Immunogenetic biomarkers in inflammatory bowel diseases: Role of the IBD3 region

Manuel Muro, Ruth López-Hernández, Anna Mrowiec

Manuel Muro, Ruth López-Hernández, Anna Mrowiec, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberhe), Immunology Service, University Hospital Virgen de la Arrixaca, 30120 Murcia, Spain

Abstract

Many studies have demonstrated the linkage between the IBD3 region (6p21.1-23), an area which encompasses the famous human leukocyte antigen (HLA) complex, and Crohn’s disease (CD) or ulcerative colitis (UC). IBD3 is the only region that meets genome-wide significance, and provides stronger evidence of the linkage than 16p13.1-16q12.2 (IBD1), the locus that contains the susceptibility gene CARD15. However, despite these findings, IBD3 susceptibility genes remain elusive and unclear due to the strong linkage disequilibrium, extensive polymorphism, and high gene density that characterize this area and also due to varying allele frequencies in populations around the world. This area presents an extremely high abundance of genes, including the classical and non-classical major histocompatibility complex (MHC) class I and II genes, and other genes, namely MHC class III genes tumor necrosis factor (TNF)-α and -β, and Hsp, whose proteins play key functions in immunological processes. To date, it is not clear which genes within the MHC family contribute to the IBD pathogenesis, although certain HLA alleles have been associated with IBD. Recent insights into the biological function of other genes encoded within the IBD3 region, such as the MHC class I chain-related molecule A (MICA) genes, have led investigators to a more comprehensive exploration of this region. MHC class I chain-related molecule A (MICA) is highly polymorphic and interacts with NKG2D, its receptor on the surface of NK, TCD4+, TCD8+, and γδ T cells (lamina propria) in patients with CD have also been reported. MICA alleles have also been associated with IBD, and a variation at amino acid position 129 of the α2-heavy chain domain seems to categorize MICA alleles into strong and weak binders of NKG2D receptor, thereby influencing the effector cells’ function. In this regard, a relevant role of MICA-129-Val/Met single nucleotide polymorphism has recently been implicated in the pathogenesis of IBD. TNF-α and -β also play an important role in inflammatory response. In fact, IBD is commonly treated with TNF-α inhibitors. Additionally, polymorphisms of TNF-α gene are known to affect the gene expression level and particular TNF-α genotypes may influence the response of IBD patients treated with TNF-α inhibitors.
MHC class I chain-related molecule B, and tumor necrosis factor-α and -β, and their role on IBD susceptibility are discussed in the light of recent publications. IBD3 gene polymorphisms and their clinical relevance for the treatment of the IBD patients are also discussed. Insights into the natural history of these complex diseases may allow in the future appropriate patient selection for early aggressive therapy aimed at modifying the course of the disease.

Muro M, López-Hernández R, Mrowiec A. Immunogenetic biomarkers in inflammatory bowel diseases: Role of the IBD3 region. World J Gastroenterol 2014; 20(41): 15037-15048. Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i41/15037.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i41.15037

INTRODUCTION

Inflammatory bowel diseases (IBD) are an idiopathic disease that seems to involve an immune reaction of the body to its own intestinal tract. The two major types of IBD are ulcerative colitis (UC) and Crohn’s disease (CD). Although both UC and CD present different pathologic findings, a significant percentage of patients are classified as indeterminate IBD. Twin studies and segregation analysis strongly support IBD, especially CD, as complex genetic traits, whose etiology also involves immunological and environmental factors. These pathologies are polygenic-related diseases that share some susceptibility loci, but differ at others. A number of studies have been performed to identify IBD susceptibility chromosomal regions, and in some of them causative genes have been found[2-5]. However, although these studies have indicated multiple regions of interest, the replication of these results has been rather limited. IBD susceptibility genes have classically been identified with different and consecutive names, depending on the chromosomes involved [e.g., 5q31 (IBD2), 6p21 (IBD3) and 19p (IBD4)], while several loci of interest (e.g., at 3p, 3q, 14q and others) require further follow-up.

From a functional point of view, active IBD is immunologically defined as an infiltration of the lamina propria by innate immune cells (neutrophils, macrophages, dendritic and NK cells) and adaptive immune cells (B and T cells). Increased numbers and activation of these cells in the intestinal mucosa enhance local levels of tumor necrosis factor (TNF), located in the IBD3 region, and other pro-inflammatory interleukins[6,7]. Cytokines are also essential mediators of the interaction between activated immune cells and non-immune cells, including epithelial and mesenchymal cells. Moreover, germ-line variation in IL23R, IL12B, JAK2 and ST/AT3 has recently been associated with IBD susceptibility, consistent with the newly described role for IL-23 signaling and Th17 cells in inflammatory disease pathogenesis. Additionally, several genes involved in different aspects of bacterial handling, such as NOD2 (from now on called CARD15) on human chromosome 16 (IBD1) and the autophagy genes ATG16L1 and IRGM, are defective only in CD. IL10 and ECM1 are associated with UC, and an inherited variation at the human leukocyte antigen (HLA) region (IBD3) is related to an inflammatory colonic phenotype[8-10]. In fact, other non-classical HLA genes, such as HLA-G and MIC (major histocompatibility complex (MHC) class I related chain), are also implicated in IBD pathology[18,17,18].

In the last years, the application of genome-wide association studies (GWAS) has been successful in defining the genetic architecture of CD and UC and in delivering genuinely novel and important insights into disease pathogenesis. The recent “Immunochip” study[19], carried out by the International IBD Genetics Consortium, has led to the validation of 163 genetic loci containing susceptibility genes for IBD[20,21], including 71 newly established, a number that is expected to double in the next years. In the “Immunochip” study, the maximal genetic association for UC at single nucleotide polymorphism (SNP) rs6927022 mapped adjacent to HLA-DQA1 class II gene (between DQB1 and DRB1). However, there was no evidence of this SNP conferring the risk of developing CD. CD maximal association mapped to HLA class I region and showed no association with UC. These results showed that the HLA region, central to autoimmunity, is especially important and broad-based (influencing a large segment of the at-risk population) to UC. On the other hand, the HLA association with CD seems to be less important and distally located, at least in Caucasians.

Finally, although important obstacles need to be overcome, the successes of the last years suggest that a detailed description of the genetic basis of IBD is a realistic goal. This has unearthed a plethora of attractive targets for the development of future therapeutics. Insights into the natural history of these complex diseases may allow in the future appropriate patient selection for early aggressive therapy aimed at modifying the course of the disease.

In this review, we will analyze the new and old immunogenetic biomarkers of the IBD3 region and their role on IBD susceptibility, in the light of recent publications. In this context, HLA, MICA, MICB, and TNF-α and -β are the most important genes involved.

HLA SYSTEM AND IBD

Since the first report of an HLA association with IBD in 1972, more than 150 studies investigating the role of HLA genes in determining susceptibility and phenotype of IBD have been published[22]. The HLA complex encodes more than 225 genes, and GWAS have shown consistent evidence of its linkage to IBD3 (6p21.1-23)[23-29]. This has been demonstrated in several independent studies of both CD and UC[26-28]. The structure of this region is shown in Figure 1. The importance of this area was further highlighted by a meta-analysis of ten published genome-wide scans[30]. IBD3 was the only locus that met
Figure 1  Localization of the \textit{MIC} genes in the major histocompatibility complex class I region on the short arm of human chromosome six. Classical human major histocompatibility complex (MHC) class I locus (HLA-A, -B and -C), non-classical MHC (HLA-E, -F and -G) (grey boxes), and \(\text{TNF}_{\alpha}\) and HFE (white box) are shown. Pseudogenes are painted in black boxes.

