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NOD2 C.3019-3020INSC AND C.2104C>T GENE VARIANTS AMONG PATIENTS FROM WESTERN UKRAINE WITH CROHN'S DISEASE AND COLORECTAL CANCER

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Aim: **To determine the frequency of *NOD2* gene c.3019-3020insC (rs5743293) and c.2104C>T (rs2066844) allelic variants in the patients with Crohn's disease (CD), colorectal cancer (CRC) and in the control groups and to study the association of these mutations with the onset time of the diseases, gender and surgical interventions.** ***Materials and Methods:*** **The diagnoses of CD and CRC were established based on standard clinical examination and laboratory tests. Molecular genetic study of a frameshift 3020insC mutations of *NOD2* gene were performed in 54 patients with CD; missense R702W mutations of the *NOD2* gene — in 41 CD patients and 38 healthy controls. In CRC group, 3020insC mutation was tested in 48 patients, R702W mutation — in 40 patients and 40 healthy controls. PCR-RFLP technique was used to identify the mutations.** ***Results:*** **The frequency of the minor allele (M) of 3020insC mutation of *NOD2* gene in the patients with CD was significantly higher than in the control group ($p = 0.01$). The age at CD onset in females carrying 3020insC mutation was significantly lower (22.5 ± 1.6 years) when compared with females without the mutation (32.7 ± 2.5 years) ($p = 0.002$). There was no significant difference in the allele frequencies and genotype distributions of R702W mutation in the patients with CD in comparison with the controls. The mean age at CD onset in the patients carrying R702W mutation was significantly lower (28.4 ± 1.4 years) compared with the patients without the mutation (39.4 ± 2.8 years) ($p < 0.01$). Surgical interventions for CD was required in 40.0% of 3020insC mutation carriers. Among patients with CRC, only 4.2% carried 3020*ins*C mutation and 20.0% R702W mutation. Our study suggests that R702W and 3020insC mutations are not associated with the risk of CRC in Ukrainian patients. There was no statistically significant difference in mean age at CRC onset in patients with/without R702W mutation. Only one patient with CRC had two mutations.** ***Conclusion:*** **The earlier age at CD onset was associated with 3020*ins*C mutation, but only in female patients. The association between R702W mutation and the earlier age of CD onset was found. Patients with 3020insC mutation showed a trend to a higher frequency of surgical interventions for CD.**

Key Words**: 3020insC and R702W mutations of *NOD2* gene, Crohn's disease, colorectal cancer, disease onset, gender, surgical interventions.**

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Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer-related death in men and women in both developed and developing countries of Europe and North America [1, 2]. The crude incidence rate of cancer of colon/rectum, and anus in Ukraine was 26.7/24.4 among men and 24.7/17.8 among women per 100,000 of population and crude mortality rate was 14.0/14.0 among men and 12.4/9.7 among women per 100,000 of population [3]. Progress in reducing CRC death rates can be achieved by improving access to screening, early diagnosis and standard treatment in all populations [4]. This disease progresses via the sequential accumulation of multiple genetic alterations [1]. The risk of developing CRC can also increase by the interaction between low-penetrance genes, environmental factors (e.g. diet, obesity, smoking) and recently recognized microbiota [5].

Several gastrointestinal cancers, especially CRC, are strongly linked to chronic inflammatory conditions. The risk of CRC may even increase depending on the degree of underlying inflammation as in the case of long-standing inflammatory bowel diseases (IBD) — Crohn`s disease (CD) and ulcerative colitis [6]. The important problem that has not been solved is why some patients with chronic inflammation develop CRC while other patients don't [7]. The literature data indicate that the incidence and prevalence of CD are increasing over time and in different populations. In Europe, the annual incidence rate of CD ranges between 0.3–12.7 per 100,000 person-years. European prevalence rates vary between 0.6–32.2 per 100,000 persons-years [8]. The peak ages for CD occurrence are 20–30 years. In Ukraine, the annual incidence rate of CD ranges between

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Abbreviations used: CD – Crohn`s disease; CRC – colorectal cancer; HWE – Hardy–Weinberg equilibrium; IBD – inflammatory bowel diseases; NOD – nucleotide-binding oligomerization domain; OR – odds ratios; RFLP – restriction fragment length polymorphism.

