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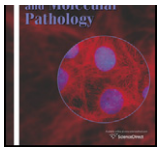

Association of c.802C>T polymorphism of *NOD2/CARD15* gene with the chronic gastritis and predisposition to cancer in *H. pylori* infected patients

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ABSTRACT

This paper shows analysis of the association of the 802C>T polymorphism of the *NOD2/CARD15* gene with the occurrence of the chronic inflammation of the gastric mucosa associated with the *Helicobacter pylori* infections, development of intestinal metaplasia and dysplasia and, in the result of this, gastric cancer. Genomic DNA samples were extracted from paraffin blocks of gastric mucosal biopsies and from peripheral blood. *H. pylori* infection was confirmed by histological analysis and urease test. Pyrosequencing of 802C>T polymorphism of the *NOD2/CARD15* gene was performed for *H. pylori* infected patients (131) and population group (100). Analysis of the *NOD2/CARD15* gene showed that frequency of the T allele was significantly higher (32.8%) in the group of patients in comparison with the population group (18.1%), with the relative risk of 1.8. In the patient group, the frequency of the CC genotype was 51.1%, CT 32.1% and TT 16.8% (relative risk: 0.7, 1.1 and 4.2, respectively), while in the population group it was 69.0%, 25.7% and 5.3% (relative risk: 1.0, 0.9 and 1.3, respectively). The increasing frequency of the T allele and CT and TT genotypes in the patients with increasingly deeper changes in the gastric mucosa becomes apparent. Our findings suggest that polymorphism 802C>T is associated with changes in gastric mucosa and plays a significant role in the initiation and the progression of carcinogenesis. The number of observed mutations in gastric mucosa correlated with severity of disease.

Introduction

Epidemiological investigations indicate that there is an association of the chronic gastritis with the occurrence of cancer predisposition (Balkwill and Mantovani, 2001). The development of inflammatory conditions of the gastric mucosa is a complex and multi-factorial process indicating disturbance of the equilibrium between aggressive and defensive factors. The process is induced by nutritional factors, bile acids, hydrochloric acid, non-steroid anti-inflammatory drugs as well as bacteria. This is well exemplified by a causative interconnection of the chronic gastritis associated with the *Helicobacter pylori* infection and the development of gastric cancer (Williams and Pounder, 1999). A similar relationship was proved to exist between chronic inflammatory intestinal diseases and the development of gastric cancer (Kurzawski et al., 2004). In the case of the chronic inflammation of the gastric mucosa, one of the most important achievements allowing the recognition of mechanisms leading to its development was the isolation and culturing in 1983 by Warren and Marshal (Warren and Marshall, 1983) of the *Campylobacter pylori* bacteria from the gastric mucosa. In the course of their investigations, they succeeded in documenting a strong link between the inflammation and the chronic active inflammation of the gastric mucosa and, at the same time, suggested a pathogenic interrelation with the gastric ulcer disease and cancer. In 1994, on the basis of numerous epidemiological studies, the International Agency on Cancer Research listed *H. pylori* as a group I carcinogen (International Agency for Research on Cancer, 1994). The interconnection between the *H. pylori* infection and gastritis and gastric cancer is indisputable (The Eurogast Study Group, 1993; Forman et al., 1991; Helicobacter and Cancer Collaborative Group, 2001). It is generally accepted that gastric cancer develops through numerous stages beginning from chronic inflammation, atrophic inflammation, intestinal metaplasia and dysplasia and later cancer. The above sequence of changes in the gastric cancer carcinogenesis is widely known as Correa cascade (Correa, 1992). Tobacco smoking, diet, high salt consumption as well as other environmental factors should be considered as etiological factors which can intensify the above process. However, the infection with *H. pylori* remains an important triggering mechanism. Furthermore,

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gastric cancer development can also be influenced individually by a genetically conditioned response to the infection. In recent years, numerous investigations have been carried out in this area.