In this respect, our group has recently found an increased frequency of \(\text{HLA-DRB1}^*03, -\text{DRB1}^*13, -\text{DRB1}^*01\) and \(-\text{DRB1}^*01:03\) alleles and a decreased frequency of \(\text{DRB1}^*15\) allele in CD patients compared with UC patients\(^{[7]}\). These data are concordant with previous studies that suggest that, among British patients, HLA-\(\text{DRB1}^*01:03\) was associated with the development of severe isolated colonic CD, defined by the requirement for infliximab (anti-\(\text{TNF}_{\alpha}\)) or colectomy\(^{[1,29]}\), while positive association of CD pathology was found with HLA-\(\text{DRB3}^*03:01\) and \(-\text{DRB1}^*13\)\(^{[26,31]}\). The association of HLA-\(\text{DRB1}^*01:03\) with UC has also been confirmed in other studies\(^{[31,33-35]}\). Moreover, HLA-\(\text{DRB1}^*15\) was also negatively associated with CD\(^{[3]}\), which explains a previous negative association with the serological antigen HLA-DR2 in the 1999 meta-analysis\(^{[31]}\). This allele seems to confer protection against all subgroups of CD pathology, in all ethnic groups, including Japanese. In addition, in our UC patients, allele frequencies of \(\text{HLA-DRB1}^*15\) were strongly increased compared with CD patients\(^{[7]}\). This data agree with other reports showing that \(\text{HLA-DRB1}^*15\) is associated with UC in the European\(^{[27]}\), North American\(^{[33]}\), Japanese\(^{[30]}\) and Korean\(^{[30]}\) populations. However, HLA-\(\text{DRB1}^*07\) frequency allele, associated in unselected patients with CD in other studies\(^{[1,25,26]}\), was not associated to CD in our study\(^{[7]}\). Additionally, in contrast to other studies\(^{[30,36-38]}\), we were not able to find an increased frequency in HLA-\(\text{DRB1}^*04\) allele in CD patients. However, the frequency of HLA-\(\text{DRB1}^*04\) was decreased in UC compared with CD patients\(^{[7]}\). Additionally, HLA-\(\text{DRB1}^*04\) allele was also negatively associated with UC in the Northern European and Japanese populations, in contrast to the positive association with CD\(^{[34]}\).

It was reported that in the Spanish population, the only DR alleles significantly associated with the disease were DR3 (very strong protection) and DR4 (weak protection)\(^{[39]}\). The strong protective effect of DR3 was evenly distributed among the haplotypes DR3-\(\text{TNF}_{\alpha}\)5\(\beta\)_5, DR3-\(\text{TNF}_{\alpha}\)2\_5, and DR3-\(\text{TNF}_{\alpha}\)other. These results showed the strong protective effect of DR3 in this population, which suggests that the relevant protective gene could be located centromeric to \(\text{TNF}_{\alpha}\) and \(\text{TNF}_{\beta}\) markers. Recent results showed that IBD Tunisian patients have an increased frequency of the homozygous \(\text{DRB1}^*07\) genotype\(^{[40]}\). In UC patients, \(\text{DQB1}^*03:02\) was also predictive of colonic extension, whereas \(\text{DRB1}^*13\) and \(\text{DQB1}^*03:01\) were associated with limited disease localization (left-sided colitis and proctitis). The same
study showed that DRB1*15 allele was also increased in patients with extra intestinal manifestations. In CD, female patients showed an increased frequency of the DRB1*13, *15, and DQB1*06 alleles and DRB1*13-DQB1*06 haplotype, whereas male patients showed a significant increase of the DRB1*07, DQB1*02 alleles, and DRB1*07-DQB1*02 haplotype. These recent results suggest a significant association of the homozygous HLA-DRB1*07 genotype with UC and CD and of several HLA-DR/DQ alleles and haplotypes with the clinical phenotypes of these diseases. At the same time, it had also been reported that IBD patients had lower odds of DQ2/8 positivity compared with healthy controls in an Italian population\(^{[41]}\). Similarly, a pathogenetic link of HLA-B27 between CD and primary sclerosing cholangitis in South Africans has been reported\(^{[42]}\).

HLA-C gene has also been reported to associate with IBD in a study that suggests that HLA-C*07 and -C*12 alleles may be strongly associated with the susceptibility to UC and CD, respectively\(^{[43]}\). Interestingly, GWAS of Japanese population\(^{[44]}\) showed that although HLA-C*12:02-B*52:01-DRB1*15:02 haplotype increases susceptibility to UC when compared with the frequency of healthy controls, this haplotype reduces the risk of non-colonic CD, whereas it has no effect on colonic CD.

HLA-A4 is another gene associated with IBD (Figure 1). HLA-G is a non-classical MHC class Ib molecule, predominantly expressed in cytotrophoblasts and, under pathological conditions, in chronically inflamed tissue.

An interesting study investigating a 14-bp deletion polymorphism (Del+/Del-) (rs16375) within exon 8 of the HLA-G gene revealed significant differences between UC and CD\(^{[18]}\). Heterozygous genotype and the homozygous Del+ phenotype were significantly increased, whereas the homozygous Del+ phenotype was significantly decreased when UC was compared with CD. The fact that the deletion in exon 8 of the HLA-A4 gene seems to influence its transcription activity suggests that the HLA-G gene might play a role in the pathogenesis of IBD.

Other polymorphisms in this region have also been associated with IBD. In this regard, significant association with the rs2395185 variant of the HLA gene and steroid-response, and positive family history in UC, patients has recently been reported\(^{[45]}\). For the HLA-rs2395185 SNP this association persisted both in the adult- and in the pediatric-onset subset of patients with a positive family history.

The unclear role of different HLA molecules in IBDs might be explained by either different affinity to the same peptide or by the HLA molecules binding with different strength to different molecules. In this respect, the IBDs might be explained by molecular mimicry hypothesis\(^{[46,47]}\). Cross-reactivity of peptides derived from bacterial luminal flora (similar in their structure to HLA antigens) with host “self” antigens present in the gut may lead to the generation of auto-reactive T lymphocytes that either stimulate or inhibit the immune system.

Recent insights into the biological function of other genes encoded within the IBD3 region have also led investigators to a more comprehensive exploration of this region. An example of this is the analysis of MIC genes presented in the next section.

**MIC GENES AND IBD**

**Polymorphism of MIC Molecules**

On the 2 Mb class I segment in the short arm of human chromosome 6, multiple MHC class I chain-related (MIC) loci have been identified\(^{[48]}\). The genes at these loci represent a second lineage of mammalian MHC class I genes. The MIC genes include seven members (MICA to MICG), five of which are pseudogenes and gene fragments, and two, namely MICA and MICB, are functional genes closely related to each other (Figure 1).

They code for stress-induced cell surface molecules, which do not associate with β2-microglobulin and do not appear to present peptides\(^{[49]}\), since the putative peptide-binding groove is too narrow to accommodate a ligand. This suggests that MICA/MICB are not antigen-presenting molecules\(^{[50]}\). MICA gene has an overall homology of 83% with MICB gene, but their homology with the classical MHC class I genes is quite low, ranging from 15% to 35%\(^{[51]}\).

MICA/MICB polymorphic residues are positioned on the outer edge of an antigen-binding cleft, apparently bordering an invariant ligand-binding site, unlike MHC class I molecules\(^{[52,53]}\). The functional significance of these polymorphisms is unknown, although certain changes in the amino acid sequence of the protein influence the abnormal expression\(^{[54]}\) or the affinity in the interaction with NKG2D/DAP10, its ligand on the surface of NK, T\(\gamma\)δ and T CD8+ lymphocytes. MIC triggers the cytolysis mediated by these NKG2D-bearing cells (Figure 2). It has been demonstrated that the allelic diversity at the MICA locus affects ligand binding between MICA and NKG2D, potentially affecting NK-cell activation and the modulation of T-cell responses\(^{[46,55]}\).

