Molecular characteristics of breast cancer according to clinicopathological factors

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Abstract. The purpose of the present study was to evaluate the correlation between molecular factors such as BRCA1 DNA repair associated (*BRCA1*), checkpoint kinase 2 (*CHEK2*) and nucleotide binding oligomerization domain containing 2 (*NOD2*) gene mutations and clinicopathological factors in patients with breast cancer (BC). Prognostic factors were analyzed in BC patients with confirmed *BRCA1* (n=73), *CHEK2* (n=51) and *NOD2* (n=31) mutations. The control group was selected from BC patients without mutations (n=392). The *BRCA*-associated cancer cases were significantly more often triple negative compared with sporadic cancer (62% vs. 14%; P=0.0001). Luminal B HER2-positive and HER2-positive non-luminal subtypes were observed more often in the control group (33 and 17%). The luminal A subtype was detected in 53% of *CHEK2* mutation carriers and 45% of *NOD2* mutation carriers. A lower histological grade was observed significantly more often in patients with *CHEK2* mutations in comparison with the control group (88 vs. 69%; P=0.003). Lymph nodes without metastases were reported more frequently in *NOD2* mutation carriers (74 vs. 54%; P=0.038), in *BRCA1* mutations (73 vs. 54%; P=0.004) and, although not significantly, in *CHEK2* mutation carriers (69 vs. 54%; P=0.071) compared with the control group. In conclusion, *BRCA1* mutation was associated with TNBC and the luminal B HER2 (-) subtype. HER2-positive subtypes were characteristic of the control group. *CHEK2* and *NOD2* mutation carriers had a more favorable profile of prognostic factors.

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Introduction

Breast cancer is a heterogeneous complex of diseases, comprising a spectrum of numerous subtypes with distinct biological features. These biological subtypes lead to differences in treatment responses and clinical outcomes (1). Traditional classification systems include biological characteristics, including tumor size, lymph node involvement, histological grade, patient age, estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2 or c-erbB2) status (2). The diagnosis of breast cancer is based on clinical examination in combination with imaging and is confirmed by pathological assessment. Clinical examination includes bimanual palpation of the breasts and locoregional lymph nodes. Imaging diagnostics facilitate the assessment of the presence of distant metastases (bones, liver and lungs). A neurological examination is only required when symptoms are present (3).

The pathological report should include the histological type, grade, immunohistochemical (IHC) evaluation of estrogen receptor (ER) status (using a standardized assessment methodology, e.g., Allred or H-score), and progesterone receptor (PR) and human epidermal growth factor 2 receptor (HER2) gene expression. HER2 gene amplification status may be determined directly from all invasive tumors using *in situ* hybridization (fluorescent, chromogenic or silver), either as a replacement for IHC or for tumors with an ambiguous (2+) IHC score (4). Proliferation markers such as Ki67 should also be assessed (5).

Breast cancer tumors are divided into subtypes according to aforementioned factors, defined by routine histology and IHC (the 2015 St Gallen Consensus Conference). This classification is very important for prognosis and treatment decisions (1,6). Luminal-A is the most common subtype and represents 50-60% of all breast cancer cases. This subtype is defined as ER-positive and/or PR-positive tumors with a negative HER2 and low Ki67 (proliferating cell nuclear antigen) index, assessed by immunohistochemistry. Patients with luminal-A breast cancer have a good prognosis and the relapse rate is significantly lower compared with that for other subtypes (7). Luminal-B tumors comprise 15-20% of breast cancer cases and have a more aggressive phenotype, higher histological grade, increased proliferative index and a worse prognosis. There are distinct HER2-negative (ER-positive; HER2-negative; Ki67% high; PR low) and HER2-positive (ER-positive; HER2-positive; any Ki67; any PR)

luminal B subtypes. HER2-positive cancer accounts for 15-20% of breast cancer subtypes. These tumors are characterized by high expression of the HER2 gene and other genes associated with the HER2 pathway and/or HER2 amplicon located on the 17q12 chromosome (8). The HER2-positive non-luminal subtype is highly proliferative, with negative steroid receptor status. The other group are basal-like tumors (triple negative, HER2-negative, ER and PR absent) (9). The progress in genetic diagnostics has led to the identification of novel molecular factors, including the *BRCA1/2, CHEK2, TP53* and *PALB2* genes (10-13). Additionally, the role of tumor-infiltrating lymphocytes (TILs) in carcinogenesis and cancer progression has been confirmed (14,15). The development of novel specific molecular targets within cancer cells is currently an important goal of oncology and part of treatment individualization. In recent years there has been a significant increase in the influence of genetic factors on the diagnostic process and the therapeutic decisions of patients with cancer, particularly breast cancer.

