

**Received:** 2007.03.12  
**Accepted:** 2008.02.08  
**Published:** 2008.09.01

**Authors' Contribution:**

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

## NOD2/CARD15 polymorphism in patients with rectal cancer

Jacek Szeliga<sup>1ABCDEF</sup>, Zbysław Sondka<sup>2ABCDEF</sup>, Marek Jackowski<sup>1AEF</sup>,  
Joanna Jarkiewicz-Tretyn<sup>2ACD</sup>, Andrzej Tretyn<sup>2ACDG</sup>, Marek Malenczyk<sup>3B</sup>

<sup>1</sup> Department of General, Gastroenterologic, and Oncologic Surgery, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń, Poland

<sup>2</sup> Department of Biotechnology, Institute of General and Molecular Biology, Nicolaus Copernicus University, Toruń, Poland

<sup>3</sup> Obstetrics, Feminine Health, and Oncologic Gynecology Unit, District Hospital, Toruń, Poland

**Source of support:** Departmental sources

### Summary

**Background:**

Reports published in the past several years have not provided conclusive evidence regarding a relationship between the development of colorectal cancer and *NOD2* gene mutations, though some geographic variability has been shown.

**Material/Methods:**

The goal of the current project was to analyze the frequency of selected *NOD2* gene variants, including P286S, R702W, G908R, and 1007fs, in the Polish population of patients with rectal cancer. Fifty-one rectal cancer patients undergoing treatment were included in the study. As a control group to provide a reference point for *NOD2* polymorphism in the population, DNA obtained from cord blood collected from the placenta of 100 patients immediately after parturition was used.

**Results:**

It was found that the aforementioned mutations were more frequent among the colorectal cancer patients and that the presence of the 1007fs variant might also be associated with young patient age.

**Conclusions:**

The analysis of the material does not allow presenting a conclusive answer as to whether the 1007fs, G908R, and R702W mutations or P268S polymorphism contribute to the development of sporadic colorectal cancer in the Polish population. Patients in some populations could likely benefit from instituting earlier colorectal cancer screening studies following the detection of the 1007fs mutation.

**key words:**

**NOD2/CARD15 • rectal cancer**

**Full-text PDF:**

<http://www.medscimonit.com/fulltxt.php?ICID=867966>

**Word count:**

2393

**Tables:**

1

**Figures:**

1

**References:**

21

**Author's address:**

Jacek Szeliga, Department of General, Gastroenterologic, and Oncologic Surgery, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, ul. Św. Józefa 53/59, 87-100 Toruń, Poland, e-mail: jacky2@wp.pl

## BACKGROUND

In 2001, two independent research groups reported that *NOD2*-encoded protein participates in innate immunity [1,2]. This system regulates responses to pathogenic microorganisms in humans and other mammalian species. The system was initially thought to be stimulated via the recognition of specific pathogens by receptors present on the surface of immune cells. This theory was revised upon the discovery of *NOD2* on chromosome 16q12, followed by the identification of an entire family of *NOD2*-related genes. *NOD1* and *NOD2* proteins were found to act as, among others, intracellular receptors of bacterial proteins and participate in nonspecific immune responses through activation of the nuclear factor  $\kappa$ B [3]. Finally, it was particularly surprising that the structure of *NOD1* and *NOD2* was homologous to that of Apaf-1 protein (which serves as an apoptosis regulator) and of plant R polypeptides, gene products underlying the innate immunity of plants to pathogenic microorganisms [4]. The highly conservative structure of *NOD2* reflects the prominent role played by this molecule in humans and animals: a murine homolog of this protein displays over 80% similarity to human *NOD2*.

*NOD2* consists of three domains. Two CARDs (caspase recruitment domains) are located at the N-terminus, one nucleotide-binding NOD (nucleotide oligomerization domain) is in the middle, and eleven repetitive leucine-rich amino-acid sequences (dubbed LRR for leucine-rich repeats) occupy the C-terminus of the polypeptide. *NOD1* shares structural similarity with *NOD2*; however, the first contains one CARD whereas the latter has two. More recent publications frequently refer to *NOD1* and *NOD2* proteins as *CARD4* and *CARD15* [4,5].

In medicine, *NOD2* mutations are generally strongly associated with the pathogenesis of Crohn's disease. Regional (distal) ileitis appears to be a complex disorder stemming, among others, from genetic abnormalities. The frameshift mutation (Leu1007fs) and two other nonsense mutations (Gly908Arg and Arg702Trp) of *NOD2* increase the risk in their carriers of developing this disease: this chance is 2–4 times higher in heterozygotes and even 20–30 times higher in homozygotes [1,2].

