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The 3020insC *NOD2* gene mutation in patients with ovarian cancer

Mutacja 3020insC w genie *NOD2* u chorych z rakiem jajnika

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Summary

Objective: *There is an increasing evidence that genetic factors play a role in the etiology of malignant tumors. Mutations of BRCA1 and BRCA2 genes are responsible for an increased risk of ovarian cancer. The role of mutations in NOD2 gene in this type of neoplasm is still under investigation.*

The aim: *The aim of this study was to determine:*
1. incidence of NOD 2 3020insC constitutional mutation in a group of consecutive women with ovarian cancer,
2. risk of developing ovarian cancer in patients with NOD2 gene mutation,
3. clinical and pathological features of ovarian cancer in NOD2 gene mutation carriers.

Patients and Methods: *Clinical and pathological data were collected from 257 non-selected patients with primary epithelial ovarian cancer. The researches identified NOD2 3020insC gene mutation. On the basis of patient source documentation we obtained the data concerning the age of patients at diagnosis, histopathological recognition, FIGO stage and morphological grade G.*

Results: *19 out of 257 women were identified with germ-line 3020insC mutation of NOD2 gene (7.39%). An increased risk of ovarian cancer in NOD2 mutation carriers was not revealed (OR=1.01; p=0.928; 95%CI=0.61-1.66). The mean age at diagnosis of patients with NOD2 mutation was 54.8 (SD=9.9), while for non-carriers it was 53.2 (SD=10.2). The difference between these frequencies was statistically irrelevant (p=0.550).*
Clinical and pathological profile of ovarian cancer was made. We assessed the following features: age at disease onset, histopathology, FIGO stage and morphological grade G. For NOD2 mutation carriers no statistically significant features of ovarian cancer were revealed.

Conclusion:
1. Despite high frequency of constitutional mutations occurrence in NOD2 gene in women with ovarian cancer, genetic testing seem not to be justified in all women diagnosed with this disease.

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Otrzymano: **27.06.2008**
Zaakceptowano do druku: **25.07.2008**

2. Due to a lack of increased risk of ovarian cancer in NOD2 gene mutation carriers, proceedings for them may not differ from recommendations for general population.
3. It is difficult to determine characteristic clinical and pathological features of ovarian cancer for NOD2 gene mutation carriers.

Key words: **ovarian cancer** / ***NOD2*** /

Streszczenie

Wstęp: *Obserwuje się coraz więcej dowodów na rolę czynników genetycznych w rozwoju nowotworów. Wiadomo, że mutacje w genie BRCA1 i BRCA2 odpowiadają za podwyższone ryzyko rozwoju raka jajnika. Znaczenie innych zaburzeń genetycznych takich jak mutacje w genie NOD2 jest ciągle jeszcze badane.*
Cel pracy: *Celem badania było określenie:*
1. częstości występowania mutacji konstytucyjnej NOD2 3020insC w grupie nieselekcjonowanych kobiet z rakiem jajnika,
2. ryzyka rozwoju raka jajnika u pacjentek z mutacją genu NOD2,
3. cech patologiczno-klinicznych raka jajnika u nosicielek mutacji NOD2.
Materiał i metoda: *Do badania włączono 257 nieselekcjonowane chore z rakiem jajnika. Wykonano oznaczenia mutacji 3020insC w genie NOD2.*
Zebrano dane z historii chorób dotyczące wieku zachorowania, rozpoznania histopatologicznego, stopnia klinicznego zaawansowania nowotworu wg FIGO i stopnia morfologicznej złośliwości G.
Wyniki: *Mutację 3020insC w genie NOD2 badano u 257 pacjentek. Zmianę wykryto u 19 kobiet uzyskując częstość 7,39%. Nie wykazano podwyższenia ryzyka rozwoju raka jajnika dla nosicielek mutacji NOD2 (OR=1,01; 95%CI=0,61-1,66; p=0,928). Średnia wieku zachorowania dla nosicielek mutacji NOD2 wynosiła 54,8 (SD=9,9) w porównaniu do 53,2 (SD=10,2) dla chorych bez mutacji. Różnica częstości nie była istotna statystycznie (p=0,550). Dokonano charakterystyki patologiczno-klinicznej raka jajnika. Ocenie poddano wiek zachorowania, typ histologiczny nowotworu, stopień morfologicznej złośliwości G i stopień zaawansowania wg FIGO.*
Nie znaleziono istotnych statystycznie cech nowotworu charakterystycznych dla nosicielek mutacji w genie NOD2.
Wnioski:
1. Pomimo wysokiej częstości występowania mutacji konstytucyjnych w genie NOD2 w grupie kobiet chorych na raka jajnika nie wydaje się zasadne wykonywanie testu u wszystkich chorych z tym nowotworem.
2. Z powodu braku podwyższonego ryzyka rozwoju raka jajnika u nosicielek mutacji w genie NOD2 postępowanie w stosunku do nich może nie odbiegać od rekomendacji dla populacji ogólnej.
3. Trudno jest określić charakterystyczne cechy patologiczno-kliniczne raka jajnika dla nosicielek mutacji NOD2.