30–50 per 100,000 population [9]. CD is characterized by flare-ups and remissions of varying duration and severity, and only a minority of patients has a chronic, continuous disease course [10]. CD may involve any part of the digestive tract, but mainly affects the distal ileum and the colon. Approximately 80% of these CD patients will require at least one intestinal surgery during their lifetime [11]. Due to early onset, fluctuating disease course, unpredictable prognosis and lack of a cure, CD poses a considerable burden on patients.

Since CD is the predisposing factor for CRC, CD-related genes might, to a certain extent, be associated with cancer initiation [1]. Pro-inflammatory stimulus may lead to continuous cell proliferation and eventually DNA damage [12]. NOD2 is a member of evolutionarily conserved Nod-like receptors family which share a tripartite structure of a C-terminal sensor domain (leucine-rich repeats), a central nucleotide-binding oligomerization domain (NOD) and an N-terminal effector domain (CARD) [13]. NOD2 participates in sensing components of microbial cell wall and has been reported to regulate apoptosis and chronic inflammatory conditions [14].

Genetic variation of *NOD2* has been shown to be associated with increased susceptibility to CD in several independent studies, although in some populations no association has been observed [15–18]. The common mutations, frameshift change (3020insC→1007fs) and missense changes (Arg702Trp), located in the leucine-rich repeat domain of the NOD2 protein have been reported to be linked with the disease [15, 16]. These NOD2 variants exhibit genetic heterogeneity between Caucasian and Asian populations [1]. However, the exact molecular function of NOD2 is not thoroughly established. Some investigators have shown that these mutations affect behavior, prognosis and response to CD treatment [4]. Several lines of evidence are compatible with a significant role of *NOD2* variants in determining an association with earlier age of disease onset [19–21]. This is consistent with genetic evidence that a younger age at diagnosis identifies families with greater linkage to the *IBD1* locus [22]. Currently, little is known about the association between *NOD2* mutations and the requirement for initial surgery and surgical recurrences in CD. Most of the published data have shown an positive association between *NOD2* mutations and ileal surgery, and the mutation with the strongest association was found to be the 3020*ins*C mutation [23, 24]. In three independent studies, one carried out in Germany [25], others — in Spain and Italy [26, 27], patients with mutations of the gene presented an increased risk of repeated surgery, and such surgery had been required earlier. These findings were even reinforced within the pediatric population. Some studies have demonstrated that *NOD2* mutations were a predictor of earlier age of surgery within a pediatric population [28]. Another study has shown that deficient *NOD2* function confers an increased risk of not only CD, but also CRC [21]. Most data addressing *NOD2* polymorphisms and CRC are essentially linkage studies concerning a specific country or region [4]. However, the frequency of *NOD2* polymorphisms shows a significant geographic variability. Recently, possible association of the *NOD2* variants R702W and 3020insC with CRC has been studied in some Caucasian CRC patients, as well as Polish, Greek and Finnish, but the results have been controversial [29, 30].

The aim of the study was to determine the frequency of *NOD2* gene c.3019-3020insC (rs5743293) and c.2104C>T (rs2066844) allelic variants in the patients with CD, CRC and in the control groups and to study the association of these mutations with the onset time of the diseases, gender and surgical interventions.

PATIENTS AND METHODS

The diagnoses of CD and CRC were established by means of physical examination, chest X-ray, computed tomography, endoscopic examination with biopsy including histology, baseline laboratory blood, urine and feces tests in 54 patients with CD and 48 patients with CRC. Among patients with CRC, 33/48 (68.7%) persons had sporadic cancer and 15/48 (31.3%) patients had familial cancer. Family history is significant if one of the first or second degree relatives had CRC. The clinical data included CD and CRC phenotypes, age of onset, age of surgical interventions, date and type of surgery, location of the disease, disease phenotype. The clinical and pathogenetic features of CD course were evaluated according to the Montreal classification (Montreal, 2005). All patients and healthy volunteers were from 3 Western regions of Ukraine, such as Lviv, Ternopil and Ivano-Frankivsk. The diagnoses were confirmed at the Proctology Clinic of Lviv Regional State Hospital.

