Clinical research

Comparison between *NOD2* gene mutation carriers (3020insC) and non-carriers in breast cancer patients: a clinicopathological and survival analysis

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Abstract

Introduction: The 3020insC mutation of *NOD2* predisposes to many types of common cancers, e.g. breast cancer. In this report we compare *NOD2* 3020insC mutation carriers with non-carriers in a similar age range at diagnosis according to clinicopathological factors and survival in breast cancer patients from the Silesia region in Poland.
Material and methods: We reviewed the medical records of 72 early breast cancer patients, who were diagnosed and treated in COI in Gliwice. Genetic diagnostics was conducted in all patients. Twenty-eight (39%) patients were *NOD2* mutation carriers and 44 (61%) were non-carriers.
Results: Triple-negative breast cancer (TNBC) was detected more often in *NOD2* mutation carriers than non-carriers (25% vs. 11.4%, $p = 0.194$). Similarly, lymph nodes without metastases (N0) were reported more frequently in patients with *NOD2* mutation (71.4% vs. 43.2%, $p = 0.029$). HER2 without overexpression was observed insignificantly more often in group with NOD2 mutation (82.1% vs. 63.6%, $p = 0.115$). Similarly, lower histological grade (G1+G2). There was no difference in tumor size (T1–T2) (89.3% vs. 86.4%, $p = 1.00$) or steroid receptor status (28.6% vs. 29.5%, $p = 1.00$) between groups. The median follow-up was 5.1 years (range: 0.6–26.1 years) for NOD2 carriers and 5.3 years (range: 2.0–19.7 years) for non-carriers. There was no difference between mutation carriers and non-carriers according to overall survival (5-year OS: 96% vs. 93%, $p = 0.427$).
Conclusions: There were no differences between NOD2 (3020insC) mutation carriers and non-carriers, according to comorbid condition, drugs, tumor size, steroid receptor status and 5-year overall survival.

Key words: breast cancer, *NOD2* mutation carriers, *NOD2* non-carriers, clinicopathological factors.

Introduction

The *NOD2* gene has been identified and mapped to chromosome 16q12 by Hugot *et al.* It consists of 12 exons, and its product, a cyto-

solic protein, consists of 1,040 amino acids [1]. The NOD2 protein plays an important role in immune system function. It is active in some types of immune system cells (including monocytes, macrophages, and dendritic cells), which help to protect the body against foreign invaders such as viruses and bacteria. The protein is also active in several types of epithelial cells, including Paneth cells, which are found in the lining of the intestine. These cells help to defend the intestinal wall against bacterial infection [2]. NOD2 is involved in the inflammatory response and the activation of the nuclear factor κB (NFκB) pathway.

At least 17 mutations in the *NOD2* gene have been found to cause Blau syndrome, an inflammatory disorder that primarily affects the skin, joints, and eyes. *NOD2* gene mutations can also cause an early-onset sarcoidosis. The mutation predisposes to Crohn's disease, a common chronic inflammatory bowel disease that is known to favor colorectal cancer development [3–6]. Approximately 40 variations in the *NOD2* gene have been associated with an increased risk of Crohn's disease.

The 3020insC mutation of *NOD2* also predisposes to many types of common cancers, e.g. breast cancer. The mutant allele is more frequently found in women with an early-onset breast cancer and in women with ductal breast cancer with an in situ component. The NOD2 3020insC allele is relatively common (7.3%) in the Polish population. The aim of this study was to compare 3020insC *NOD2* mutation carriers with non-carriers in a similar age range at diagnosis according to clinicopathological factors and survival.

Material and methods

A retrospective analysis was conducted on the medical records of 72 breast cancer patients who were diagnosed and treated with chemotherapy, hormone therapy and/or immunotherapy at Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch in Poland (COI). The patients were Caucasian women from the southern part of Poland – the Silesian region. Twenty-eight (39%) of the patients were *NOD2* mutation carriers and 44 (61%) of them were non-carriers (control group). The inclusion criterion in the control group was age at diagnosis which was comparable to the age of *NOD2* mutation carriers. All patients in the control group were *BRCA* non-carriers. Genetic diagnostics were conducted in the years 2012–2016. All patients gave written informed consent for genetic examination. The median age at diagnosis of patients was 46 years (range: 27–64 years). All of them were in good performance status (ZUBROD 0-1). The complete characteristics of patients with regard to demographic and clinicopathological features are presented in Tables I and II.

