Chapter

Single-Nucleotide Polymorphisms in Inflammatory Bowel Disease

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Abstract

Inflammatory bowel disease (IBD) mainly includes ulcerative colitis (UC) and Crohn's disease (CD). Both conditions are characterized by chronic inflammation of the gastrointestinal tract, with alternating periods of relapse and remission. Both forms of IBD involve an uncontrolled inflammatory process in the intestines, leading to worsening quality of life and requiring long-term medical and/or surgical intervention. Epidemiological and clinical studies suggest that the pathogenesis of inflammatory bowel disease is strongly linked to genetic predisposition. CD and UC are considered polygenic diseases in which familial clustering is observed in 5–10% of patients. Among genetic factors associated with IBD development, it has been found that many single nucleotide polymorphisms are associated with susceptibility to IBD progression. SNP can affect the production or function of a protein and thus affect the development of the disease. However, although the overall role of genes involved in the development of IBD is already in most cases known, as of today it is unclear how the SNPs in these genes affect cellular function, or how such changed cellular functions would contribute to the development of IBD. In the present work several selected polymorphisms in genes involved in IBD development are discussed.

Keywords: ulcerative colitis, Crohn's disease, single-nucleotide polymorphisms

1. Introduction

Inflammatory bowel disease (IBD) mainly includes ulcerative colitis (UC) and Crohn's disease (CD). Both conditions are characterized by chronic inflammation of the gastrointestinal tract, with alternating periods of relapse and remission. Both forms of IBD involve an uncontrolled inflammatory process in the intestines, leading to worsening quality of life and requiring long-term medical and/or surgical intervention [1].

The pathomechanism of IBD is still not well explain, but evidence suggests that it results from perturbation of the homeostasis between the intestinal microbiota and the mucosal immune system, with the involvement of both genetic and environmental factors [2].

Ulcerative colitis is a chronic inflammatory disease, which mainly affects the large intestine. Typical clinical symptoms of UC are diarrhea, rectal bleeding, and abdominal pain. Nonspecific symptoms include fever, appetite loss, and weight loss. The disease significantly affects the patient's quality of life due to its repeated remissions and relapses [3].

UC is characterized by recurring episodes of inflammation limited to the mucosal layer of the colon and practically invariably involves the rectum and may extend in a proximal and continuous fashion to involve other portions of the colon. Endoscopic features of inflammation include loss of vascular markings, granularity and friability of the mucosa, and erosions. In the setting of severe inflammation is observed deep ulcerations and spontaneous bleeding [4] (**Figure 1**). CD is described by transmural rather than superficial mucosal inflammation and by skip lesions rather than continuous disease [5].

In Crohn's disease, inflammatory changes may occur in all parts of the gastrointestinal tract, from the oral cavity to the rectum, but are usually localized in the terminal segment of the small intestine, that is, the ileum. Inflammatory changes are discontinuous and affect the entire thickness of the intestinal wall. The most common symptoms of CD are abdominal pain, fever, weight loss, and diarrhea [6].

The commonly used instrument for evaluating the disease severity of CD is Crohn's Disease Activity Indicator (CDAI; remission <150 score, severe disease >450 score). Whereas the Mayo score (0—normal, 3—severe disease) is used to assess the severity of ulcerative colitis [7].

The optimal goal of management in IBD is a durable period of steroid-free remission and the induction and subsequent maintenance of mucosal healing [4]. Mucosal healing is predominantly defined by endoscopic assessment of intestinal inflammation and is referred to as the absence of mucosal ulcerations in CD, while in UC, an international consensus defined it as the absence of friability, blood, erosions, and ulcers of the gut mucosa. However, these indices allow to determine improvements of endoscopic lesions, even when the rather rigid endpoint of mucosal healing, and thereby the total disappearance of all mucosal ulcerations is not met [8] (**Figure 2**).

Many genes, which may be linked to IBD, are believed to be associated with microbiological defense mechanisms involving the epithelial barrier and innate, adaptive immune systems [9].

