World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i30.10262

World J Gastroenterol 2014 August 14; 20(30): 10262-10278
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory bowel disease in pediatric and adolescent patients: A biomolecular and histopathological review

Luciana Rigoli, Rosario Alberto Caruso

Luciana Rigoli, Department of Pediatrics, University of Messina, I-98125 Messina, Italy
Rosario Alberto Caruso, Department of Human Pathology, University of Messina, I-98125 Messina, Italy
Author contributions: Rigoli L and Caruso RA jointly contributed to this paper; Both authors read and approved the final version.
Correspondence to: Luciana Rigoli, MD, Department of Pediatrics, University of Messina, Via Consolare Valeria, 1, I-98125 Messina, Italy. lrigoli@unime.it
Telephone: +39-90-2212120 Fax: +39-90-2213788
Received: November 4, 2013 Revised: March 4, 2014
Accepted: April 15, 2014
Published online: August 14, 2014

Abstract

Crohn's disease (CD) and ulcerative colitis (UC) are the two main forms of inflammatory bowel disease (IBD) with both overlapping and distinct clinical, pathological and biomolecular features. It has been suggested that pediatric IBD is a distinct disease entity, with probably different disease subtypes.The aim of this study is to review and summarize the evolution of the current concept of pediatric IBD. The results of this review reinforce the idea that pediatric CD and UC may be further classified in various clinicopathologic entities. For clinicians and pathologists convenience, practical algorithms for the distinction of the various subphenotypes of pediatric IBD are also provided.

Key words: Pediatric inflammatory bowel disease; Crohn's disease; Ulcerative disease; Histopathology; Molecular biology

Core tip: The review contains the most recent data of the literature and suggests a clinical- pathological heterogeneity of the inflammatory bowel disease (IBD) in childhood. It provides diagrams that display the main anatomo-clinical and biomolecular correlations and that may be encountered in IBD children. These diagnostic patterns and correlations may be useful in clinical practice for pediatric IBD.

Rigoli L, Caruso RA. Inflammatory bowel disease in pediatric and adolescent patients: A biomolecular and histopathological review. *World J Gastroenterol* 2014; 20(30): 10262-10278 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i30/10262.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i30.10262

INTRODUCTION

Inflammatory bowel disease (IBD) represents a group of idiopathic, chronic, inflammatory intestinal conditions in which complex interactions among genetic, immune, and environmental factors are involved. Crohn's disease (CD) and ulcerative colitis (UC) are the two most common forms of IBD with both overlapping and distinct clinical, pathological and biomolecular features. Traditionally, UC is defined as a disease involving the colonic mucosa in a diffuse, continuous manner, always affecting the rectum. In contrast, CD may involve any part of the gastrointestinal tract and frequently shows discontinuous or segmental involvement. However, IBD may be better considered as a syndrome of complex disorders with a significant heterogeneity in disease presentation and course[1-5]. It has also been suggested that pediatric IBD is characterized by distinct phenotypic differences including disease type, disease location, disease behaviour, gender preponderance and genetically attributable risk, compared to adult-onset IBD[6-11]. The incidence of IBD in childhood is rising worldwide[12,13]. Rates are highest in North America and Europe, with rapid increases noted in developing nations adopting a Westernized lifestyle.

Childhood and adolescent IBD accounts for nearly 30% of total cases[14-17]. Pediatric IBD demonstrates a pattern with CD predominating over UC. In particular, the incidence of CD has risen markedly, while a rather stable incidence of pediatric onset UC has been reported[18]. A recent study based on the Swedish population found that the incidence of CD was 9.2 per 100000 per person years. The incidence of UC in children over the same time period was 2.8 per 100000 per person years[19]. Similar rates have been reported by other studies[20-24].

Recent population based studies have demonstrated a significant male excess in incidence of pediatric CD[25]. Pediatric CD more often involves the ileocolonic/colonic regions, whereas adult CD does not demonstrate a high proportion of colonic disease[7]. Furthermore, a variety of phenotypic characteristics have been described in pediatric UC thanks to increasing diagnostic accuracy[26,27]. Reflecting these trends, IBD classification has been changed from the Vienna statement[28], through the Montreal classification[29] to the recent pediatric Paris classification[30].

Although the precise etiology of IBD remains elusive, both animals and human studies point towards a strong genetic susceptibility. Genome wide association studies (GWAS) identified over 160 susceptibility loci/genes that are significantly associated with IBD[31-33]. However, newer genomics technologies are now beginning to complement GWAS findings and add to our understanding of the molecular genetic universe of IBD. Genetic studies have provided detailed appreciation of the molecular architecture of IBD, and, in particular, the areas of overlap between CD and UC (such as Th17 pathways) and the pathways which are disease-specific. Moreover, the genes implicated in childhood-onset and adult-onset IBD often overlap, suggesting similar contributory genetic predisposition and pathophysiological pathways. For CD, gene discoveries have focused on defective processing of intracellular bacteria, autophagy, and innate immunity. For UC, the focus has been on barrier function.

A further addition to the complexity of understanding disease mechanisms is that a susceptibility allele often requires other genetic and non-genetic cues to manifest the disease[34]. The variable concordance rate in monozygotic twins of 27%-50% in CD compared with 15%-19% in UC suggests that non-genetic factors may have an even more important role in UC than in CD[35,36].

In the present review, we summarize current knowledge concerning the correlation between clinicopathologic features and genetic profiles of pediatric IBD in order to offer practical algorithms for the categorization of the vast majority in this group of lesions.

PARIS CLASSIFICATION

The issue of subclassification of IBD by phenotype has been reviewed in recent years. The World Congress of Gastroenterology in Vienna, in 1998, considered age of onset (A), disease location (L), and disease behaviour (B) as predominant phenotypic elements[28]. The Montreal revision of the Vienna classification did not changed the three predominant parameters of age at diagnosis, location, and behaviour, but modification within each of these categories was made[29]. However, the criteria of Montreal Classification has inherent limitations with respect to classification of pediatric IBD. In the pediatric Paris classification[30] growth failure in the patient at any time was added as G1 *vs* G0 (never growth failure) to classic phenotypic elements (age at diagnosis, disease location, and disease behaviour). The comparisons between the Montreal and Paris classifications for CD and UC are shown in Tables 1 and 2, respectively.

IBD UNCLASSIFIEDAND "INDETERMINATE" COLITIS

The term "indeterminate colitis" was originally coined for IBD resections with features of both UC and CD, usually in the setting of severe acute or "fulminant" colitis[37]. Over the years the term has been adopted by clinicians to describe patients in whom a diagnosis of UC or CD cannot be made based on standard clinical testing, including colonoscopy, imaging, laboratory tests and biopsy[38]. However, this term has been used incorrectly with considerable confusion among clinicians and pathologists. Recently, it has been recommended that the term "indeterminate colitis" be reserved only for patients for whom a surgical specimen is available and the term "colonic IBD type unclassified" (IBD-U) for patients with no surgical specimen available and for whom endoscopy is inconclusive and histological changes do not fit with either CD or UC[29]. It remains controversial whether IBD-U constitutes a problem of classification or an IBD subtype distinct from CD and UC. Some authors believe that IBD-U is not a third form of IBD with specific diagnostic criteria, being a provisional diagnosis of exclusion used until a diagnosis of UC or CD is made with certainty[39,40]. Instead, other authors consider IBD-U as a distinct phenotype of IBD for the following reasons: (1) A recent meta-analysis showed that IBD-U is more common in children accounting for 12.7% of all cases of IBD *vs* 6% in adults[41]; (2) Children with IBD-U have a disease that rapidly progresses to pancolitis[42,43]; (3) Although many patients with IBD-U will be reclassified as having CD or UC on long-term follow-up evaluation, a significant proportion of them will still carry the diagnosis of indeterminate colitis[44]; (4) Epidemiologic data have shown that clinical course and prognosis of IBD-U could be worse compared with UC, especially concerning outcome of surgery with greater risk of pouchitis[45]; and (5) As will be discussed in the next section, IBD-U is diagnosed in a large subgroup of patients at a very

Table 1 Montreal and Paris classifications for Crohn's disease

	Montreal classification		Paris classification	
Age at diagnosis	A1	< 17 yr	A1a	< 10 yr
			A1b	10-16 yr
	A2	17-40 yr	A2	17-40 yr
	A3	> 40 yr	A3	> 40 yr
Location	L1	Ileal disease	L1	Distal 1/3 ileum ± limited cecal disease colonic disease ileocolonic disease isolated upper disease
	L2	Colonic disease	L2	
	L3	Ileocolonic disease	L3	
	L4	Isolated upper disease	L4	
			L4a	Upper disease proximal to ligament of Treitz
			L4b	Upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum
Behavior	B1	Non-stricturing, non-penetrating	B1	Non-stricturing, non-penetrating
	B2	Stricturing	B2	Stricturing
	B3	Penetrating	B3	Penetrating
			B2B3	both stricturing and penetrating
	P	Perianal disease modifier	P	Perianal disease modifier
Growth	Not applicable		G0	No evidence of growth delay
			G1	Growth delay

Table 2 Montreal and Paris classifications for ulcerative colitis

	Montreal classification		Paris classification	
Extent	E1	Ulcerative proctitis	E1	Ulcerative proctitis
	E2	Leftsided colitis distal to splenic flexure	E2	Leftsided colitis distal to splenic flexure
	E3	Extensive colitis distal to hepatic flexure	E3	Extensive colitis proximal to splenic flexure
Severity	S0	Clinical remission	E4	Pancolitis, proximal to hepatic flexure
	S1	Mild ulcerative colitis	S0	Never severe
	S2	Moderate ulcerative colitis	S1	Ever severe
	S3	Severe ulcerative colitis		

early age (0-2 years, infantile IBD)[30-46].