Table I. Patients' characteristics according to the presence of *NOD2* (3020insC) mutation

Risk factor	*NOD2* mutation carriers *N* = 28	*NOD2* non-carriers *N* = 44	OR (95% CI)	*P*-value
Age median [years]	46.0 (27–64)	45.5 (30–63)		
Cigarette smoking:				0.728
Yes, 9 (12.5%)	4 (14.3%)	5 (11.4%)	1.3 (0.23–6.69)	
No, 63 (87.5%)	24 (85.7%)	39 (88.6%)	1	
Comorbid condition:				0.612
Yes, 23 (31.9%)	10 (35.7%)	13 (29.5%)	1.32 (0.42–4.06)	
No, 49 (68.1%)	18 (64.3%)	31 (70.5%)	1	
Diabetes:				1.00
Yes, 3 (4.2%)	1 (3.6%)	2 (4.5%)	0.78 (0.01–15.68)	
No, 69 (95.8%)	27 (96.4%)	42 (95.5%)	1	
Cardiovascular diseases:				0.767
Yes, 14 (19.4%)	6 (21.4%)	8 (18.2%)	1.23 (0.31–4.65)	
No, 58 (80.6%)	22 (78.6%)	36 (81.1%)	1	
Viral diseases:			–	0.389
Yes, 1 (1.4%)	1 (3.6%)	0		
No, 71 (98.6%)	27 (96.4%)	44 (100%)		

Table II. Cancers in family history according to presence of NOD2 (3020insC) mutation carriers

Risk factor	*NOD2* mutation carriers *N* = 28	*NOD2* non-carriers *N* = 44	OR (95% CI)	*P*-value
Breast cancer in family history:				0.178
Yes, 19 (26.4%)	10 (35.7%)	9 (20.5%)	2.16 (0.65–7.17)	
No, 53 (73.6%)	18 (64.3%)	35 (79.5%)	1	
Renal cancer in family history:				0.389
Yes, 1 (1.4%)	1 (3.6%)	0	–	
No, 71 (98.6)	27 (96.4%)	44 (100%)		
Lung cancer in family history:				0.698
Yes, 7 (9.7%)	2 (7.1%)	5 (11.4%)	0.6 (0.05–4.04)	
No, 65 (90.3%)	26 (92.9%)	39 (88.6%)	1	
Gynecological cancer in family history:				0.235
Yes, 7 (9.7%)	1 (3.6%)	6 (13.6%)	0.23 (0.00–2.14)	
No, 65 (90.3%)	27 (96.4%)	38 (86.4%)	1	
Gastrointestinal cancer in family history:				0.373
Yes, 14 (19.4%)	7 (25%)	7 (15.9%)	1.76 (0.45–6.76)	
No, 58 (80.6%)	21 (75%)	37 (84.1%)	1	
Larynx cancer:				0.518
Yes, 2 (2.8%)	0	2 (4.5%)	0 (0–3.03)	
No, 70 (97.2%)	28 (100%)	42 (95.5%)	1	
Cancer in family history:				0.809
Yes, 42 (58.3%)	17 (60.7%)	25 (56.8%)	1.17 (0.40–3.47)	
No, 30 (41.7%)	11 (39.3%)	19 (43.2%)	1	

Anthracycline-based chemotherapy was applied to 37 (51.4%) patients. Nineteen (26.4%) of the women received anthracycline and taxanes together (at the same time or taxanes after anthracyclines). Sixteen (22.2%) patients had no systemic treatment. Adjuvant and neoadjuvant therapy were given to 32 (44.5%) and 24 (33.3%) in the whole group, respectively. Trastuzumab was administered to patients with tumor HER2 (human epidermal growth factor) overexpression. All women with positive steroid receptor status (estrogen (ER), progesterone receptor (PR)) received hormone therapy (53, 73.6% of patients). Radiotherapy was applied to 49 (61%) including all patients after breast preserving treatment. Treatment strategies are presented in Table III. The analysis of patients' medical records was performed according to the national legal regulation.

All patients were tested for the presence of 3020insC mutation of NOD2. Mutation analysis was carried out by a multiplex allele-specific polymerase chain reaction assay. Each patient signed informed consent before venous blood collection for a genetic test. Genomic DNA was isolated from peripheral blood leucocytes.