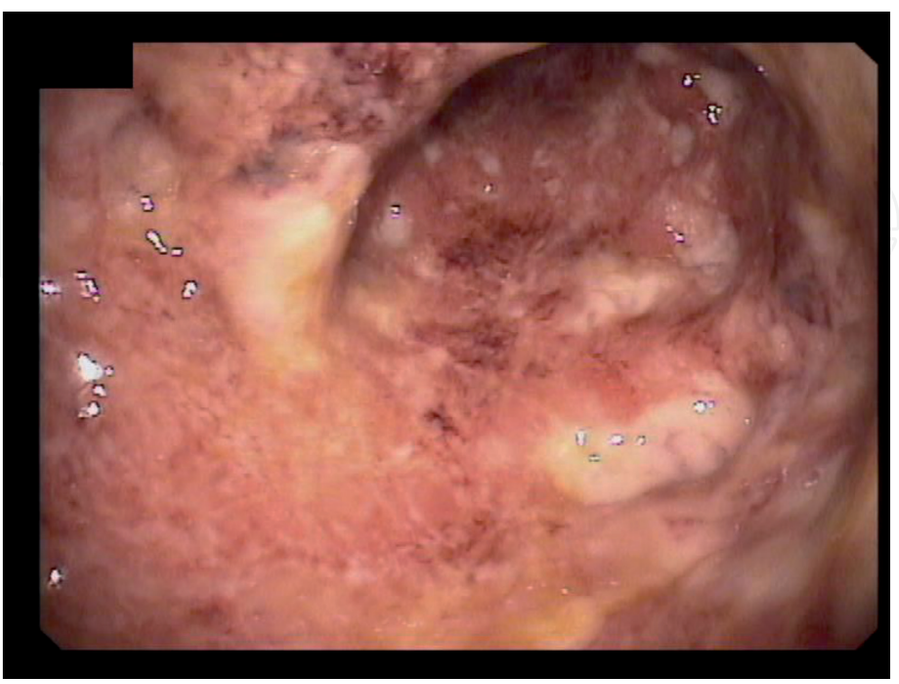

Figure 1.
Endoscopic images of ulcerative colitis according to the Mayo score (Grade 3—severe disease). (Source: Own study.)

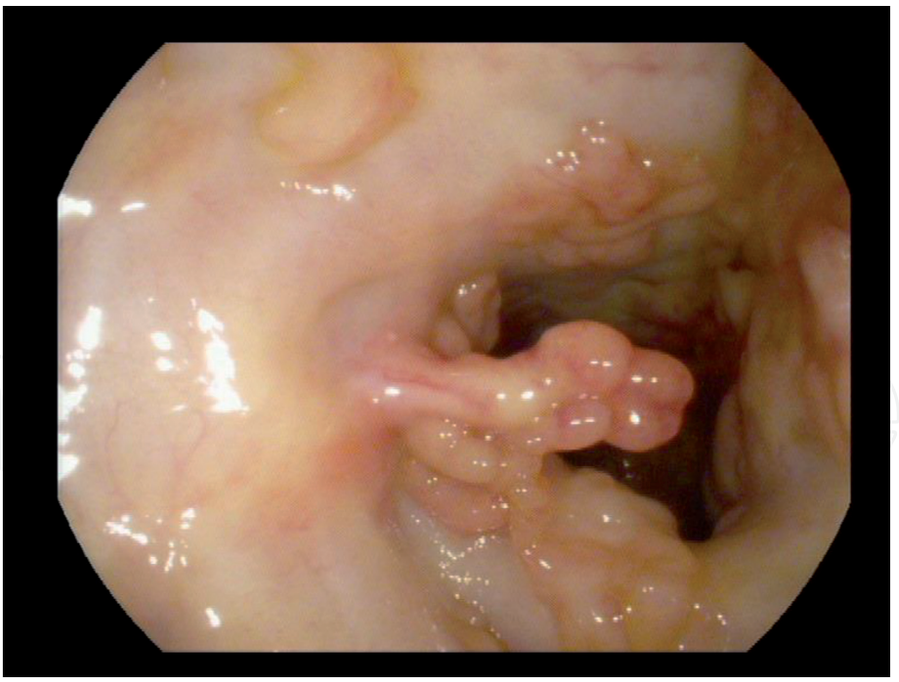

Figure 2.
Endoscopic assessment of the large intestine was observed by mucosal bridging and pseudopolyps, which confirms regeneration of the mucosa in the course of remission of ulcerative colitis. (Source: Own study.)

In the result of large cohort genome-wide association studies (GWAS) of cases and controls, over 200 IBD susceptibility loci have now been reported [10].

GWAS research was aimed at searching the SNPs that are over-represented in IBD patients when compared with healthy controls. SNPs that occurred more frequently in IBD patients are thus called disease-associated variants [11].

Among the genetic factors involved, there are several single-nucleotide polymorphisms (SNP) associated with the susceptibility to IBD progression. Many of these mutations regulate immune responses with several being enriched in immune cells, in particular CD4+ T cells and dendritic cells [12].