General prevalence of asymptomatic *H. pylori* infection in US population was estimated to reach approximately 52%. Infection increases with age and was closely correlated with socioeconomic status. The prevalence of *H. pylori* infection increased rapidly with age at 1% per year for the overall population. The frequency of *H. pylori* infection was higher in black (70%) than Caucasian population (34%) (Graham et al., 1991). Prevalence of asymptomatic *H. pylori* infection in Korean and Japan population reaches 46.6–59.6% and 40.6%, respectively (Kim, 2005; Yim et al., 2007; Hirai et al., 2009). Detailed studies for Polish population have not been performed.

Ogura et al. (Ogura et al., 2001a) selected the *NOD2/CARD15* (nucleotide-binding-oligomerization domain/N-terminal caspase-activating and recruitment domains) as the potential gene in the susceptibility to the Crohn's disease and determined its function as an intracellular receptor for bacterial products and for the participation in signal transduction leading to the activation of the nuclear transcription factor-κB (NF-κB). On the basis of the knowledge concerning the role of the NOD2 protein in the process of recognition of the bacterial component and taking into consideration its location within the inflammatory bowel disease (IBD1) (Ogura et al., 2001b), the above researchers sequenced the *NOD2/CARD15* gene in patients suffering from the Crohn's disease and confirmed the link of the mutation in the change of the reading frame 3020insC in the LRR (leucine-rich repeat) region of protein (Dobrowolska-Zachwieja et al., 2005). Recently, reports have been published suggesting a link of the mutation within the *NOD2/CARD15* gene with the occurrence of the predisposition for the ulcerating inflammation of the large intestine (Kurzawski et al., 2004; Papaconstantinou et al., 2005).

This paper analyzes the association of the c.802C>T polymorphism (Pro268Ser) in the exon 4 of the *NOD2/CARD15* gene with the occurrence of the chronic inflammation, atrophic inflammation, intestinal metaplasia and dysplasia, their intensification and later cancer. Studies were undertaken based on our initial observation of correlation of changes within the *NOD2/CARD15* gene with the occurrence of the chronic inflammation of the gastric mucosa associated with the infection by *H. pylori* bacteria, development of intestinal metaplasia and dysplasia and, in the result of this, gastric cancer (Hnatyszyn et al., 2005).

Materials and methods

Patients

From 1430 patients aged from 8 to 89 examined gastroscopically (Endoscope Unit, Department of Internal Medicine and Gastroenterology, Regional Hospital, Slubice) due to dyspepsia complaints, a group of patients was selected with a co-existing infection with *H. pylori* bacteria and chronic gastritis, chronic atrophic gastritis, intestinal metaplasia and dysplasia. Patients subjected to analysis represent the group of consecutive cases. In addition, the authors identified a group of patients with gastric cancer (intestinal type) as well as a group of patients in whom normal gastric mucosa without infection with *H. pylori* bacteria was found. The investigations excluded patients taking, on a long-term basis, non-steroid anti-inflammatory drugs, drugs reducing gastric secretion, antibiotics and anti-coagulants during the previous 2 months as well as patients abusing alcohol and smoking tobacco, patients with serious somatic ailments (diseases of the liver, kidneys, cardiovascular and respiratory systems etc.), patients after operations on bile ducts and gastric resections and patients with active ulcer disease (gastric ulcer and duodenal ulcer). In addition, a population group of 100 persons was included in part of molecular investigations. All groups – patient, control and population – were derived from the same geographical area in Western Poland. The authors have not observed any MALT lymphoma cases, probably due to very low prevalence, not exceeding 1 case per 140,000 individuals in 1 year. Individuals from population group did not undergo endoscopy since at blood drawing they have not reported any symptoms from digestive tract. Characterization of the examined groups is presented in Table 1.

Gastroscopic examinations

All endoscope examinations of the stomach were performed in local anesthesia (2% Lignocainum) using videogastroscope (GiF Q 145, Olympus, Tokyo, Japan) by the same endoscopist. None of the patients was subjected to general anesthesia. The macroscopic assessment of the endoscope image of the inflammatory changes of the gastric mucosa (edema, hyperemia, granulation of mucosa, presence of erosions and ulcers, hypertrophy or atrophy of gastric folds) was carried out in accordance with Sydney classification (Working Party Report to the World Congresses of Gastroenterology, 1991; Dixon et al., 1996). The following five tissue samples were taken in the course of the performed examinations: from the region of the pylorus, angle and corpus of the stomach from the larger and smaller curvature.