The purpose of the present study was to evaluate the correlation between molecular factors, including *BRCA1, CHEK2* and *NOD2* gene mutations, and well known clinicopathological factors in patients with breast cancer. As a follow-up study, it sought to assess the usefulness of molecular factors in the traditional classification systems of breast cancer.

Patients and methods

Patients. The present study retrospectively analyzed a data from a previous study conducted between the years 2007 and 2016 in the MSC Memorial Cancer Centre and Institute of Oncology, Gliwice Branch (COI; Poland), clinicopathological prognostic factors were analyzed in patients with breast cancer with confirmed *BRCA1* (n=73), *CHEK2* (n=51) and *NOD2* (n=31) mutations. The control group was selected from breast cancer patients without mutations (n=392). The patients in all groups (*BRCA1, CHEK2* and *NOD2* mutation carriers, and the control group) were treated according to the same protocol. All patients provided written informed consent allowing their biological material to be used to clinical research.

All patients were women diagnosed, treated and followed-up at the COI in Gliwice. Patient underwent clinical follow-up examinations every 3 months in the first 2 years, every 6 months thereafter until the 5th year following diagnosis, and every year subsequently. Inclusion criteria were as follows: Breast cancer confirmed by microscopic examination; performance status ZUBROD 0-1; age >18 years; and normal values of renal and liver function, bone marrow. Data for age at onset, menopausal status, surgical procedures, disease stage according to TNM classification (T-the size of the tumor; N-spread of cancer to nearby lymph nodes; and M-metastasis), histology, estrogen and progesterone receptor status, HER2 status and contralateral breast cancer were gathered from hospital records and pathology reports. The analysis of patient medical records was performed according to national legal regulation. The complete characteristics of patients with regard to demographic and clinicopathological features are presented in Tables I and II. Treatment strategies are illustrated in Table III. Preliminary results of this study for *BRCA, CHEK2* and *NOD2* mutations have been presented in our previous publications (16-18).

Methods. The status of *CHEK2**1100delC and I157T mutations (GenBank NM_007194.3) was assessed by ASA-PCR and RFLP-PCR techniques, respectively. The present study examined the most common mutations in *BRCA1* (c.68_69delAG, c.181T>G, c.4034delA, c.5266dupC and c.3700_3704del5; GenBank NM_007294.3) and *BRCA2* (c.5946delT and c.9403delC; GenBank NM_000059.3) present in the Silesian population. The presence of the c.3016_3017insC mutation of *NOD2* (GenBank NM_022162.1) was also evaluated in the study group (Table IV). Each patient provided informed consent prior to venous blood collection for a genetic test. Genomic DNA was isolated from peripheral blood leucocytes.

Statistical analysis. Statistical analysis was performed using STATISTICA 13 software (StatSoft, Inc., Tulsa, OK, USA). The frequency of side effects was counted. The qualitative features are presented as the percentage of their occurrence and were evaluated with Fisher's test and χ^2 test with the Yates correction. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical factors and mutations. *BRCA1* mutation carriers were significantly younger in comparison with patients without detected mutations (P=0.0001). The median age of *BRCA1* mutation carriers was 43 years (range, 25-74 years) and for the control group it was 53 years (range, 26-78 years). Patients with *BRCA*-associated breast cancer were also significantly more often in the premenopausal age range compared with the control group (71 vs. 49%; P=0.0005). The median age at menarche was 14 years old, which was similar in the two groups (P=0.559). However, the median number of births was significantly lower in patients with *BRCA1* mutations (P=0.0001). The median age of *CHEK2* carriers was 50 years (range, 26-71). In the present analysis, *CHEK2* carriers were younger, although not significantly, compared with the control group (P=0.081). No significant differences were identified between *CHEK2* mutation carriers and the control group according to postmenopausal status (47 vs. 51%; P=0.656). The median age at breast cancer diagnosis for the carriers of the *NOD2* mutation was 47 years (range, 27-68). All mutation carriers were younger compared with patients in the control group. The youngest patients were *BRCA1* mutation carriers (median age, 43 years), followed by those with the *NOD2* mutation (median age, 47 years) and those with the *CHEK2* mutation (median age, 50 years) (Table I). There were no differences in age between *BRCA1* and *NOD2* mutation carriers (P=0.338) or between patients with *CHEK2* and *NOD2* mutations (P=0.268). *BRCA1* mutation carriers were younger in comparison with patients with *CHEK2* mutation (P=0.015). *BRCA1* mutation carriers were more frequently in the premenopausal period compared with patients with the *CHEK2* (P=0.037) or *NOD2* mutation (P=0.054).