2. Single-nucleotide polymorphisms in innate and adaptive immunity genes in IBD

The latest research on genetics and immunology has confirmed that the innate immune system is of great importance in inducing intestinal inflammation [13], and the publication of Dudzińska et al. [14], "Single nucleotide polymorphisms in selected genes in inflammatory bowel disease," is dedicated to this issue. This paper attempts to demonstrate polymorphisms *NOD2/CARD15* (*nucleotide-binding oligomerization domain containing 2/caspase recruitment domain family number 15*) and the *DLG5* gene (*discs large homolog 5*) in patients with IBD hospitalized at the Department of Gastroenterology, Regional Specialist Hospital, SPZOZ im. Stefan Cardinal Wyszynski in Lublin.

NOD2/CARD15 is located on chromosome 16q12.1 and was the first disease-susceptibility gene discovered for CD. *NOD2/CARD15* is a pattern-recognition receptor that is involved in the homeostasis of intestinal immunity [15].

CARD15/NOD2 plays an important role in immune function. In response to bacterial infection, *CARD15/NOD2* acts as an intracellular bacterial receptor and activates the kappa B nuclear factor (NF-κB), particularly after recognizing the bacterial wall component muramyl dipeptide (MDP) [16].

NOD2/CARD15 mutations lead to dysregulation of host-microbe interactions, contributing to the development of inflammation in the ileum, which is characteristic of CD [17].

Today more than 60 polymorphisms of this gene have been identified; however, three common mutations Leu1007fsinsC, Arg702Trp, and Gly908Arg have been specifically associated with ileal involvement, stricturing complications, and earlier age of onset [18].

While the gene *DLG5* encodes scaffold proteins belonging to the MAGUK family, which participate in the formation of cellular connections, maintenance of cell shape, and intracellular signal transduction [19]. Expression of this gene is widely expressed in the tissues of the small and large intestines. *DLG5* gene polymorphisms have been shown to increase susceptibility to IBD, including both CD and UC [20]. *DLG5* has been shown to be localized at cell-cell contact sites and is involved in maintaining epithelial integrity. Different variants of *DLG5* may contribute to the loss of cell polarization complexes and adhesion complexes, so that epithelial cell polarity is not maintained, and epithelial-mesenchymal transition (EMT) is induced [20]. EMT is a process involving the transformation of immobile, polarized cells with an epithelial phenotype into cells with a mesenchymal phenotype. The characteristic features of EMT include lack of polarity and cell adhesion, reduced expression of E-cadherin, and increased mobility and invasion capacity [21].

Thus, it can be assumed that *DLG5* polymorphisms may impair the epithelial barrier in the gastrointestinal tract and lead to abnormal epithelial structure, making it more susceptible to IBD (CD and UC). Furthermore, the *DLG5* scaffold protein also belongs to the CARD family of proteins (like *CARD15*/*NOD2*). Thus, *DLG5* is probably involved in the regulation of NF-κB activation or caspase activation within the host defense mechanisms [22]. Therefore, both the *NOD2* and *DLG5* genes may interact functionally to contribute to the risk of developing CD.

Our research in the group of patients diagnosed with CD and UC is related to the occurrence of polymorphisms in the *NOD2* and *DLG5* genes.

The test material was blood collected from patients on an empty stomach after 12 hours of rest. In addition, medical history was taken with regard to the occurrence of extraintestinal symptoms and autoimmune diseases in the family. The family history of all subjects was negative. DNA was isolated using a QIAamp DNA Blood Mini Kit (QIAgen), followed by quantitative and qualitative evaluation of the isolated DNA samples.

The following primers were used for PCR: (F) GACTCTTTTGGCCTTTTCAGATT and (R) CCAATGGTCTTTTTTCCTTACTCC for *CARD15/NOD2* and (F) TTATTCCCCTTCCACAGGCACTAC and (R) GCCGCAGCTGAATGGAGA for *DLG5*.

The PCR product was sequenced, and the sequences obtained were recorded in FASTA format. The nucleotide sequences of the *CARD15/NOD2* and *DLG5* gene fragments were compared using DNA Baser software.

The size of the analyzed *CARD15/NOD2* gene fragment was 243 bp. No SNPs were observed in this fragment in patients with CD or UC.

Although numerous reports confirm that *CARD15/NOD2* gene polymorphisms are associated with a predisposition to IBD [23], our research did not show this relationship. This may be linked to the absence of parenteral symptoms in the subjects.

One of the most common single-nucleotide polymorphisms of the *CARD15/NOD2* gene is P268S (SNP5), where the cytosine residue at position 802 is replaced by thymine. In the Polish population, P268S polymorphism has been found in 49.5% of patients with CD, and its presence in both alleles is associated with earlier

Figure 3.
SNP at position rs1248696, T > C substitution in a patient with Crohn's disease (source: Dudzińska et al. 2018).

onset of disease symptoms and increased risk of parenteral symptoms such as joint abnormalities, iritis, and erythema nodosum [24].