Słowa kluczowe: **rak jajnika / *NOD2*** /

Introduction

Ovarian cancer is a major women's health problem in gynecologic oncology. So far, the analyses have shown poor efficiency of screening procedures in decreasing mortality rates from this disease. A hope is put on indicating women with an increased risk of this neoplasm. It has been assessed, that technological progress in molecular research will certainly lead to the development of quick tests, which will enable detecting mutations in patients and their families. Finding mutation carriers in families with ovarian cancer may be of great importance in prevention of this disease.

It is claimed, that the leading cause of high genetic predisposition towards ovarian cancer is carrying of mutations in constitutional genes *BRCA1* and *BRCA2*. However, it has been observed, that in many cases of familial incidence, the genetic cause of this neoplasm is unknown. This provoked further research into other genes like *NOD2*, whose mutations or polymorphism variants are responsible for an increased risk of this disease.

In order to broaden our knowledge about ovarian cancer it is extremely important to determine the incidence of genetic disturbances as well as clinical and pathological features of this disease in mutation carriers. It is also crucial to appoint families with an increased risk of ovarian cancer by doing tests for genetic disturbance carrier state and by analysing pedigree and clinical data of each patient. Some data suggest also a relationship between the mutation incidence and sensitivity to cytostatics.

The *NOD2* gene was identified and mapped on chromosome 16q12 by Hugot et al. [1].

It consists of 12 exons and codes cytoplasmatic protein composed of 1040 aminoacids. The *NOD2* protein has three functional domains: EBD *(amino-terminal effector-binding domain)* with two CARDs *(caspase –recruitment domain)*, a centrally placed NOD *(nucleotide-binding oligomerization domain)* and LRD *(carboxy-thermal ligand-recognition domain)* which includes 10 leucine repeats (LRRs) on the carboxy therm [2].

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Ginekol Pol. 2008, 79, 544-549

The *NOD2* protein is involved in inflammatory response and activation of nuclear factor kappa-B (NFκB) through interactions with CARD domains after stimulation of monocytes by bacterial products [3]. The *NOD2* protein recognises muramyl dipeptide (MDP) deriving from bacterial peptidoglygan (PGN) through LRRs and additionally activates NFκB through the connection of CARDs with an adequate CARD from Rip2/RICK/CARDIAK [4]. The analysis of *NOD2* evolution has shown strong conservatism of the encoded protein, which is similar from 44.5% in fugu fish protein to 99.1% in chimpanzee [5].

The activation of NFκB is stronger if 3020insC mutation occurs in *NOD2*. Insertion of cytosine in exon 11 causes a frameshift and, consequently, substitution of leucine in the position 1007 (Leu1007fsinsC) in *NOD2* protein. This mutation, due to premature termination of protein synthesis, leads to 33 aminoacids shortening in the protein sequence.