Therefore, the presence of methionine or valine at codon 129 of \(\alpha\)2 domain could confer strong and weak affinity, respectively. Alleles at the MICA locus can be defined as strong or weak binders depending on their capacity to bind NKG2D. The strong NKG2D binding alleles share methionine at position 129, whereas weak binding alleles have valine at this position. In this respect, high-affinity alleles include MICA*001, *002, *007 and *017, while low-affinity alleles include MICA*004, *006, *008, *009 and *010\(^{[5]}\). Although the significance of high/low affinity for NKG2D in terms of immune activation is unclear, knowledge of specific haplotypes may provide valuable information with regard to modern population’s ancestry, admixture and the selective pressures maintaining such as linkage.

The crystal structure of the MICA-NKG2D complex has also revealed that NKG2D binds as a homodimer to one molecule of MICA\(^{[4,55]}\). One of the NKG2D
molecules binds mostly to the α1 domain of MICA, while the other NKG2D molecule binds mostly to the α2 domain. The NKG2D homodimer overlays MICA diagonally, and αβTCR overlays MHC class I molecules in a similar way. The hypothetical binding pockets of MICA remain free of any ligand confirming that MICA is not an antigen-presenting molecule. The half-life for the MICA-NKG2D complex indicates that it is more stable than the complexes formed by TCR and MHC class I molecules[56].

Furthermore, some authors have also suggested that MICA should be considered a cell homeostasis sensor rather than a cell stress sensor whose up-regulated expression is induced not only by cell distress but also by strong proliferation and pro-inflammatory stimuli that disrupt the cellular homeostasis and elicits a cytotoxicity that eliminates altered cells, thus contributing to the restoration of the normal homeostasis[56].

MICA has also been shown to play a role in very different aspects of the immune response, such as solid transplant rejection, immune response against viruses and intracellular bacteria, inflammation, homeostasis of epithelia, mother-fetus tolerance and immune response against tumors[55-59].

**MICA/MICB Molecules in Intestinal Pathology**

Different studies have demonstrated that MIC genes are implicated in intestinal homeostasis. In fact, MICA is a stress-inducible cell surface antigen that is recognized by intestinal epithelial V61 γδ T cells, NK cells and CD8⁺ T cells with their NKG2D receptor participating in the immunological reaction in intestinal mucosa. With regard to intestinal pathology, patients suffering active celiac disease with villous atrophy showed a strong MICA expression on the cells of crypts[60]. MICA is also expressed in villous epithelial cells of the gut in normal or disease-free individuals, but its localization is mostly intracellular.

IL-15, which is over-expressed in the intestine of patients with celiac disease, appears to be involved in this up-regulation of MICA expression and contributes to the cytotoxicity of NKG2D+ intraepithelial lymphocytes (IELs)[61-63]. These cells lyse target epithelial cell lines in a NKG2D-dependent way, developing villous atrophy.

On the other hand, MICA-A5.1 polymorphism has been associated with the risk of atypical celiac disease, while a double dose of MICA 5.1 allele could predispose to celiac disease with gastrointestinal symptoms[64,65]. Another study has shown that MICB gene is also associated with celiac disease and that both MICB*008 and MICB*002 alleles are part of the celiac disease susceptibility extended haplotypes B8/DR3/DQ2, B18/DR3/DQ2, and DR4/DQ8[66].

With respect to IBD pathology, increased expression of MICA in intestinal epithelial cells and increased expression of NKG2D in CD4⁺ T cells (lamina propria) in patients with CD have been reported[67].

Lü et al[68] investigated the association of the microsatellite polymorphisms in the intron 1 of MICB and the MICA-MICB haplotype with the susceptibility to UC in the Chinese population. All the subjects were of Chinese Han ethnicity. The frequency of MICB-C-A18 was significantly higher in UC patients compared with healthy controls and was increased in the female patients compared with the female healthy controls. Thus, MICB-C-A18 seems to be positively associated with UC in the Chinese population.

The same group generated MICA*A5.1-expressing Raji cells by gene transfection in a later study[69] which also showed that the frequency of MICA*A5.1 was significantly higher in UC patients compared with the healthy controls and that the frequency of a MICA*A5.1/A5.1 homozygous genotype was increased in UC patients. Raji cells with MICA*A5.1 expression produced indeed more soluble MICA than Raji cells with full-length...
MICA expression in culture supernatant. Raji cells with MICA*A5.1 expression were more resistant to killing by NK cells than Raji cells with full-length MICA expression. Thus, the MICA*A5.1 allele and MICA*A5.1/A5.1 genotype seem to be significantly associated with Chinese UC patients in central China. The authors suggested that MICA*A5.1 may play a role in the development of UC by producing more soluble MICA and resistance to NK cells. The association of MICB exon 2-4 polymorphisms and sMICA expression with the susceptibility to UC in central China was also investigated by the group[74]. Their results showed that allele frequency of MICB0106 was significantly higher in UC patients than in healthy controls, especially in patients with extensive colitis, moderate and severe disease, extraintestinal manifestations, male patients, and patients over the age of 40 years. The sMICA level was also significantly higher in UC than in healthy controls but not associated with the MICB0106 genotypes. In 2011, they obtained intestinal mucosal biopsies from patients with UC[71] and observed that the relative amount of MICA mRNA in the colonic mucosa of UC patients was significantly higher compared with controls, as were the MICB and NKG2D mRNA expressions. Confocal microscopy resonance scanning also showed that MICA was localized predominantly on the basolateral membranes of the epithelium. Further flow cytometry confirmed that the percentage of IFN-γ producer NK cells that expressed NKG2D in peripheral blood lymphocytes was higher in UC patients than in the healthy controls. Thus, MICA, MICB and NKG2D were up-regulated in the colonic mucosa of UC and were associated with activating NK cells with enhanced NKG2D and IFN-γ production.

On the other hand, our group found no statistical differences in the distribution of MICA alleles between the IBD, CD and UC patients in the Spanish population, in a recent study[76]. Our data corroborated the first reported studies showing no association of CD pathology and MICA allele polymorphism and contradict other studies showing that UC is mostly associated with MICA*007, and Type 2 IBD peripheral arthropathy with MICA*008[17,67,72].

The association between the polymorphism of the transmembrane region of MICA and the genetic susceptibility in Tunisian patients with IBD has been recently studied[70]. No MICA alleles were significantly increased in IBD compared with controls. The MICA-A5.1 allele was significantly decreased in CD patients. In UC, MICA-A6 was associated with the presence of extraintestinal manifestations, whereas MICA-A5 was associated with late age of onset. In CD, MICA-A6 was significantly increased in active disease patients when compared with moderately active or inactive disease. In conclusion, MICA seems to play a disease-modifying role, rather than being an important gene in the susceptibility to IBD in the South Tunisian population.

Finally, a recent meta-analysis shows that MICA alleles are associated with ulcerative colitis in Asians[4,53].

On the other hand, a variation at amino acid position 129 of the α2-heavy chain domain (MICA-129 Val and Met) seems to categorize MICA alleles into strong and weak binders of the NKG2D receptor and thereby to influence effector cells’ function[4,53]. Evidence supporting this hypothesis was provided by Zhao et al[74]. The authors showed that the frequency of MICA-129 Val/Val genotype, as well as serum sMICA levels were significantly higher in UC patients than in the controls. Moreover, higher levels of sMICA were associated with severe colitis in the patients.