AGE OF ONSET

An important modification recommended by the Paris classification[30] includes age at onset as A1a (0 to < 10 years) and A1b (10 to < 17 years). In both the Montreal[29] and Paris[30] Classification systems, A2 and A3 account for age of diagnosis at 17-40 years, and > 40 years, respectively.

Although rare, IBD may occur before the age of 2 years. Therefore, the Paris classification[30] suggested the possibility of distinguishing a separate group of children diagnosed with IBD at a very early age (0-2 years, infantile IBD). This subgroup is characterized by a high rate of consanguinity, more severe disease course, association with primary immunodeficiency and resistance to immunosuppressive treatment[45,46]. Abscess formation, anal fissures and enterocutaneous or rectovaginal fistulae complicate the disease and frequently require partial or total colectomy[30,47].

The suspicion of a monogenetic cause of these early onset forms was recently confirmed by the discovery of mutations in the genes coding for one of the two IL10 receptors causing impaired IL10 signalling[48-52].

The region encoding IL10 was originally identified by a German GWAS in UC, and the association documented with non-coding variants upstream of the IL10 gene[53]. Subsequently, an international GWAS meta-analysis showed that this region is associated with CD[54]. Through a genetic-linkage analysis and candidate-gene sequencing on samples from two unrelated consanguineous families with children affected by early-onset IBD, Glocker *et al*[55] identified three distinct homozygous mutations in genes IL10RA and ILRB. These genes encode the IL10R1 and IL10R2 proteins, respectively, which form a heterotetramer to make up the IL10 receptor. Functional experiments have shown that the *IL10RA* and *ILRB* gene mutations abrogate IL10 signalling and lead to severe intestinal inflammation. Loss of function mutation in IL10 and IL10R was also identified in 66 patients with very early onset IBD. In this study, it has been found that in 5 patients with IL10R deficiency, the allogenic hematopoietic stem cell transplantation induced sustained clinical remission with a median follow-up time of 2 years[49]. Recently, Moran *et al*[50] have identified a novel homozygous, splice-site point mutation in IL10RA in an infantile-onset IBD Caucasian female. The patient was also affected by significant arthritis and folliculitis. The mutation caused a premature stop codon (P206X) and IL10 insensitivity. Moreover, 188 children with early-onset IBD and 188 healthy subjects have been studied. In the discovery cohort, five IL10RA polymorphisms associated with UC have been found[50]. These studies

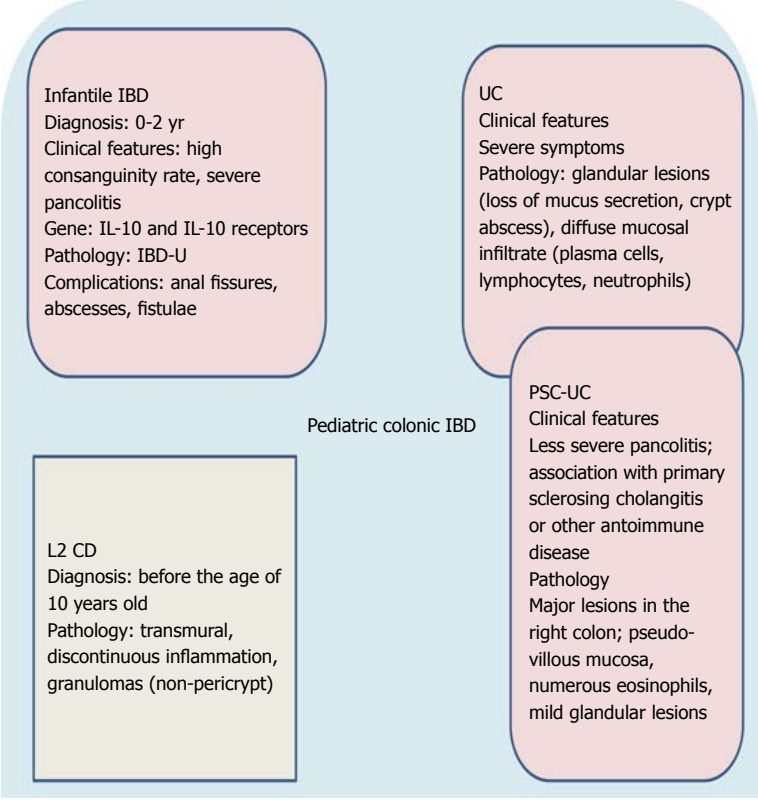

Figure 1 Distinct clinicopathological and biomolecular features of the pediatric colonic inflammatory bowel disease. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

show the role of immune pathways in early onset IBD pathogenesis. Indeed, IL10 is an anti-inflammatory cytokine secreted by a variety of cell types and is critical for maintaining immune homeostasis in the gastrointestinal (GI) tract. IL10 restricts excessive immune response[56]. In particular, IL10 limits the secretion of proinflammatory cytokines, such as tumour necrosis factor α (TNF-α) and IL12[57]. Moreover, the assembly of IL10R1 results in the activation of the receptor-associated Janus tyrosine kinases, JAK1 and Tyk2, leading to the phosphorylation of STAT3 (signal transducer and activator of transcription 3) and the induction of STAT3-dependent genes[58]. A severe enterocolitis has been found in mice that are deficient in either IL10 or IL10R2. These data underline the pivotal role of IL10 in the mediation of signalling that controls inflammation in the gut.

Other monogenic primary immunodeficiencies showing IBD-like gastrointestinal pathology include Wiskott-Aldrich syndrome[47], chronic granulomatous disease[47], XIAP deficiency[55,59], X-linked (IPEX) syndrome[60] and nuclear factor kB essential modulator (NEMO) deficiency[61]. It is also noteworthy that a large subgroup of these patients have IBD-U[30,42]. Therefore, we think that IBD-U is an IBD subtype distinct from CD and UC as it constitutes a histopathological substrate of a clinicopathologic entity with characteristic epidemiological (< 2 years old), clinical (severe clinical course with pancolitis), and genetic features (*i.e.*, mutation in interleukin-10 receptor and interleukin gene or other primary immunodeficiencies). It is, therefore, plausible that infantile IBD (< 2 years) may be incorporated into future modifications of the Paris classification. Figure 1 summarizes the main clinicopathologic characteristics of infantile IBD.

CD

Disease location

According to the Paris classification[30], CD location is categorized as follows: (L1) involvement of the terminal ileum only, with limited or no cecal disease; (L2) colonic involvement only; (L3) involvement of both the terminal ileum and colon; (L4) isolated upper gastrointestinal disease. L4 is further separated into esophagogastroduodenal disease (L4A), jejuna/proximal ileal disease (L4B), or both L4A and L4B. Adolescents more often present with ileal disease (L1), whereas children have a tendency to present with isolated colonic disease (L2). Pediatric patients with L2 disease are less likely to have esophagogastroduodenal involvement or stricturing disease behaviour than patients with L1 and L3 disease[62]. These data support the existence of discrete subtypes of CD, which are, in part, defined by the predominant anatomical location of disease[7] and summarized in Table 3 and Figure 2.

Paneth cells are protagonist in the L1CD

Histologic, immunologic and biomolecular evidence strongly suggests a key role for Paneth cells in L1 CD. Histologically, Paneth cells are abundant in the small

Table 3 Salient histopathological and biomolecular features in pediatric inflammatory bowel disease

Gene	Genetic alterations	Histopathology	Clinical features	Ref.
IL10RA	G141R	IBD-U	Infantile IBD	[48-50]
IL10RB	W159X			
NOD2	R702W, G908R, L1007fsinsC	Abnormal Paneth cells, granuloma-poor	L1 CD	[72]
ATG16L1	T300A			
NOD2	R702W, G908R, L1007fsinsC		L1 CD,	[81-87]
			Structuring (B2) behavior	[82,83,87]
			Early surgery	[83]
			Growth delay	[83,84]
			Higher disease activity	[83,88]
NOD2	R702W, G908R, L1007fsinsC	Diminished immunohistochemical Expression of alpha-defensin in small intestinal Paneth cells	L1, L3 CD	[106-108]
TLRs	TLR-4 Asp299gly		CD, UC	[111]
IRGM	rs4958847		Fistulizing (B3) CD	[135]
ILRL1 and *IL33*	Upexpression of mRNA		Extensive UC	[138-140,150]
β-defensin	Low gene copy number	Diminished immunohistochemical expression of beta-defensin 2	L2 CD	[104,105]
HBD2				
NCF4	Rs4821544 polymorphism		Perianal CD	[157]

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

intestine and are occasionally found in the cecum and proximal ascending colon[63]. In parallel, L1 (distal 1/3 ileum ± limited cecal disease) CD location corresponds to the normal distribution of Paneth cells.

From an immunological viewpoint, Paneth cells, by virtue of their vast repertoire of effector molecules, are multifunctional cells. They produce antimicrobial proteins such as alpha-defensins HD5 and HD6, lysozyme, secretory phospholypase A2 and the lectinRegⅢγ[64]. Wehkamp *et al*[65] showed that ileal (L1) but not isolated colonic (L2) CD is associated with a diminished synthesis of Paneth cell defensins. Wehkamp and Stange[66], therefore, proposed the term "Paneth's disease" to describe a complex disease in which Paneth cells might explain the poor antimicrobial capability of (L1) CD. Paneth cells have also been shown to contain TNF-α transcripts. With ultrastructural immunogold methods, Beil *et al*[67] showed TNF-α in mature and immature secretory granules of Paneth cells. One of the major biological roles of TNF-α is in the host defence to bacterial, viral and parasitic infections, and Paneth cells are considered to be the major source of TNF-α in the normal bowel[67]. Clinical and molecular studies implicate TNF-α as a key mediator in the initiation and propagation of CD[68]. This is evidenced by an increased amount of TNF in inflammatory cells infiltrating ileal tissue (eosinophils, mast cells, neutrophils, macrophages, fibroblasts) and a marked clinical response of TNF-α antagonists in patients with active CD[69]. Therefore, abnormal production of TNF-α protein by Paneth cell may be involved in the pathogenesis of L1 CD[69].