The size of the *DLG5* gene fragment was 107 bp. One SNP at position 1,248,696 was found in the gene fragment. A T > C substitution occurred in one sample (Patient 3) from the group of patients with Crohn's disease (**Figure 3**). Other authors note that the R30Q (Rs1248696) variant of *DLG5*, where amino acid 30 in exon 3 changes from arginine to glutamine, is associated with the development of IBD [25].

Despite numerous reports indicating the presence of polymorphisms of the *NOD2/CARD15* gene, our studies did not show the position of SNP in patients with IBD from the Lublin region. The study suggests that SNPs (T > C substitution) affect the function of the *DLG5* protein and thus play a role in the development of IBD, in particular Crohn's disease.

3. The role of autophagy genes in the pathogenesis of IBD

Autophagy is a conserved lysosome-dependent catabolic process, degrading and recycling protein aggregates or damaged organelle. Autophagy affects the pathogenesis of IBD in multiple ways, including secretion of antimicrobial materials from Paneth cells, clearance of invading pathogens, presentation of antigen, or proinflammatory cytokine production by macrophages [26].

SNPs of autophagy genes such as autophagy-related gene 16 like 1 (*ATG16L1)* and immunity-related GTPase family M (*IRGM*) have been identified to be associated with CD. *ATG16L1* and *IRGM* genes affect cellular autophagy processes and bacterial clearance in immune cells and may affect bacterial composition of the gut in patients with IBD [27].

There is some overlap in these autophagy-related genetic variants in both CD and UC, but majority of the identified variants are more associated with ileal CD. Despite this association, the positive predictive value for disease development in individuals carrying autophagy variants is low because the *ATG16L1* T300A polymorphism is linked with CD susceptibility, which is also present in a large proportion of healthy individuals who do not develop IBD [28].

However, some of the studies show that a knock-in mouse model expressing A*TG16L1* T300A does not develop spontaneous inflammation, but it exhibits morphological defects in both Paneth cells and goblet cells, and the presence of the T300A mutation in *ATG16L1* leads to aberrant functionality of Paneth cells. These findings indicate a close relationship between *ATG16L1* variants and Paneth cells [15].

Recent data GWAS identified the single-nucleotide polymorphism (SNP) rs13361189—a SNP lying immediately upstream of the autophagy gene *IRGM*, and many studies have investigated *IRGM* gene variants both in adult and pediatric and in UC, confirming its role in the IBD pathogenesis. It was shown that SNP rs13361189, the deletion allele, modulated the expression of *IRGM* in transformed cells [29].

Other study demonstrated functional effects of the synonymous SNP rs10065172 (c.313C > T). This synonymous variant rs10065172 in *IRGM* alters a binding site for certain microRNAs, miR-196, and causes deregulation of *IRGM*-dependent xenophagy of bacteria in patients with CD [29].

4. AHR and its role in regulating intestinal inflammation

Tryptophan metabolism plays important roles in the pathogenesis and therapeutics of IBD [30]. Recent study investigates the interaction between Card9 and the gut microbiota in the generation of the microbiota-derived tryptophan metabolite [2].

Tryptophan can be metabolized either by the gut bacteria into indole derivatives, such as indole-3-acetic acid (IAA), or by host cells into kynurenine (Kyn) via indoleamine 2,3-dioxygenase 1 (IDO1) [31].

Kyn derived from host metabolism, and indole-derived tryptophan metabolites produced by gut microbiota are endogenous ligands of aryl hydrocarbon receptor (AHR), an important regulator of immune response. AHR disturbance results in disordered immune responses, including decreased Treg cell levels and increased TNF-α levels, and a modified timeframe of IL-10 and IL-12 secretion. Elevated the serum levels of tryptophan metabolites including kynurenic acid (KA) is potential aryl hydrocarbon receptor (AHR) ligands to impact colitis. Several findings suggest that KA and other tryptophan metabolites inhibit the colonic inflammation [30].

Although polymorphisms in AHR have not yet been associated with IBD, AHR is known to play a central role in the regulation of intestinal inflammation and is upregulated in the inflamed gut [12].