On the contrary, we have recently found a higher frequency of MICA-129 Met/Met and a lower frequency of MICA-129 Val/Met genotypes in IBD patients (mainly in UC) compared with control subjects[74]. All these preliminary data suggest a relevant role of MICA-129-Val/Met SNP (weak/strong binders of NKG2D receptor) in the pathogenesis of IBD[75]. However, MICA may be in linkage disequilibrium with another gene or genes that are in fact responsible for the protection against IBD, in which case it would involve a secondary disease association. In any case, these facts need to be confirmed by further studies. In conclusion, a relevant role of MICA-129-Val/Met SNP (weak/strong binders of NKG2D receptor) in the pathogenesis of IBD may imply an association with the protection against the disease, although whether it confers primary or secondary protection remains to be determined.

**TNF-α AND TNF-β**

TNF-α and -β are key cytokines known to play a role in inflammatory responses, and the loci for these genes are found in the IBD3 region on chromosome 6p21, which is known to be associated with an increased risk for IBD. TNF-α/TNF receptor interactions do not only play a pivotal role in the pathogenesis of the inflammatory response, but also cause apoptosis, cell proliferation, and differentiation[76]. Alterations in the regulation of TNF-α, especially TNF-α over-production, have been implicated in a variety of symptoms associated with autoimmune disorders, including IBD, and especially CD[77]. Inflammation, anorexia, and weight loss are also all associated with increased levels of circulating TNF-α that are seen in CD[13].

In fact, three anti-TNF agents, namely infliximab (IFX), adalimumab and certolizumab pegol have been approved by the US Food and Drug Administration for the treatment of luminal CD[76]. IFX has also been approved for fistulising CD and UC. The advent of these anti-TNF-α agents has also changed the way of treating IBD refractory to standard medications[76,78]. Clinical trials showed that IFX response varies among patients. It would be beneficial if the efficacy could be predicted by genetic factors since these treatments are very expensive. In this sense, a recent report shows a relationship between the TNF receptor polymorphism and the IFX response in Japanese patients[78].
Several SNPs in the TNF-α promoter region are known to affect its gene expression (e.g., -238G/A and -308G/A)\[^{[79]}\]. Such variations in the TNF-α promoter region have previously been associated with the susceptibility to a range of autoimmune disorders, asthma, psoriasis and rheumatoid arthritis\[^{[79-82]}\]. The -238G/A SNP is associated with a lower production of TNF-α in patients with ulcerative colitis\[^{[83]}\]. Conversely, the -308A allele is associated with enhanced TNF-α production in cells in vitro and in CD patients in vivo\[^{[84,85]}\].

The TNF-α -308G/A and the TNF-β NcoI polymorphisms have also been associated with survival in sepsis or septic shock of various origins. Polymorphisms of the TNF-α -308 and TNF-β +252 do not correlate with age, gender, disease activity or lesion site\[^{[86,87]}\] in the Chinese population.

In a recent study, we did not find differences in individual TNFα -238G/A and -308G/A SNPs allele and genotype frequencies between CD, UC and IBD patients. These data contrast with other reports showing that the -308 G/A SNP could be associated with IBD susceptibility\[^{[79]}\], while they agree with Cao et al\[^{[86]}\]. A recent meta-analysis showed that TNF-α -308 polymorphisms are also associated with ulcerative colitis in Asians\[^{[79]}\], whereas another study from Northern India [evaluating TNF-α (-1031T > C, -863C > A and -857C > T)] showed that the high-producing genotype of TNF-α (-863AA) was associated with increased risk of IBD, and more so with UC\[^{[88]}\]. Similarly, the combined effect of TNF-α polymorphisms in haplotype analysis demonstrated additionally increased risk of IBD.

However, in the TNF-α promoter gene polymorphism (-308G/A SNP) study, we found an increased frequency of the -308A allele and -308GA genotype in the non-responders to anti-TNF treatment compared with responder patients\[^{[89]}\]. This -308GA genotype has been classified as a high producer of TNF-α cytokine\[^{[80,82]}\], which could explain the different response of IBD patients to TNF-α inhibitor treatment. It can be argued that in situations with a higher production of TNF-α, its inhibitor could not be completely inhibitory, thus promoting a worse clinical response to TNF-α inhibitors. In addition, no differences were found between the two drugs used\[^{[89]}\]. In any case, more polymorphisms affecting the biological response and the responder/non-responder status of anti-TNF should be investigated, since the contribution of these genotypes to the immune response may be weak. It should also be mentioned that no analysis of the biological response of anti-TNF has been performed in this study.

Given the inter-individual variability in the response to the anti-TNF monoclonal antibody IFX, the predictive value of TNF and/or IL1β as surrogate markers of IFX response was recently studied\[^{[90]}\]. Baseline serum concentrations of TNF and IL1β were higher in UC patients than in CD patients. CD patients showing < 0.64 pg/mL IL1β at baseline were more frequently responders than non-responders, and the C allele of the IL1B polymorphism was associated with higher IL1β serum concentrations and with poorer clinical remission after 14 wk of IFX treatment. No significant association was found between serum TNF concentration or TNF polymorphisms and patient response to IFX.

New data on adaptive immunity are emerging, indicating that: (1) the mucosal Th1 and Th2 responses of IBD may be actually secondary to defects of the innate immune response; (2) the dysfunction of regulatory T cells may be contributing to mucosal immune abnormalities; and (3) the newly described Th17 cells are also prominently involved in the gut inflammatory response in both forms of IBD\[^{[91]}\]. In fact, TNF is synthesized by different cells, mostly of monocyte line and T lymphocytes\[^{[80]}\], and also intestinal epithelial cells (IEC). All known responses to TNF are triggered by binding to one of two distinct receptors, designated TNFR1 (also known as TNFRSF1A, CD120α, p55) and TNFR2 (also known as TNFRSF1B, CD120b, p75), which are differentially regulated on various cell types in normal and diseased tissue\[^{[92]}\].

TNF-α induces the expression of various genes, such as urokinase plasminogen activator, cyclooxygenase II (COX II) and vascular endothelial growth factor by activating the nuclear factor-κB\[^{[92]}\]. In this way TNF-α performs multiple biological functions, such as increasing leukocyte recruitment (induction of leukocyte adhesion molecules)\[^{[92,93]}\], modulation of nitric oxide (NO) production (by increasing vascular permeability)\[^{[93,94]}\], induction of secretion of proinflammatory cytokines\[^{[86]}\], and the proliferation and differentiation of immune cells.

Chronic ulcerative colitis (CUC) is characterized by increased IEC apoptosis associated with elevated TNF, inducible nitric oxide synthase (iNOS), and p53. In addition, p53 has been reported to be increased in crypt IECs in human colitis and is needed for IEC apoptosis in chronic dextran sulfate sodium-colitis\[^{[95]}\]. Goretsky et al\[^{[96]}\] examined the roles of TNF and iNOS in the regulation of p53-induced IEC apoptosis in CUC. The IEC TUNEL staining, caspases 3, 8, and 9, and p53 protein levels, induced by anti-CD3 mAb activation of T cells, were markedly reduced in TNF receptor 1 and 2 gene knockout mice. Induction of IEC apoptosis correlated with increased p53, which was attenuated in iNOS(-/-) mice. IEC p53 levels and apoptosis were also reduced in IL-10 (-/-) colitic mice treated with neutralizing TNF mAb and the iNOS inhibitor, aminoguanidine, further suggesting that TNF and iNOS are upstream of p53 during colitis-induced IEC apoptosis. IEC apoptosis and p53 levels were assessed in control vs untreated or anti-TNF-treated CUC patients with equivalent levels of inflammation. In this study, IEC apoptosis and p53 levels were clearly higher in untreated CUC but markedly reduced in patients treated with anti-TNF mAb. Therefore, TNF-induced iNOS activates a p53-dependent pathway of IEC apoptosis in CUC. The inhibition of IEC apoptosis may be an important mechanism for mucosal healing in anti-TNF-treated CUC patients.