A history of cancer in the family was reported in 123 (31%) in control group and 80 (52%) patients with mutations (P=0.0001). A family history of cancer was observed more frequently in *CHEK2* mutation carriers (61 vs. 31%; P=0.0001) and *NOD2* mutation carriers (58 vs. 31%; P=0.005) compared with control group patients. There was a detected tendency

Table I. Clinicopathological characteristics of the patient according to mutation.

Clincopathological criteria	BRCA1, n=73	P-value (BRCA1 vs. Control group)	CHEK2, n=52	P-value (CHEK2 vs. Control group)	NOD2, n=31	P-value (NOD2 vs. Control group)	Control group, n=392
Age (median 52 years)	43 (24-74)	0.0001	50 (26-71)	0.081	47 (27-68)	0.005	53 (26-78)
Menopausal status		0.0005		0.656		0.853	
Postmenopausal	21 (29%)		24 (47%)		15 (48%)		200 (51%)
Premenopausal	52 (71%)		27 (53%)		16 (52%)		192 (49%)
Cardiovascular diseases		1.00		0.513		0.681	
Yes	4 (5%)		4 (8%)		2 (6%)		21 (5%)
No	69 (95%)		47 (92%)		29 (94%)		371 (95%)
Infectious diseases		1.00		0.412		0.302	
Yes	2 (3%)		3 (6%)		2 (6%)		13 (3%)
No	71 (97%)		48 (94%)		29 (94%)		379 (97%)
Diabetes		0.661		0.605		0.499	
Yes	2 (3%)		0 (0%)		1 (3%)		8 (2%)
No	71 (97%)		51 (100%)		30 (97%)		384 (98%)
Hypertension		0.159		0.116		0.620	
Yes	7 (10%)		13 (25%)		6 (19%)		64 (16%)
No	66 (90%)		38 (75%)		25 (81%)		328 (84%)
History of cancer in family							
Cancer	31 (42%)	0.065	31 (61%)	0.0001	18 (58%)	0.005	123 (31%)
Breast cancer	18 (25%)	0.0009	14 (27%)	0.001	11 (35%)	0.0003	39 (10%)
Colorectal cancer	1 (1%)	0.336	7 (14%)	0.017	3 (10%)	0.193	18 (5%)
Gastric cancer	3 (4%)	0.726	5 (10%)	0.044	2 (6%)	0.302	13 (3%)
Lung cancer	5 (7%)	0.579	4 (8%)	0.505	1 (3%)	1.00	20 (5%)
Larynx cancer	1 (1%)	1.00	3 (6%)	0.096	0 (0%)	1.00	7 (2%)
CNS	1 (1%)	1.00	4 (8%)	0.013	0 (0%)	1.00	5 (1%)

BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2; CNS, central nervous system.

towards a family history of cancer in patients with BRCA1 mutations (42 vs. 31%; P=0.065). A family history of breast cancer was reported in patients with mutations in BRCA1 (25 vs. 10%; P=0.0009), CHEK2 (27 vs. 10%; P=0.001) or NOD2 (35 vs. 10%; P=0.0003) in comparison with the control group. Colorectal cancer (14 vs. 5%; P=0.017) and gastric cancer (10 vs. 3%; P=0.044) within the family history were observed more frequently in patients with CHEK2 mutation compared with the control group. Similarly, a significant family history of CNS tumors was identified in patients with CHEK2 mutations (8 vs. 1%; P=0.013) (Table I). There was reported no association between other types of cancer in family history and BRCA1, CHEK2 or NOD2 mutations.