In a number of surveys it was observed, that mutations in *NOD2* gene are connected with a predisposition to Crohn's disease. People suffering from this disease have shown an increased risk of colon cancer. However, it is still to explain how exactly the neoplasm develops. It is suggested, that genes involved in inflammatory response such as INFγ and TNFα cause neutrophile activation, which generates significant amount of free oxygen radicals [6]. It is thought that long-term exposition of gastrointestinal tract epithelium to free oxygen radicals may be related to an increased mutation incidence and, in consequence, susceptibility to develop neoplasms.

Not only patients with Crohn's disease have an increased NFκB activity level. This factor also plays a role in chronic pulmonary diseases and lung cancer development in smokers [7]. It has been proven that cytokines which cause an increase of NFκB level lead to ovarian cancer cells growth and that inhibition of NFκB activity may suppress this growth [8, 9].

The aim

The aim of this study was to determine:

1. Incidence of *NOD2* 3020insC constitutional mutation in a group of consecutive women with ovarian cancer.
2. Risk of developing ovarian cancer in patients with *NOD2* gene mutation.
3. Clinical and pathological features of ovarian cancer in *NOD2* gene mutation carriers.

Material and methods

The focus group consisted of 257 women with ovarian cancer treated at the Division of Gynecologic Oncology, Poznań University of Medical Sciences in the years 2003 - 2006. Each patient signed informed consent before venous blood collection for a genetic test. The aim of the test was to detect 3020insC mutation in *NOD2* gene. Genomic DNA was isolated from peripheral blood leucocytes. Analyses were performed in the Department of Genetics and Pathology –International Hereditary Cancer Center, Pomeranian Medical University, Szczecin. Blood samples were stored until analyses were done. The data collected from patient source documentation included: age at diagnosis, pathological recognition, FIGO stage and morphological grade G.

Statistic methods

For statistic analyses STATISTICA v. 7.0 from StatSoft and Microsoft Excel were used. Verification of a relationship between the two qualitative features was carried by means of the Chi^2 test, alternatively with Yates correction. The U Mann-Whitney test was used to compare age distribution in patient groups. The outcome was recognized as statistically significant when $p<0.05$. Odds ratio (OR) and 95%CI for OR were calculated by means of MS Excel function.

Methods of mutation detection

The incidence of each mutation was also determined for a control group from the Polish population. Genetic tests were done by researches from the Department of Genetics and Pathology – Hereditary Cancer Center in Szczecin [10].

It has been assessed, that 3020insC mutation in *NOD2* gene occurred in 140/1910 women from this group (7.32%).

Methods of mutation detection in *NOD2* gene

Genomic DNA was extracted from peripheral blood leukocytes by the standard method with proteinase K. Molecular investigations of 3020insC mutations were carried out by an allele-specific PCR assay using a standard kit (patent no. P-364412, Poland).

For genomic DNA amplification by means of PCR, primers framing the basic region 533 which surrounds 3020insC alleles were applied. Each PCR reaction included two additional primers. The first was to detect the wild allele and the second to recognize 3020insC allele.

For exposure purposes 5µl of PCR product was put to the electrophoresis on 1.5% agar gel stabilised with etidine bromide (Seatem FMC, buffer 1X TBE, 25µg/ml etidine bromide) at the voltage 6V/cm for 30 minutes. Separated products were exposed in UV light.

Results

The test for 3020insC mutation in *NOD2* gene was done in 257 patients. The mutation was detected in 19 women, reaching the incidence 7,39%. An increased risk of ovarian cancer for *NOD2* mutation carriers was not revealed (OR=1.01; 95%CI=0.61-1.66; p=0.928).

The mean age at diagnosis was 54,8 (SD=9.9) for *NOD2* mutation carriers of and 53.2 (SD=10,2) for patients without this mutation. The difference in occurrence frequency was statistically insignificant (p=0.550).

