.Asn1747Ter)	0	2 (66.7%)	1 (33.3%)	3 (0.2%)
BRCA2	c.658_659del (p.Val220fs)	1 (33.3%)	1 (33.3%)	1 (33.3%)	3 (0.2%)
BRCA2	c.9097dup (p.Thr3033fs)	0	2 (66.7%)	1 (33.3%)	3 (0.2%)
BRCA1	c.1016dup (p.Val340fs)	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.1813dup (p.Ile605fs)	0	0	2 (100%)	2 (0.1%)
BRCA1	c.3627dup (p.Glu1210fs)	0	2 (100%)	0	2 (0.1%)
BRCA1	c.3700_3704del (p.Val1234fs)	0	0	2 (100%)	2 (0.1%)
BRCA1	c.3756_3759del (p.Ser1253fs)	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.3975_3978dup (p.Ala1327fs)	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.468dup (p.Lys157Ter)	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA1	c.5145 C > G (p.Ser1715Arg)	0	0	2 (100%)	2 (0.1%)
BRCA1	c.5152 + 1G > T	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA1	c.5251 C > T (p.Arg1751Ter)	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.6998dup (p.Pro2334fs)	0	0	2 (100%)	2 (0.1%)
BRCA2	c.7251_7252del (p.His2417fs)	0	0	2 (100%)	2 (0.1%)
BRCA2	c.7758G > A (p.Trp2586Ter)	0	1 (50.0%)	1 (50.0%)	2 (0.1%)
BRCA2	c.9253dup (p.Thr3085fs)	1 (50.0%)	0	1 (50.0%)	2 (0.1%)
BRCA1	c.1505T > A (p.Leu502Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.1647dup (p.Asn550Ter)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.1794_1798del (p.Ser599fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.1796_1800del (p.Thr598_Ser599insTer)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.1999 C > T (p.Gln667Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2099_2108del (p.Leu700fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.220 C > T (p.Gln74Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.2228dup (p.His743fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.2241dup (p.Lys748fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2429del (p.Asn810fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2479_2480del (p.Glu827fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.2806_2809del (p.Asp936fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.2808_2811del (p.Ala938Profs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.3132del (p.Asn1045fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.329dup (p.Glu111fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3410dup (p.Leu1137fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3545_3546del (p.Gln1181_Phe1182insTer)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3682_3685del (p.Asn1228fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.3779del (p.Leu1260fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3847_3848del (p.Val1283fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.3861TAA[1] (p.Asn1288del)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.4190_4191insGGATACCATGCAACATAACCTGA (p.Ile1405fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.4240del (p.Thr1414fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.4354 C > T (p.Gln1452Ter)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.4516del (p.Asp1506fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.4675G > A (p.Glu1559Lys)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.470_474del (p.Lys157fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.4986 + 3G > C	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5073dup (p.Trp1692fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.5117G > A (p.Gly1706Glu)	0	0	1 (100.0%)	1 (0.07%)
Continued					

Gene	*gBRCA1/2* variants	HER2+	HER2low	HER2neg	Number of carriers*
BRCA1	c.5136G > A (p.Trp1712Ter)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.5154G > T (p.Trp1718Cys)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.517G > T (p.Gly173Cys)	1 (100.0%)	0	0	1 (0.07%)
BRCA2	c.5279 C > G (p.Ser1760Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5351dup (p.Asn1784fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.53T > C (p.Met18Thr)	0	0	1 (100.0%)	1 (0.07%)
BRCA1	c.5497G > A (p.Val1833Met)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.5503 C > T (p.Arg1835Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5564del (p.Ser1855fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA1	c.5566 C > T (p.Arg1856Ter)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.5734G > T (p.Glu1912Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.5946_5949del (p.Ser1982fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.6298 C > T (p.Gln2100Ter)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.6352_6353del (p.Val2118fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.6408_6414del (p.Asn2137fs)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.6468_6469del (p.Gln2157fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.6593del (p.Thr2199fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7069_7070del (p.Leu2357fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7538_7547del (p.Lys2514fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7540_7549del (p.Lys2514fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7792G > T (p.Glu2598Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.7868 A > G (p.His2623Arg)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.7879 A > T (p.Ile2627Phe)	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.7975 A > G (p.Arg2659Gly)	1 (100.0%)	0	0	1 (0.07%)
BRCA2	c.8633-1G > A	0	1 (100.0%)	0	1 (0.07%)
BRCA2	c.8969G > A (p.Trp2990Ter)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.9275_9278del (p.Tyr3092fs)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.9371 A > T (p.Asn3124Ile)	0	0	1 (100.0%)	1 (0.07%)
BRCA2	c.9795 C > A (p.Cys3265Ter)	0	0	1 (100.0%)	1 (0.07%)