However, the mechanisms of action of anti-TNF-α
agents are still unclear\cite{59,98,99}. The neutralization of TNF-α in the inflamed mucosa is unlikely to be a sufficient explanation. Antibody-dependent cytotoxicity also induces apoptosis or lysis of TNF-α-producing cells\cite{59,100}. This mechanism involves the Fc portion of antibodies that increases the pro-apoptotic factor caspase-3\cite{100}.

Finally, RNA interference (RNAi) holds great promise for the specific and selective silencing of aberrantly expressed genes, such as TNF-α in IBD. A very recent study investigates the efficacy of an amphiphilic cationic cyclodextrin (CD) vector for effective TNF-α siRNA delivery to macrophage cells and to mice with induced acute-colitis\cite{101}. RAW264.7 cells were transfected with CD.TNF-α siRNA, stimulated with lipopolysaccharide (LPS). Female C57BL/6 mice were exposed to dextran sodium sulphate (DSS) and treated by intra-rectal administration with either CD. TNF-α siRNA or a control solution. In vitro, siRNA in CD nanocomplexes remained intact and stable in both fed and fasted simulated colonic fluids. RAW264.7 cells transfected with CD.TNF-α siRNA and stimulated with LPS displayed a significant reduction in both gene and protein levels of TNF-α and IL-6. CD. TNF-α siRNA-treated mice revealed a mild amelioration in clinical signs of colitis, but significant reductions in total colon weight and colonic mRNA expression of TNF-α and IL-6 compared to DSS-control mice were detected. This data seems to indicate the clinical potential of a local CD-based TNF-α siRNA delivery system for the treatment of IBD.

In sum, a number of IBD immunogenetic markers have been identified\cite{102,103,104}, and further investigation may establish new approaches to these enigmatic pathologies. To conclude, further large-scale and functional studies of IBD3 genes and polymorphisms are required in order to determine specific associated variations within the loci responsible for the gene dysfunction that confers the risk of IBD, and to identify gene variants that may alter the response to the treatment.

REFERENCES

1. Ahmad T, Marshall SE, Jewell D. Genetics of inflammatory bowel disease: the role of the HLA complex. *World J Gastroenterol* 2006; 12: 3628-3635 [PMID: 16773677 DOI: 10.3748/wjg.v12.i23.3628]

2. Pierik M, Yang H, Barmada MM, Cavanaugh JA, Annese V, Brant SR, Cho JH, Duerer RH, Hugot JP, McGovern DP, Paavola-Sakkii P, Radford-Smith GL, Pavli P, Silverberg MS, Schreiber S, Taylor KD, Vlietinck R. The IBD international genetics consortium provides further evidence for linkage to IBD4 and shows gene-environment interaction. *Inflamm Bowel Dis* 2005; 11: 1-7 [PMID: 15674107 DOI: 10.1097/00054725-200501000-00001]

3. van Heel DA, Udalova IA, De Silva AP, McGovern DP, Kinouchi Y, Hull J, Lench NJ, Cardon LR, Carey AH, Jewell DP, Kwiatkowski D. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF(-kappa)B transcription factors. *Hum Mol Genet* 2002; 11: 1281-1289 [PMID: 12019209 DOI: 10.1093/hmg/ddi1281]

4. Bouguen G, Chevaux JB, Peyrin-Biroulet L. Recent advances in cytokines: therapeutic implications for inflammatory bowel diseases. *World J Gastroenterol* 2011; 17: 547-556 [PMID: 21350703 DOI: 10.3748/wjg.v17.i5.547]

5. Muro M, López-Hernández R, Campillo JA, Salama H, Martinez P, Lucas D, Valdes M, Botella C, Minguela A, Miras M, Carballo F, Alvarez-Lopez R. The Role of Human Leukocyte Antigen (HLA) Complex in IBD: Crohn’s Disease and Ulcerative Colitis. *Adv Stud Biol* 2009; 1: 37-41

6. van Heel DA, Udalova IA, De Silva AP, McGovern DP, Kinouchi Y, Hull J, Lench NJ, Cardon LR, Carey AH, Jewell DP, Kwiatkowski D. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF(-kappa)B transcription factors. *Hum Mol Genet* 2002; 11: 1281-1289 [PMID: 12019209 DOI: 10.1093/hmg/ddi1281]

7. Barnarda MM, Brant SR, Nicolae DL, Achkar JP, Panhuysen CI, Bayless TM, Cho JH, Duerer RH. A genome scan in 260 inflammatory bowel disease-affected relative pairs. *Inflamm Bowel Dis* 2004; 10: 15-22 [PMID: 15085521 DOI: 10.1097/00054725-200401000-00002]

8. Bonen DK, Cho JH. The genetics of inflammatory bowel disease. *Gastroenterology* 2003; 124: 521-536 [PMID: 12557156 DOI: 10.1053/gast.2003.50045]

9. Duerer RH, Barnarda MM, Zhang L, Pfützer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. *Am J Hum Genet* 2000; 66: 1857-1862 [PMID: 10747815 DOI: 10.1086/302947]

10. Sonwallakan SA, James RM, Ahmad T, Zhang L, Verbeke CS, Barnard DL, Jewell DP, Hull MA. Fulminant Crohn’s colitis after allogeneic stem cell transplantation. *Gut* 2003; 52: 1518-1521 [PMID: 12970148 DOI: 10.1136/gut.52.10.1518]

11. Fisher SA, Hampe J, Macpherson AJ, Forbes A, Lennard-Jones JE, Schreiber S, Curran ME, Mathew CG, Lewis GW. Sex stratification of an inflammatory bowel disease genome screen search shows male-specific linkage to the HLA region of chromosome 6. *Eur J Hum Genet* 2002; 10: 259-265 [PMID: 12032734 DOI: 10.1038/sj.ejhg.5200792]

12. Lantermann A, Hampe J, Kim WH, Winter TA, Kidd M, Nagy M, Fölsch UR, Schreiber S. Investigation of HLA-DPA1
genotypes as predictors of inflammatory bowel disease in the German, South African, and South Korean populations. Int J Colorectal Dis 2002; 17: 238-244 [PMID: 12073072]

Muro M, López-Hernández R, Campillo JA, Martínez P, Lucas D, Botella C, Minguela A, Álvarez-López R. Recent advances in basic and clinical aspects in inflammatory bowel diseases, as Crohn’s disease and ulcerative colitis: Role of gastrointestinal granulocytes. In: Berhardt LV, editor. Advances in Medicine and Biology. New York: Nova Science Publishers, Inc., 2010: 133-154

López R, Salgado G, Lucas D, Valdés M, López M, Salama H, Campillo JA, Martínez P, Minguela A, Miras M, Álvarez-López MR, Carballo F, Muro M. Immunogenetics of inflammatory bowel disease (IBD): Influence of IBD3 genomic loci. In: Terasaki PI, editor. Visuals of European Clinical Histocompatibility. Canoga Park: OneLambda., 2009: 69-71

Glas J, Martin K, Brünnler G, Kopp R, Folwaczny C, Weiss EH, Albert ED. MICA, MICB and C1_4_1 polymorphism in Crohn’s disease and ulcerative colitis. Tissue Antigens 2001; 58: 243-249 [PMID: 11782275 DOI: 10.1034/j.1399-0039.2001.580404.x]