Experiments were accepted by the Bioethic Commission of the Poznan University of Medical Sciences. All patients as well as parents and children had access to full information concerning the experiments and submitted their written consent for their performance.

Histological examinations

Preparations for the histological assessment were subjected to routine methods treating them with hematoxylin and eosin. The intensity of gastric mucosal inflammation, atrophy of glands, intestinal metaplasia and dysplasia were evaluated in each section in accordance with the Sydney classification (Working Party Report to the World Congresses of Gastroenterology, 1991; Dixon et al., 1996). Atrophic inflammation, intestinal metaplasia and dysplasia were classified at two levels (present or absent). The measure of the intensity of the inflammation was the degree of the infiltration of the gastric mucosa by lymphocytes and plasmacytes and the level of process activity was indicated by the accumulation of neutrophil granulocytes. Atrophy was described as the loss of glands typical for the gastric mucosa, while the intestinal metaplasia was described as

Table 1
Characteristics of the groups of the examined patients.

Group of patients	Number of patients (*n*)	Gender		Age (years)
		Females	Males	
Control group, without lesions and without infection with *H. pylori* in gastric mucosa	13	7 (54%)	6 (46%)	16–57 37.8 (±12.9)
Chronic gastritis	40	20 (50%)	20 (50%)	16–75 35.6 (±14.6)
Chronic gastritis with atrophy	36	19 (53%)	17 (47%)	11–76 41.0 (±18.5)
Chronic gastritis with intestinal metaplasia	17	8 (47%)	9 (53%)	13–81 38.4 (±17.2)
Chronic gastritis with dysplasia	21	12 (57%)	9 (43%)	17–87 52.5 (±18.7)
Gastric cancer intestinal type	17	5 (29%)	12 (71%)	42–84 62.2 (±12.5)
Population group	100	50 (50%)	50 (50%)	21–30 25.5 (±2.9)

the substitution of glands and/or gastric foveolas by mucous cells of the small or large intestine. Dysplasia was described as histological gland disturbances regarding their architecture and cell nuclei. Gastric cancer was described as the infiltration of the lamina of the proper mucosa by the tumor cells of the epithelium. Single pathomorphologist was involved in the interpretation of biopsies.

Detection of the H. pylori infection

The initial presence of the *H. pylori* bacteria was confirmed by the urease test (Institute of Food and Nutrition, Warsaw) which was read after 2, and in doubtful cases, after 24 h. In addition, the presence of the *H. pylori* bacteria in the gastric mucosa was confirmed by histological examinations by staining sample sections employing the Warthin–Starry method modified by Giemsa. Enrolled patients had both tests performed. The most essential result was histopathology, considering lower sensitivity and specificity of rapid urease test. None of the diagnostic method alone gives 100% recognition, but a combination of two is called “the golden standard.” Serological analyses were not used in the presented studies since they are not recommended in studies other than epidemiological ones.

Molecular analysis

In case of patients and control groups, DNA was isolated from paraffin blocks of gastric mucosal biopsies using Roche High Pure PCR template preparation kit and from peripheral blood using the method with guanidine isothiocyanate (GTC). In case of the population group, DNA was extracted from the peripheral blood using the method with guanidine isothiocyanate (GTC). After precipitation, the DNA pellet was diluted in sterile water. Genotyping was performed by pyrosequencing using PSQ96MA system. In order to amplify 114 bp fragment of the exon 4 encompassing the c.802C>T polymorphism in the *NOD2/CARD15* gene prior to pyrosequencing, the PCR reaction was performed in 20 μl of the reaction mixture containing 100 ng genomic DNA, 15 pmol of F and R primers each, 0.125 mM dNTP, and 1.0 U Taq polymerase. Primer sequences were as follows: CARD802CTF biotin-GGAGGTCTGGGCAGATGTG, CARD802CTR ACAGTGTCCGCATCGTCA and for sequencing CARD802CTS GGGCTCTTCTGCGGG. Reaction conditions are initial denaturation 94 °C, 4 min; 50 cycles: denaturation 94 °C, 30 s, primer annealing 63 °C, 45 s, synthesis 72 °C, 30 s and final synthesis 72 °C, 5 min. Single strand separation and pyrosequencing analysis were performed according the manufacturer's recommendations using PyroGold reagents.