Molecular genetic testing of two *NOD2* gene variants c.3019-3020insC (rs5743293) and c.2104C>T (rs2066844) allelic variants was performed in two study groups (patients with CD; patients with CRC) and two healthy controls cohorts. DNA was extracted from the leukocytes of the peripheral blood using salting out purification method. Polymerase chain reaction — restriction fragment length polymorphism (PCR-RFLP) technique was used to identify the gene variants. The cytosine insertion mutation (3020insC) was genotyped by RFLP-analysis with the primers (F 5′-TCCGTCTTAGCTGAGTGGCGTAGGCAGAAGCCCTCCTGCAGGGCC-3′ and R 5′-TCACTGAATGTCAGAATCAGAAG-3′) [30]. PCR assays were performed in a 25 μl of reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 250 μM dNTPs, 0.20 μM of each primer, 200 ng of genomic DNA and 2.5U of Taq DNA Polymerase (Thermo Fisher Scientific, USA). The digestion of the PCR product was done by adding ApaI (10 U/μl) restriction enzyme mix and overnight incubation at 37 °C. Then 5 μl of loading buffer was added and the digested product and separated in agarose gel (3%) at 9 V/cm for 30 min. The digested product sizes was 200 bp for wild type homozygous, 155 and 45 bp for mutated homozygous and 200 bp +

155 bp + 45 bp for heterozygous. Separated products were visualized in UV light and genotype was assessed for each sample.

The allelic variant c.2104C>T (rs2066844) was genotyped by a PCR using the primers Forward: 5'-TTCCTGGCAGGGCTGTTGTC-3' and Reverse: 5'- AGTGGAAGTGCTTGCGGAGG-3'. The PCR profile was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 53 °C for 40 s and extension at 72 °C for 30 s and a final incubation at 72 °C for 10 min. The digestion of the PCR product was done by adding Msp I (10 U/mu) restriction enzyme mix and overnight incubation at 37 °C. Then 5 µl of loading buffer was added and the digested product was separated in agarose gel (3%) at 9 V/cm for 30 min. The digested product sizes are 22 bp and 55 bp for wild type homozygous, 22 bp and 110 bp for mutated homozygous and 110 bp, 55 bp and 22 bp for heterozygous (Figure). Separated products were visualized in UV light and genotype assessed for each sample [31].

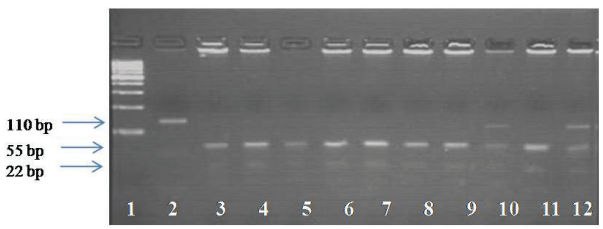

Figure. The RFLP analysis of NOD2 c.2104C>T (rs2066844) variant. The PCR product was digested with Msp I and electrophoresed in agarose gel. The digested product sizes are 22 bp and 55 bp for wild type homozygous (lanes 3-9, 11), 110 bp, 55 bp and 22 bp for heterozygous (lanes 10, 12). The PCR product without restriction (lane 2). 100 bp Ladder (lane 1)

Four groups of patients were included in the molecular genetic study:

1) the distribution of genotype and alleles frequencies of mutation 3020insC of *NOD2* gene was assessed in 54 patients with CD (24 males, mean age 38.17 ± 3.10 years; 30 females, mean age 32.03 ± 2.33 years);