Histopathological factors and mutations. Clinicopathological analysis was conducted. BRCA1-associated cancer had significantly more frequent negative steroid receptor status compared with the control group (62 vs. 31%; P=0.0001). HER2 overexpression was significantly more frequently detected in women without mutations compared with BRCA1 carriers (51 vs. 7%;

P=0.0001; Table II, Fig. 1). Histological type G3 was detected more frequently in patients with BRCA1 mutations (55 vs. 31%; P=0.0001; Fig. 2). There were observed differences between two of the analyzed groups (CHEK2 carriers and the control group) with respect to ER-positive status (82 vs. 66%; P=0.001), PR-positive status (78 vs. 59%; P=0.009) and HER2 overexpression (18 vs. 51%; P=0.0001; Table II, Figs. 1, 3 and 4). The histological grade of breast cancer differed between patients with CHEK2 mutations and the control group (12% G3 vs. 31% G3; P=0.003; Table II, Fig. 2). The absence of HER2 overexpression was observed significantly more frequently in NOD2 mutation carriers (90 vs. 49%; P=0.0001) compared with the control group (Table II, Fig. 1). By contrast, there were no differences between NOD2 mutation carriers and the control group with respect to ER (29 vs. 34%; P=0.253) and PR (32 vs. 41%; P=0.447) negative steroid receptor status (Table II, Figs. 3 and 4). A lower histological grade, G1-G2, was observed with similar frequency in NOD2 mutation carriers and the control group (74 vs. 69%; P=0.687; Table II, Fig. 2). G3 tumors were detected in 26% of mutation carriers and 31% of subjects in the control group.

Table II. Pathological characteristics of the patients.

Pathological features	BRCA1, n=73 (%)	P-value (BRCA1 vs. Control group)	CHEK2, n=51 (%)	P-value (CHEK2 vs. Control group)	NOD2, n=31 (%)	P-value (NOD2 vs. Control group)	Control group, n=392 (%)
HER2 overexpression		0.0001		0.0001		0.0001	
Positive	5 (7)		9 (18)		3 (10)		199 (51)
Negative	68 (93)		42 (82)		28 (90)		193 (49)
Tumor grade		0.0001		0.003		0.687	
G1-G2	33 (45)		45 (88)		23 (74)		269 (69)
G3	40 (55)		6 (12)		8 (26)		123 (31)
Estrogen status		0.0001		0.001		0.253	
Positive	25 (34)		42 (82)		22 (71)		258 (66)
Negative	48 (66)		9 (18)		9 (29)		134 (34)
Progesterone status		0.0001		0.009		0.447	
Positive	21 (29)		40 (78)		21 (68)		231 (59)
Negative	52 (71)		11 (22)		10 (32)		161 (41)
Clinical staging nodes		0.004		0.071		0.038	
Positive	20 (27)		16 (31)		8 (26)		179 (46)
Negative	53 (73)		35 (69)		23 (74)		213 (54)
Tumor size		0.002		0.186		0.327	
T1-T2	48 (66)		38 (75)		28 (90)		322 (82)
T3-T4	25 (34)		13 (25)		3 (10)		70 (18)
Clinical staging		0.484		0.253		0.142	
I	12 (16)		16 (31)		12 (39)		87 (22)
II	46 (63)		23 (45)		14 (45)		221 (56)
III	15 (21)		12 (24)		5 (16)		84 (21)
Histological type		0.018		0.033		0.252	
Ductal invasive carcinoma	56 (77)		32 (63)		27 (87)		301 (77)
Lobular invasive carcinoma	1 (1)		11 (22)		3 (10)		38 (10)
Other	16 (22)		8 (16)		1 (3)		53 (14)

BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2; HER2, human epidermal growth factor receptor 2.

Lymph nodes metastases occurred more frequently in the control group of patients compared with the group with BRCA1 mutations (46 vs. 27%; P=0.004). There was an observed tendency towards the presence of lymph node metastases in patients of the control group compared with CHEK2 mutation carriers (46 vs. 31%; P=0.071). Lymph nodes without metastases (N0) were reported more frequently in patients with NOD2 mutations compared with the control group (74% vs. 54%; P=0.038) (Table II, Fig. 5).

In the present study, patients with BRCA1 mutations had significantly more frequent large tumor sizes (T3-T4) compared with the control group (34 vs. 18%; P=0.002). CHEK2 mutation carriers were slightly more likely to present with locally advanced breast cancer (T3-T4) compared with the control group (25 vs. 18%; P=0.186) and patients with NOD2 mutations exhibited no differences in T grade (Table II, Fig. 6).