Clinical and pathological features of ovarian cancer were described. The collected data included: age at diagnosis, pathological recognition, FIGO stage and morphological grade G.

The age at diagnosis was analyzed as a feature in the studied group. Statistic significance was revealed neither for mutation carriers aged ≤50 years (OR=0.74; 95%CI=0.28-1.94; p=0.538) nor >50 years (OR=1.35; 95%CI=0.51-3.56; p=0.538).

Histological type of ovarian cancer was analyzed as a feature in the studied group.

Table I. Age at diagnosis for carriers of 3020insC mutation of *NOD2* gene in the group of patients with ovarian cancer.

Age at diagnosis	*NOD2* 3020insC
≤ 40	0/19 p = 0.337
41-49	7/19 p = 0.939; OR = 1.09; (95%CI=0.41-2.87)
≤ 50	7/19 p = 0.538; OR = 0.74;(95%CI=0.28-1.94)
> 50	12/19 p = 0.538; OR = 1.35; (95%CI=0.51-3.56)
50-60	6/19 p = 0.973; OR = 0.99; (95%CI=0.33-2.44)
> 60	6/19 p = 0.489; OR = 1.65; (95%CI=0.60-4.56)

Table II. Histological type of ovarian cancer in carriers of 3020insC mutation in *NOD2* gene.

Histological type of ovarian cancer	*NOD2* 3020insC
Adenocarcinoma serosum	7/19 p = 0.335; OR = 0.62; (95%CI=0.24-1.64)
Adenocarcinoma endometrioides	5/19 p = 0.245; OR = 1.88; (95%CI=0.64-5.53)
Adenocarcinoma solidum	4/19 p = 0.564; OR = 1.40; (95%CI=0.44-4.46)
Adenocarcinoma mucinosum	1/19 p = 0.622; OR = 0.52; (95%CI=0.07-4.07)
Adenocarcinoma clarocellulare	0/19 p = 0.616
Adenocarcinoma	2/19 p = 0.558; OR = 2.43; (95%CI=0.50-11.85)
Total	19

No characteristic histological type was found in carriers of the mutation. Statistic significance was not revealed for any type of ovarian cancer. Thus, for *adenocarcinoma serosum* (OR=0.62; 95%CI=0.24-1.64; p=0.335), for *adenocarcinoma endometrioides* (OR=1.88; 95%CI=0.64-5.53; p=0.245) and for *adenocarcinoma solidum* (OR=1.40; 95%CI=0.44-4.46; p=0.564).

Morphological grade G of ovarian cancer was analyzed in the studied group. No statistic significance was revealed for any morphological stage in mutation carriers.

It was assessed as follows: G1 (OR=1.02; 95%CI=0.31-3.33; p=0.980), G2 (OR=0.74; 95%CI=0.24-2.25; p=0.595) and G3 (OR=1.33; 95%CI=0.46-3.90; p=0.599).

Table III. Morphological grade – G of ovarian cancer in carriers of 3020insC mutation in *NOD2* gene.

Morphological grade G	*NOD2* 3020insC
G1	4/19 p = 0.980; OR = 1.02; (95%CI=0.31-3.33)
G1/G2	9/19 p = 0.599; OR = 0.75; (95%CI=0.26-2.19)
G2	5/19 p = 0.595; OR = 0.74; (95%CI=0.24-2.25)
G2/G3	11/19 p = 0.980; OR = 0.98; (95%CI=0.30-3.23)
G3	6/19 p = 0.599; OR = 1.33; (95%CI=0.46-3.90)
None	4

Table IV. FIGO stage in carriers of 3020insC mutation in *NOD2* gene in the group of patients with ovarian cancer.