2) the distribution of genotype and alleles frequencies of mutation R702W of *NOD2* gene was assessed in 41 patients with CD (21 males, mean age 38.86 ± 3.46 years; 20 females, mean age 33.05 ± 3.17 years);

3) the distribution of genotype and alleles frequencies of mutation 3020insC of *NOD2* gene was assessed in 48 patients with CRC (27 males, mean age 54.96 ± 2.53 years; 21 females, mean age 54.04 ± 2.61 years);

4) the distribution of genotype and alleles frequencies of mutation R702W of *NOD2* gene was assessed in 40 patients with CRC (21 males, mean age 56.38 ± 3.22 years; 19 females, mean age 54.32 ± 2.28 years).

The study included two groups of healthy subjects-volunteers with different mean age matching the age at disease onset in groups of patients with CD and CRC:

1) 38 subjects (19 males, mean age 34.94 ± 1.73 years; 19 females, mean age 33.47 ± 1.91 years) to detect the frequency distribution of genotype and alleles frequencies of mutations 3020insC and R702W of *NOD2* gene compared to the patients with CD;

2) 40 subjects (22 males, mean age 53.68 ± 1.14 years; 18 females, mean age 51,67 ± 1.39 years) to detect the frequency distribution of genotype and alleles frequencies of mutations 3020insC and R702W of *NOD2* gene compared to the patients with CRC.

The Ethics Committee of Danylo Halytsky Lviv National Medical University approved this study and written informed consent was obtained from all the subjects.

Statistical analysis. Frequency of the mutations among CD and CRC patients in comparison with healthy controls was evaluated by means of odds ratios (OR) and their 95% confidence intervals (95% CI). The comparison of mean age at CD onset was performed using Mann — Whitney U-test. Hardy — Weinberg equilibrium (HWE) in both groups was analyzed by χ^2 test. OR with the corresponding χ^2 distribution test and 95% CI were used to assess the association between the investigated *NOD2* variant and susceptibility to CRC and CD. The relationship between the *NOD2* variants and the risk was analyzed under genotype and allelic models. Homozygous genotype for the wild-type allele in Caucasians was used as the reference category. Only P values < 0.05 were considered significant. If $\chi^2 \leqslant 3.84$ and $p \geqslant 0.5$, the population was considered in HWE.

RESULTS

The presence of the mutant alleles was examined in the patients with CD, CRC without previous diagnosis of CD and CD-associated CRC as described above. The distribution of genotypes and alleles frequencies of 3020insC mutation in patients with CD and healthy controls is shown in Table 1. We have found a statistically significant difference in the allele frequencies and genotype distributions of c.3020insC mutation in the patients with CD in comparison with the control group. The frequency of the wild type was significantly lower in the patients with CD in comparison with the control group.

Table 1. Distribution of genotypes and alleles frequencies of 3020insC mutation of *NOD*2 gene in patients with CD and healthy controls

Genotypes/ Alleles	CD (n = 54)		Control group 1 (n = 38)		*P* value	OR (95% CI)
	n	%	n	%		
Wild type	39	72.2	36	94.7	0.01	0.14 (0.03–0.68)
Heterozygous	13	24.1	2	5.3	0.03	5.71 (1.21–27.01)
Homozygous	2	3.7	0	0	0.41	3.67 (0.17–78.57)
Allele N	91	84.3	74	97.4	0.01	0.14 (0.03–0.65)
Allele M	17	15.7	2	2.6		

The frequency of the minor allele "M" of the c.3020insC mutation in the patients with CD was significantly higher than in the control group. The observed NOD2 c.3020insC genotype frequencies in the patients with CD and controls were in agreement with HWE (cases: $\chi^2 = 0.46$, $p = 0.5$; controls: $\chi^2 = 0.03$, $p = 0.9$), suggesting no population stratification.

The associations of age at CD onset and gender in the carriers of 3020insC mutation or the patients without mutation are shown in Table 2.