Glas J, Török HP, Tonenchí L, Wetzes M, Beynon V, Te-shome MY, Cotofana S, Schiennan U, Griga T, Klein W, Epplen JT, Folwaczny C, Folwaczny M, Mussack T, Weiss EH. The 14-bp deletion polymorphism in the HLA-G gene displays significant differences between ulcerative colitis and Crohn’s disease and is associated with ileocolonic resection in Crohn’s disease. Int Immunol 2007; 19: 621-626 [PMID: 17446213 DOI: 10.1093/intimm/dxm027]

Jostins L, Ripek S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schump LM, Sharma Y, Anderson CA, Eisers J, Mitrovic M, Ning K, Clynen I, Theatre E, Spain SL, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Eisers J, Mitrovic M, Ning K, Clynen I, Theatre E, Spain SL, Silverberg MS, Gu X, Zhang Q, Lazarro A, Steinhardt AH, Greenberg GR, Griffiths AM, McLeod RS, Cohen Z, Fernández-Viña M, Amos CI, Siminovitch K. CARD15 and HLA DRB1 alleles influence susceptibility and disease localization in Crohn’s disease. Am J Gastro-enterol 2004; 99: 306-315 [PMID: 15046222 DOI: 10.1111/j.1572-0241.2004.04038.x]

Ahmad T, Arzumia A, Neville M, Bunce M, Ling KL, Welsh KL, Marshall SE, Jewell DP. The contribution of human leukocyte antigen complex genes to disease phenotype in ulcerative colitis. Tissue Antigens 2003; 62: 527-535 [PMID: 14617036 DOI: 10.1046/j.1399-0039.2003.00129.x]

Shina T, Inoko H, Kulski JK. An update of the HLA genomic region, locus information and disease associations: 2004. Tissue Antigens 2004; 63: 631-649 [PMID: 15546336 DOI: 10.1111/j.1399-0039.2004.00327.x]

Silverberg MS, Mirea L, Bull SB, Murphy JE, Steinhart AH, Greenberg GR, McLeod RS, Cohen Z, Wade JA, Siminovich KA. A population- and family-based study of Canadian families reveals association of HLA DRB1*0103 with colonic involvement in inflammatory bowel disease. Inflamm Bowel Dis 2003; 9: 1-9 [PMID: 12656131 DOI: 10.1097/01.MIB.000010000.00001]

Ahmad T, Arzumia A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KL, Jewell DP. The molecular classification of the clinical manifestations of Crohn’s disease. Gastroenterology 2002; 122: 854-866 [PMID: 11910336 DOI: 10.1016/S0016-5085(02)02413]

Stokkers PC, Reitsma PH, Tytgat GN, van Deventer SJ. HLA-DR and -DQ phenotypes in inflammatory bowel disease: a meta-analysis. Gut 1999; 45: 395-401 [PMID: 10446108 DOI: 10.1136/gut.45.3.395]

Lappalainen M, Halme L, Turunen U, Saavalainen P, Einarssdottir E, Färkkilä M, Kontula K, Paavola-Sakki P. Association of IL23R, TNFRSF1A, and HLA-DRB1*0103 allele variants with inflammatory bowel disease phenotypes in the Finnish population. Inflamm Bowel Dis 2008; 14: 1118-1124
33 Trachtenberg EA, Yang H, Hayes E, Vinson M, Lin C, Targar SR, Tyan D, Erlich H, Rotter JI. HLA class II haplotypes associated with inflammatory bowel disease in Jewish (Ashkenazi) and non-Jewish caucasian populations. *Hum Immunol* 2000; 61: 326-333 [PMID: 1069124 DOI: 10.1016/S0198-8859(99)00134-2]

34 Futami S, Aoyama N, Honsako Y, Tamura T, Morimoto S, Nakashima T, Ohmoto A, Okano H, Miyamoto M, Inaba H. HLA-DRB1*1502 allele, subtype of DR15, is associated with susceptibility to ulcerative colitis and its progression. *Dig Dis Sci* 1995; 40: 814-819 [PMID: 7720475 DOI: 10.1007/BF02036495]

35 Myung SJ, Yang SK, Jung HY, Chang HS, Park B, Hong WS, Kim JH, Min I. HLA-DRB1*1502 confers susceptibility to ulcerative colitis, but is negatively associated with its intractability: a Korean study. *Int J Colorectal Dis* 2002; 17: 233-237 [PMID: 12073071 DOI: 10.1007/s00034-001-0381-4]

36 Matake H, Okabe N, Naito S, Y. An HLA study on 149 Japanese patients with Crohn’s disease. *Gastroenterol Jpn* 1992; 27: 496-501 [PMID: 1526431]

37 Nakajima A, Matsushashi N, Kodama T, Yazaki Y, Taka-zoe M, Kimura A. HLA-linked susceptibility and resistance genes in Crohn’s disease. *Gastroenterology* 1995; 109: 1462-1467 [PMID: 7557126 DOI: 10.1016/0016-5085(95)00631-2]

38 Yoshitake S, Kimura A, Okada M, Yossato S, Sasaizuki T. HLA class II alleles in Japanese patients with inflammatory bowel disease. *Tissue Antigens* 1999; 53: 350-358 [PMID: 10323339 DOI: 10.1034/j.1399-0039.1999.530405.x]

39 Gómez-García M, Oliver J, Márquez A, Mendoza JL, López-Nevot MA, Fernández-Arquero M, González-Escribano MF, Díaz-Rubio M, de la Concha EG, Urcelay E, Martín J, Gómez-García M. Antigen class II alleles in Japanese patients with inflammatory bowel disease: susceptibility and disease heterogeneity revealed by human leukocyte antigen genotyping. *Genet Test Mol Biomarkers* 2012; 16: 482-487 [PMID: 22224365 DOI: 10.1089/gtmb.2011.0132]

40 Di Giacomo D, Santoniola A, Zingone F, Troncone E, Caria MC, Borgheresi P, Parrilli G, Ciacci C. Human leukocyte antigen (HLA) B27 prevalence in non-celiac patients with gastrointestinal diseases. *World J Gastroenterol* 2013; 19: 2507-2513 [PMID: 23674852 DOI: 10.3786/wjg.v9.i16.2507]

41 Buchel OC, Bosch FJ, Janse van Rensburg J, Bezuhenhout E, de Vries CS, van Zyl JH, Middlecote BD, de K Grundling TP. Functional MICA-129 polymorphism and serum levels of soluble MICA are correlated with ulcerative colitis in a Tunisian population. *Hum Immunol* 2009; 70: 43-48 [PMID: 19000729 DOI: 10.1016/j.jhimun.2008.10.008]

42 Zhao J, Jiang Y, Lei Y, Zou K, Wang C, Huang S, Yi F, Xia B. Functional MICA-129 polymorphism and serum levels of soluble MICA are correlated with ulcerative colitis in Chinese patients. *J Gastroenterol Hepatol* 2011; 26: 593-598 [PMID: 21155878 DOI: 10.1111/j.1440-352X.2011.06524.x]

43 Muro M, Llorente S, López-Hernández R, Boix F, Bolarín JM, Egija I, Jordana ES, Martí S, Collpilao I, Salgado G, Moya-Quiles MR, Minguella A, García-Alonso AM, Álvarez-López M. MICA molecules in disease and transplantation, a double-edged sword? *Current Immunol Review* 2012; 8: 307-325 [DOI: 10.2174/174399912804080233]

44 Arreygue-García NA, Daneri-Navarro A, del Toro-Arreola A, Cid Arregui A, Gonzalez-Ramella O, Jave-Suarez LF, Aguilar-Lemarroy A, Troyo-Sanroman R, Bravo-Cuellar A, Cid-Arregui A, Gonzalez-Ramella O, Jave-Suarez LF, Arreygue-García NA. Association between HLA-Cw polymorphism and inflammatory bowel disease. *Zhouggu Neike Zazhi* 2011; 50: 856-858 [PMID: 22321328]