FIGO stage	*NOD2* 3020insC
I	5/19 p = 0.892; OR = 1.08; (95%CI=0.37-3.14)
II	2/19 p = 0.931; OR = 1.53; (95%CI=0.33-7.10)
I/II	7/19 p = 0.660; OR = 1.24; (95%CI=0.47-3.28)
III/IV	12/19 p = 0.660; OR = 0.80; (95%CI=0.30-2.12)

FIGO stage was analyzed in the studied group. No characteristic feature was found for carriers of 3020insC mutation in *NOD2* gene. The estimation was made for stage I (OR=1.08; 95%CI=0.37-3.14; p=0.822), for stages I/II together (OR=1.24; 95%CI=0.47-3.28; p=0.660) as well as for stages III/IV together (OR=0.80; 95%CI=0.30-2.12; p=0.660).

Discussion

In a number of surveys it has been observed that mutations in *NOD2* gene are connected with predisposition to Crohn's disease. Researchers continuously have been interested in the role of chronic inflammation in carcinogenesis. According to the recent studies, patients with this disease have an increased risk of colon cancer as well as of other neoplasms.

In the focus group of 257 patients we looked for 3020insC mutation. This genetic change was revealed in 19 women (7.39%). Thus an increased risk of ovarian cancer was not proved for this mutation (OR=1.01; 95%CI=0.61-1.66; p=0.928).

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However, in the light of the results obtained in the presented study, it is worth to recall the outcome of the study by Lubiński et al, which showed statistically significant increase of ovarian cancer risk (OR=1.6; p=0.03) [11]. In a group of 317 patients mutation carrying was found in 35 women (11%). In our focus group the frequency of mutation carrying was lower and not statistically significant.

There have been few studies concerning the relationship between *NOD2* mutation and ovarian cancer. Below we would like to reckon some interesting results obtained in some of them.

In the study by Lubiński et al. the relationship between 3020insC *NOD2* mutation and neoplasms incidence was assessed in a group of 2604 patients with neoplasms and in a control group of 1910 people. The 3020insC *NOD2* mutation frequency in the group with neoplasms was higher in 8 out of 12 analyzed subgroups with different cancers. A significant relationship was found for colon cancer (OR=1.8), especially in the age group of >50 years (OR=2.2; p=0.006), for lung cancer (OR=1.7) and for ovarian cancer (OR=1.6). An association with low stage larynx cancer was also observed in the age group of <50 years (OR=2,9; p=0,009).

No relationship was found between the studied mutation and breast cancer in general however, the predisposition to intraductal breast cancer (DCIS) was noticed (OR=2.1; p=0.01). This relationship was especially distinct in women aged <50 years with DCIS (OR=3.0; p=0.01). Also, an association between kidney cancer and *NOD2* mutation was found (OR=0.4; p=0.03). The control group at Lubiński et al. study consisted of adults and newborns from five Polish cities. The frequency of *NOD2* 3020insC mutation occurrence in the group of newborns (7.8%) was similar to the group of adults (6.9%). No statistic difference in mutation occurrence frequency depending on the Polish cities was found.

For the inhabitants of Szczecin the observed frequency was 8.2%, while for other cities 7.3%. It may be explained by small differences in ethnic background in the Polish population compared to the societies of North America and Western Europe. The homogenic ethnicity of Polish population has already been proved in some research [12].

3020insC mutation is also present in other European populations, so it is expected, that similar studies will be conducted in other countries. For example, in the Greek population a research into colon cancer revealed an association with *NOD2* [13]. However, there are exceptions. For instance, a Finnish study proved no association between *NOD2* mutation and colon cancer [14].

To sum up, *NOD2* mutations are quite common. In Lubiński's study 7.3% people from the control group are carriers. The study proves that carrying *NOD2* 3020insC mutation causes 30% higher risk of developing neoplasms [15].

In the study by Dębniak et al. a correlation between the *NOD2* mutation and colon cancer was found (31/255; 12.2%; OR=1.8; p=0.01), especially in an older age (OR=2.2). The correlation was also found in: breast cancer (18/126; 14.3%), DCIS (OR=2.1; p=0.009), kidney cancer (8/245; 3.2%; OR=0.4; p=0.02); lung cancer (30/258; 11.6%; OR=1.7; p=0.03) and ovarian cancer (35/317; 11.0%; OR=1.6; p=0.03) [16].