Table 2. Associations of age at CD onset and gender in patients with and without 3020insC of *NOD2* gene mutation

Gender	Patients with mutation: The mean age at CD onset (in years), M ± m (n)	Patients without mutation: The mean age at CD onset (in years), M ± m (n)	*p*
Males	34.1 ± 7.3 (n = 7)	37.7 ± 3.8 (n = 17)	0.66
Females	22.5 ± 1.6 (n = 8)	32.7 ± 2.5 (n = 22)	0.002
Total	27.9 ± 3.7 (n = 15)	34.6 ± 2.1 (n = 39)	0.02

The age at CD onset in the female carriers of 3020insC mutation was significantly lower compared to the females without the mutation. The age of the males with this mutation was not significantly different compared to the age of the males without the mutation.

Surgical interventions required 30/54 (55.6%) of all CD patients and among those who underwent surgery 12/30 (40.0%) were carriers of 3020insC mutation.

The mutation in hetero- and homozygous states has been detected in 7/9 (77.8%) of patients who underwent right hemicolectomy. The mean age of the patients in this group (3 men and 4 women) was 17.7 ± 2.2 years (8–22 years). 3020insC mutation in heterozygous state was detected only in 3/24 (12.5%) patients without CD surgery. These finding suggest that the proportion of patients requiring surgical interventions for CD and carrying 3020insC mutation was 12/18 (66.7%) and higher compared to those without the mutation (3/21) (14.3%).

The presence of the mutant alleles of c.3020insC mutation was examined in two groups of patients: with CRC without previous diagnosis of CD and with CRC, associated with CD. The distribution of genotypes and alleles of c.3020insC mutation of *NOD2* gene in patients with CRC without previous diagnosis of CD is shown in Table 3.

Table 3. Distribution of genotypes and alleles of 3020insC mutation of *NOD2* gene in patients with CRC and healthy controls

Genotype/ Allele	CRC (n = 48)		Control group 2 (n = 40)		*P* value	OR (95% CI)
	n	%	n	%		
Wild type	46	95.8	38	95.0	0.85	1.21 (0.16 –9.00)
Heterozygous	1	2.1	2	5.0	0.47	0.40 (0.04–4.63)
Homozygous	1	2.1	0	0	0.57	2.56 (0.10–64.53)
Allele N	93	96.9	78	97.5	0.80	0.79 (0.13–4.88)
Allele M	3	3.1	2	2.5		

There was no statistically significant difference in the allele frequencies and genotype distributions of 3020*ins*C mutation in the patients with CRC in comparison with the control group. One homozygous genotype was detected in the patient with CRC. Also, we compared allele frequencies in two groups and no difference was found. The *NOD2* 3020insC allele was found in 2.5% of individuals in the Western Ukraine control population in comparison with 7.3% of individuals in the Polish controls [32]. Only 2/48 (4.2%) of the patients with CRC carried 3020*ins*C mutation. These two patients had adenocarcinomas and 3–10 adenomatous polyps of large bowel. One patient was a 70 year old male with cancer of splenic flexure of large bowel, who was a homozygous carrier of the mutation. Another patient was a 53 years old female with cancer of rectum. She was a heterozygous carrier of the mutation. Her father (62 years old) had cancer of rectum and her brother (42 years old) and sister (40 years old) had polyps of sigma and rectum.

The observed *NOD2* c.3020insC genotype frequencies in patients with CRC were in disagreement with HWE (cases: $\chi^2 = 20.65$, $p = 0.01$). 3020insC genotype frequencies in controls were in agreement with HWE (controls: $\chi^2 = 0.03$, $p = 0.9$), suggesting no population stratification.

This mutation was not found in the group of 8 patients with CRC, associated with CD.

The presence of the mutant alleles of R702W mutation of *NOD2* gene was examined in the patients with CD. The distribution of genotypes and alleles of R702W mutation in these patients is shown in Table 4. There was no statistically significant difference in the allele frequencies and genotype distributions of R702W mutation in the patients with CD in comparison with control group.