Statistical analysis

Statistical analysis was carried out using Statistica 7 software. The qualitative features were presented as the percentage of their occurrence and evaluated with Fisher's test and the χ^2 test with Yates' correction. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated as measures of association between the analyzed factors and the presence of *NOD2* mutation. Overall survival was estimated using the Kaplan-Meier method. The differences were considered significant if the *p*-value was ≤ 0.05.

Results

We analyzed a group of 72 breast cancer patients (28 *NOD2* mutation carriers and 44 non-carriers). The median age at breast cancer diagnosis

Table III. Treatment strategies according to presence of *NOD2* (3020insC) mutation

Risk factor	*NOD2* mutation carriers, *N* = 28	*NOD2* non-carriers, *N* = 44	*P*-value
Radiotherapy:			0.796
Yes, 49 (68.1%)	20 (71.4%)	29 (65.9%)	
No, 23 (31.9%)	8 (28.6%)	15 (34.1%)	
Hormone therapy			0.788
Yes, 53 (73.6%)	20 (71.4%)	33 (75%)	
No, 19 (26.4%)	8 (28.6%)	11 (25%)	
Chemotherapy			0.147
Yes, 56 (77.8%)	19 (67.9%)	37 (84.1%)	
No, 16 (22.2%)	9 (32.1%)	7 (15.9%)	

was 46.0 (27–64) years for the carriers of the *NOD2* mutation and 45.5 (30–63) years for non-carriers. There was no difference between these two groups in cigarette smoking (14.3% vs. 11.4%, $p = 0.728$; OR = 1.3). Co-morbid conditions were observed in 35.7% of patients with NOD2 mutation and 29.5% of patients without mutation ($p = 0.612$; OR = 1.32). Similarly, there was no association between the presence of mutation and cardiovascular diseases (21.4% vs. 18.2%, $p = 0.767$; OR = 1.23) or diabetes (3.6% vs. 4.5%, $p = 1.00$; OR = 0.78) or viral diseases (3.6% vs. 0, $p = 0.389$) (Table I).

Cancer in family history was reported in 60.7% of *NOD2* mutation carriers and in 56.8% of non-carriers, $p = 0.809$; OR = 1.17. The presence of breast cancer (35.7% vs. 20.5%, $p = 0.178$; OR = 2.16), renal cancer (3.6% vs. 0, $p = 0.389$) and gastrointestinal cancers (25% vs. 15.9%, $p = 0.373$, OR = 1.76) were detected insignificantly more often in family history of *NOD2* mutation carriers in comparison to non-carriers. In contrast, lung (11.4% vs. 7.1%, $p = 0.698$; OR = 0.6), gynecological cancers (13.6% vs. 3.6%, $p = 0.235$; OR = 0.23) and larynx cancer (4.5% vs. 0, $p = 0.518$) were described insignificantly more often in non-carriers (Table II).

Triple-negative breast cancer (TNBC) tumors were detected insignificantly more often in *NOD2* mutation carriers than non-carriers (25% vs. 11.4%, $p = 0.194$; OR = 2.6). Similarly, lymph nodes without metastases (N0) were reported more frequently in patients with *NOD2* mutation in comparison to non-carriers (71.4% vs. 43.2%, $p = 0.029$; OR = 0.30). Most patients had tumors of a lower grade, T1-T2 (87.5%). There was no difference in tumor size between groups (T1-T2) (89.3% vs. 86.4%, $p = 1.00$; OR = 0.76). HER2 without overexpression was observed insignificantly more often in *NOD2* mutation carriers (82.1% vs. 63.6%, $p = 0.115$; OR = 0.38). In contrast, there were no differences between mutation carriers and non-carriers according to ER (28.6% vs. 29.5%, $p = 1.00$; OR = 1.05) and PR (32.1% vs. 27.3%, $p = 0.791$; OR = 0.79) negative steroid receptor status. The lobular type of breast cancer was observed insignificantly more often in *NOD2* mutation carriers (7.1% vs. 4.5%). Most patients in both groups had ductal invasive carcinoma. Lower histological grade (G1+G2) was observed insignificantly more frequently in *NOD2* mutation carriers than non-carriers (78.6% vs. 63.6%, $p = 0.202$; OR = 0.48). Grade 3 were detected in 21.4% of mutation carriers and 36.4% of non-carriers. Pathological characteristics of the tumors according to the presence of *NOD2* mutation are shown in Table IV.