No statistic significance was found for: urine bladder cancer (18/172; 10.5%; OR=1.5), larynx cancer (23/223; 3.2%; OR=1.5), malignant melanoma (10/198; 5.1%; OR=0.7), pancreas cancer (6/127; 4.7%; OR=0.6), prostate cancer (17/298; 5.7%; OR=0.6), stomach cancer (20/213; 9.4%; OR=1.3) or thyroid cancer (8/82; 9.8%; OR=1.4). In the control group of 1910 people 140 mutation carriers were revealed (7.3%).

In the study by Janiszewska et al. carried on a group of 148 patients with breast cancer 3020insC mutation was found in 8.8%. The mean age at disease was 43 years [17]. In the group of patients older than 50, the mutation was not revealed. The association between *NOD2* mutation and the familial breast cancer was also not proved. However, it was observed that the mutation frequency was twice higher (11.4%) in the group of women from families with sporadic breast cancer and with aggregation of other neoplasms, especially in gastrointestinal tract. In 104 out of 148 examined families (70.3%), a concentration of following cancers was revealed: colon, pancreas, stomach, larynx, lung, ovarian, endometrial, prostate, kidney, malignant melanoma, leukemia and sarcoma.

Kurzawski et al. also observed an association between Crohn's disease and *NOD2* 3020insC gene mutation carrying in the group of 556 patients with colon cancer [18].

A significant relationship between age and cancer incidence in general was not proved. In patients with larynx cancer in the study by Lubiński et al. the association was stronger for young people, while in patients with colon cancer the relationship was stronger for the older than 50. In the group with ovarian cancer for *NOD2* 3020insC carriers no significance of age at disease was observed.

What surprises, is an association of *NOD2* mutation with Crohn's disease, as well as with other neoplasms. It should be remembered that *NOD2* penetration for Crohn's disease incidence amounted below 1%, while for colon cancer it was about 10%. This is the reason why suggestion that *NOD2* mutation does not cause evident predisposition to these diseases was made.

In the study by Irmejs et al. *NOD2* mutation incidence in Latvia was assessed [19]. The *NOD2* 3020insC mutation was present in 7.7% (18/235) patients with colon cancer, in 9.2% (17/185) with breast cancer and in 7.7% (75/975) people from an control group. The *NOD2* 3020insC mutation was associated with a higher risk of breast cancer (OR=2.5; p<0.05) for patients diagnosed at the age between 51 and 60.

It has been thought, that *NOD2* 3020insC mutation carrying is responsible for higher risk of different neoplasms incidence, including cancer of colon, lung, larynx and ovary. It has been also assumed, that mutation carrying increases the risk of cancer by 25-35%.

However, a significant raise of the risk of any neoplasms was not confirmed by further research. Consequently, there are no genetic or clinical recommendations available. The *NOD2* gene is involved in inflammatory processes regulations, but it is not obvious if suppression of control mechanisms is responsible for higher cancer incidence.

Early diagnostics and neoplasm prevention procedures are now a priority in the health care all over the world. Genetic studies have an immense significance, as it is assumed that

most of neoplasms have their genetic basis. Identification of higher predisposition towards cancer gives a chance for effective treatment and higher survival rates. The research into detection of inherited predisposition to cancer is focused on discovering the incidence pattern related to genetic disturbances. Identification of people with higher cancer risk would be more effective if databases and population registries were implemented and used.

Conclusion

1. Despite high frequency of constitutional mutations occurrence in *NOD2* gene in women with ovarian cancer, genetic testing seem not to be justified in all women diagnosed with this disease.
2. Due to lack of increased risk of ovarian cancer in *NOD2* gene mutation carriers, proceedings for them may not differ from recommendations for general population.
3. It is difficult to determine characteristic clinical and pathological features of ovarian cancer for *NOD2* gene mutation carriers.