A different issue is an association between *NOD2* mutations and the process of oncogenesis. Lubinski et al. (2005) analyzed the frequency of the Leu1007fs mutation in a Polish population of healthy volunteers versus subjects suffering from one of twelve types of cancer. This mutation was found to be very common, as approximately 7% of the control subjects were its carriers. Among patients diagnosed with cancer of the thyroid gland, stomach, ovary, larynx, lungs and, primarily, breast and colorectum, the percentage of 3020insC mutation carriers ranged from 9.4 (stomach) to 12.2 (colorectum) and even 14.3% (the DCIS form of breast cancer) [6].

The aforementioned correlations, although not well established in the current body of literature, led us to analyze the link between selected changes in the *NOD2/CARD15* gene and colorectal cancer (adenocarcinoma). According to the Polish National Cancer Registry, this is second most common type of cancer (after lung cancer) in the Polish population. In 2005 there were 13,987 new cases of colorectal

cancer in Poland. In 44% of those cases (6094) the cancer was located within the distal colon, from the rectosigmoid junction to the anus. Independent research of this type of malignant colonic neoplasm seems to be justified taking into account distinct diagnostic methods, different therapy approaches, and, consequently, prognosis.

## MATERIAL AND METHODS

Fifty-one patients of the Cancer Genetics Clinic, District Hospital, Toruń, Poland, diagnosed with rectal adenocarcinoma were included in the study. This group consisted of patients diagnosed with rectal cancer in 2005 and 2006 who accepted the invitation to join the study. Overall, 60 invitations were sent, which gives an acceptance rate of 87%. As a control group providing a reference point for the *NOD2* polymorphism in the population, we used DNA obtained from cord blood collected from the placenta of 100 patients (Obstetrics, Feminine Health, and Oncologic Gynecology Unit, District Hospital, Toruń, Poland) immediately after parturition. All persons included in the study were ethnic Poles. The study was approved by the Nicolaus Copernicus University Bioethics Committee at the Collegium Medicum in Bydgoszcz (No. KB/174/2006).

The P286S, R702W, G908R, and 1007fs mutations were analyzed. The PCR-RFLP technique was utilized to establish P286S polymorphism as well as the R702W and G908R mutations. The presence of the 1007fs *NOD2* mutation was determined with the allele-specific amplification (ASA) technique at the Department of Biotechnology, Nicolaus Copernicus University, Toruń, Poland.

## RESULTS

### Control group

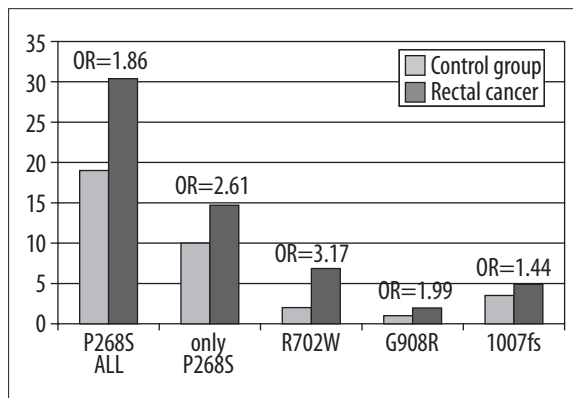
All the investigated variants of the *NOD2* gene were present in the control group. P268S polymorphism was detected in 30 of the 100 persons (30%), the R702W mutation in 4 persons (4%), G908R mutation in two (2%), and the 1007fs mutation in 7 (7%).

### P268S Polymorphism

Twenty-seven (52.9%) of the 51 colorectal cancer patients were P268S polymorphism carriers in the *NOD2* gene. These included 9 females and 18 males. Three males and one female had this polymorphism in two alleles of the gene. The average age of the polymorphism carriers was 62.6 years, in females 66.6 years and in males 60.6 years. The differences in frequency of the 268S allele between the study group and controls (Figure 1) were statistically significant ( $OR=2.61$ ,  $p=0.0078$ ; R language, Bonferroni correction for multiple testing of the same data (in this case: four times), statistically significant when  $p<0.05/4=0.0125$ ). However, P268S polymorphism also accompanies the three other mutations studied. The comparison of cases when the mutation of interest was not accompanied by any of the others did not yield any significant differences.