From a histopathological viewpoint, Paneth cell depletion occurs in the most heavily inflamed areas of the ileal mucosa[70]. Recently, Günther *et al*[71] showed a significant decrease in the number of Paneth cells and a high number of dying cells with shrunken eosinophilic cytoplasm at the crypt base in histological samples from terminal ileum of patients with active CD. Paneth cells showed ultrastructural signs of non-apoptotic cell death, such as organelle swelling, vacuole formation and the lack of blebbing. The data of Günther *et al*[71] suggest that necroptosis (a form of non-apoptotic cell death) of Paneth cells might be involved in the pathogenesis of CD.

Recently, VanDussen *et al*[72] have studied the correlation between Paneth cell phenotype (based on lysozyme-positive secretory granule morphology) and NOD2 and ATG16L1 genotype. They observed an inverse correlation between abnormal Paneth cells (with disordered, diminished, diffuse, or excluded granule phenotypes) and the presence of granulomas[72]. The cumulative number of NOD2 and ATG16L1 risk alleles had an additive effect on the proportion of abnormal Paneth cells. Moreover, high proportions of abnormal Paneth cells were associated with shorter time to disease recurrence after surgery. VanDussen *et al*[72] suggest stratifying CD based on Paneth cell phenotypes rather than the presence of granulomas. In fact, granulomas could be sparse and more likely to be undersampled, especially in biopsy specimens, whereas Paneth cell phenotypes are more easily analyzed within limited samples. Therefore, these authors concluded that histologic analysis of Paneth cell phenotypes can be used to divide patients with CD into subgroups with distinct pathognomonic and clinical features[49]. However, further correlative histopathologic and biomolecular studies are necessary to clarify the role of necroptosis and/or granule changes of Paneth cells in L1 CD characterized by abundant or few granulomas (so-called granuloma-rich or granuloma-poor CD).

Biomolecular studies of antimicrobial function in Paneth cells

Defective antimicrobial function in Paneth cells has been described by a variety of mechanisms including mutations in the innate immune receptor NOD 2, defensins, Toll-like receptors (TLRs), the autophagy protein ATG16L1, *IRGM* gene.

NOD2 gene

With the pivotal study of Hugot *et al*[73], who discovered the very first and also strongest susceptibility gene for CD in NOD2/CARD15, "IBD gene hunting" was opened. NOD2 is an intracytoplasmic member of the family of intracellular NLR (NOD like receptors) able to recognize pathogen associated molecular patterns (PAMPs) and to modulate an inflammatory response through enhanced NF-κB activation[74,75]. NOD2 is highly expressed in cells of the phagocyte system and is involved in the production of defensins in response to gut microbiota. Three single nucleotide polimorphisms (SNPs) within the NOD2/CARD15 gene (R702W, G908R, and 1007fsinsC) have been established as independent risk factors for CD in Caucasians[76,77] and they represent 82% of the mutations in NOD2[78]. Interestingly, the NOD2/CARD15 mutations are absent or very rare in Asians, Arabs, Africans, and African Americans[79]. NOD2/CARD15 variants have been associated to more severe CD, a greater need for surgery and a younger age at onset[80]. In children, a correlation with ileal (L1) localization[81-87], stricturing behavior[82,83,87], early surgery[83], growth delay[83,84] and higher disease activity[83,88] has been found. However, these results have not been confirmed by other studies[89-91]. The precise mechanistic relationship between NOD2/CARD15 and CD remains controversial. Interestingly, a study by Zelinkova *et al*[92] suggested that NOD2 mutations may result in perpetuation of mucosal inflammation through insufficient pathogen elimination.

The important role of NOD2 in the pathogenesis of CD has been underlined by recent studies on microRNAs (miRNAs)[93]. miRNAs are short non-coding RNAs that have emerged as key modulators of various cellular processes at the post-transcriptional level[94-96]. There are recent reports on their role in the regulation of intestinal permeability as the loss of intestinal miRNAs impairs the epithelial barrier function, and causes acute inflammation[97]. Some Authors showed that miRNA-122 regulates intestinal permeability tight junctions (TJ) by targeting occluding mRNA degradation[98-102]. Moreover, Chen *et al*[102] found that NOD2 is a functional target of miRNA-122.

Other mechanisms have been proposed for NOD2/CARD15 mutations in recent years, ranging from abnormal Paneth cell function with reduced defensin secretion, altered modulation of Toll-like receptor signaling and a reduced ability to trigger autophagy[103]. Several studies have shown the role of defensins in the pathogenesis of CD[104-107]. In particular, ileal (L1) and ileal colonic (L3) CD is characterized by a specific decrease in small intestinal Paneth cell human α-defensin HD-5 and-6[106-108]. In a group of pediatric CD, Perminow *et al*[109] studied the role of HD5 and TCF-4, a Wnt-signaling transcription factor which controls Paneth cell defensin expression. They showed a low intestinal expression of HD5 and TCF4 mRNA in ileal CD, confirming the important role of antimicrobial host defense in pediatric patients.

Toll-like receptors (TLRs) have an important role in the pathogenesis of CD. They are crucial components of innate immunity and cell surface molecules that also detect normal and pathogenic microbial agents and can trigger antimicrobial host defense responses. TLRs are abundantly expressed on the surface of monocytes, macrophages, and dendritic and epithelial cells[110]. In IBD mucosa, dendritic cells are activated and there are increased levels of TLR2 and TLR4. These TLRs mediate the recognition of bacterial lipoproteins and LPS, respectively. An association has been described between the TLR4 D299G SNP and IBD, in UC as well as CD patients[111]. This SNP is associated with impaired LPS signaling and increased susceptibility to gram-negative infections, thus supporting the role of gram-negative bacteria in the pathogenesis of IBD. Polymorphisms have been described in IBD with regard to the TLR9 gene, but their functional significance is still unclear[112].

Genes of the autophagy: A key role in IBD

The autophagy 16-like 1 (*ATG16L1*), immunity-related guanosine triphosphate M (IRGM), and leucine-rich repeat kinase 2 (*LRRK2*) genes, which regulate autophagy, have been associated with CD in GWAS[113,114].

Autophagy describes the sequestration of intracellular material such as obsolete organelles or large unfolded protein within membranes, and their trafficking to fuse with lysosome with a subsequent degradation of the contents. Autophagy is now recognized as playing a key role in innate immunity against intracellular microorganisms.

ATG16L1 is essential for all forms of autophagy as it has a role in the clearance of intracellular bacteria[115]. It interacts with two other autophagy proteins, ATG5 and ATG12, to forms a complex essential for the process of autophagy[116]. The association at ATG16L1 with CD seems to be entirely accounted for by the *T300A* coding variant which maps to a highly conserved part of the gene adjacent to the coiled-coil domain. ATGL16L1 mutations cause a deficiency of the correspondent protein and disrupt the recruitment of the ATG12-ATG5. Therefore, autophagosome formation and degradation of proteins with a long half-life are severely impaired in ATG16L1-deficient cells[117,118]. The decreased autophagy impairs immune tolerance by autoantigen presentation on major histocompatibility complex class II molecules and causes immune inflammation[119].

ATG16L1 gene mutations could also impair the mechanisms that involve autophagy and apoptosis as there is an acceleration in the rate of epithelial cell apoptosis and an inhibition of inflammatory cell apoptosis in CD and UC[120,121]. Several studies have been performed on the role of *ATG16L1* gene in the pathogenesis of IBD.

The *T300A* allele was correlated with the incidence of CD in three populations from Germany, Hungary and the Netherlands[122]. In contrast, no correlation between T300A allele and UC was detected. However, the results of other studies on the association of T300A

allele with predisposition to CD and UC, are inconsistent[122], also in child-onset IBD cases[123]. Recently, a meta-analysis performed on twenty-five studies of CD, 14 of which involved cases of UC[124] showed that the T300A allele confers a susceptibility to CD and to UC. However, ATG6L1 was associated with the risk of child-onset CD, but not with child-onset UC probably because there are few studies on pediatric UC. Recently, a functional link between NOD2/CARD15, ATG16L1 and autophagy has been provided[125-128].

IRGM gene

In the Wellcome Trust Case-Control Consortium (WTCCC) GWA scan, a highly significant association between variant flanking IRGM and susceptibility to CD has been shown. The *IRGM* gene is located on chromosome 5q33.1, and is required during the initiation phase of autophagy, when it localizes to bacteria- containing autophagic vacuoles. IRGM and autophagy are involved in clearance of intracellular organisms such as *M. tubercolusis*[129,130] and the CD-associated IRGM variant is predicted to affect autophagic control of Salmonella typhimurium[131].

The IRGM risk alleles for CD are non-coding and appear to affect mRNA transcription or stability. Over the last 5 years three distinct mechanisms have been identified which might explain the impact on IRGM expression. Cooney *et al*[131] discovered a large copy number variant upstream of IRGM which correlated with tissue-specific expression effects. Prescott *et al*[132] reported a disruption of a transcription factor binding site in the IRGM promoter. Most recently, Brest *et al*[133] found that a synonymous coding variant of IRGM alters the binding domain of miRNA196, a family of miRNAs, hence affecting mRNA stability and gene translation. In this study, it has been shown that microRNA196 is overexpressed in CD patients and that it downregulates an IRGM protective variant but not the risk-associated allele.