45 Okada Y, Yamazaki K, Uemomo J, Takahashi A, Kumasaka N, Ashikawa K, Aoi T, Takazoe M, Matsu T, Hirano A, Matsu-moto T, Kanatami N, Nakamura Y, Yamamoto K, Kubo M. HLA-Cw*1202-B*5201-DRB1*1502 haplotypes increase risk for ulcerative colitis but reduces risk for Crohn’s disease. *Gastroenterology* 2011; 141: 864-871.e1 [PMID: 21699788 DOI: 10.1053/j.gastro.2011.05.048]

46 Latino A, Palmieri O, Latino T, Corritore G, Bossa F, Martino G, Biscaglia G, Scimeda C, Valvano MR, Pastore M, Marsiglia A, D’Incà R, Andriulli A, Annese V. Investigation of multiple susceptibility loci for inflammatory bowel disease in an Italian cohort of patients. *PLoS One* 2011; 6: e22688 [PMID: 21818367 DOI: 10.1371/journal.pone.0022688]

47 Muro M, López-Hernández R, Campillo JA, Lucas D, Martínez-García P, Botella C, Minguela A, Alvarez-López R. MICA gene and relevance to immune responses in organ transplants and inflammatory, tumoral and autoimmune diseases. *Curr Immunol Rev* 2009; 5: 161-166 [DOI: 10.2174/157395997581063072]
eloma. *Proc Natl Acad Sci USA* 2008; 105: 1285-1290 [PMID: 18202175 DOI: 10.1073/pnas.0711293105]

59 Suárez-Alvarez B, López-Vázquez A, Gonzalez MZ, Fdez-Morera JL, Díaz-Molina B, Blanco-Gelaz MA, Pascual D, Martínez-Borra J, Muro M, Alvarez-López MR, López-Larrea C. The relationship of anti-MICA antibodies and MICA expression with heart allograft rejection. *Am J Transplant 2007;* 7: 1842-1848 [PMID: 17511763 DOI: 10.1111/j.1600-6143.2007.01838.x]

60 Hüb S, Mention J, Monteiro RC, Zhang S, Cellier C, Schmitz J, Verkarre V, Fodil N, Bahram S, Berens-Sussenaerts N, Caillat-Zucman S, Kühbacher T, Herfarth HH, Krawczak M. Pharmacogenetic investigation of toll-like receptor 1 and 2 polymorphisms in patients with inflammatory bowel disease. *Ann Acad Med Singapore* 2002; 31: 1025-1030 [PMID: 12305161 DOI: 10.1142/S0120868X02007821]

61 Rodriguez-Rodero S, Rodrigo L, Díaz-Morera JL, Martínez-Borra J, López-Vázquez A, Fuentes D, López-Arbesu R, López-Soto A, Gonzalez S, López-Larrea C. MHC class I chain-related gene A (MICA) and MHC class I chain-related gene B promoter polymorphisms and celiac disease. *Hum Immunol* 2006; 67: 208-214 [PMID: 16698444 DOI: 10.1016/j.humimm.2006.02.011]

62 Liu M, Li J, Ye M, Zhang X, Tan Q, MIIB microsatellite polymorphism is associated with ulcerative colitis in Chinese population. *Clin Immunol* 2006; 120: 199-204 [PMID: 16679067 DOI: 10.1016/j.clim.2006.03.001]

63 Li Y, Xia B, Lu M, Ge L, Zhang X. MICB0106 gene polymorphism is associated with ulcerative colitis in central China. *Int J Colorectal Dis* 2010; 25: 153-159 [PMID: 19662431 DOI: 10.1007/s00384-009-0767-y]

64 Ge LQ, Jiang T, Zhao J, Chen ZT, Zou F, Xia B. Upregulated mRNA expression of major histocompatibility complex class I chain-related gene A in colon and activated natural killer cells of Chinese patients with ulcerative colitis. *J Dig Dis* 2011; 12: 329-334 [PMID: 21091928 DOI: 10.1111/j.1751-2990.2010.00464.x]

65 Orchard TR, Dhar A, Simmons JD, Vaughan R, Welsh KI, Jewell DP. MHC class I chain-like gene (A) (MICA) and its associations with inflammatory bowel disease and peripheral arthropathy. *Clin Exp Immunol 2001;* 126: 437-440 [PMID: 11735709 DOI: 10.1046/j.1365-2241.2001.01662.x]

66 Kamoun A, Bouzid D, Mahfoudh N, Amouri A, Gaddour H, Hakim F, Taha N, Masmoudi H, Makni H. Association study of MICA-TM polymorphism with inflammatory bowel disease in the South Tunisian population. *Genet Test Mol Biomarkers* 2013; 17: 615-619 [PMID: 23822824 DOI: 10.1089/gtmb.2012.0423]

67 Ng SC, Tssei KK, Kamm MA, Bai X, Wu J, Chan FK, Sung JJ. Genetics of inflammatory bowel disease in Asia: systematic review and meta-analysis. *Inflamm Bowel Dis 2012, 18: 1164-1176 [PMID: 21887729 DOI: 10.1002/ibd.21845]

68 Lucas D, Campillo JA, López-Hernández R, Martínez-García P, López-Sánchez M, Botella C, Salgado G, Minguela A, Alvarez-López MR, Muro M. Allelic diversity of MICA gene and MICA/HLA-B haplotype variation in a population of the Murcia region in southeastern Spain. *Hum Immunol* 2008; 69: 655-660 [PMID: 18718866 DOI: 10.1016/j.humimm.2008.07.011]

69 Stokkers PC, Hommes DW. New cytokine therapeutics for inflammatory bowel disease. *Cytokine* 2004; 28: 167-173 [PMID: 15588691 DOI: 10.1016/j.cyto.2004.07.012]

70 Bradley JR. TNF-mediated inflammatory disease. *J Pathol* 2008; 214: 149-160 [PMID: 18161752 DOI: 10.1002/path.2287]

71 Matsukura H, Ikeda S, Yoshimura N, Takazoe M, Muramatsu M. Genetic polymorphisms of tumour necrosis factor receptor superfamily 1A and 1B affect responses to infliximab in Japanese patients with Crohn’s disease. *Aliment Pharmacol Ther 2008;* 27: 765-770 [PMID: 18248655 DOI: 10.1111/j.1365-2036.2008.03630]

72 Ferguson LR, Huebner C, Petermann I, Garry RB, Barclay ML, Demmers P, McCulloch A, Han DY. Single nucleotide polymorphism in the tumor necrosis factor-alpha gene affects inflammatory bowel diseases risk. *World J Gastroenterol* 2008; 14: 4652-4661 [PMID: 18698679 DOI: 10.3748/wjg.14.4652]

73 Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; 94: 3195-3199 [PMID: 9096369 DOI: 10.1073/pnas.94.7.3195]

74 Rioux JD, Goyette P, Vyas TJ, Hammarström L, Fernando MM, Green T, De Jager PL, Foissy S, Wang J, de Bakker PI, Leslie S, McVean G, Padyukov L, Alfredsson L, Annese V, Haifler DA, Pan-Hammarström Q, Mattei R, Sawyer SJ, Compston AD, Cree BA, Mirel DB, Daly MJ, Behrens TW, Klareskog L, Gregersen PK, Oksenberg JR, Hauser SL. Medium-scale association studies for inflammatory bowel disease identify multiple susceptibility variants within the MHC region 7. *Nat Genet* 2006; 38: 241-248 [PMID: 16500066 DOI: 10.1038/ng.1108]