### R702W mutation

Six (11.7%) of the 51 colorectal adenocarcinoma patients were carriers of the R702 mutation in the *NOD2* gene. These



**Figure 1.** *NOD2* allele frequency in patients suffering from rectal cancer (dark gray) and healthy controls (gray). *OR* – ratio of the chance of observing the mutated allele in cancer patients and the chance of observing the same mutated allele in healthy controls. The “P268S only” group includes those individuals who showed only P268S polymorphism and none of the studied mutations.

included 2 females and 4 males. One male had this mutation in both alleles of the gene. The average age of the mutation carriers was 66.16 years, in females 64 years and in males 67.25 years. All R702W mutation carriers were also P268S polymorphism carriers. The two-tailed Fisher’s test showed that the increase in 702W allele frequency in colorectal cancer patients compared with controls was not statistically significant ( $OR=3.17$ ,  $p=0.09$ , greater than the Bonferroni-corrected  $p$  of 0.0125 and even greater than 0.05) (Figure 1). It was shown, however, that in persons aged 65 or older (21 individuals in the group studied, including 4 mutation carriers) the difference in the frequency of the mutation of interest was statistically significant ( $OR=4.96$ ,  $p=0.04$ ), though the Bonferroni correction negates this conclusion.

#### G908R mutation

Two (3.9%) of the 51 colorectal cancer patients were carriers of the G908R mutation in the *NOD2* gene. These included one male and one female. The age of the female mutation carrier was 82 years and of the male 55 years (Table 1).

All G908R mutation carriers were also P268S polymorphism carriers. The G908R mutation-containing *NOD2* allele was more frequent in the study group than in controls ( $OR=1.99$ , Figure 1); however, Fisher’s test did not show significance between the observed values ( $p=0.6$ ).

#### 1007fs mutation

Five (9.8%) of the 51 colorectal adenocarcinoma patients were carriers of the 1007fs mutation in the *NOD2* gene. These included 3 females and 2 males. The characteristic feature of this group was a significantly lower patient age when diagnosed with cancer. It was 50.6 years, on average 12.5 years lower than for the entire group studied. One patient with this mutation (female patient #5) already underwent surgery at the age of 39. All 1007fs mutation carriers were also P268S polymorphism carriers. A relatively higher frequency of the 1007fs allele was observed in the study group than in the controls (Figure 1,  $OR=1.44$ ). The two-tailed Fisher’s test showed that the increase in 1007fs allele frequency in the operated patients compared with the controls was not statistically significant. In the colorectal patients aged 56 years or older (17 individuals, including 5 carriers) there was a significant increase in the frequency of this mutation ( $OR=5.41$ ,  $p=0.015$ ). Bonferroni correction does not support the conclusion that the observed frequency differences are significant ( $p>0.0125$ , Table 1).

#### DISCUSSION

In eukaryotes, nuclear factor  $\kappa$ B, whose activity is regulated by, among others, NOD2 protein, plays an essential role in the regulation of basic processes of the organism, including immune response, apoptosis, cell cycle control, and the development of individual cell lines [7]. Due to its crucial involvement in the regulation of cell division mechanisms, it was attributed with an uncertain role in the process of cancer development, where its activity is significantly elevated. Already in the mid-1990s its importance was described in relation to cancer of the thyroid, breast, lung, and colorectum [8–11].

The most recent data leave no doubt that alterations in the *NOD2* gene participate in the development of inflammato-

**Table 1.** The numbers, percentages, and average ages of patients suffering from rectal cancer and displaying the *NOD2* gene mutations.

Test type	P268S	R702W	G908R	1007fs
Number of persons with the mutation	27.0	6.0	2.0	5.0
Number of women with the mutation	9.0	2.0	1.0	3.0
Number of men with the mutation	18.0	4.0	1.0	2.0
% of persons with the mutation	52.9	11.7	3.33	9.8
% of women with the mutation	42.85	9.52	4.76	14.28
% of men with the mutation	60.0	13.33	3.33	6.66
Average age of persons with the mutation	62.6	66.16	68.5	50.6
Average age of women with the mutation	66.6	64.0	82.0	49.0
Average age of men with the mutation	60.6	67.25	81.0	53.0

ry Crohn's disease, though reports to the contrary do sporadically surface. *NOD2* involvement in the development of colorectal cancer appears more dubious, although Crohn's disease itself is a recognized pre-cancer state [12]. One of the first reports defining the importance of *NOD2* mutation in oncogenesis was the publication by Lubinski et al. describing a correlation between the 1007fs *NOD2* mutation and an elevated risk of colorectal cancer in older people (over 50 years old) [13]. Those observations were not confirmed by other studies. Alhopuro et al. analyzed a large population of over 1000 Finnish patients and found that the 1007fs mutation could not by itself be regarded as a factor accompanying an increased risk of colorectal cancer and that the statistically significant elevated frequency of the mutation in Polish people aged 50 and over could not be reproduced in Finns [14]. There were attempts to explain this phenomenon through the heterogeneity of mutations, some environmental factors, and other oncogenesis-related phenomena within the European population [14,15].