Lapaquette *et al*[134] found that the reduced *IRGM* gene expression correlated with impaired clearance by macrophages of CD-associated adherent-invasive *Escherichia coli*. A recent Italian study[135] reported the association between CD and two risk SNPs (rs1000113 and rs4958847) for IRGM, irrespective of age, but not in UC. In addition, a trend to B3 (penetrating) disease behavior in patients with IRGM SNPs was suggested[135]. Moon *et al*[136] showed that IRGM SNP rs10065172 was significantly associated with CD susceptibility. They also reported a protective role of SNP rs72553867.

By suppressive subtractive hybridization (SSH) technique, Sim *et al*[137] studied the differential expression gene profiles in ileal biopsies from CD children. Twenty-eight genes previously reported in association with adult CD, and 47 new genes were identified. It is significant that some adult CD genes have also been found in early-onset CD. Indeed, it underlines that, in some cases, there is a common genetic pathway between pediatric and adult CD cases. Several genes reported in the study are involved in microbial pathogenesis, antigen presentation, inflammation, regulation of epithelial barrier function, vesicular transport or cell differentiation and proliferation. Recent studies have found that IL33 expression is enhanced in the inflamed colonic mucosa of IBD, especially in UC[138,139], and the IL33/IL1RL1 signalling axis has been implicated in the IBD pathogenesis[140]. Recently, new genes have been identified by GWAS in IBD[141-150]. In particular, in 2008, Kugathasan *et al*[141] performed the first GWAS in a cohort with pediatric disease onset, identifying two new loci on 20q13 and 21q22. This paper reported the associations with two intronic SNPs (rs2315008 and rs4809330) at the 20q13 locus. These two SNPs map to the zinc finger CCCH-type with G patch domain (ZGPAT) gene which is located in a region containing eight potential candidate genes for CD. Moreover, an association, at the 21q22 region, of CD with the intergenic SNP rs2836878 has been shown that implicated the nearby PSMG1 gene[141].

A follow-up scan on an extended cohort of 3426 childhood-onset IBD (European/North America collaboration) identified five more new loci associated with pediatric IBD[147]. These loci included 16p11 near the cytokine gene IL27, 22q12, 10q22, 2q37 and 19q13.11. The results of this study showed that IL27 is a promising candidate gene for pediatric CD. Recently, Latiano *et al*[149] have studied a large Italian cohort of adult and early-onset IBD to verify the role of new genes involved in the immune response and inflammation (PTGER4, HLA-BTNL2, TNFSF15, NKX2-3, ZNF365, IFNG, PTPN2, and PSMG1).

Role of colonic epithelial cells producing β-defensins in colonic (L2) CD location.

Since Paneth cells are rare in the colon, the contribution of α-defensins to antimicrobial defence in the large intestine is only limited. In contrast, β-defensins HDB1-3 are secreted by columnar and goblet cells in the colon[151,152]. A deficiency of β-defensin HDBD2 has been shown by molecular biology in L2 (colonic) CD and confirmed on the protein level by immunohistochemistry[104]. This observed colonic defect of the antimicrobial barrier caused by a diminished expression of β-defensins may allow luminal microbes to attach to and invade the mucosa triggering the inflammation[153].

CD behaviour

The Montreal classification[29] describes three behaviours for CD: nonstricturing nonpenetrating disease (B1), stricturing disease (B2), penetrating/fistulizing disease (B3). In addition, the Paris classification[30] proposes a new classification B2B3 to identify patients with both B2 and B3 phenotypes (either at the same or different times). Disease locations are the most important factor identified in determining the risk of developing either a structuring or penetrating complication (more complications with ileal, less with colonic disease)[154]. To date,

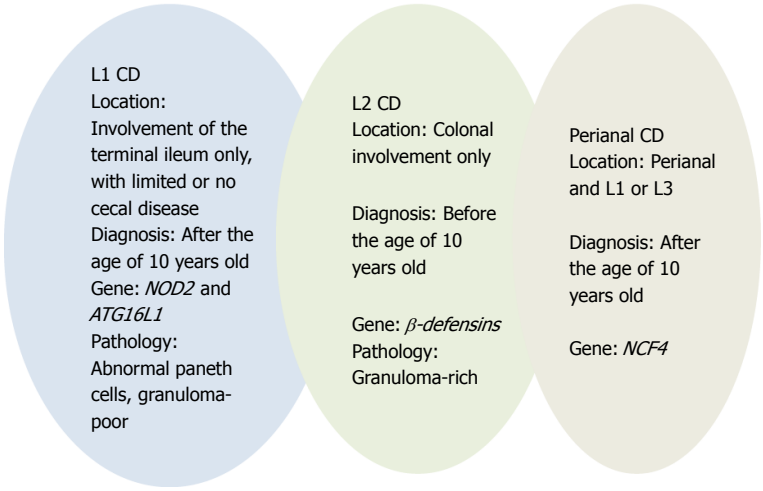

Figure 2 Subtypes of Crohn's disease. CD: Crohn's disease.

there have been few pediatric studies that have evaluated the association between CD behavior and genotype with prolonged follow-up. In a study by Shaoul *et al*[155], pediatric CD at the end of follow-up (mean 4.9 years) was classified as inflammatory (78%), stricturing (17%) and penetrating (7%). Moreover, a role for NOD2 in CD behavior (as opposed to location) was not supported in this study. NOD2 genotype was clearly associated with ileal involvement (L1), but not as an independent risk factor for stricturing, penetrating disease or a need for surgery[155]. A recent study showed that L1 CD and stricturing disease behavior are more common in children diagnosed after 10 years of age than in younger patients[39].

Approximately 10% of newly diagnosed pediatric patients shows perianal CD at time of diagnosis[156]. The Vienna classification did not distinguish luminal and perianal fistulising disease into different categories[28]. Subsequent evidence suggested that perianal and luminal fistulae often occur completely independently of each other. According to the Montreal and Paris classifications[29,30], a separate perianal modifier was added that can coexist with any disease behaviour. Perianal CD is defined as inflammation at or near the anus, including tags, fissures, fistulae, abscesses, or stenosis. A recent study by Eglinton *et al*[157] suggests specific associations with perianal CD patients compared with patients without perianal involvement. These associations include younger age at diagnosis, male gender, ileal (L1 or L3) location, and complicated disease behaviour (B2 + B3). In addition, an association with the NCF4 gene was demonstrated[157]. Taken together, literature data suggest that perianal CD represents a distinct disease phenotype, summarized in Figure 2.

UC

UC is classically defined as a chronic inflammation characterized by a continuous involvement of the colonic mucosa without granulomas on biopsy, affecting the rectum and a variable extent of the colon in continuity, and characterized by a relapsing, remittent course[158]. However, several studies suggest that pediatric UC may present with atypical phenotypes, such as macroscopical rectal sparing, macroscopic skip lesions in the colon, periappendiceal inflammation, backwash ileitis, limited upper gastrointestinal inflammation, and extensive colitis with less severe and less diffuse architectural abnormalities[159,160]. Thus, diagnosis of UC in pediatric patients may be particularly difficult. The recent Paris classification[30], which examined this issue, adds a new category (E4) for pancolitis (inflammation extending proximal to the hepatic flexure) (Table 2). Moreover, a disease behavior classification of S0 or S1 was adopted, with the latter denoting the presence of severe disease at any time in patient history (Table 2). The classification of UC based on anatomical extent has clinical relevance, as it affects the choice of therapy and the mode of delivery of drugs. For example, oral therapy is the first choice for UC extending above the splenic flexure[161].

Primary sclerosing cholangitis (PSC) is a cholestatic liver disease of unknown etiology, commonly associated with IBD and characterized by inflammation, fibrosis, and stenoses of the biliary tree leading to liver cirrhosis[162]. In most patients (80%-90%) the IBD can be classified as UC. Several features of UC in PSC differ from those of a general UC population (increased frequency of pancolitis, "backwash ileitis", rectal sparing and colorectal carcinoma), and these observations have led to the hypothesis that UC in PSC may represent a specific phenotypic entity (PSC-UC)[163]. There are a few studies on PSC-UC in pediatric patients. Ordonez *et al*[164] studied twenty-eight consecutive children with UC associated with PSC, celiac disease, or autoimmune hepatitis comparing them with a matched control group of 27 children with isolated UC. At diagnosis, pancolitis was seen in 18/28 UC associated with autoimmunity patients compared with 8/27 in UC. Pathological findings were also different from isolated UC: (1) major lesions predominantly located in the right colon; (2) pseudovillous appearance of the mucosa, and strong infiltration

with eosinophils; and (3) mild glandular lesions. Evolution in UC associated with autoimmune disease was less aggressive, requiring less corticosteroids/immunomodulators[164]. In conclusion, clinical, histological, and molecular analyses reveal marked differences between pediatric patients with isolated UC and those with associated autoimmune phenomena, supporting the hypothesis of a distinct autoimmune presentation of UC (Figure 1).

Goblet cells as protagonist in the UC

Mucus produced by goblet cells forms a key component of the mucosal barrier. Gel-forming mucins of intestinal mucus are arranged into a bilayer with a firm inner layer devoid of bacteria and a looser outer layer with MUC2 being major constituent of both[165]. The mucus layer has a crucial role in intestinal homeostasis, as decreased levels of goblet cells, leading to reduced mucin secretion, are a hallmark of human UC[166]. The thickness of the mucus layer in the healthy colon is between 100 and 300 μm, increasing from the ascending colon to the rectum, whereas in UC, this mucus layer is thinner, more variable and in part denuded[167-169]. Taken together, several lines of evidence strongly suggest a key role for goblet cells in UC.