The majority of patients in studied groups had the ductal invasive carcinoma subtype: 77% of BRCA1 mutation carriers, 63% of

CHEK2 mutation carriers and 87% of patients with NOD2 mutations. The lobular type of breast cancer was detected significantly more frequently in CHEK2 mutation carriers compared with the control group (22 vs. 10%; P=0.033) (Table II).

Compared with the control group, BRCA1 mutation carriers were younger, and more frequently had higher tumor sizes (T3-T4), G3 tumors, negative steroid receptor status (ER-) and tumors without HER2 overexpression. CHEK2 mutation carriers more frequently had tumors without HER2 overexpression, ER-positive receptor status and lower histological grade (G1-G2). Patients with NOD2 mutation were younger and frequently had tumors without HER2 overexpression, when compared with the control group.

Molecular subtypes of breast cancer in patients with mutations. The distributions of the molecular types in breast cancer patients with the BRCA1, CHEK2 and NOD2 mutations differed significantly from the distributions of the subtypes in

Table III. Treatment strategy according to the presence of mutation.

Treatment strategy	BRCA1, n=76 (%)	CHEK2, n=51 (%)	NOD2, n=31	Control group, n=392 (%)
Chemotherapy regimen				
AC\FAC	48 (66)	22 (44)	12 (40)	328 (84)
AC + taxanes	18 (25)	8 (16)	5 (17)	41 (10)
CMF	5 (7)	2 (4)	1 (3)	3 (1)
Without	0 (0)	18 (35)	12 (39)	20 (5)
Trastuzumab therapy				
Yes	1 (1)	8 (16)	2 (6)	179 (46)
No	72 (99)	43 (84)	29 (94)	213 (54)
Hormonotherapy				
Yes	28 (38)	44 (86)	22 (71)	255 (65)
No	45 (62)	7 (14)	9 (29)	137 (35)
Local treatment				
Mastectomy	51 (70)	35 (69)	17 (55)	264 (67)
Breast conservation surgery (BCT)	14 (19)	15 (29)	14 (45)	106 (27)
Without surgery	8 (11)	1 (2)	0 (0)	22 (6)
Radiotherapy				
Yes	51 (70)	27 (53)	24 (77)	282 (72)
No	22 (30)	24 (47)	7 (23)	110 (28)

BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2; AC, anthracycline and cyclophosphamide; FAC, 5-Fluorouracil, anthracycline and cyclophosphamide; CMF, cyclophosphamide, mitoxantrone, 5-Fluorouracil; BCT, breast conserving therapy.

Table IV. Mutation sites of all analyzed molecular factors.

Mutation sites	n	%
CHEK2	51	
c.470T>C	48	94
c.1100delC	3	6
NOD2	31	
c.3016_3017insC	31	100
BRCA1	73	
c.5266dupC	41	56
c.181T>G	25	34
c.68_69delAG	2	3
c.3700_3704delGTAAA	2	3
c.1692_1693delTG	1	1
c.213-12A>G	1	1
c.5346G>A	1	1

BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2.

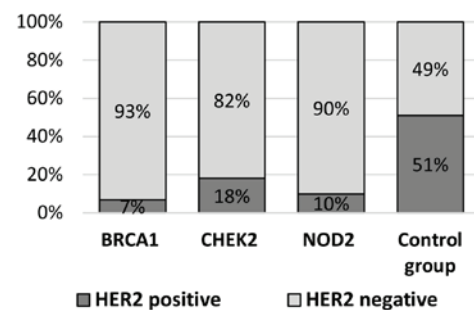Figure 1. Correlation between molecular factors and HER2 overexpression. HER2, human epidermal growth factor receptor 2; BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2.

B HER2-positive subtypes, were reported more frequently in the control group (56 and 33%, respectively) in comparison with the mutation carriers: BRCA1 (34 and 7%, respectively); CHEK2 (33 and 12%, respectively) and NOD2 (26 and 6%, respectively). Luminal A type breast cancer was diagnosed more frequently in CHEK2 mutation carriers (53%) and NOD2 mutation carriers (45%) compared with the control group (13%) (Table V).