Statistical analysis

If it is not indicated otherwise, the χ^2 Pearson's test was utilized for the analysis. The analysis of the compliance with the Hardy-Weinberg distribution for the population group, the χ^2 analysis for the trend and the calculation of the odds ratio were conducted with the assistance software at site: http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. The remaining analyses were carried out using the Statistica 6.0 (StatSoft Inc.) program.

Results

Molecular studies of association of c.802C>T polymorphism of the *NOD2/CARD15* gene with the chronic gastritis and predisposition to cancer in *H. pylori* infected patients were performed for patients with clinical diagnosis of different stages of chronic inflammation confirmed by gastroscopic and histological analyses (Fig. 1). *H. pylori* infections were detected using urease test and staining of histopatological slides using Warthin–Starry method modified by Giemsa.

Genotyping of the c.802C>T polymorphism (Pro268Ser) of the *NOD2/CARD15* gene was conducted using pyrosequencing that allowed unequivocal determination of all three genotypes (CC, CT and TT). In the case of the population group, the genotype distribution was in Hardy–Weinberg equilibrium $p = 0.607$.

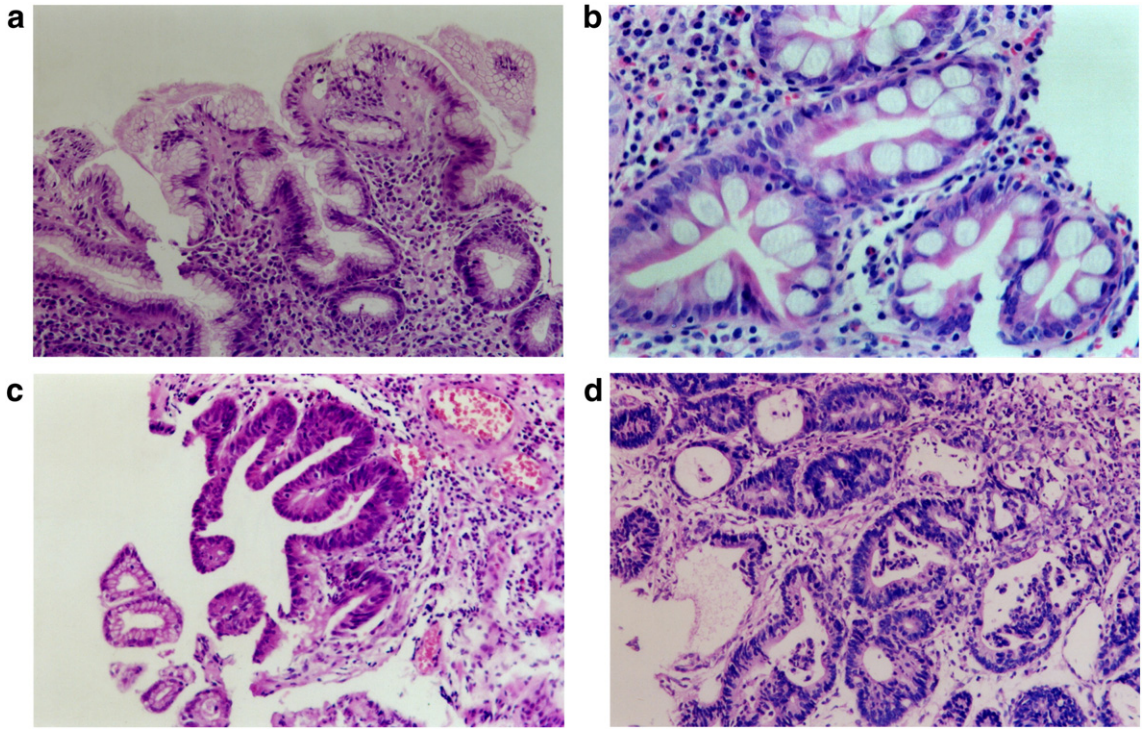

Fig. 1. Microscope photographs of the gastric mucosa with different stages of chronic inflammation associated with the *H. pylori* infections: (a) chronic inflammation (magnification 200×), (b) intestinal metaplasia (magnification 400×), (c) dysplasia (magnification 200×) and (d) gastric cancer (magnification 200×). Histological preparation was stained with hematoxylin and eosin. The presence of the *H. pylori* was confirmed by the Warthin–Starry method modified by Giemsa.

During the first stage, we compared the frequency of occurrence of alleles of the c.802C>T polymorphism between the expanded population group (the population group combined with the control group) ($n=113$) and the group which comprised all patients with the concomitant *H. pylori* infection and chronic gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer (intestinal type) ($n=131$). The frequency of the T allele was significantly higher (32.8%) in the group of patients in comparison with the expanded population group (18.1%) $\chi^2=13.59$, $p=0.0002$ (χ^2 with Yates' correction $\chi^2=12.84$, $p=0.0003$), $df=1$, OR = 2.2 [95%CI 1.4-3.3], with relative risk 1.8 (Fig. 2). In addition, the genotype distribution between these groups showed significant difference. In the patient group, the frequency of the CC genotype was 51.1%, CT 32.1% and TT 16.8%, with relative risks of 0.7, 1.1 and 4.2, respectively, while in the expanded population group it was 69.0%, 25.7% and 5.3%, respectively, with relative risks of 1.0, 0.9 and 1.3, respectively, $\chi^2=11.09$, $df=2$, $p=0.0039$ and χ^2 for the trend = 10.92, $df=1$, $p=0.00095$ (Fig. 3).