Table 4. Distribution of genotypes and alleles of R702W mutation of *NOD2* gene in patients with CD and healthy controls

Genotype/ Allele	CD (n = 41)		Control group 1 (n = 38)		*P* value	OR (95% CI)
	n	%	n	%		
Wild type	37	90.2	36	94.7	0.46	0,51 (0.09–2.98)
Heterozygous	4	9.8	2	5.3	0.46	1.95 (0.34–11.29)
Homozygous	0	0	0	0	0.97	0.93 (0.02–47.91)
Allele N	78	95.1	74	97.4	0.47	0.53 (0.09–2.96)
Allele M	4	4.9	2	2.6		

The observed *NOD2* R702W genotype frequencies in the patients with CD and controls were in agreement with HWE (cases: $\chi^2 = 0.11$, $p = 0.9$; controls: $\chi^2 = 0.03$, $p = 0.9$), suggesting no population stratification.

The mean age at CD onset in the patients carrying R702W mutation was significantly lower (28.4 ± 1.4 years) comparing with that in the patients without the mutation (39.4 ± 2.8 years) ($p < 0.01$). Among the patients who carried R702W mutation in heterozygous state, one was a male and three patients were females. The distribution of genotypes and alleles of R702W mutation of *NOD2* gene in the patients with CRC without previous diagnosis of CD is shown in Table 5. There was no statistically significant difference in the allele frequencies and genotype distributions of R702W mutation in the patients with CRC in comparison with control group. R702W mutation was identified in 8 (20.0%) patients with CRC (3 males : 5 females). There was no statistically significant difference of the mean age at CRC onset in the patients carrying R702W mutation (50.5 ± 6.0 years) and in the patients without the mutation (55.8 ± 2.4 years) ($p > 0.05$). Only one patient with CRC carried two mutations: he was a heterozygous carrier of R702W mutation and a homozygous carrier of 3020*ins*C mutation. None of the controls had more than one mutation.

The observed *NOD2* R702W genotype frequencies in the patients with CRC and controls were in agreement with HWE (cases: $\chi^2 = 0.49$, $p = 0.5$; controls: $\chi^2 = 0.06$, $p = 0.9$), suggesting no population stratification.

Table 5. Distribution of genotypes and alleles of R702W mutation of *NOD2* gene in patients with CRC and healthy controls

Genotype/ Allele	CRC (n = 40)		Control group 2 (n = 40)		*P* value	OR (95% CI)
	n	%	n	%		
Wild type	32	80.0	37	92.5	0.12	0.32(0.08–1.33)
Heterozygous	8	20.0	3	7.5	0.12	3.08 (0.75–12.61)
Homozygous	0	0	0	0	1.00	1.00 (0.02–51.63)
Allele N	72	90.0	77	96.3	0.13	0.35 (0.09–1.37)
Allele M	8	10.0	3	3.8		

Among heterozygous carriers of R702W mutation, 4/8 (50.0%) patients had a familial cancer anamnesis. Patients from these families meet two Amsterdam criteria of diagnosis, at least one other family member has been diagnosed with CRC and/or other malignancies, and 3/8 (37.5%) patients, mutation carriers, had multiple cancers of large bowel. In 8 patients with CRC, the disease was associated with CD. One of them, a 57 years old male, was a heterozygous carrier of R702W mutation. He had undifferentiated squamous cell carcinoma of hepar flexure of large bowel (T4N0M1G3).

DISCUSSION

In the patients with CD in comparison with the control group, we have found a significantly higher frequency of the minor allele "M" of mutation c.3020insC and no significant difference in the allele frequencies and genotype distributions of R702W mutation.

These mutations exhibit genetic heterogeneity between Caucasian and Asian populations [33]. The contribution of the c.3020insC and R702W mutations to CD has been studied in some European countries with positive associations in CD patients [17, 34–36], but not in other European and in Asian countries [37–40]. However, a recent genome-wide association study in a Japanese population reported a significant association with these mutations and identified two new susceptibility loci for CD [41].