Discussion

In previous studies, patients with BRCA1 mutation were characterized by their younger age, negative steroid receptor status

the control group (Table V, Fig. 7). The BRCA1-associated cancers were significantly more often triple negative (TNBC) compared with the tumors in the sporadic cancer cases (62 vs. 14%; P=0.0001). Luminal B subtypes, particularly Luminal
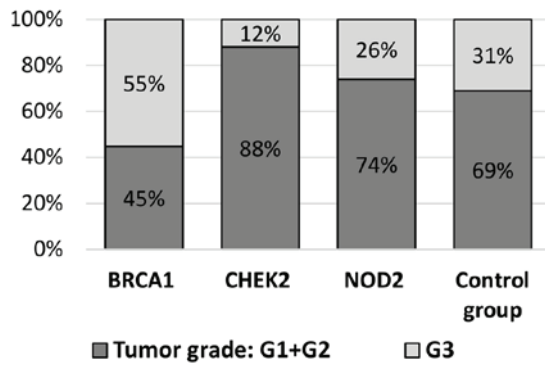
Figure 2. Correlation between molecular factors and tumor grade. *BRCA1*, BRCA1 DNA repair associated; *CHEK2*, checkpoint kinase 2; *NOD2*, nucleotide binding oligomerization domain containing 2.
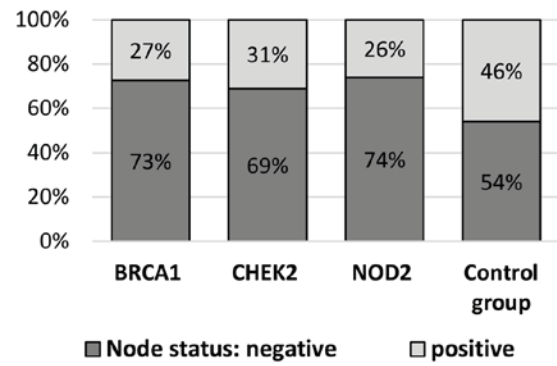

Figure 5. Correlation between molecular factors and node status. *BRCA1*, BRCA1 DNA repair associated; *CHEK2*, checkpoint kinase 2; *NOD2*, nucleotide binding oligomerization domain containing 2.
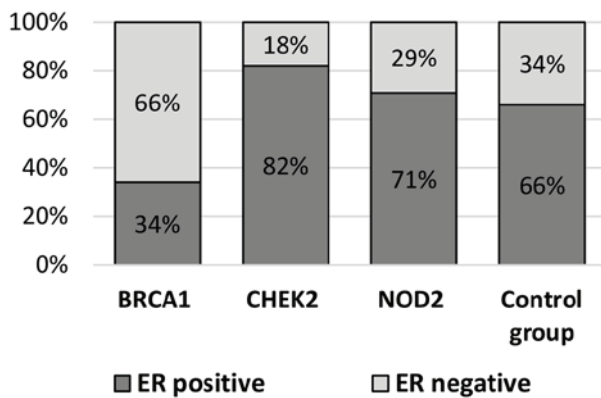

Figure 3. Correlation between molecular factors and ER status. ER, estrogen receptor; *BRCA1*, BRCA1 DNA repair associated; *CHEK2*, checkpoint kinase 2; *NOD2*, nucleotide binding oligomerization domain containing 2.
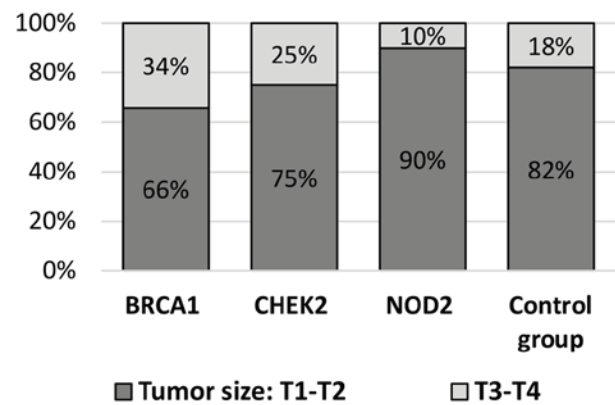