Our studies aimed at a more thorough clarification of this issue. We analyzed the potential influence of the most frequently described *NOD2/CARD15* gene mutations, namely 1007fs, G908R, and R702W as well as P268S polymorphism, on the occurrence of only rectal cancer. These mutations had been examined prior to this study and analyses of similar relationships have been performed (Zaahl et al.), although they concerned only classical cases of intestinal inflammatory diseases (ulcerative colitis and Crohn's disease). The studies were of epidemiological nature and their results were not corroborated by statistically significant findings in other populations, including in South Africa [16]. The authors noted primarily a low frequency (<20%) of the aforementioned alterations in the gene, which confirmed a variability in the geographic distribution of the mutations. Similar studies were performed by a group of researchers in New Zealand who unequivocally showed that the combination of three of the mutations described above, namely R702W, G908R, and 1007fs, is associated with an increased frequency of sporadic colorectal cancer in the population, though no link with age and tumor dynamics was defined [17]. This was one of the first reports delineating the importance of the above-mentioned *NOD2* alterations in oncogenesis.

In the current study we found that P268S was the most common form of *NOD2/CARD15* polymorphism; we detected it in more than half of patients suffering from colorectal adenocarcinoma (52.9%) and it was always present when one of the remaining mutations was demonstrated. This corroborates the hypothesis put forth by Bonen et al. that this variant exhibits profound activity in various intestinal diseases [18]. This polymorphism by itself, as a lone alteration, did not prove to have a statistically significant impact on cancer development. It was one of the first reports on the aforementioned changes within the *NOD2* gene as an underlying cause in oncogenesis. This relationship was not corroborated by the findings in several European populations. Lakatos et al. did not observe a correlation between clinical characteristics and the frequency of sporadic colorectal cancer (CRC) and selected *NOD2/CARD15* variants in the Hungarian population [18]. Furthermore, data obtained by Tupanen and colleagues in 1042 Finnish CRC patients did not show a link between the R702 and

G908R mutations and contradicted the earlier findings in the Finnish population [19].

We observed that P268S was the most frequently occurring *NOD2/CARD15* polymorphism as it was present in more than half of colorectal adenocarcinoma cases (52.9%) and was always found when any of the remaining mutations existed. This may support the notion put forth by Bonen et al. that this variant displays a vast array of actions in various intestinal diseases [20]. By itself, this polymorphism did not turn out to have a statistically significant effect on the development of cancer, nor did the remaining mutations within the *NOD2* gene. As visualized through statistical analyses, the 1007fs and R702W mutations do not seem to affect cancer development. These two changes have been previously associated with the severity of the intestinal inflammatory condition observed in Crohn's disease, which is thought to elevate the risk of colorectal adenocarcinoma development. These mutations have also been found to predispose the patient to display a more severe course of inflammatory disease with serious complications that typically require surgical intervention [21]. G908R appears to have a similar influence, although this mutation also seemed more frequent in cancer patients than in controls. The fact that the 1007fs mutation is present in relatively young people is an interesting observation. The average age of 50 years in patients belonging to this group was much different from that of the remaining groups (Table 1). The youngest surgically treated patient was not yet 40 years old. It is extremely difficult to explain this phenomenon based on just one study, especially because thus far this phenomenon has not been supported by statistics, and only one publication (Kurzwaski et al.) showed a correlation between sporadic cancer with the *NOD2* mutation and patient age [13]. Although the current study does not prove the presumed (in some European populations, including the Polish one) relationship between composite mutations in the *NOD2* gene and colorectal cancer, knowledge of this potential but not fully recognized correlation may aid, following large population studies, in selecting a subgroup of patients who require increased oncological alertness and, possibly, earlier endoscopic screening, similarly to FAP or HNPCC. The geographic variability observed in previous epidemiological studies may be an obstacle in confirming this relationship. Therefore, enriching the patient database with new analyzed cases may likely lead to defining the geographic range of this potential risk of cancer development.

## CONCLUSIONS

The analysis of the material does not allow us to present a conclusive answer as to whether 1007fs, G908R, and R702W mutations as well as P268S polymorphism contribute to the development of sporadic colorectal cancer in the Polish population. Considering the many contradictory reports, this still undefined mechanism can be explained only by including additional cases in the analysis and performing meta-analyses.