In the second part of study, the frequency of alleles and genotypes and relative risks between groups of patients with the increasing intensity of changes in the gastric mucosa was compared. Because of small numbers, when comparing genotype frequencies, patients with the CT and TT genotypes were pooled together (Figs. 4 and 5). Both in the case of the analysis of the allele frequency and the comparison of genotype frequency and their relative risks, the increasing frequency of the T allele and CT and TT genotypes in the patients with increasingly deeper changes in the gastric mucosa becomes apparent. In the case of the control group, the T allele constituted 19.2%, in patients with chronic gastritis and chronic atrophy gastritis – 28.9%, in patients with intestinal metaplasia – 29.4%, in patients with dysplasia – 38.1% and in patients with gastric cancer – 47.1%, with relative risks of 1.5, 1.5, 2.0 and 2.5, respectively. The proportion of patients with the CT and TT genotypes in individual groups increased similarly and amounted to 23.1%, 43.4%, 47.1%, 52.4% and 70.6%, respectively, with relative risks of 1.9, 2.0, 2.3 and 3.1, respectively. Genotypes and allele relative risks in patients and control group indicate the prevalence of allele T and genotypes CT+TT in the patient group as compared with the control group (Figs. 4 and 5). The number of individuals in the control group is the smallest due to formal problems with examinations but is comparable with the number of patients in intestinal metaplasia, dysplasia and gastric cancer patients.

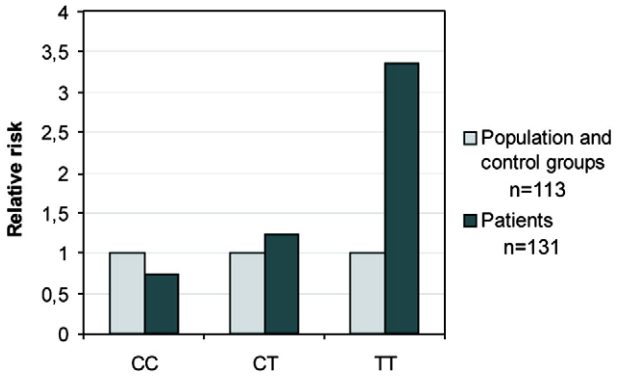

Fig. 3. Relative risk of disease associated with polymorphism c.802C>T of *NOD2/CARD15*. Relative risk was analyzed in the expanded population group encompassing population and control groups and in patients with chronic gastritis, chronic gastritis with atrophy of gastric mucosa, chronic gastritis with intestinal metaplasia, chronic gastritis with dysplasia, and patients with gastric cancer. Genotype distribution showed significant difference; risk of disease occurrence is about four times higher in patients with TT genotype compared to patients with CC genotype ($\chi^2=11.09$, $p=0.0039$, χ^2 for trend = 10.92, $p=0.001$).