Figure 6. Correlation between molecular factors and tumor size. *BRCA1*, BRCA1 DNA repair associated; *CHEK2*, checkpoint kinase 2; *NOD2*, nucleotide binding oligomerization domain containing 2.
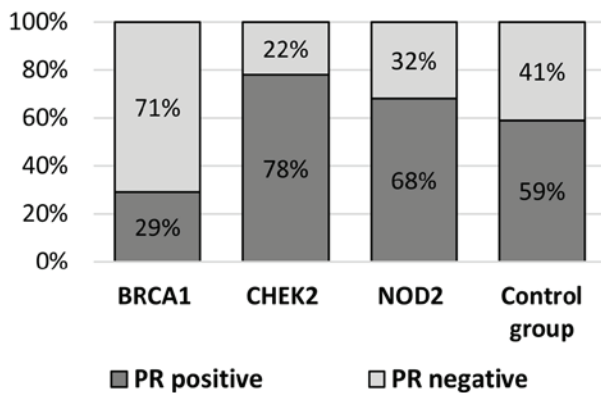

Figure 4. Correlation between molecular factors and PR status. PR, progesterone receptor; *BRCA1*, BRCA1 DNA repair associated; *CHEK2*, checkpoint kinase 2; *NOD2*, nucleotide binding oligomerization domain containing 2.

(ER-), HER2 negative and triple negative tumors (19). The basal type of cancer was also significantly associated with *BRCA1* expression (20). The present results confirmed those of previous studies. In the analyzed group, the median age of patients with *BRCA1* mutations was significantly lower compared with that of patients in the control group (43 vs. 53 years; P<0.0001). These mutation carriers were also more frequently in the premenopausal period (71 vs. 49%; P=0.0005), had more locally

advanced primary tumors (34 vs. 18%; P=0.002) and were more often triple negative (62 vs. 14%; P=0.0001). The more advanced disease stage and TNBC subtype in *BRCA1* mutation carriers may be associated with a more aggressive clinicopathological tumor type. These observations are consistent with those from previous studies (21-24).

A study conducted by de Bock *et al* (25) demonstrated that patients with a *CHEK2* mutation were significantly younger than patients without this mutation (49.0 vs. 53.2 years; P=0.03). Patients with a germline CHEK2*1100delC mutation more frequently had tumors with positive steroid receptor status [ER (91 vs. 69%; P=0.03); PR (81 vs. 53%; P=0.04)] in comparison with non-carriers. By contrast, no significant differences between these two groups were reported with respect to tumor size, histological subtype, grade, or surgical procedure, or in the choice of adjuvant systemic therapy or radiotherapy (25). In patients with early-onset breast cancer from Poland, ER-positive status was observed more frequently in carriers of *CHEK2* truncating mutations compared with non-carriers (72 vs. 58%; P=0.01). Women with a *CHEK2* mutation had a four-fold increased risk of ER-positive breast cancer in the Polish population (26). A correlation between *CHEK2**1100delC mutation status and tumor characteristics was also reported in trial conducted by Kilpivaara *et al* (27). In that study, no association was observed between this mutation and hormone

Table V. Molecular subtype of breast cancer according to St Gallen.

Molecular subtype	BRCA1, n=76 (%)	P-value BRCA1 vs. Control group	CHEK2, n=51 (%)	P-value, CHEK2 vs. Control group	NOD2, n=31 (%)	P-value NOD2 vs. Control group	Control group, n=392 (%)
Luminal A	3 (4)		27 (53)		14 (45)		49 (13)
Luminal B HER2 negative	20 (27)		11 (22)		6 (19)		90 (23)
Luminal B HER2 positive	5 (7)	0.0001	6 (12)	0.0001	2 (6)	0.0001	131 (33)
HER2 positive non luminal	0 (0)		3 (6)		1 (3)		68 (17)
Triple negative	45 (62)		4 (8)		8 (26)		54 (14)

BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2; HER2, human epidermal growth factor receptor 2.
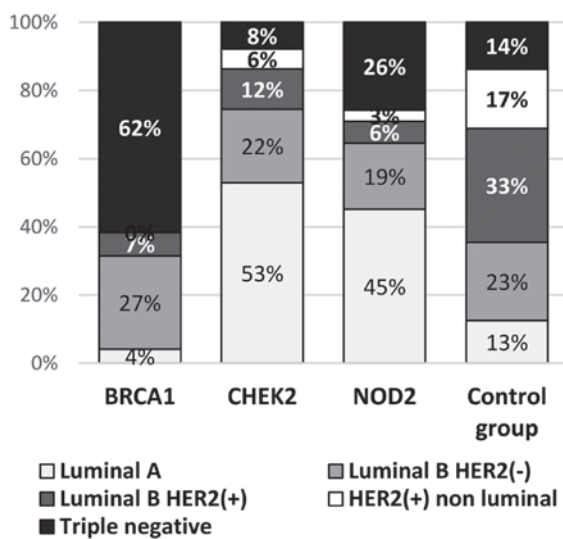

