Genetic background of inflammatory bowel disease

Jarosław Kierkus¹, Michał Szczepański, Edyta Szymańska², Sylwia Szymańska², Maciej Dądalski¹, Piotr Socha¹

¹ Department of Gastroenerology, Hepatology and Immunology
The Children’s Memorial Heal Institute
Warsaw, Poland
² Medical University of Warsaw
Warsaw, Poland

Abstract
Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the colon and small intestine consisting of two major types – Crohn’s disease (CD) and ulcerative colitis (UC). It can strike people in any age but is most frequently diagnosed in children or teenagers. IBD exact etiology is still not known but several causes including genetic susceptibility, environmental factors and bacterial or viral infections, psychical trauma or constant stress have been suggested. can determine the illness as well. The aim of treatment such as proper nutrition, drugs, biological therapy and finally surgery is to induce and sustain periods of remission. Therefore, more and more advanced genetic examinations are helpful not only in deepen our knowledge about IBD pathogenesis but also in discover new methods of treatment.

Key words: Crohn’s disease, genetics, IBD5, inflammatory bowel disease, NOD2

Introduction
Inflammatory bowel disease (IBD) is a heterogeneous group of chronic conditions of the gastrointestinal tract which major types are Crohn’s disease (CD) and ulcerative colitis (UC). The incidence of IBD is increasing systematically in well developed countries although in Poland it is still less common than in northern areas and the United States. According to primary epidemiological researches published, which concerned the frequency of IBD among children, the disease has affected 529 individuals younger than 18 years old within 2 years (2002-2004) [10]. The manifestations of IBD are different, it can present with any of the following symptoms: abdominal pain, vomiting, diarrhea, hematochezia (bright red blood in stools), weight loss and various associated complaints or diseases like arthritis, pyoderma gangrenosum, and primary sclerosing cholangitis. They are nonspecific therefore it is difficult to diagnose it quickly and introduce proper treatment which is of fundamental importance. Currently there is no cure for IBD but as it goes through periods of flare-ups and remissions, the aim of treatment (nutrition, medications, surgery and biological therapy) is first treating the acute problem, then maintaining remission. The exact cause of IBD is still unknown, a combination of environmental factors, genetic predisposition, bacteria from the intestinal walls and activation of the immune system seems play the role in development of the disease [7].

The role of genetic factors
The first susceptibility gene called NOD2 (nucleotide-binding oligomerization domain) was found in 2001, and 3 years later new genes such as DLG5 on 10q23, OCTN1 and OCTN2 on 5q31, NOD1 (CARD4) were identified [3]. The HLA and TLR4 associations were also reported [18]. Recently, the multiple genome-wide association studies included new genes – IL23R, IRGM, PTGER4, ATG16L1 as a potential IBD risk factors [27].
**NOD-like receptors (NLR) family**

NOD2 (currently CARD15 – caspase activating recruitment domain) or NOD2/CARD15 on 16q12, locus IBD1 is highly associated with ileal CD not UC. It functions as an intracellular sensor for bacterial peptidoglycan and can be activated by a minimal bioactive component, muramyl dipeptide (MDP) presented in both gram positive and negative bacteria [18]. Its expression is increased on the blood monocytes, dendritic cells and the epithelial cells of ileum. The function mechanism is related with activation of NF-κB and MAP kinase pathways. CARD-CARD domains joint RICK kinase (RIP-like interacting CLARP kinase) providing to nuclear NF-κB translation and activation of proinflammatory molecules [18].

Three common NOD2/CARD15 mutations including one frame-shift type (SNP13) and two missense type (SNP12) were detected so far. Its carriage has been specifically associated with ileal involvement, structuring complications and a modestly earlier age of onset but not with extraintestinal symptoms or infliximab (anti-TNFα agent) response [32]. The presence of SNP13 mutation increases the risk of colon cancer in patients above 50th years old [18].