All the patients were followed up in the Cancer Center and Institute of Oncology in Gliwice. The median follow-up was 5.1 years (range: 0.6–26.1 years) for *NOD2* carriers and 5.3 years (range: 2.0–19.7 years) for non-carriers. There was no difference between mutation carriers and non-carriers in overall survival (5 years OS: 96% vs. 93%, $p = 0.427$). In the case of contralateral breast cancer the follow-up was counted from the date of the first diagnosis of breast cancer. Second primary cancers were found in four patients: in three *NOD2* mutation carriers (2 patients with breast cancer and one patient with endometrial cancer) and in one patient in the control group (lung cancer).

Discussion

In the present study, we compare NOD2 mutation carriers and non-carriers according to clinicopathological factors such as hormone status (estrogen (ER), progesterone receptor (PR)), human epidermal growth factor (HER2), tumor size, the presence of lymph node metastases, co-morbid conditions and history of cancer in the family.

The *NOD2* 3020insC allele is relatively common (7.3%) in the Polish population [7]. In a previous study it was reported in 8.0% of 462 breast cancer patients from Szczecin [8] and in 8.8% of 148

Table IV. Pathological characteristics of tumors according to presence of NOD2 (3020insC) mutation

Risk factor	*NOD2* mutation carriers *N* = 28	*NOD2* non-carriers *N* = 44	OR (95% CI)	*P*-value
Clinical staging nodes:				0.029
N positive, 33 (45.8%)	8 (28.6%)	25 (56.8%)	0.30 (0.10–0.93)	
N0, 39 (54.2%)	20 (71.4%)	19 (43.2%)	1	
Tumor size:				1.00
T3-4, 9 (12.5%)	3 (10.7%)	6 (13.6%)	0.76 (0.11–3.98)	
T1-2, 63 (87.5%)	25 (89.3%)	38 (86.4%)	1	
Grade G:				0.202
G3, 22 (30.6%)	6 (21.4%)	16 (36.4%)	0.48 (0.13–1.57)	
G1 + G2 50 (69.4%)	22 (78.6%)	28 (63.6%)	1	
ER:				1.00
Positive, 51 (70.8%)	20 (71.4%)	31 (70.5%)	1.05 (0.33–3.48)	
Negative, 21 (29.2%)	8 (28.6%)	13 (29.5%)	1	
PR:				0.791
Positive, 51 (70.8%)	19 (67.9%)	32 (72.7%)	0.79 (0.25–2.57)	
Negative, 21 (29.2%)	9 (32.1%)	12 (27.3%)	1	
HER2 overexpression:				0.115
Positive, 21 (29.2%)	5 (17.9%)	16 (36.4%)	0.38 (0.10–1.32)	
Negative, 51 (70.8%)	23 (82.1%)	28 (63.6%)	1	
Triple negative:				0.194
Yes, 12 (16.7%)	7 (25%)	5 (11.4%)	2.6 (0.61–11.61)	
No, 60 (83.3%)	21 (75%)	39 (88.6%)	1	
Histological type:				0.622
Ductal invasive carcinoma, 59 (81.9%)	24 (85.7%)	35 (79.5%)	1	
Lobular invasive carcinoma, 4 (5.6%)	2 (7.1%)	2 (4.5%)	1.46 (0.19–11.08)	
Other, 9 (15.9%)	2 (7.1%)	7 (15.9%)	0.42 (0.08–2.18)	
Luminal B type, 29 (40.3%)	8 (28.6%)	21 (47.7%)	1	0.099
Luminal A type, 24 (33.3%)	12 (42.9%)	12 (27.3%)	2.63 (0.84–8.22)	
Triple negative, 12 (16.7%)	7 (25%)	5 (11.4%)	3.68 (0.90–15.01)	
Non-luminal, 7 (9.7%)	1 (3.6%)	6 (13.6%)	0.44 (0.05–4.23)	

breast cancer patients from Bydgoszcz [9]. The population risk of breast cancer before the age of 50, associated with the *NOD2* mutation, is approximately 1% [8]. In the present study, the mutation was detected in 28 women, reaching the incidence of 6.1%.