Figure 7. Correlation between molecular factors and molecular breast cancer type. BRCA1, BRCA1 DNA repair associated; CHEK2, checkpoint kinase 2; NOD2, nucleotide binding oligomerization domain containing 2.

receptor status, tumor histology or lymph node status. Another analyzed risk factor was ionizing radiation treatment. In a study conducted by Broeks et al (28), an association was observed between BRCA1, BRCA2 and CHEK2 germline mutation carriers and the risk of radiation-induced contralateral breast cancer in comparison with non-carriers [odds ratio (OR), 2.51; 95% confidence interval, 1.03-6.10; P=0.049]. In the present analysis, the presence of luminal A type breast cancer was reported in CHEK2 mutation carriers more frequently than in the control group (53% vs. 13%; P=0.0001). HER2 overexpression was detected more frequently in the control group. In the present study, there was observed tendency towards a family history of cancer in patients of the control group compared with mutation carriers (46 vs. 31%; P=0.071). The present study also observed a tendency towards a family history of cancer, particularly gastric, colorectal or CNS cancer, in the carrier groups. Breast cancer within the family history was observed significantly more often in CHEK2 mutation carriers in comparison with the control group (27 vs. 10%; P=0.001). Lower histological grade was observed significantly more often

in patients with CHEK2 mutations compared with the control group (88 vs. 69%; P=0.003). The carriers also had locally advanced breast cancer (T3-T4) slightly more frequently than the control group (25 vs. 18%; P=0.186). Domagala et al (29) reported a significant association between CHEK2 mutations and molecular breast cancer subtype classification (P=0.004). Patients with mutations in the CHEK2 gene primarily had luminal subtypes of breast cancer (108/117=92.3%). The CHEK2-I157T variant was associated with the luminal A subtype (P=0.01), whereas CHEK2-truncating mutations were associated with the luminal B subtype (P=0.005).

In certain studies, there was an observed association between the NOD2 3020insC mutation and early breast cancer (OR=1.9; P=0.01) (30). Similarly, ductal invasive carcinoma breast cancer with an in-situ component was more frequently reported in mutation carriers (OR=2.2; P=0.006) (30). In the present group, all patients had early breast cancer. Ductal invasive carcinoma was also observed more frequently in NOD2 mutation carriers (87 vs. 77%; P=0.252) in comparison with the control group. Other clinicopathological factors were also analyzed. Janiszewska et al (31) did not report any NOD2 mutations in patients diagnosed with breast cancer after the age of 50 years. There was no reported association between NOD2 mutations and a strong family history of breast cancer. This mutation frequency (11.4%) was two times higher in women from families with a single case of breast cancer. The association of NOD2 mutations with other common types of cancer, including digestive tract cancer, was described. The median age at breast cancer diagnosis in the present group of patients was 47 years (range, 26-68) for the carriers of the NOD2 mutation and 53 years (range, 26-78) for the control group. Differences were observed with respect to age between the two groups. The other factors associated with NOD2 mutations in the present study were: HER2 negative tumors (HER2-) and lymph nodes without metastases (N-). The most common type of breast cancer in this group was the Luminal type A and the TNBC subtype.

In conclusion, the presence of mutations was associated with a younger age of disease diagnosis, independent of mutation type (BRCA1, CHEK2 and NOD2). BRCA1 mutation was associated with TNBC cancer and the Luminal B HER2-negative breast cancer subtype, CHEK2 mutation with

the Luminal A and Luminal B HER2-negative subtypes, and *NOD2* mutation with Luminal A breast cancer and the TNBC subtype. *CHEK2* and *NOD2* mutation carriers had favorable prognostic profiles, such as G1-G2, N (-) and HER2-negative tumors.

Authors' contributions

JH is responsible for the study design, preparation of the manuscript and final approval of the version to be published. ZK conducted the statistical analysis, corrected the manuscript, provided intellectual content and gave final approval of the version to be published.

Ethics approval and consent to participate

Not applicable. The present study was retrospective.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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