Hisamatsu et al. revealed that product of NOD2/CARD15 expression can be antibacterial factor in CaCo2 cells of intestinal epithelium [14]. Wild-type cells with NOD2/CARD15 can not be infected by Salmonella typhimurium while those changed ones lost its function. Additionally, studies in mice with changed NOD2 variants did not reveal a spontaneous CD development which prove that NOD2 mutations alone are insufficient to produce IBD [17]. It is also thought that NOD2/CARD15 expression influences the regulation of Paneth cells degranulation in small intestine. According to Kobayashi et al. and Wehkamp et al. people with lower NOD2/CARD15 expression may display down-regulation of α-defensin [3, 31].

**Genes influencing mucosal integrity and transport – IBD5**

Genes within the IBD5 linkage region OCTN1/SLC22A4 and OCTN2/SLC22A5 have been reported as associated with IBD [8]. They encode the carnitine (required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy) and organic cation transporters. OCTN1 mutation reduces carnitine transport and increases organic cation transport, while OCTN2 is responsible for inhibit the carnitine and tetaethylammonium (TEA) uptake [8]. A univariate analysis detected association between IBD5 and steno/fistulizing behavior in CD patients and presence of more extensive colitis in UC patients [8]. However, none of the representative SNPs in IBD5 was associated with CD or UC in the Japanese subjects which demonstrate population-specific differences. Furthermore, the frequency of the IBD5 homozygous mutant genotype (5q31 polymorphisms) significantly increased in CD patients lacking response to infliximab.

Another gene suspected as associated with IBD is DLG5 (Disks large homolog 5) is a member of membrane-associated guanylate kinase (MAGUK) family. The protein encoded by this gene interacts with components of adherens junctions and the cytoskeleton. It may play a role in the transmission of extracellular signals to the membrane and cytoskeleton and in the maintenance of epithelial cell structure. Genetic variation in the DLG5 gene (R30Q) is associated with CD and UC combined [8].

**TLR family**

Toll-like receptors (TLR) family members play essential roles in the recognition of pathogens by the innate immune system, and their signaling pathways play an important role in the gene induction involved in inflammation and immune responses. Stimulation of TLRs induces not only proinflammatory cytokine genes but also type I interferon genes. TLR characterized so far activate the MyD88/IRAK signaling cascade, which bifurcates and leads to NF-kappaB and c-Jun/ATF2/TCF activation [24, 34]. TLR2 and TLR4 for example, are involved in lipopolysaccharide (LPS) recognition and signaling. Accumulating evidence suggests that some TLR molecules are also involved in signaling receptor complexes that recognize components of gram-positive bacteria and mycobacteria. TLR-mediated innate responses and immunological tolerance to commensals are regulated by a multitude of mechanisms including production of transforming growth factor (TGF)-β, interleukin (IL)-10 and stimulation of peroxisome proliferator-activated receptor (PPAR)-γ and de-ubiquitinating enzymes, all of which are altered in IBD. Therefore, considerable evidence suggests that patients with IBD may carry some of the polymorphisms of TLR genes such as D299G for TLR4, R80T and R753G for TLR1 for example [12, 34].

**Genes influencing immune system**

IL23R (interleukin 23 receptor) represents both a CD and a UC susceptibility gene. This protein is embedded in the cell membrane of several types of immune system cells, including T cells, natural killer (NK) cells, monocytes, and dendritic cells which identify foreign substances and defend the body against infection and disease. WHEREAS Interleukin 23 is a cytokine, which is a type of protein that regulates the activity of immune system cells. When interleukin 23 binds to its receptor, it triggers a series of chemical signals inside the cell. These signals promote inflammation and help coordinate the immune system’s response to foreign invaders such as bacteria and viruses [26]. Several variations in or near the IL23R gene have been found to influence the risk of developing CD. These associations have been found primarily in Caucasian (white) populations. Arg381Gln, for example, appears to reduce the risk of developing CD but it is unclear how this change protects against the disease. A weaker effect was seen in UC. Analysis accounting for Arg381Gln
suggested that other loci within IL23R also influence IBD susceptibility. It is believed that the receptor’s role in triggering inflammation in the intestinal walls may underlie its connection with those disorders which would suggest that incapacity to synthesize properly IL23R (SNP rs1004819 and rs2201841) may result in clinical manifestations of IBD [1, 9, 29].