The presence of the *NOD2* 3020insC allele increases the lifetime risk of cancer by approximately 25% to 35% [7]. In the study conducted by Huzarski *et al.*, the authors found a modest, not statistically significant, association of the *NOD2* 3020insC mutation with family history of breast cancer [8]. Janiszewska *et al.* suggested that the *NOD2* 3020insC mutation might predominantly increase the risk of developing digestive tract cancer rather than breast cancer [9]. In a study conducted by Kurzawski *et al.* the frequency of the 3020insC mutation in a consecutive series of 250 non-hereditary nonpolyposis colorectal cancer patients > 50 years of age was significantly elevated compared to the control population (OR = 2.23; $p = 0.0046$) [10]. Teodorczyk *et al.* showed that for *NOD2* 3020insC carriers over 50 years of age the risk of gastric cancer more than doubled

(OR = 2.479, p = 0.022) and among women almost trebled [11]. In our analysis, the presence of breast cancer (35.7% vs. 20.5%, p = 0.178; OR = 2.16), renal cancer (3.6% vs. 0, p = 0.389) and gastrointestinal cancers (25% vs. 15.9%, p = 0.373, OR = 1.76) were detected insignificantly more often in family history of NOD2 mutation carriers in comparison to non-carriers.

In some studies there was observed an association between *NOD2* mutation and early breast cancer (OR = 1.9; p = 0.01) [8]. Similarly, ductal invasive carcinoma breast cancer with an in situ component was more often reported in mutation carriers (OR = 2.2; p = 0.006) [8]. In our group all patients had early breast cancer. No significant difference was found between *NOD2* mutation carriers and non-carriers according to the presence of ductal invasive carcinoma (85.7% vs. 79.5%). The lobular type of breast cancer was observed insignificantly more often in *NOD2* mutation carriers (7.1% vs. 4.5%). The other clinicopathological factors were also analyzed. Janiszewska *et al.* did not find any *NOD2* mutation in patients diagnosed with breast cancer after the age of 50 years. The median age at breast cancer diagnosis in our patients was 46.0 (27–64) years for carriers of the *NOD2* mutation and 45.5 years (30–63) for non-carriers. We did not observe a difference according to age between the groups due to the selection of the control group (in a similar age range at diagnosis as *NOD2* mutation carriers). The other factors associated with *NOD2* mutation in our study are: HER2 negative tumors (HER2–), lymph nodes without metastases (N–) and lower histological grade (<G3), which is in agreement with our previous results [12].

In conclusion, there were no differences between *NOD2* (3020insC) mutation carriers and non-carriers according to comorbid condition, drugs, tumor size, steroid receptor status and 5-year overall survival. The presence of *NOD2* mutation was found to be associated with an increased risk of breast, renal and colorectal cancer in family history. *NOD2* mutation in breast cancer women was associated with lymph nodes without metastasis (N0), lower histological grade (G<3) and negative HER2 receptor status (HER2–).

Conflict of interest

The authors declare no conflict of interest.

References

1. Hugot JP, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 1996; 29: 821-3.
2. Inohara N, Nuñez G. NODs intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 2003; 3: 371-82.
3. Zeybek C, Basbozkurt G, Gul D, Demirkaya E, Gok F. A new mutation in Blau syndrome. Case Rep Rheumatol 2015; 2015: 463959.
4. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. Nature 2001; 411: 603-6.
5. Hampe J, Cuthbert A, Croucher PJ, et al. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. Lancet 2001; 357: 1925-8.
6. Branquinho D, Freire P, Sofia C. NOD2 mutations and colorectal cancer – Where do we stand? World J Gastrointest Surg 2016; 8: 284-93.
7. Lubiński J, Huzarski T, Kurzawski G, et al. The 3020insC allele of NOD2 predisposes to cancer of multiple organs. Hered Cancer Clin Practice 2005; 3: 59-63.
8. Huzarski T, Lener M, Domagala W, et al. The 3020insC allele of NOD2 predisposes to early-onset breast cancer. Breast Cancer Res Treat 2005; 89: 91-3.
9. Janiszewska H, Haus O, Lauda-Swieciak A, et al. The NOD2 3020insC mutation in women with breast cancer from the Bydgoszcz region in Poland. First results. Hered Cancer Clin Pract 2006; 4: 15-9.
10. Kurzawski G, Suchy J, Kładny J, et al. The NOD2 3020insC mutation and the risk of colorectal cancer. Cancer Res 2004; 64: 1604-6.
11. Teodorczyk U, Cybulski C, Jakubowska A, et al. Mutations and polymorphisms of genes moderate increase in gastric cancer risk. Cancer Clin Pract 2012; 10 (Suppl 3): A22.
12. Huszno J, Grzybowska E, Kołosza Z, et al. The 3020insc allele of the NOD2 gene in breast cancer patients – a clinicopathological analysis. Int J Res Stud Med Health Sci 2017; 2: 3-7.