Discussion

Our investigations revealed a significant interrelationship between the c.802C>T polymorphism (Pro268Ser) of the *NOD2/CARD15* gene and the intensity of the chronic inflammation of the gastric mucosa associated with the *H. pylori* infection. It was shown that the frequency of the T allele of this polymorphism increased in the groups of the examined patients with the intensity of morphological changes observed in the mucosa. Beginning with the group with inflammatory and atrophic changes, through the intestinal metaplasia and dysplasia it increased significantly and reached the highest index in patients with the gastric cancer of intestinal type. The immunological response of the gastric mucosa to the *H. pylori* infection and the course of the inflammatory reaction are the main factors responsible for the development of the chronic inflammation of the gastric mucosa and, consequently, carcinogenesis (Williams and Pounder, 1999).

Interactions between the bacteria and cells of the gastric mucosa result in the development of an inflammatory infiltration made up of neutrophils, lymphocytes, plasmatic cells and macrophages. The *H. pylori* bacteria stimulate directly the intracellular signaling cascade leading to the activation of numerous transcriptional factors. One of the important roles in this process is played by the activation of the

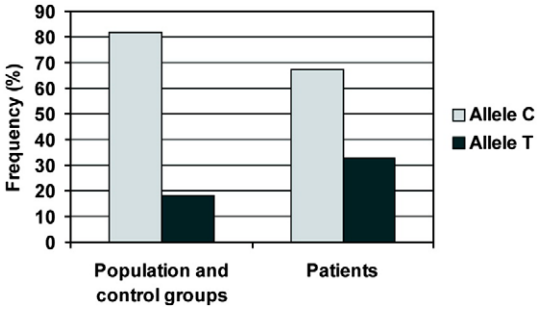

Fig. 2. Frequency of C and T alleles of polymorphism c.802C>T of *NOD2/CARD15*. Frequency was analyzed in the expanded population group encompassing population and control groups and in patients with chronic gastritis, chronic gastritis with atrophy of gastric mucosa, chronic gastritis with intestinal metaplasia, chronic gastritis with dysplasia, and patients with gastric cancer. Risk of disease occurrence is about twice higher in patients with allele T compared to patients with allele C ($\chi^2=13.59$, $p=0.0002$ (χ^2 with Yates' correction $\chi^2=12.84$, $p=0.0003$), OR = 2.2 [95%CI 1.4–3.3]).

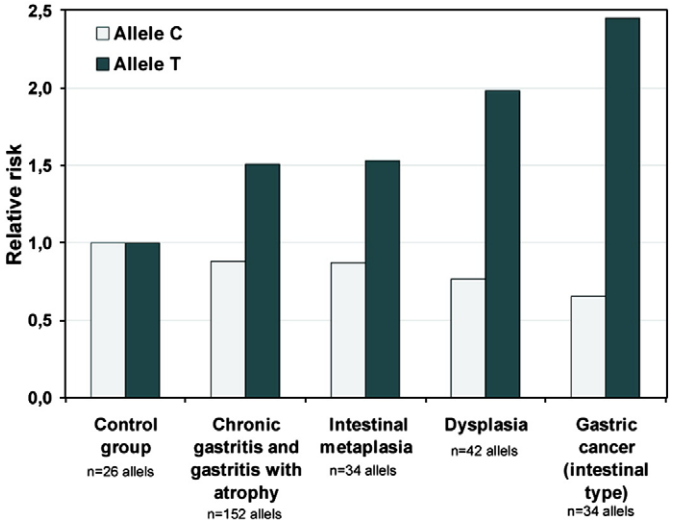

Fig. 4. Association of C and T alleles of polymorphism c.802C>T of the *NOD2/CARD15* gene with changes in gastric mucosa. Relative risk of disease in analyzed groups with increased severity of changes in gastric mucosa was studied showing increasing values for T allele from 1.5–2.5 times in the patients with increasingly deeper changes in the gastric mucosa ($\chi^2=6.99$, $p=0.136$, χ^2 for trend = 6.44, $p=0.011$).