PTGER4 gene encodes G-protein coupled receptor family including prostaglandin E receptor 4 (EP4) which maintain intestinal homeostasis by suppressing colitis, mucosal damage and CD4 cell activation. Studies in mice revealed that Prostaglandin E2 receptors EP2 and EP4 are up-regulated in peritoneal macrophages and modulate TNF-alpha and IL-6 production. In human purified naïve T cells they mediate signaling and cyclic AMP pathways to up-regulate IL-23 and IL-1 receptor expression. Their receptor-selective synthetic agonists cause down-regulation of induced TNFα production, but up-regulation on the pro-inflammatory cytokine IL-10 production. These results confirmed the importance of COX-2/PTGER2 in the healing mechanism of gastric ulcers and further suggested that the healing-promoting action of PTGER2 is mediated by the activation of EP4 receptors and is associated with VEGF expression. Additionally, E2 (PTGER2) can induce expression of early growth response 1 (EGR1) and lead to the phosphorylation of glycogen synthase kinase-3. Therefore, SNPs in PTGER4 gene have a negative effect on intestinal transport which is probably related with CD development (not UC) and its severe manifestation [8].

**Genes influencing autophagosome pathway**

Autophagy is a cytoplasmic process that keeps a cell stable – it is used to surround and destroy foreign invaders such as bacteria and viruses as well as to recycle worn-out cell parts and break down certain proteins when they are no longer needed. It also plays an important role in controlled cell death (apoptosis). Until recently, it was considered to be only a cell maintenance mechanism, but last researches has uncovered a role for autophagy in innate and adaptive immunity, which sheds light on the potential mechanism of the correlation between this gene mutation and an increased risk for IBD as exact role that ATG16L1 plays in its etiology is still unknown [6].

In 2007 Hampe et al. identified ATG16L1 gene as associated a higher risk for CD. This amino acid polymorphisms, Thr300Ala within the ATG16L1 gene is a part of the autophagosome pathway and has been implicated in the processing of intracellular bacteria [13]. Numerous meta-analysis address the relationship between rs2241880/T300A polymorphism of ATG16L1 and IBD. For childhood disease however, this variant was related to CD only. Other studies revealed that rs6431660 variant was weakly associated with UC but no evidence for epistasis between the ATG16L1 gene and other susceptibility genes (IL23R, CARD15, SLC22A4/5) were found. It seems that ATG16L1 is mainly a CD susceptibility gene without epistatic interaction with other IBD susceptibility genes and is not upregulated in intestinal inflammation [5, 6].

Several variations in or near the IRGM (immunity-related GTPase protein type M) gene (especially rs1000113 and rs4958847 variants) have been associated with an increased risk of developinng CD [11]. Normally, the IRGM protein helps trigger autophagy in cells infected with certain kinds of bacteria (mycobacteria), including the type that causes tuberculosis. SNPs in regions of DNA that regulate IRGM production may disrupt the autophagy process, preventing the immune system from destroying harmful bacteria effectively. An abnormal immune response to bacteria in the intestinal walls may lead to chronic inflammation and the digestive problems [19].