Table 3. The rate of gBRCA1 and gBRCA2 in breast cancer with respect to HER2 status. *For the whole number of carriers, the percentage is provided as a frequency in the whole study population (1412 patients).

expression[32]. Some reports noticed differences in age at diagnosis and tumor grade, with HER2-low tumors having less aggressive features compared to HER2-zero cases, though results can vary by population[34,35]. Assessment of genetic profile of HER2-low tumors demonstrated also a higher rate of genetic alteration in PI3K-Akt signaling pathway as compared to HER2-positive and HER2-zero BC[30]. In contrast HER2-zero BC showed more alterations in checkpoint factors, Fanconi anemia genes and p53 signaling, as well as cell cycle pathway compared to HER2-low breast tumors.

Important, HR positive BC with *gBRCA1/2* were shown to have more aggressive tumor phenotypes as compared to sporadic cancer. Adjuvant hormone therapy (tamoxifen) is indicated for patients HR-positive BC[36,37]. However, according to recent studies the beneficial effect of adjuvant hormone therapy on decreasing local recurrence and death rate was not achieved in *BRCA2*-associated breast cancer[38,39]. Moreover, it was demonstrated that *gBRCA2* carriers who received adjuvant hormone therapy had a higher risk of death compared to sporadic BC[40].

It is also worth noting that *gBRCA1* and *gBRCA2* have differently affected HER2-low status rate. In general, *gBRCA1* was more often related to HER-2 negative status, while *gBRCA2* variants were more commonly associated with HER2-low status. To note these data correlate with other study demonstrating relatively high rate of HER2-low status in hereditary cancer[33]. It was shown that despite the lower frequency of HER2-positive BC among individuals with *BRCA1/2* and *PALB2* PVs, hereditary BC demonstrated similar proportion of HER2-low expression as compared to sporadic BC[33]. In the retrospective observational study performed using TCGA data, researchers showed that HER2-low breast cancer associated with defects in HRR related genes demonstrated elevation of HRD score as compared to sporadic BC. Importantly, HER2-low status was mostly associated with mutations in *ARID1A*, *ATM*, and *BRCA2* genes[29]. This finding aligns with our results uncovering the close association between *gBRCA2* variants and HER2-low status. From the clinical perspective, the presence of HRR mutations in HER2-low tumors opens up potential therapeutic avenues and defines a group of *gBRCA2m BC* with HER2-low status who can benefit both from PARPi and ADC (trastuzumab deruxtecan).

Finally, we found that some particular variants of *BRCA1* and *BRCA2* were differently related to the age of BC onset, HR and HER2 status. These findings allow us to generate a hypothesis that some *BRCA1/2* variants

are differently associated with expression of hormone receptors and HER2. However, the interpretation of these data requires further studies.

Limitations of the study

Although our study included a relatively high sample size, it has a cross-sectional design that impacts the limitations of the results. About half of the patients underwent pre-test genetic counselling. There was no available follow up data enabling us to assess patients` outcomes and the efficiency of treatment strategies. We also did not have a whole spectrum of family history data from all patients for deeper analysis of hereditary cancer risks. Due to allocation to one laboratory of pathology and genetic data reports, that could be bias of selection, though the patients from various regions of Ukraine were included in the study.