Molecular biology of UC

GWAS and candidate gene association studies identified several UC susceptibility loci, including 7 that overlap with CD (*e.g.*, IL23 pathways genes, NKX2-3 and IL10)[170]. In a recent study, three new UC specific loci (*HNF4A*, *CDH1* and *LAMB1*) has been found that are involved in the regulation of barrier function[171].

An association was seen at rs6017342 SNP which maps within a recombination hotspot on chromosome 20q13 in which the 3' untraslated region (UTR) of the HNF4A gene is located. This gene encodes the transcription factor hepatocyte nuclear factor 4 α which regulates the expression of multiple components within the adherens junction, the tight junction and desmosome[172].

Cell-cell junctions have an important role on epithelial organization and barrier function. Moreover, in the embryonic age, the *HNF4A* gene participates in the development of mammalian gastrointestinal tract[173]. *CDH1* gene which encodes E-cadherin has been located on chromosome 16q22. E-cadherin is a transmembrane glycoprotein, a key component of the adherens junction and mediates intercellular adhesion in the intestinal epithelium[171]. It also participates in processes of epithelial restitution and repair following the damage of mucosa. Indeed, there is a significant reduced expression of CDH1 in areas of active UC[174]. Interestingly, two CDH1 variants associated with UC[171] have also been associated with colorectal cancer[175], a possible complication of UC. The recent hypothesis is that HNF4A and E-cadherin co-participate to maintain the integrity of the epithelial intestinal barrier. *LAMB1* gene is located on chromosome 7q31[171] and encodes the laminin β 1 subunit, which is detected in laminins-1, -2 and-10. In UC, the expression of laminins in the intestinal basement membrane is downregulated[171].

In 2011, Anderson *et al*[176] identified 29 additional UC risk loci from a meta-analysis and IL1R2, IL8RA/B, IL7R, IL12B, DAP, PRDM1, JAK2, IRF5, GNA12 and LSP1 were proposed as new important candidate genes in UC pathogenesis. Several genes were associated with cytokines and cytokine receptors, key regulators of citokyne-mediated signalling pathways, innate and adaptive immune response, macrophage activation and regulation of apoptosis. Interestingly, an association with *DAP* gene (death-associated protein) has been found. The expression of DAP kinase increases with inflammation in UC[177] and is a negative regulator of autophagy[178]. Therefore, the association with DAP suggests a possible link between autophagy and UC.

PRDM1, *IRF5* and *NKX2-3* genes could have a key role for transcriptional regulation in UC pathogenesis. GNA12 which plays a fundamental role in the tight junction assembly in epithelial cells has been identified at the 7p22 locus[179]. It is the most likely UC candidate gene from those described in the meta-analysis of Anderson *et al*[176]. Barrier integrity is important in UC pathogenesis given previous associations to *HNF4A*, *CDH1* and *LAMB1* genes[171]. Further studies are, however, need to verify the role of the genes in pediatric UC. The salient histopathological and biomolecular features of pediatric IBD are shown in Table 3.

CONCLUSION

In conclusion, our review reinforce the idea that pediatric UC and CD may be further classified into various clinicopathologic entities, based on genotype-phenotype correlation reported in recent literature. Therapy for complex genetic diseases such as IBD is difficult. For example, treatment with ant-TNF-α monoclonal antibodies does not induce remission in the majority of CD cases. It is becoming increasingly apparent that novel strategies to define and stratify IBD patients, that are based on serum, DNA and histopathology, will be needed to progress towards improved diagnostics, prognostics and therapeuthics.

REFERENCES

1 **Walfish A**, Sachar D. Phenotype classification in IBD: Is there an impact on therapy? *Inflamm Bowel Dis* 2007; **13**: 1573-1575 [PMID: 17763470]
2 **Louis E**, Van Kemseke C, Reenaers C. Necessity of phenotypic classification of inflammatory bowel disease. *Best Pract Res Clin Gastroenterol* 2011; **25** Suppl 1: S2-S7 [PMID: 21640927 DOI: 10.1016/S1521-6918(11)7003-8]
3 **Targan SR**, Hawkey CJ. Indeterminate colitis. In: Hawkey CJ, Bosch J, Richter JE, Garcia-Tsao G, Chan FKL. Textbook of Clinical Gastroenterology and Hepatology. Chichester: Wiley-Blackwell, 2012: 394-398
4 **Sachar DB**, Walfish A. Inflammatory bowel disease: one or two diseases? *Curr Gastroenterol Rep* 2013; **15**: 298 [PMID: 23250698 DOI: 10.1007/s11894-012-0298-9]

5 **Kaser A**, Zeissig S, Blumberg RS. Genes and environment: how will our concepts on the pathophysiology of IBD develop in the future? *Dig Dis* 2010; **28**: 395-405 [PMID: 20926863 DOI: 10.1159/000320393]

6 **Kelsen J**, Baldassano RN. Inflammatory bowel disease: the difference between children and adults. *Inflamm Bowel Dis* 2008; **14** Suppl 2: S9-S11 [PMID: 18816756 DOI: 10.1002/ibd.20560]

7 **Levine A**. Pediatric inflammatory bowel disease: is it different? *Dig Dis* 2009; **27**: 212-214 [PMID: 19786743 DOI: 10.1159/000228552]

8 **Sagiv-Friedgut K**, Karban A, Weiss B, Shaoul R, Shamir R, Bujanover Y, Reif S, Boaz M, Shani I, Levine A, Leshinsky-Silver E. Early-onset Crohn disease is associated with male sex and a polymorphism in the IL-6 promoter. *J Pediatr Gastroenterol Nutr* 2010; **50**: 22-26 [PMID: 19934771 DOI: 10.1097/MPG.0b013e3181b7a6a4]

9 **Gupta N**, Bostrom AG, Kirschner BS, Ferry GD, Winter HS, Baldassano RN, Gold BD, Abramson O, Smith T, Cohen SA, Heyman MB. Gender differences in presentation and course of disease in pediatric patients with Crohn disease. *Pediatrics* 2007; **120**: e1418-e1425 [PMID: 18055660]

10 **Biank V**, Broeckel U, Kugathasan S. Pediatric inflammatory bowel disease: clinical and molecular genetics. *Inflamm Bowel Dis* 2007; **13**: 1430-1438 [PMID: 17600381]

11 **Van Limbergen J**, Russell RK, Drummond HE, Aldhous MC, Round NK, Nimmo ER, Smith L, Gillett PM, McGrogan P, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Satsangi J, Wilson DC. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. *Gastroenterology* 2008; **135**: 1114-1122 [PMID: 18725221 DOI: 10.1053/j.gastro.2008.06.081]

12 **Heyman MB**, Kirschner BS, Gold BD, Ferry G, Baldassano R, Cohen SA, Winter HS, Fain P, King C, Smith T, El-Serag HB. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. *J Pediatr* 2005; **146**: 35-40 [PMID: 15644819]

13 **Ponder A**, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. *Clin Epidemiol* 2013; **5**: 237-247 [PMID: 23922506 DOI: 10.2147/CLEP.S33961]

14 **Russel MG**. Changes in the incidence of inflammatory bowel disease: what does it mean? *Eur J Intern Med* 2000; **11**: 191-196 [PMID: 10967506]

15 **Pappa HM**, Semrin G, Walker TR, Grand RJ. Pediatric inflammatory bowel disease. *Curr Opin Gastroenterol* 2004; **20**: 333-340 [PMID: 15703661]

16 **Hait E**, Bousvaros A, Grand R. Pediatric inflammatory bowel disease: what children can teach adults. *Inflamm Bowel Dis* 2005; **11**: 519-527 [PMID: 15905698]

17 **Murch SH**, Baldassano R, Buller H, Chin S, Griffiths AM, Hildebrand H, Jasinsky C, Kong T, Moore D, Orsi M. Inflammatory bowel disease: Working Group report of the second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2004; **39** Suppl 2: S647-S654 [PMID: 15184765]

18 **Kugathasan S**, Judd RH, Hoffmann RG, Heikenen J, Telega G, Khan F, Weisdorf-Schindele S, San Pablo W, Perrault J, Park R, Yaffe M, Brown C, Rivera-Bennett MT, Halabi I, Martinez A, Blank E, Werlin SL, Rudolph CD, Binion DG. Epidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: a statewide population-based study. *J Pediatr* 2003; **143**: 525-531 [PMID: 14571234]

19 **Malmborg P**, Grahnquist L, Lindholm J, Montgomery S, Hildebrand H. Increasing incidence of paediatric inflammatory bowel disease in northern Stockholm County, 2002-2007. *J Pediatr Gastroenterol Nutr* 2013; **57**: 29-34 [PMID: 23459320 DOI: 10.1097/MPG.0b013e31828f21b4]

20 **Benchimol EI**, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis* 2011; **17**: 423-439 [PMID: 20564651 DOI: 10.1002/ibd.21349]

21 **Grieci T**, Bütter A. The incidence of inflammatory bowel disease in the pediatric population of Southwestern Ontario. *J Pediatr Surg* 2009; **44**: 977-980 [PMID: 19433182 DOI: 10.1016/j.jpedsurg.2009.01.038]

22 **Bernstein CN**, Wajda A, Svenson LW, MacKenzie A, Koehoorn M, Jackson M, Fedorak R, Israel D, Blanchard JF. The epidemiology of inflammatory bowel disease in Canada: a population-based study. *Am J Gastroenterol* 2006; **101**: 1559-1568 [PMID: 16863561]