75 Gonzalez S, Rodrigo L, Martinez-Borra J, Lopez-Vazquez A, Fuentes D, Niño P, Cadahía V, Saro C, Dieguez MA, Lopez-Vazquez A, Rodrigo L, Martinez-Borra J, Lopez-Vazquez A, Fuentes D, Lopez-Arbesu R, Lopez-Soto A, Gonzalez S, Lopez-Larrea C. MHC class I chain-related gene A promoter polymorphisms and celiac disease. *Hum Immunol* 2006; 67: 208-214 [PMID: 16698444 DOI: 10.1016/j.humimm.2006.02.011]

76 Allez M, Tieng V, Nakazawa A, Treton X, Pacault V, Dulphey N, Caillat-Zucman S, Paul P, Ornert JM, Douay C, Raval S, Tamouza R, Charron D, Lémann M, Mayer L, Toubert A, CD4+CD25+ T cells in Crohn’s disease mediate inflammatory and cytotoxic responses through MICA interactions. *Gastroenterology* 2007; 132: 2346-2358 [PMID: 17570210 DOI: 10.1053/j.gastro.2007.03.025]

77 Liu M, Xia B, Li J, Ye M, Zhang X, Tan Q, MICB microsatellite polymorphism is associated with ulcerative colitis in Chinese population. *Clin Immunol* 2006; 120: 199-204 [PMID: 16679067 DOI: 10.1016/j.clim.2006.03.001]

78 Liu M, Xia B, Li J, Ye M, Zhang X, Tan Q, MICB microsatellite polymorphism is associated with ulcerative colitis in Chinese population. *Clin Immunol* 2006; 120: 199-204 [PMID: 16679067 DOI: 10.1016/j.clim.2006.03.001]

79 Li Y, Xia B, Lu M, Ge L, Zhang X. MICB0106 gene polymorphism is associated with ulcerative colitis in central China. *Int J Colorectal Dis* 2010; 25: 153-159 [PMID: 19662431 DOI: 10.1007/s00384-009-0767-y]
Luis E, Vermeire S, Rutgeerts P, De Vos M, Van Gossum A, Pescatore P, Fiasse R, Pelckmans P, Reynaert H, D’Haens G, Malaise M, Baelde J. A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. Scand J Gastroenterol 2002; 37: 818-824 [PMID: 12190096 DOI: 10.1080/gas.37.7.818.824]

Fowler EV, Eri R, Hume G, Johnstone S, Pandeyya N, Lincoln AT, Templeton D, Radford-Smith GL. TNF-α and IL10 SNPs act together to predict disease behaviour in Crohn’s disease. J Med Genet 2005; 42: 523-528 [PMID: 15937090 DOI: 10.1136/jmg.2004.027245]

Cao Q, Zhu Q, Wu ML, Hu WL, Gao M, Si JM. Genetic susceptibility to ulcerative colitis in the Chinese Han ethnic population: association with TNF polymorphisms. Chin Med J (Engl) 2006; 119: 1198-1203 [PMID: 16863613]

Song Y, Wu KC, Zhang L, Hao ZM, Li HT, Zhang LX, Qiao TD, Li CN, Fan DM. Correlation between a gene polymorphism of tumor necrosis factor and inflammatory bowel disease. Chin J Dig Dis 2005; 6: 170-174 [PMID: 16246225 DOI: 10.1111/j.1443-9573.2005.00226.x]

Ahirwar DK, Kesavarani P, Singh R, Ghoshal UC, Mittal RD. Role of tumor necrosis factor-alpha (C-863A) polymorphism in pathogenesis of inflammatory bowel disease in Northern India. J Gastroenterol Cancer 2012; 43: 196-204 [PMID: 22149467 DOI: 10.1007/s12029-010-9238-9]

López-Hernández R, Valdés M, Campillo JA, Martínez-Garcia P, Salama H, Salgado G, Boix F, Moya-Quiles MR, Minguela A, Sánchez-Torres A, Miras M, García A, Carballo F, Álvarez-López MR, Muro M. Genetic polymorphisms of tumour necrosis factor alpha (TNF-α) promoter gene and response to TNF-α inhibitors in Spanish patients with inflammatory bowel disease. Int J Immunogenet 2014; 41: 63-68 [PMID: 23950430 DOI: 10.1111/iji.12059]

Lacruz-Guzmán D, Torres-Moreno D, Pedrero F, Romero-Cara P, García-Tercero I, Trujillo-Santos J, Conesa-Zamora P. Influence of polymorphisms and TNF and IL1β serum concentration on the infliximab response in Crohn’s disease and ulcerative colitis. Eur J Clin Pharmacol 2013; 69: 431-438 [PMID: 22960943 DOI: 10.1007/s00228-012-1389-0]

Paul AT, Gohil VM, Bhutani KK. Modulating TNF-alpha signaling with natural products. Drug Discov Today 2006; 11: 725-732 [PMID: 16846800 DOI: 10.1016/j.drudis.2006.06.002]

Pober JS, Lapierre LA, Stolpen AH, Brock TA, Springer TA, Fiers W, Bevilacqua MP, Mendrik DL, Gimbrone MA. Activation of cultured human endothelial cells by recombinant lymphotixin: comparison with tumor necrosis factor and interleukin 1 species. J Immunol 1987; 138: 3319-3324 [PMID: 3494766]

Slownik MR, De Luca LG, Fiers W, Pober JS. Tumor necrosis factor activates human endothelial cells through the p55 tumor necrosis factor receptor but the p75 receptor contributes to activation at low tumor necrosis factor concentration. Am J Pathol 1993; 143: 1724-1730 [PMID: 7504889]

Estrada C, Gómez C, Martín C, Moncada S, González C. Nitric oxide mediates tumor necrosis factor-alpha cytotoxicity in endothelial cells. Biochem Biophys Res Commun 1992; 186: 475-482 [PMID: 15378278 DOI: 10.1016/S0006-291X(05)83822-0]

Yoshizumi M, Perrella MA, Burnett JC, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. Circ Res 1993; 73: 205-209 [PMID: 7685252 DOI: 10.1161/01.res.73.1.205]

Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417-429 [PMID: 12166785 DOI: 10.1056/NEJMra028311]

Goretsky D, Brant SR. Recent insights into the genetics of inflammatory bowel disease. J Gastrointest Cancer 2011; 42: 170-174 [PMID: 2136636 [DOI: 10.1111/j.1365-2036.2011.04612.x]

Steenholdt C, Svensson M, Bendtz K, Thomsen OØ, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2011; 33: 987-995 [PMID: 21366636 DOI: 10.1111/j.1365-2036.2011.04612.x]

Cromer WE, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. World J Gastroenterol 2011; 17: 578-593 [PMID: 21350707 DOI: 10.3748/wjg.v17.i7.578]

McCarthy J, O’Neill MJ, Bourre L, Walsh D, Quinlan A, Hurley G, Ogier J, Shanahan F, Melgar S, Darcy R, O’Driscoll CM. Gene silencing of TNF-alpha in a murine model of acute colitis using a modified cyclodextrin delivery system. J Control Release 2013; 168: 28-34 [PMID: 23500058 DOI: 10.1016/j.jconrel.2013.03.004]

Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology 2011; 140: 1704-1712 [PMID: 21503736 DOI: 10.1053/j.gastro.2011.02.046]

Yun J, Xu CT, Pan BR. Epidemiology and gene markers of ulcerative colitis in the Chinese. World J Gastroenterol 2009; 15: 788-803 [PMID: 19230040 DOI: 10.3748/wjg.v15.i15.788]

Lees CW, Barrett JC, Parkes M, Satsangi J. New IBD genetics: common pathways with other diseases. Gut 2011; 60: 1739-1753 [PMID: 21300624 DOI: 10.1136/gut.2009.199679]

P- Reviewer: Hann HW, Peng CY S- Editor: Zhai HH L- Editor: A E- Editor: Ma S