Moreover, only *BRCA1* and *BRCA2* germline variants with no CNVs assessment were assessed, without additional analysis of other genes with high penetrance (such as *PALB2*, *TP53* and *STK11*) and other HRR genes, that could affect the heterogeneity of the comparison group that included non-carriers of *gBRCA1/2*. In addition, lack of other HRR gene information precludes the analysis of other HRR genes` relation to HER2-low status.

Conclusions

gBRCA1 and *gBRCA2* variants differed in their association with breast carcinoma biology, molecular subtype and HER2-status. Patients with *gBRCA1* demonstrated the prevalence of TNBC type with predominance of HER2 zero status. In contrast, *gBRCA2* carriers had a higher rate of HER-low breast cancer.

Materials and methods

Ethics statement

The requirement of ethical approval was waived by IRB of Medical Laboratory CSD (Kyiv, Ukraine) for the studies on humans because of the anonymous nature of the retrieved retrospective data. The studies were conducted in accordance with the local legislation and institutional requirements. Due to the retrospective nature of the study, IRB of Medical Laboratory CSD waived the need to obtain informed consent.

Patient population

This was a retrospective multicenter cross-sectional study that included data about 1412 cases of invasive BC from all regions of Ukraine. Histopathology, immunohistochemistry (IHC) and genetic testing of patients` blood was performed in ISO15189 certified laboratory (CSD LAB) during the period from 2021 to 2024. The following inclusion criteria were applied: women aged 18–75 years old, full set of demographic and clinical data, histologically confirmed invasive breast carcinoma, available data on IHC for ER, PR, HER2 and Ki-67 and FISH in case of IHC2 + HER2 score, as well as available report on testing for germline *BRCA1/2* variants. Both primary and metastatic BC cases were included in the study. Exclusion criteria included lack of full set of data, age under 18 or over 75, lobular carcinoma and ductal in situ cases. Depersonalized data was retrieved and used for further analysis. There was no patient involvement in the design of the study. The oncologist obtained consent for testing and secondary data use for research before diagnostics. The collected clinicodemographic data included the patient`s gender, age at BC diagnosis, and location of BC. Demographic characteristics of BC patients enrolled in the study are summarized in Table 1.

Pathology and IHC

Tissue samples were fixed in 10% neutral buffered formalin for 24 h and processed automatically (Milestone LOGOS Microwave Hybrid Tissue Processor, Milestone, Italy). Paraffin-embedded blocks were cut at 4 µm thickness (ThermoScientific HM 340E microtome, USA). Sections were stained by hematoxylin and eosin using Dako Cover Stainer (Agilent, USA). Histological assessment was performed by trained pathologists according to the WHO classification of breast tumors (5th ed)[41]. Tumor grade was defined according to the Nottingham grading system. The Nottingham combined histologic grade assessment was based on assigning a score from 1 to 3 to three parameters: amount of tubule formation, extent of nuclear pleomorphism and mitosis count. The final histologic grade was based on a sum of the individual scores of these parameters. Grade 1 was assigned for tumors with final 3–5 score, Grade 2 – for score 6 or 7, and Grade 3 – for neoplasms with 8 or 9 score.

IHC was conducted according to the standard protocol using Autostainer Link 48 (Agilent, USA). The following antibodies were used: ER (Agilent, clone EP1), PR (Agilent, clone PgR 636), HER2 (Agilent, polyclonal, cat.no A0485) and Ki-67 (Agilent, clone MIB-1) to assess the molecular subtype of BC.

For biomarkers assessment the ASCO/CAP guidelines were applied. ER-negative and PR-negative status was defined as < 1% staining in the nuclei via IHC[42]. ER and PR status was considered positive in case of 1% and higher positive nuclear staining in tumor cells. As for Ki-67, the percentage of cells with immunopositive nuclei was counted and the cut-off of 20% was applied in reporting. HER2 status was graded as negative in case of IHC score 0, HER2-low was assigned when HER2 was assessed as 1 + via IHC and HER2 with IHC score 2 + with no *ERBB2* amplification in FISH testing[43]. HER2-positive tumors included strong positive IHC 3 + cases and HER2 with equivocal results supported by *ERBB2* amplification by FISH[44].

