NOD2/CARD15 polymorphism in patients with rectal cancer

Jacek Szeliga1, Zbysław Sondka2, Marek Jackowski1, Joanna Jarkiewicz-Tretyn2, Andrzej Tretyn2, Marek Malenczyk3

1 Department of General, Gastroenterologic, and Oncologic Surgery, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń, Poland
2 Department of Biotechnology, Institute of General and Molecular Biology, Nicolaus Copernicus University, Toruń, Poland
3 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 κB [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 (caspace 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, Femailine 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 cases (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 accompanied 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
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=1.99, Table 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=1.96, 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 kB, 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.

<table>
<thead>
<tr>
<th>Test type</th>
<th>P268S</th>
<th>R702W</th>
<th>G908R</th>
<th>1007fs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of persons with the mutation</td>
<td>27.0</td>
<td>6.0</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Number of women with the mutation</td>
<td>9.0</td>
<td>2.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Number of men with the mutation</td>
<td>18.0</td>
<td>4.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>% of persons with the mutation</td>
<td>52.9</td>
<td>11.7</td>
<td>3.33</td>
<td>9.8</td>
</tr>
<tr>
<td>% of women with the mutation</td>
<td>42.85</td>
<td>9.52</td>
<td>4.76</td>
<td>14.28</td>
</tr>
<tr>
<td>% of men with the mutation</td>
<td>60.0</td>
<td>13.33</td>
<td>3.33</td>
<td>6.66</td>
</tr>
<tr>
<td>Average age of persons with the mutation</td>
<td>62.6</td>
<td>66.16</td>
<td>68.5</td>
<td>50.6</td>
</tr>
<tr>
<td>Average age of women with the mutation</td>
<td>66.6</td>
<td>64.0</td>
<td>82.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Average age of men with the mutation</td>
<td>60.6</td>
<td>67.25</td>
<td>81.0</td>
<td>53.0</td>
</tr>
</tbody>
</table>
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 Tapanen 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 (Kurzawski 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:**

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
One Stop Shop in Science

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