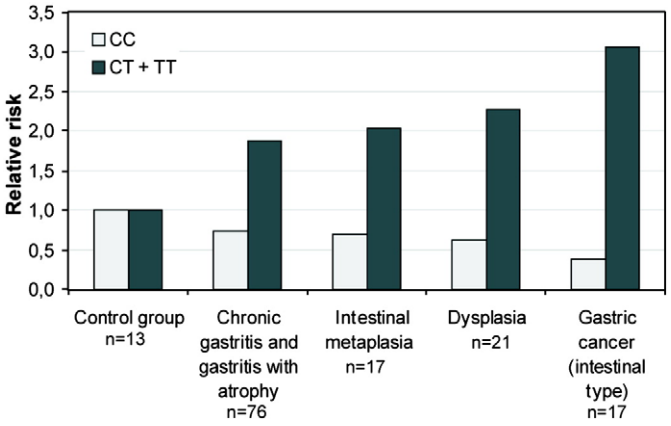

Fig. 5. Association of CT and TT genotypes of polymorphism c.802C>T of the *NOD2/CARD15* gene with changes in gastric mucosa. Relative risk of disease associated with polymorphism c.802C>T of the *NOD2/CARD15* gene in groups with increased severity of changes in gastric mucosa. Increasing relative risk of the CT and TT genotypes in patients with increasingly deeper changes in the gastric mucosa was observed. The presence of CC and TT genotypes increases two to three times the risk of severity of disease ($\chi^2 = 7.42$, $p = 0.116$, χ^2 for trend $= 6.36$, $p = 0.012$).

nuclear factor NF-κB (Keates et al., 1997) enhanced by the tumor necrosis factor alpha (TNF-α) and interleukin-1 (Peek, 2001). It can also be activated by the *H. pylori* infection of the cells of the gastric mucosa via the mitogen-activated protein kinase (MAPK) (Peek, 2001; Naumann et al., 1999; Keates et al., 1999). The increased activity of the nuclear factor NF-κB correlates with the degree of progress of histological changes of the stomach mucous inflammation associated with this infection (Isomoto et al., 2000). It is capable to activate numerous genes associated with the inflammatory and immunological response of the host, including gene coding several dozens of different cytokines, chemokines as well as receptors involved in the process of immunological identification (Mori et al., 2001). Our preliminary observation suggests lack of correlation of severity of gastritis with age of patients in estimated groups, but it requires thoroughly examinations (data not shown). We postulate this correlation with the duration of *H. pylori* infection. In recent years, researchers have been focusing their attention on the link between the genetic polymorphism in humans and the occurrence of diseases. The existence of such link was shown, among others, in the interleukin-1 gene (IL-1) (El-Omar et al., 2000) as well as within the tumor necrosis factor alpha gene (TNF-α) (Kustmann et al., 1999; Machado et al., 2003). The risk of gastric cancer is complementary enhanced by the presence of various genotypes of pro-inflammatory cytokines such as TNF-α, interleukin-1β and receptor antagonist interleukin-1 (El-Omar et al., 2003). Polymorphism found within the suppressor gene p53 is of similar significance (Hiyama et al., 2002). It can create favorable circumstances for the development of pathogenic conditions associated with the *H. pylori* infection.

The *NOD2/CARD15* gene plays an important role in the above-described processes. As indicated, changes within the *NOD2/CARD15* gene are advantageous for the development of the Crohn's disease (Ogura et al., 2001a, Nagy et al., 2005), ulcerating inflammation of the large intestine (Juyal et al, 2007) as well as cancer of the large intestine (Kurzawski et al., 2004; Papaconstantinou et al, 2005).