SNPs are common, single-nucleotide genetic variants that can influence protein function, protein stability, or gene expression. Genome-wide association studies and candidate gene studies have identified SNPs near AHR target genes that are significantly associated with AHR-regulated phenotypes, such as psoriasis (CYP1A1) and systemic lupus erythematous (CYP1A1). These findings suggest that SNPs near AHR-binding sites might impact AHR target gene expression and contribute to individual variation in disease risk and pharmacotherapy phenotypes. Of interest, that SNP is distant from an AHR response element (AHRE) but still influences AHR binding and CYP1A1 expression after AHR agonist treatment, which suggests that it may influence the stability of the AHR complex and its ability to regulate CYP1A1 gene expression [32].

It is also important that over the last years, some functions of noncoding DNA have been discovered, and the role of regulatory sequences in transcriptional regulation, development of the disease process, and determination of cell type specificity is nowadays widely appreciated [11].

In the latest research, Boyd et al. [33] presented Cap Analysis of Gene Expression (CAGE) analysis on biopsies from the descending colon from 94 IBD patients and controls. These data enabled annotation of IBD-regulated enhancers and transcription start sites (TSSs) and characterization of IBD-associated

SNPs in such regions. Researchers have shown that clear overrepresentation of IBD-associated SNPs in both IBD upregulated enhancer and promoter regions also presented regions that had the largest IBD heritability enrichment compared to other genomic regions. The results of these studies carried out many resources for interpretation of the functional impact of noncoding genetic variants [33].

5. SNPs in the multi-drug resistance 1 gene

Another of the genes whose mutations may play a role in the pathogenesis of IBD is multi-drug resistance 1 (*MDR1)* gene. *MDR1* encodes P-glycoprotein 170 (P-gp), an ATP-dependent drug transport efflux pump, which highly expressed in many cells and epithelial surfaces, including the epithelium of GI tract [34].

In the gut, P-gp is expressed on the apical surfaces of the superficial columnar epithelial cells in the intestine with the levels of expression gradually rising from the duodenum to the distal parts of the intestine with the highest levels of expression in the distal small bowel and colon [35].

It is now known that substrates for the P-gp pump include a variety of structurally and pharmacologically distinct hydrophobic compounds, such as drugs and toxins, and P-gp might also play a critical role in host bacterial interactions in the gastrointestinal tract and maintenance of intestinal homeostasis [34].

SNPs of *MDR1* can occur naturally in humans, but some of them were related to altered P-gp expression and function. Three most common SNPs that have been repeatedly shown to predict changes in the function of P-gp are synonymous SNPs C1236T (rs1128503) in exon 12 and C3435T (rs1045642) in exon 26 and nonsynonymous, triallelic SNP G2677T/A (Ala893Ser/Thr or rs2032582) located in exon 21. In German, studies have been shown that both T allele and TT genotype of C3435T polymorphism were more frequently present among UC subjects, and the association of G allele of 2677 and IBD was also shown in North American study. However, further research that analyzed different allele combinations of those SNPs in IBD patients gave conflicting results [34].

6. SNPs in the neutrophil cytosolic factor 4 gene

More recently, GWAS has identified a number of new genetic susceptibility factors for IBD. Of these, new candidates have confirmed the association of the proinflammatory cytokine interleukin-23 receptor subunit (IL-23R) with CD and UC and a second gene, neutrophil cytosolic factor 4 (*NCF4*) with CD [36].

Researchers, Nuij et al. [27], have demonstrated that an IBD-associated SNP in the neutrophil cytosolic factor 4 (*NCF4*) gene results in a decreased antimicrobial function of granulocytes, as demonstrated by a reduced production of reactive oxygen species by these cells [27]. Moreover, as recently shown, SNPs in the interleukin 23 receptor gene (IL23R) have affected to express the anti-microbial peptide DMBT1 in intestinal epithelial cells in IBD [27].

7. Conclusion

Chronic inflammatory bowel disease is a subject of great interest among researchers because the pathomechanism of these conditions is difficult to explain, and thus far there is no optimal therapeutic process completely eliminating the symptoms and effects of the disease [37, 38].

Epidemiological and clinical studies suggest that the pathogenesis of inflammatory bowel disease is strongly linked to genetic predisposition [39, 40].

CD and UC are considered polygenic diseases in which familial clustering is observed in 5–10% of patients [41].

Among genetic factors associated with IBD development, it has been found that many single-nucleotide polymorphisms are associated with susceptibility to IBD progression. SNP can affect the production or function of a protein and thus affect the development of the disease [14].

However, although the overall role of genes involved in the development of IBD is already in most cases known, as of today it is unclear how the SNPs in these genes affect cellular function, or how such changed cellular functions would contribute to the development of IBD [27]. Therefore, further research is needed to demonstrate how gene polymorphism leads to the development of IBD.

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