The following molecular subtypes of BC were defined based on the available data about hormone receptors, HER2 status, Ki-67 expression: Luminal-like (ER positive and PgR positive, HER2 negative (including 0, 1 + and 2 + with no amplification by FISH), triple negative (ER negative, PgR negative, HER2 negative), or HER2 positive (any ER and PgR status, HER2 positive)[45–47].

Next-generation sequencing (NGS) testing for *gBRCA1/2*

In this study, all patients underwent germline NGS testing to detect single nucleotide variations (SNV) and indel variants in *BRCA1* and *BRCA2* genes. Genomic DNA was extracted from whole blood using the E.Z.N.A.® Blood DNA Mini Kit (Omega Bio-tek, USA). Quantification of DNA was performed via a spectrofluorometric DeNovix dsDNA Broad Range Assay on a Denovix QFX fluorometer (DeNovix, USA). NGS library preparation was performed using the *BRCA* Pro Panel kit (AmoyDx, China) according to the manufacturer's instructions. Sequencing was set up on the Illumina NextSeq 550Dx platform and NGS data analysis was performed via ANDAS ADXHS-*gBRCA* v1.5.0 (AmoyDx, China). The bioinformatic data analysis pipeline was provided by sequencing reagent kit manufacturer to be used specifically with AmoyDx reagents (AmoyDx, China). The data analysis pipeline includes the following steps: fastq file quality control, sequence alignment to reference hg19 human genome, variant calling and genetic variant functional annotation. Sequencing and NGS data analysis were performed at CSD LAB, Kyiv, Ukraine. Only genetic variants in heterozygous and homozygous (>25% depth of sequencing cutoff) states were analyzed to prevent potential mosaic variants from being included in the dataset. The method is validated for clinical diagnostic use and each library preparation batch is evaluated after sequencing to identify artifact patterns, i.e. a singular genetic variant present in all cases within a batch. We do not expect any false positive/false negative results to be included. In this study, only variants of Class 5 Pathogenic (PV) and Class 4 Likely pathogenic (LPV) were considered, defined by ACGS Best Practice Guidelines (2020). Patients who lack PV or LPV gBRCA1/2 were assigned as wild-type (WT) in this study.

Statistical data analysis

Statistical analysis was conducted using MedCalc® Statistical Software version 23.1.7 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2025) and GraphPad Prism (GraphPad Prism Version 10.4.1 (627 GraphPad Software, San Diego, California, USA; www.graphpad.com). Lollipop graphs were constructed via G3viz R package[48]. Descriptive statistics were provided as Median and interquartile range (IQR; $Q_I - Q_{III}$). For comparing continuous variables, the Kruskal-Wallis test was applied. Categorical data were assessed as frequency (%). χ^2 test was used to compare frequencies. The P value of < 0.05 was considered statistically significant.

Data availability

The data represented in the study are deposited at NCBI Sequence Read Archive, BioProject Accession: PRJNA1331801, submissions SUB15651797 and SUB15637069.

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Author contributions
SL, DK, OK, OS and OSul conceived and designed research; SL, AK, AM, OK, OS retrieved and analyzed the data; OK, OS, NK, AM, YaS, NV, KK, AKh, VZ, AA, NO interpreted the results and prepared the figures; SL, DK, AK, OK, OS, NK, YaS, NV, VZ and OSul drafted the manuscript; all the authors contributed to editing, revision, and approved the final version of the manuscript.

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Declarations

Conflict of interest
The authors declare no competing interests.

Ethics declarations
The requirement of ethical approval was waived by IRB of Medical Laboratory CSD (Kyiv, Ukraine) for the studies on humans because of the anonymous nature of the retrieved retrospective data. The studies were conducted in accordance with the local legislation and institutional requirements. Due to the retrospective nature of the study, IRB of Medical Laboratory CSD waived the need to obtain informed consent.

Additional information

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