The gene undergoes expression in monocytes. It plays the function of the intracellular receptor for the protein products of bacterial origin (Ogura et al., 2001a; Chamaillard et al., 2003) and it participates in the transduction of the signal which leads to the activation of the NF-κB transcription factor and transcription of regulatory genes (Ogura et al., 2001b). The consequence of the reactions, both auto immunological and inflammatory, is chronic inflammation of gastric mucosa. The occurrence of the c.802C>T polymorphism (Pro268Ser) within the *NOD2/CARD15* gene can predispose to an altered response to *H. pylori* proteins. It can also be presumed that it predisposes to the enhanced inflammatory response of the gastric mucosa.

Results of our investigations suggest that the frequency of the T allele occurrence of the examined polymorphism correlates with the intensification of pathomorphological changes and constitutes an important element of both initiation and development of carcinogenesis. Therefore, if this allele occurs and coexists in patients with the *H. pylori* infection, they should require endoscope supervision and, within the program of gastric cancer prevention, they should undergo early eradication treatment even if only slight endoscope and histological changes of the gastric mucosa or their absence were diagnosed. In our studies we estimated severity of changes in gastric mucosa in *H. pylori* infected patients according to Correa cascade (Correa, 1992). Patients with active gastric and duodenal ulcer diseases were excluded. Their pathogenesis differs from chronic gastritis, atrophy and metaplasia, despite correlations with *H. pylori* infections. Wong et al. published very interesting results of a comprehensive prospective study comprising 1630 healthy carriers of the *H. pylori* bacteria in which they showed that the eradication treatment of patients without precancerous changes prevents significantly the development of gastric cancer (Wong et al., 2004). This is in agreement with earlier reports from investigations carried out by Uemura et al. (2001). Therefore, it can be presumed that endoscope examinations of the upper part of the gastrointestinal tract accompanied by a histological assessment of patients infected with the *H. pylori* bacteria carried out in high risk populations would be advantageous. Eradication treatment can result in the regression of histological changes and act on the molecular level (Nardone et al., 1999). However, a question arises whether there is “a point of no return” at which advantages resulting from the eradication treatment can be limited by the development of atrophic changes and intestinal metaplasia as well as molecular changes which can no longer be reversed. In the publication of Rosenstiel et al. (2006), association of mutations of *NOD2/CARD15* with gastritis or gastric ulcer development was not found. However, significant association of the Arg702Trp mutation in the *NOD2/CARD15* gene with gastric lymphoma was observed. In the same study, the authors indicate that carriers of the rare allele T had more than doubled risk to develop lymphoma than controls (Rosenstiel et al., 2006).

The population group included 100 individuals and the number seems to be sufficient (200 chromosomes) for studies of allele frequency in polymorphic locus. Individuals from population group did not undergo endoscopy since at blood drawing they have not reported any symptoms from digestive tract. Our estimations of allele C and T frequencies in Polish populations may be useful for further relevant studies. What proportion of this group proceed to develop mucosal abnormalities including carcinoma cannot be answered at this moment and prospective studies are required.

Rosenstiel et al. (2006) tested the influence of genetic variants in the *NOD1* and *NOD2* genes on the clinical outcome of *H. pylori* infection on a cohort of 428 consecutive patients with *H. pylori* infection and 83 patients with low-grade *H. pylori* associated gastric lymphoma from a prospective multicentre trial. Neither haplotype analysis of *NOD1* nor single marker analysis of *NOD2* revealed an association with the development of gastric ulcer in patients with chronic *H. pylori* infection. Our study involved single pathologist interpretation instead of multicenter, using the same criteria.

At the present time, it is difficult to recommend *NOD2/CARD15* genotyping as a routine procedure. The problem requires further investigations including association studies in other populations and the examination of the effect of the c.802C>T polymorphism (Pro268Ser) on the functioning of the NOD2 protein. However, it cannot be ruled out that the analysis of this polymorphism will be applied in diagnostics, especially in populations characterized by a high incidence of gastric cancer and high index of occurrence of *H. pylori* infections.

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