23 **Perminow G**, Brackmann S, Lyckander LG, Franke A, Borthne A, Rydning A, Aamodt G, Schreiber S, Vatn MH. A characterization in childhood inflammatory bowel disease, a new population-based inception cohort from South-Eastern Norway, 2005-07, showing increased incidence in Crohn's disease. *Scand J Gastroenterol* 2009; **44**: 446-456 [PMID: 19117240 DOI: 10.1080/00365520802647434]

24 **Lehtinen P**, Ashorn M, Iltanen S, Jauhola R, Jauhonen P, Kolho KL, Auvinen A. Incidence trends of pediatric inflammatory bowel disease in Finland, 1987-2003, a nationwide study. *Inflamm Bowel Dis* 2011; **17**: 1778-1783 [PMID: 21744433 DOI: 10.1002/ibd.21550]

25 **Sawczenko A**, Sandhu BK. Presenting features of inflammatory bowel disease in Great Britain and Ireland. *Arch Dis Child* 2003; **88**: 995-1000 [PMID: 14612366]

26 **Yantiss RK**, Odze RD. Diagnostic difficulties in inflammatory bowel disease pathology. *Histopathology* 2006; **48**: 116-132 [PMID: 16405661]

27 **Magro F**, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, Villanacci V, Becheanu G, Borralho Nunes P, Cathomas G, Fries W, Jouret-Mourin A, Mescoli C, de Petris G, Rubio CA, Shepherd NA, Vieth M, Eliakim R; European Society of Pathology (ESP); European Crohn's and Colitis Organisation (ECCO). European consensus on the histopathology of inflammatory bowel disease. *J Crohns Colitis* 2013; **7**: 827-851 [PMID: 23870728 DOI: 10.1016/j.crohns.2013.06.001]

28 **Gasche C**, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000; **6**: 8-15 [PMID: 10701144]

29 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544]

30 **Levine A**, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, Fell J, Ruemmele FM, Walters T, Sherlock M, Dubinsky M, Hyams JS. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; **17**: 1314-1321 [PMID: 21560194 DOI: 10.1002/ibd.21493]

31 **Cho JH**, Brant SR. Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1704-1712 [PMID: 21530736 DOI: 10.1053/j.gastro.2011.02.046]

32 **Denson LA**, Long MD, McGovern DP, Kugathasan S, Wu GD, Young VB, Pizarro TT, de Zoeten EF, Stappenbeck TS, Plevy SE, Abraham C, Nusrat A, Jobin C, McCole DF, Siegel CA, Higgins PD, Herfarth HH, Hyams J, Sandborn WJ, Loftus EV, Kappelman MD, Lewis JD, Parkos CA, Sar-

tor RB. Challenges in IBD research: update on progress and prioritization of the CCFA's research agenda. *Inflamm Bowel Dis* 2013; **19**: 677-682 [PMID: 23448796 DOI: 10.1097/MIB.0b013e31828134b3]
33 **Khor B**, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011; **474**: 307-317 [PMID: 21677747 DOI: 10.1038/nature10209]
34 **Graham DB**, Xavier RJ. From genetics of inflammatory bowel disease towards mechanistic insights. *Trends Immunol* 2013; **34**: 371-378 [PMID: 23639549 DOI: 10.1016/j.it.2013.04.001]
35 **Brant SR**. Update on the heritability of inflammatory bowel disease: the importance of twin studies. *Inflamm Bowel Dis* 2011; **17**: 1-5 [PMID: 20629102 DOI: 10.1002/ibd.21385]
36 **Halfvarson J**. Genetics in twins with Crohn's disease: less pronounced than previously believed? *Inflamm Bowel Dis* 2011; **17**: 6-12 [PMID: 20848478 DOI: 10.1002/ibd.21295]
37 **Price AB**. Overlap in the spectrum of non-specific inflammatory bowel disease--'colitis indeterminate'. *J Clin Pathol* 1978; **31**: 567-577 [PMID: 670413]
38 **Geboes K**, Colombel JF, Greenstein A, Jewell DP, Sandborn WJ, Vatn MH, Warren B, Riddell RH. Indeterminate colitis: a review of the concept--what's in a name? *Inflamm Bowel Dis* 2008; **14**: 850-857 [PMID: 18213696 DOI: 10.1002/ibd.20361]
39 **Martland GT**, Shepherd NA. Indeterminate colitis: definition, diagnosis, implications and a plea for nosological sanity. *Histopathology* 2007; **50**: 83-96 [PMID: 17204023]
40 **Feakins RM**. Inflammatory bowel disease biopsies: updated British Society of Gastroenterology reporting guidelines. *J Clin Pathol* 2013; **66**: 1005-1026 [PMID: 23999270 DOI: 10.1136/jclinpath-2013-201885]
41 **Prenzel F**, Uhlig HH. Frequency of indeterminate colitis in children and adults with IBD - a metaanalysis. *J Crohns Colitis* 2009; **3**: 277-281 [PMID: 21172287 DOI: 10.1016/j.crohns.2009.07.001]
42 **Carvalho RS**, Abadom V, Dilworth HP, Thompson R, Oliva-Hemker M, Cuffari C. Indeterminate colitis: a significant subgroup of pediatric IBD. *Inflamm Bowel Dis* 2006; **12**: 258-262 [PMID: 16633047]
43 **Romano C**, Famiani A, Gallizzi R, Comito D, Ferrau' V, Rossi P. Indeterminate colitis: a distinctive clinical pattern of inflammatory bowel disease in children. *Pediatrics* 2008; **122**: e1278-e1281 [PMID: 19047226 DOI: 10.1542/peds.2008-2306]
44 **Malaty HM**, Mehta S, Abraham B, Garnett EA, Ferry GD. The natural course of inflammatory bowel disease-indeterminate from childhood to adulthood: within a 25 year period. *Clin Exp Gastroenterol* 2013; **6**: 115-121 [PMID: 23901288 DOI: 10.2147/CEG.S44700]
45 **Ruel J**, Ruane D, Mehandru S, Gower-Rousseau C, Colombel JF. IBD across the age spectrum-is it the same disease? *Nat Rev Gastroenterol Hepatol* 2014; **11**: 88-98 [PMID: 24345891 DOI: 10.1038/nrgastro.2013.240]
46 **Ruemmele FM**, El Khoury MG, Talbotec C, Maurage C, Mougenot JF, Schmitz J, Goulet O. Characteristics of inflammatory bowel disease with onset during the first year of life. *J Pediatr Gastroenterol Nutr* 2006; **43**: 603-609 [PMID: 17130735]
47 **Cannioto Z**, Berti I, Martelossi S, Bruno I, Giurici N, Crovella S, Ventura A. IBD and IBD mimicking enterocolitis in children younger than 2 years of age. *Eur J Pediatr* 2009; **168**: 149-155 [PMID: 18546019 DOI: 10.1007/s00431-008-0721-2]
48 **Glocker EO**, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, Perro M, Diestelhorst J, Allroth A, Murugan D, Hätscher N, Pfeifer D, Sykora KW, Sauer M, Kreipe H, Lacher M, Nustede R, Woellner C, Baumann U, Salzer U, Koletzko S, Shah N, Segal AW, Sauerbrey A, Buderus S, Snapper SB, Grimbacher B, Klein C. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* 2009; **361**: 2033-2045 [PMID: 19890111 DOI: 10.1056/NEJMoa0907206]
49 **Kotlarz D**, Beier R, Murugan D, Diestelhorst J, Jensen O, Boztug K, Pfeifer D, Kreipe H, Pfister ED, Baumann U, Puchalka J, Bohne J, Egritas O, Dalgic B, Kolho KL, Sauerbrey A, Buderus S, Güngör T, Enninger A, Koda YK, Guariso G, Weiss B, Corbacioglu S, Socha P, Uslu N, Metin A, Wahbeh GT, Husain K, Ramadan D, Al-Herz W, Grimbacher B, Sauer M, Sykora KW, Koletzko S, Klein C. Loss of interleukin-10 signaling and infantile inflammatory bowel disease: implications for diagnosis and therapy. *Gastroenterology* 2012; **143**: 347-355 [PMID: 22549091 DOI: 10.1053/j.gastro.2012.04.045]
50 **Moran CJ**, Walters TD, Guo CH, Kugathasan S, Klein C, Turner D, Wolters VM, Bandsma RH, Mouzaki M, Zachos M, Langer JC, Cutz E, Benseler SM, Roifman CM, Silverberg MS, Griffiths AM, Snapper SB, Muise AM. IL-10R polymorphisms are associated with very-early-onset ulcerative colitis. *Inflamm Bowel Dis* 2013; **19**: 115-123 [PMID: 22550014 DOI: 10.1002/ibd.22974]
51 **Begue B**, Verdier J, Rieux-Laucat F, Goulet O, Morali A, Canioni D, Hugot JP, Daussy C, Verkarre V, Pigneur B, Fischer A, Klein C, Cerf-Bensussan N, Ruemmele FM. Defective IL10 signaling defining a subgroup of patients with inflammatory bowel disease. *Am J Gastroenterol* 2011; **106**: 1544-1555 [PMID: 21519361 DOI: 10.1038/ajg.2011.112]
52 **Shah N**, Kammermeier J, Elawad M, Glocker EO. Interleukin-10 and interleukin-10-receptor defects in inflammatory bowel disease. *Curr Allergy Asthma Rep* 2012; **12**: 373-379 [PMID: 22890722 DOI: 10.1007/s11882-012-0286-z]
53 **Franke A**, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, Domingues FS, Albrecht M, Nothnagel M, Ellinghaus D, Sina C, Onnie CM, Weersma RK, Stokkers PC, Wijmenga C, Gazouli M, Strachan D, McArdle WL, Vermeire S, Rutgeerts P, Rosenstiel P, Krawczak M, Vatn MH, Mathew CG, Schreiber S. Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat Genet* 2008; **40**: 1319-1323 [PMID: 18836448 DOI: 10.1038/ng.221]
54 **Franke A**, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellinghaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latiano A, Mathew CG, Montgomery GW, Prescott NJ, Raychaudhuri S, Rotter JI, Schumm P, Sharma Y, Simms LA, Taylor KD, Whiteman D, Wijmenga C, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Cohen A, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D'Inca R, Dubinsky M, Edwards C, Florin T, Franchimont D, Gearry R, Glas J, Van Gossum A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Laukens D, Lawrance I, Lemann M, Levine A, Libioulle C, Louis E, Mowat C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinhart AH, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson D, Walters T, Targan SR, Brant SR, Rioux JD, D'Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annese V, Hakonarson H, Daly MJ, Parkes M. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010; **42**: 1118-1125 [PMID: 21102463 DOI: 10.1038/ng.717]
55 **Glocker E**, Grimbacher B. Inflammatory bowel disease: is it a primary immunodeficiency? *Cell Mol Life Sci* 2012; **69**: 41-48 [PMID: 21997382 DOI: 10.1007/s00018-011-0837-9]
56 **Moore KW**, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19**: 683-765 [PMID: 11244051]
57 **Fiorentino DF**, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; **147**: 3815-3822 [PMID: 1940369]