The age of CD onset in the Ukrainian females who carried 3020insC mutation in a heterozygous state was significantly lower compared with the females without the mutation. We confirmed that the c.3020insC mutation is associated with early-onset CD in female carriers. The mean age at CD onset in the patients carrying R702W mutation was significantly lower comparing with the age of the patients without the mutation ($p < 0.01$). A significant role of *NOD2* variants in determining an association with earlier age at CD onset was confirmed in some studies [19, 20, 25]. This is consistent with genetic evidence that the younger age of diagnosis identifies families with greater linkage to the IBD1 locus [22].

Surgical interventions for CD were confirmed in 40.0% of carriers of 3020insC mutation and among them were 77.8 % of patients who underwent right hemicolectomy. The same trends were also demonstrated in several studies [21]. European scientists revealed that carriers of this mutation have a more aggressive disease that requires more frequent and earlier surgery [4]. Homozygosity for the *NOD2* frameshift 3020insC mutation predicts early onset of CD and frequent need for surgical intervention with high risk of re-stenosis [24]. Two homozygous genotypes were detected in our patients with early onset of CD. One patient, an 18-year-old woman, with ileal stenosis and sigmoido-vaginal fistula underwent one-stage operation: right-sided hemicolectomy and sigmoid resection. One year later this patient developed a recto-vaginal fistula. The second patient, a 30-year-old man, with entero-enteral fistulas and ileal stenosis underwent proximal subtotal colectomy and two years later underwent extirpation of rectum and sigmoid bowel.

The population-based studies tried to link 3020insC and R702W mutations and an increase in CRC risk [42]. In the group of patients from Western Ukraine with CRC, no statistically significant difference in allele frequencies and genotype distribution of 3020*ins*C mutation was confirmed compared to healthy controls. Only one homozygous genotype was detected in the patient with CRC. NOD2 involvement in the development of CRC appears more dubious, although CD itself is a recognized precancer state [12]. In the patients with CD, CRC was predominant malignancy and the most common localization of CRC was sigmoid colon [43]. We observed 8 patients with CRC, associated with CD, and 3020*ins*C mutation was not found among them.

One of the first reports defining the importance of *NOD2* mutations in oncogenesis was the publication on the Polish population [30] describing a correlation between the 3020*ins*C *NOD2* mutation and an elevated risk of CRC in older patients. These observations have not been confirmed by other studies [42]. The studies in different ethnic populations have produced controversial results regarding the involvement of these mutations of *NO2* gene in CRC [30, 42, 44, 45]. Some published data have confirmed the importance of these mutations in the predisposition of their carriers to the development of CRC [32], other studies have not confirmed this statement [42, 43]. As a complex disease, cancer is strongly influenced by environmental and genetic factors, and the gene polymorphisms represent a critical cause of the difference in individual susceptibility to cancer [45]. Different sensitivity to chemotherapy of CRC patients with the 3020insC mutation has been described in the literature. In particular, it is noted that non-adjuvant chemotherapy is required for the treatment of CRC in carriers of this mutation [46]. However, according to a group of scientists [42], the presence of this mutation, as a factor in the predisposition to the development of CRC, was confirmed only in patients with non-hereditary CRC. These results could be explained by the high percentage of patients with a hereditary burden of this disease, as well as different molecular mechanisms of sporadic CRC and cancer associated with CD.

The R702W variant might be a predisposing factor to sporadic CRC in Portugal, particularly in patients under 60 years of age and in female patients. However, other researchers found no evidence of *NOD2* muta-

tions in CRC patients diagnosed under the age of 60 and in women. They did not find any associations between *NOD2* mutations and family history, symptoms or CRC pathologic characteristics [47]. Our study has shown that in Ukraine half of the carriers of R702W mutation with CRC had a familial cancer anamnesis. This phenomenon is difficult to explain on the basis of a single study, because there were only 8 patients in this group. Individual polymorphisms possibly have increased the risk of CRC only in the selected groups. Our study suggests that R702W and 3020insC variants are not associated with CRC risk in the Ukrainian patients. Additional genetic or environmental factors may play a role in the development of CRC in *NOD2* variant carriers. More and more research has focused on the association between *NOD2* polymorphisms and the risks of various cancers, such as gastric, endometrial cancer, CRC, etc., but the results of individual studies were contradictory [48]. Identification of key gene polymorphisms that are associated with risk of cancer is essential for predicting an individual risk [49]. Further work is needed to establish the possible role of *NOD2* mutations in CRC predisposition.