**Recently discovered genes**

The multidrug resistance gene (ABCB1/MDR1), a member of the ATP-binding cassette (ABC) transporters, is an attractive candidate gene for the pathogenesis of inflammatory bowel diseases (IBD) and perhaps for response to therapy. It encodes an ATP-dependent efflux transporter pump (P-glycoprotein) that is highly expressed in various tissues, including the epithelial surfaces of the intestine. The level of expression of P-glycoprotein has implications not only for the development of resistance to various pharmaceutical agents but also for disease susceptibility such as various types of cancer, HIV, hypercholesteremia, Parkinson's disease. Several SNPs in the MDR1 gene have also, arguably, been associated with IBD [12, 21]. So far, only limited data are available on MDR1 and ABCG2 polymorphisms in East European IBD patients, but six haplotype tagging single nucleotide polymorphisms (SNPs) representing the haplotypic variations of the ABCB1/MDR1 gene (specially one intronic variant rs3789243) were identified as POSSIBLE predisposition to UC not to CD. Schwab et al suggested for example, that the Ser893 variant increased susceptibility in ulcerative colitis but not Crohn’s disease [25]. Brant et al. described that the reference genotype (G2677) increased risk, whereas other studies have failed to show an association [4]. Hungarian studies revealed that the frequency of the ABCG2 and MDR1 SNPs was not significantly different among CD, UC patients and controls [11]. There was no difference in risk for steroid resistance in CD patients carrying variant ABCG2 or MDR1 3435T alleles. In addition, carriage of the variant allele was not associated with disease phenotype, presence of extra-intestinal manifestations, smoking, response to infliximab therapy or the need for surgery. In UC, the carriage of variant ABCG2 alleles seemed to be preventive for arthritis (15.5% versus 31.7%; OR: 0.39, 95% CI: 0.16-0.98). Therefore, these recent findings still provide closer investigations as new insights into the localization of the critical susceptibility determinants within the gene may have potentially important implications in the application of pharmacogenetics across not only IBD but also a range of common diseases, including HIV, epilepsy and colorectal cancer [11, 19, 23]. Last studies in a Japanese IBD cohort revealed association between IBD...
and haplotypes within the TNFSF15 which is the human gene for vascular endothelial growth inhibitor (VEGI). This cytokine belongs to TNF ligand family and is abundantly expressed in endothelial cells, but is not expressed in either B or T cells. Its expression is inducible by TNF and IL-1α. It can activate NF-kappaB and MAP kinases, and acts as an autocrine factor to induce apoptosis in endothelial cells. This cytokine is also found to inhibit endothelial cell proliferation, and thus may function as an angiogenesis inhibitor [29]. The fact that TNFSF15 and its mRNA and protein expression is upregulated in IBD, particularly in CD was first reported by Japanese researchers and were confirmed in the study of two European IBD cohorts. Interestingly, a core TNFSF15 haplotype showing association with increased risk to the disease was common in the two ethnic groups. However, recent large scale case control studies performed by Yamazaki et al. conferred susceptibility to CD in both Japanese and UK populations but no evidence for an association with Japanese UC was observed, although a potential association with Caucasian UC was reported [16]. Ethnic differences in genetic susceptibility may be explained by differences in the haplotype background. Some TNFSF15 polymorphisms identified in the Japanese were monomorphic or nearly monomorphic in the Caucasian population. Thus it seems likely that population specific patterns of haplotypes may contribute to differences in UC susceptibility. Thus to identify the pathogenic SNP, a functional study is clearly needed [22].

Association with human leukocyte antigens (HLA)

The HLA region has been implicated in determining the disease susceptibility or the clinical phenotype of IBD, because their products play a central role in the immune response. Multiple studies have reported associations between HLA-DR or -DQ phenotypes and IBD. Some clinical features of CD and UC may be influenced by specific HLA-DR alleles. In UC some alleles (particularly DR3) appear to segregate with more aggressive disease, whereas in CD different alleles (particularly DR13) co-segregate in patients with colonic disease and extraintestinal manifestations [2, 8, 28].

Summary

According to numerous epidemiological studies and research works, the role of genetic factor in IBD can not be challenge. Every year new susceptibility genes and their mutations are discovered. They are associated mostly with CD but recent findings revealed also some genes unique to UC. Such a considerable progress of genome-wide association studies have important implications in the therapeutic approaches. Therefore, a more complete pathophysiologic genetic definition of IBD will require integration of clinical observations, genetic data, statistical analyses, and delineation of underlying biologic processes.

References