58 **Williams L**, Bradley L, Smith A, Foxwell B. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. *J Immunol* 2004; **172**: 567-576 [PMID: 14688368]

59 **Worthey EA**, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, Serpe JM, Dasu T, Tschannen MR, Veith RL, Basehore MJ, Broeckel U, Tomita-Mitchell A, Arca MJ, Casper JT, Margolis DA, Bick DP, Hessner MJ, Routes JM, Verbsky JW, Jacob HJ, Dimmock DP. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genet Med* 2011; **13**: 255-262 [PMID: 21173700 DOI: 10.1097/GIM.0b013e3182088158]

60 **Agarwal S**, Mayer L. Diagnosis and treatment of gastrointestinal disorders in patients with primary immunodeficiency. *Clin Gastroenterol Hepatol* 2013; **11**: 1050-1063 [PMID: 23501398 DOI: 10.1016/j.cgh.2013.02.024]

61 **Orange JS**, Geha RS. Finding NEMO: genetic disorders of NF-[kappa]B activation. *J Clin Invest* 2003; **112**: 983-985 [PMID: 14523034]

62 **de Bie CI**, Paerregaard A, Kolacek S, Ruemmele FM, Koletzko S, Fell JM, Escher JC. Disease phenotype at diagnosis in pediatric Crohn's disease: 5-year analyses of the EUROKIDS Registry. *Inflamm Bowel Dis* 2013; **19**: 378-385 [PMID: 22573581 DOI: 10.1002/ibd.23008]

63 **Stappenbeck TS**. Paneth cell development, differentiation, and function: new molecular cues. *Gastroenterology* 2009; **137**: 30-33 [PMID: 19497398 DOI: 10.1053/j.gastro.2009.05.013]

64 **Ouellette AJ**. Paneth cell alpha-defensin synthesis and function. *Curr Top Microbiol Immunol* 2006; **306**: 1-25 [PMID: 16909916]

65 **Wehkamp J**, Wang G, Kübler I, Nuding S, Gregorieff A, Schnabel A, Kays RJ, Fellermann K, Burk O, Schwab M, Clevers H, Bevins CL, Stange EF. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007; **179**: 3109-3118 [PMID: 17709525]

66 **Wehkamp J**, Stange EF. Paneth's disease. *J Crohns Colitis* 2010; **4**: 523-531 [PMID: 21122555 DOI: 10.1016/j.crohns.2010.05.010]

67 **Beil WJ**, Weller PF, Peppercorn MA, Galli SJ, Dvorak AM. Ultrastructural immunogold localization of subcellular sites of TNF-alpha in colonic Crohn's disease. *J Leukoc Biol* 1995; **58**: 284-298 [PMID: 7665984]

68 **Beisner J**, Stange EF, Wehkamp J. Paneth cell function--implications in pediatric Crohn disease. *Gut Microbes* 2011; **2**: 47-51 [PMID: 21637018 DOI: 10.4161/gmic.2.1.14649]

69 **Keshav S**. Paneth cells: leukocyte-like mediators of innate immunity in the intestine. *J Leukoc Biol* 2006; **80**: 500-508 [PMID: 16793911]

70 **Kelly P**, Feakins R, Domizio P, Murphy J, Bevins C, Wilson J, McPhail G, Poulsom R, Dhaliwal W. Paneth cell granule depletion in the human small intestine under infective and nutritional stress. *Clin Exp Immunol* 2004; **135**: 303-309 [PMID: 14738460]

71 **Günther C**, Martini E, Wittkopf N, Amann K, Weigmann B, Neumann H, Waldner MJ, Hedrick SM, Tenzer S, Neurath MF, Becker C. Caspase-8 regulates TNF-α-induced epithelial necroptosis and terminal ileitis. *Nature* 2011; **477**: 335-339 [PMID: 21921917 DOI: 10.1038/nature10400]

72 **VanDussen KL**, Liu TC, Li D, Towfic F, Modiano N, Winter R, Haritunians T, Taylor KD, Dhall D, Targan SR, Xavier RJ, McGovern DP, Stappenbeck TS. Genetic variants synthesize to produce paneth cell phenotypes that define subtypes of Crohn's disease. *Gastroenterology* 2014; **146**: 200-209 [PMID: 24076061 DOI: 10.1053/j.gastro.2013.09.048]

73 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603 [PMID: 11385576]

74 **Hisamatsu T**, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology* 2003; **124**: 993-1000 [PMID: 12671896]

75 **Kobayashi KS**, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nuñez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005; **307**: 731-734 [PMID: 15692051]

76 **Ahmad T**, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002; **122**: 854-866 [PMID: 11910336]

77 **Rigoli L**, Romano C, Caruso RA, Lo Presti MA, Di Bella C, Procopio V, Lo Giudice G, Amorini M, Costantino G, Sergi MD, Cuppari C, Calabro GE, Gallizzi R, Salpietro CD, Fries W. Clinical significance of NOD2/CARD15 and Toll-like receptor 4 gene single nucleotide polymorphisms in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 4454-4461 [PMID: 18680223]

78 **Parkes M**. The genetics universe of Crohn's disease and ulcerative colitis. *Dig Dis* 2012; **30** Suppl 1: 78-81 [PMID: 23075873 DOI: 10.1159/000341130]

79 **Chamaillard M**, Iacob R, Desreumaux P, Colombel JF. Advances and perspectives in the genetics of inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2006; **4**: 143-151 [PMID: 16469672]

80 **Weersma RK**, Stokkers PC, van Bodegraven AA, van Hogezand RA, Verspaget HW, de Jong DJ, van der Woude CJ, Oldenburg B, Linskens RK, Festen EA, van der Steege G, Hommes DW, Crusius JB, Wijmenga C, Nolte IM, Dijkstra G. Molecular prediction of disease risk and severity in a large Dutch Crohn's disease cohort. *Gut* 2009; **58**: 388-395 [PMID: 18824555 DOI: 10.1136/gut.2007.144865]

81 **Wine E**, Reif SS, Leshinsky-Silver E, Weiss B, Shaoul RR, Shamir R, Wasserman D, Lerner A, Boaz M, Levine A. Pediatric Crohn's disease and growth retardation: the role of genotype, phenotype, and disease severity. *Pediatrics* 2004; **114**: 1281-1286 [PMID: 15520108]

82 **Kugathasan S**, Collins N, Maresso K, Hoffmann RG, Stephens M, Werlin SL, Rudolph C, Broeckel U. CARD15 gene mutations and risk for early surgery in pediatric-onset Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 1003-1009 [PMID: 15551253]

83 **Russell RK**, Drummond HE, Nimmo EE, Anderson N, Smith L, Wilson DC, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset M, Mahdi G, Satsangi J. Genotype-phenotype analysis in childhood-onset Crohn's disease: NOD2/CARD15 variants consistently predict phenotypic characteristics of severe disease. *Inflamm Bowel Dis* 2005; **11**: 955-964 [PMID: 16239840]

84 **Tomer G**, Ceballos C, Concepcion E, Benkov KJ. NOD2/CARD15 variants are associated with lower weight at diagnosis in children with Crohn's disease. *Am J Gastroenterol* 2003; **98**: 2479-2484 [PMID: 14638352]

85 **Cucchiara S**, Latiano A, Palmieri O, Staiano AM, D'Incà R, Guariso G, Vieni G, Rutigliano V, Borrelli O, Valvano MR, Annese V. Role of CARD15, DLG5 and OCTN genes polymorphisms in children with inflammatory bowel diseases. *World J Gastroenterol* 2007; **13**: 1221-1229 [PMID: 17451203]

86 **Levine A**, Kugathasan S, Annese V, Biank V, Leshinsky-Silver E, Davidovich O, Kimmel G, Shamir R, Palmieri O, Karban A, Broeckel U, Cucchiara S. Pediatric onset Crohn's colitis is characterized by genotype-dependent age-related susceptibility. *Inflamm Bowel Dis* 2007; **13**: 1509-1515 [PMID: 17763471]

87 **Ferraris A**, Knafelz D, Torres B, Fortina P, Castro M, Dallapiccola B. Analysis of CARD15 gene variants in Italian pediatric patients with inflammatory bowel diseases. *J Pediatr* 2005; **147**: 272-273 [PMID: 16126067]