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ВАРІАНТИ ГЕНА *NOD2* C.3019-3020INSC І C.2104C>T СЕРЕД ПАЦІЄНТІВ ІЗ ХВОРОБОЮ КРОНА ТА КОЛОРЕКТАЛЬНИМ РАКОМ, МЕШКАНЦІВ ЗАХІДНИХ ОБЛАСТЕЙ УКРАЇНИ

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Мета: Визначити частоту алельних варіантів c.3019-3020insC (rs5743293) та c.2104C>T (rs2066844) гена *NOD2* у пацієнтів із хворобою Крона, колоректальним раком та в осіб контрольної групи і дослідити зв'язок цих мутацій з віком виникнення захворювань, статтю та операційними втручаннями. ***Матеріали та методи:*** Діагноз хвороби Крона та колоректального раку встановлювали на підставі клінічного огляду, рентгенографії грудної клітки, комп'ютерної томографії, ендоскопічного дослідження з біопсією, включаючи гістологію, лабораторних аналізів крові, сечі та калу. За допомогою молекулярно-генетичних досліджень, проведених у групах пацієнтів із хворобою Крона, досліджено розповсюдженість мутації зсуву рамки зчитування 3020insC гена *NOD2* у 54 пацієнтів, міссенс-мутації R702W гена *NOD2* у 41 пацієнта та у 38 здорових осіб контрольної групи. Визначено розповсюдженість мутації 3020insC серед 48 хворих на колоректальний рак, мутації R702W — серед 40 хворих на рак та серед 40 осіб контрольної групи. Для ідентифікації мутацій було використано методику полімеразної ланцюгової реакції — поліморфізму довжин рестрикційних фрагментів. ***Результати:*** Встановлено, що частота мінорного алеля (M) 3020insC мутації гена *NOD2* у пацієнтів із хворобою Крона є істотно вищою, ніж у контрольній групі (p = 0,01). Вік виникнення хвороби Крона у жінок із мутацією 3020insC вірогідно нижчий (22,5 ± 1,6 року) порівняно з віком початку хвороби у жінок без мутації (32,7 ± 2,5 року) (p = 0,002). Не виявлено статистично значущої різниці у частотах але-

лей та розподілу генотипів мутації R702W у пацієнтів із хворобою Крона в порівнянні з контрольною групою. Середній вік виникнення хвороби Крона у пацієнтів із мутацією R702W був істотно нижчим (28,4 ± 1,4 року) порівняно з віком пацієнтів без мутації (39,4 ± 2,8 року) ($p < 0,01$). Хірургічних втручань при хворобі Крона потребували 40,0% носіїв мутації 3020insC. Серед хворих на колоректальний рак лише у 4,2% відмічали мутацію 3020insC і у 20,0% пацієнтів — R702W. Результати наших досліджень свідчать про те, що мутації 3020insC та R702W не пов'язані з ризиком виникнення колоректального раку в українських пацієнтів. Не було статистично достовірної різниці в середньому віці виникнення колоректального раку у пацієнтів з мутацією R702W та без неї. Лише у одного хворого на рак цієї локалізації було виявлено дві мутації. ***Висновок***. Більш ранній вік початку хвороби Крона асоціюється з мутацією 3020insC, але лише у пацієнтів жіночої статі. Виявлено зв'язок між мутацією R702W і більш раннім віком виникнення хвороби Крона. У пацієнтів із мутацією 3020insC спостерігали тенденцію до більшої частоти операцій з приводу хвороби Крона.

Ключові слова: хвороба Крона, колоректальний рак, вік початку захворювання, хірургічні втручання, мутації 3020insC і R702W гена *NOD2*.