Acknowledgments

We would like to thank the team of nurses from the Division of Gynecologic Oncology, especially Irena Jurzynek and Anna Szwed for blood collection. This study was supported by the Polish National Scientific Committee, grant KBN No 2PO5E07330.

References

1. Hugot J, Laurent-Puig P, Gower-Rousseau C, [et al.]. Mapping of a susceptibility locus for Crohn_s disease no chromosome 16. *Nature.* 1996, 379, 821-823.

2. Inohara N, Nuoez G. NODs: intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol.* 2003, 3, 371-382.

3. Hampe J, Cuthbert A, Croucher P, [et al.]. Associacion between insertion mutation in NOD2 gene and Crohn_s disease in German and British population. *Lancet.* 2001, 357, 1925-1928.

4. Inohara N, Ogura Y, Chen F, [et al.]. Human NOD1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem.* 2001, 276, 2551–2554.

5. King K, Sheikh M, Cuthbert A, [et al.]. Mutation selection and evolution of the Crohn disease susceptibility gene CARD15. *Hum Mutat.* 2006, 27, 44-54.

6. Van Der Woude C, Kleibeukar J, [et al.]. Chronic inflammation, apoptosis and (pre-) malignant lesions in gastrointestinal tract. *Apoptosis.* 2004, 9, 123-130.

7. Wright J, Christman J. The role of nuclear factor kappa B in the pathogenesis of pulmonary diseases: implications for therapy. *Am J Respir Med.* 2003, 2, 211-219.

8. Liu J, Yang G, Thompson-Lanza J, [et al.]. A genetically defined model for human ovarian cancer. *Cancer Res.* 2004, 1, 1655-1663.

9. Huang S, Robinson J, Deguzman A, [et al.]. Blockade of nuclear factor-kappa B signaling inhibits angiogenesis and tumorigencity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. *Cancer Res.* 2000, 60, 5334-5339.

10. Górski B, Cybulski C, Huzarski T, [et al.]. Breast cancer predisposing allele in Poland. *Breast Cancer Res Treat* 2005, 92, 19-24.

11. Lubiński J, Huzarski T, Kurzawski G, [et al.]. The 3020insC allele of NOD2 predispose to cancer of multiple organs. *Hered Cancer Clin Pract.* 2005, 3, 59-63.

12. Górski B, Byrski T, Huzarski T, [et al.]. Founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer. *Am J Hum Genet.* 2000, 66, 1963-1968.

13. Papaconstatiou I, Theodoropoulos G, Gazouli M, [et al.]. Associacion between mutations in the CARD15/NOD2 gene and colorectal cancer in a Greek population. *Int J Cancer.* 2005, 114, 433-435.

14. Alhopuro P, Ahvenainen T, Mecklin J, [et al.]. NOD2 3020insC alone is not sufficient for colorectal cancer predisposition. *Cancer Res.* 2004, 64, 7245-7247.

15. Lubiński J, Huzarski T, Kurzawski G, [et al.]. The 3020insC allele of NOD2 predispose to cancer of multiple organs. *Hered Cancer Clin Pract.* 2005, 3, 59-63.

16. Dębniak T, Cybulski C, Kurzawski G, [et al.]. Low-risk genes and multi-organ cancer risk in the polish population. *Hered Cancer Clin Pract.* 2006, 4, 52-55.

17. Janiszewska H, Haus O, Lauda-Świeciak 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, 15-19.

18. Kurzawski G, Suchy J, Kładny J, [et al.]. The NOD2 3020insC mutation and the risk of colorectal cancer. *Cancer Res.* 2004, 64, 1604-1606.

19. Irejs A, Miklasevics E, Boroschenko V, [et al.]. Pilot study on low penetrance breast and colorectal cancer predisposition markers in Latvia. *Hered Cancer Clin Pract.* 2006, 4, 48-51.

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