## REFERENCES:

1. Hugot JP, Chamaillard M, Zouali H et al: Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*, 2001; 411: 599-603

2. Ogura Y, Bonen DK, Inohara N et al: A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*, 2001; 411(6837): 603–6
3. Ogura Y, Inohara N, Benito A et al: Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF- $\kappa$ B. *J Biol Chem*, 2001; 276: 4812–18
4. Kambe N, Nishikomori R, Kanazawa N: The cytosolic pattern-recognition receptor Nod2 and inflammatory granulomatous disorders. *J Dermatol Sci*, 2005; 39: 71–80
5. Kufer TA, Fritz JH, Philpott DJ: NACHT-LRR proteins (NLRs) in bacterial infection and immunity. *Trends Microbiol*, 2005; 13: 381–88
6. Lubiński J, Huzarski T, Kurzawski G et al: The 3020insC allele of NOD2 predisposes to cancers of multiple organs. *Heredit. Cancer Clin Pract*, 2005; 3: 59–63
7. Chen F, Castranova V, Shi X: New Insights into the Role of Nuclear Factor- $\kappa$ B in Cell Growth Regulation. *Am J Pathol*, 2001; 159: 387–97
8. Sovak MA, Bellas RE, Kim DW et al: Aberrant nuclear factor- $\kappa$ B/Rel expression and the pathogenesis of breast cancer. *J Clin Invest*, 1997; 100: 2952–60
9. Mukhopadhyay T, Roth JA, Maxwell SA: Altered expression of the p50 subunit of the NF- $\kappa$ B transcription factor complex in non-small cell lung carcinoma. *Oncogene*, 1995; 11: 999–1003
10. Gilmore TD, Koedood M, Piffat KA, White DW: Rel/NF- $\kappa$ B/I $\kappa$ B proteins and cancer. *Oncogene*, 1996; 13: 1367–78
11. Dejardin E, Deregowski V, Chapelier M et al: Regulation of NF- $\kappa$ B activity by I $\kappa$ B-related proteins in adenocarcinoma cells. *Oncogene*, 1999; 18: 2567–77
12. Guo QS, Xia B, Jiang Y et al: NOD2 3020insC frameshift mutation is not associated with inflammatory bowel disease in Chinese patients of Han nationality. *World J Gastroenterol*, 2004; 10: 1069–71
13. Kurzawski G, Suchy J, Kladny J et al: The NOD2 3020insC Mutation and the Risk of Colorectal Cancer. *Cancer Res*, 2004; 64(5): 1604–6
14. Alhopuro P, Ahvenainen T, Mecklin JP et al: NOD2 3020insC alone is not sufficient for colorectal cancer predisposition. *Cancer Res*, 2004; 64(20): 7245–47
15. Hugot JP, Chamaillard M, Zouali H et al: Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature (Lond)*, 2001; 411: 599–603
16. Zaahl MG, Winter T, Warnich L, Kotze MJ: Analysis of the three common mutations in the CARD15 gene (R702W, G908R and 1007fs) in South African colored patients with inflammatory bowel disease. *Mol Cell Probes*, 2005; 19(4): 278–81
17. Roberts RL, Geary RB, Allington MD et al: Caspase recruitment domain-containing protein 15 mutations in patients with colorectal cancer. *Cancer Res*, 2006; 66(5): 2532–35
18. Lakatos PL, Hitre E, Szalay F et al: Common NOD2/CARD15 variants are not associated with susceptibility or the clinicopathologic characteristics of sporadic colorectal cancer in Hungarian patients. *BMC Cancer*, 2007; 7: 54
19. Tuupanen S, Alhopuro P, Mecklin JP et al: No evidence for association of NOD2 R702W and G908R with colorectal cancer. *Int J Cancer*, 2007; 121(1): 76–79
20. Bonen DK, Ogura Y, Nicolae DL et al: Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. *Gastroenterology*, 2003; 124(1): 140–46
21. Barreiro M, Nunez C, Dominguez-Munoz JE et al: Association of NOD2/CARD15 mutations with previous surgical procedures in Crohn's disease. *Rev Esp Enferm Dig*, 2005; 97(8): 547–53

www.IndexCopernicus.com

One Stop Shop

in Science

This copy is for personal use only - distribution prohibited.



- Scientists networking & collaboration
- Online Research Team
- Scientists profiles
- Individual career monitor
- Personalized information delivery
- Information intergration:  
literature/grants/patents/jobs