88 **Roesler J**, Thürigen A, Sun L, Koch R, Winkler U, Laass MW, Gahr M, Rösen-Wolff A, Henker J. Influence of CARD15 mutations on disease activity and response to therapy in 65 pediatric Crohn patients from Saxony, Germany. *J Pediatr Gastroenterol Nutr* 2005; **41**: 27-32 [PMID: 15990626]

89 **Shaoul R**, Karban A, Weiss B, Reif S, Wasserman D, Pacht A, Eliakim R, Wardi J, Shirin H, Wine E, Leshinsky-Silver E, Levine A. NOD2/CARD15 mutations and presence of granulomas in pediatric and adult Crohn's disease. *Inflamm Bowel Dis* 2004; **10**: 709-714 [PMID: 15626887]

90 **Weiss B**, Shamir R, Bujanover Y, Waterman M, Hartman C, Fradkin A, Berkowitz D, Weintraub I, Eliakim R, Karban A. NOD2/CARD15 mutation analysis and genotype-phenotype correlation in Jewish pediatric patients compared with adults with Crohn's disease. *J Pediatr* 2004; **145**: 208-212 [PMID: 15289769]

91 **Ideström M**, Rubio C, Granath F, Finkel Y, Hugot JP. CARD15 mutations are rare in Swedish pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2005; **40**: 456-460 [PMID: 15795594]

92 **Zelinkova Z**, van Beelen AJ, de Kort F, Moerland PD, Ver Loren van Themaat E, te Velde AA, van Deventer SJ, de Jong EC, Hommes DW. Muramyl dipeptide-induced differential gene expression in NOD2 mutant and wild-type Crohn's disease patient-derived dendritic cells. *Inflamm Bowel Dis* 2008; **14**: 186-194 [PMID: 17941075]

93 **Iborra M**, Bernuzzi F, Correale C, Vetrano S, Fiorino G, Beltrán B, Marabita F, Locati M, Spinelli A, Nos P, Invernizzi P, Danese S. Identification of serum and tissue micro-RNA expression profiles in different stages of inflammatory bowel disease. *Clin Exp Immunol* 2013; **173**: 250-258 [PMID: 23607522 DOI: 10.1111/cei.12104]

94 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438]

95 **Kloosterman WP**, Plasterk RH. The diverse functions of microRNAs in animal development and disease. *Dev Cell* 2006; **11**: 441-450 [PMID: 17011485]

96 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

97 **McKenna LB**, Schug J, Vourekas A, McKenna JB, Bramswig NC, Friedman JR, Kaestner KH. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function. *Gastroenterology* 2010; **139**: 1654-1664, 1664.e1 [PMID: 20659473 DOI: 10.1053/j.gastro.2010.07.040]

98 **Lagos-Quintana M**, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol* 2002; **12**: 735-739 [PMID: 12007417]

99 **Boutz DR**, Collins PJ, Suresh U, Lu M, Ramírez CM, Fernández-Hernando C, Huang Y, Abreu Rde S, Le SY, Shapiro BA, Liu AM, Luk JM, Aldred SF, Trinklein ND, Marcotte EM, Penalva LO. Two-tiered approach identifies a network of cancer and liver disease-related genes regulated by miR-122. *J Biol Chem* 2011; **286**: 18066-18078 [PMID: 21402708 DOI: 10.1074/jbc.M110.196451]

100 **Ye D**, Guo S, Al-Sadi R, Ma TY. MicroRNA regulation of intestinal epithelial tight junction permeability. *Gastroenterology* 2011; **141**: 1323-1333 [PMID: 21763238 DOI: 10.1053/j.gastro.2011.07.005]

101 **Kanaan Z**, Rai SN, Eichenberger MR, Barnes C, Dworkin AM, Weller C, Cohen E, Roberts H, Keskey B, Petras RE, Crawford NP, Galandiuk S. Differential microRNA expression tracks neoplastic progression in inflammatory bowel disease-associated colorectal cancer. *Hum Mutat* 2012; **33**: 551-560 [PMID: 22241525 DOI: 10.1002/humu.22021]

102 **Chen Y**, Wang C, Liu Y, Tang L, Zheng M, Xu C, Song J, Meng X. miR-122 targets NOD2 to decrease intestinal epithelial cell injury in Crohn's disease. *Biochem Biophys Res Commun* 2013; **438**: 133-139 [PMID: 23872065 DOI: 10.1016/j.bbrc.2013.07.040]

103 **Noomen CG**, Hommes DW, Fidder HH. Update on genetics in inflammatory disease. *Best Pract Res Clin Gastroenterol* 2009; **23**: 233-243 [PMID: 19414149 DOI: 10.1016/j.bpg.2009.02.005]

104 **Wehkamp J**, Fellermann K, Herrlinger KR, Baxmann S, Schmidt K, Schwind B, Duchrow M, Wohlschläger C, Feller AC, Stange EF. Human beta-defensin 2 but not beta-defensin 1 is expressed preferentially in colonic mucosa of inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2002; **14**: 745-752 [PMID: 12169983]

105 **Fellermann K**, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL, Reinisch W, Teml A, Schwab M, Lichter P, Radlwimmer B, Stange EF. A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *Am J Hum Genet* 2006; **79**: 439-448 [PMID: 16909382]

106 **Wehkamp J**, Harder J, Weichenthal M, Schwab M, Schäffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schröder JM, Bevins CL, Fellermann K, Stange EF. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004; **53**: 1658-1664 [PMID: 15479689]

107 **Wehkamp J**, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, Shen B, Schaeffeler E, Schwab M, Linzmeier R, Feathers RW, Chu H, Lima H, Fellermann K, Ganz T, Stange EF, Bevins CL. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc Natl Acad Sci USA* 2005; **102**: 18129-18134 [PMID: 16330776]

108 **Zilbauer M**, Jenke A, Wenzel G, Goedde D, Postberg J, Phillips AD, Lucas M, Noble-Jamieson G, Torrente F, Salvestrini C, Heuschkel R, Wirth S. Intestinal alpha-defensin expression in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 2076-2086 [PMID: 21910169 DOI: 10.1002/ibd.21577]

109 **Perminow G**, Beisner J, Koslowski M, Lyckander LG, Stange E, Vatn MH, Wehkamp J. Defective paneth cell-mediated host defense in pediatric ileal Crohn's disease. *Am J Gastroenterol* 2010; **105**: 452-459 [PMID: 19904243 DOI: 10.1038/ajg.2009.643]

110 **Cook DN**, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. *Nat Immunol* 2004; **5**: 975-979 [PMID: 15454920]

111 **Franchimont D**, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Devière J, Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; **53**: 987-992 [PMID: 15194649]

112 **Török HP**, Glas J, Tonenchi L, Bruennler G, Folwaczny M, Folwaczny C. Crohn's disease is associated with a toll-like receptor-9 polymorphism. *Gastroenterology* 2004; **127**: 365-366 [PMID: 15236225]

113 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchi-

mont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962 [PMID: 18587394 DOI: 10.1038/ng.175]

114 **Parkes M**, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, Soars D, Drummond H, Lees CW, Khawaja SA, Bagnall R, Burke DA, Todhunter CE, Ahmad T, Onnie CM, McArdle W, Strachan D, Bethel G, Bryan C, Lewis CM, Deloukas P, Forbes A, Sanderson J, Jewell DP, Satsangi J, Mansfield JC, Cardon L, Mathew CG. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007; **39**: 830-832 [PMID: 17554261]

115 **Rioux JD**, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596-604 [PMID: 17435756]

116 **Ishihara S**, Aziz MM, Yuki T, Kazumori H, Kinoshita Y. Inflammatory bowel disease: review from the aspect of genetics. *J Gastroenterol* 2009; **44**: 1097-1108 [PMID: 19802731 DOI: 10.1007/s00535-009-0141-8]

117 **Saitoh T**, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, Tanaka K, Kawai T, Tsujimura T, Takeuchi O, Yoshimori T, Akira S. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 2008; **456**: 264-268 [PMID: 18849965 DOI: 10.1038/nature07383]

118 **Cadwell K**, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S, Stone CD, Brunt EM, Xavier RJ, Sleckman BP, Li E, Mizushima N, Stappenbeck TS, Virgin HW. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008; **456**: 259-263 [PMID: 18849966 DOI: 10.1038/nature07416]

119 **Münz C**. Enhancing immunity through autophagy. *Annu Rev Immunol* 2009; **27**: 423-449 [PMID: 19105657 DOI: 10.1146/annurev.immunol.021908.132537]

120 **Zeissig S**, Bojarski C, Buergel N, Mankertz J, Zeitz M, Fromm M, Schulzke JD. Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by tumour necrosis factor alpha antibody treatment. *Gut* 2004; **53**: 1295-1302 [PMID: 15306588]

121 **Maiuri MC**, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007; **8**: 741-752 [PMID: 17717517]

122 **Büning C**, Durmus T, Molnar T, de Jong DJ, Drenth JP, Fiedler T, Gentz E, Todorov T, Haas V, Buhner S, Sturm A, Baumgart DC, Nagy F, Lonovics J, Landt O, Kage A, Büning H, Nickel R, Büttner J, Lochs H, Schmidt HH, Witt H. A study in three European IBD cohorts confirms that the ATG16L1 c.898A& gt; G (p.Thr300Ala) variant is a susceptibility factor for Crohn's disease. *J Crohns Colitis* 2007; **1**: 70-76 [PMID: 21172187 DOI: 10.1016/j.crohns.2007.08.001]

123 **Zhang HF**, Qiu LX, Chen Y, Zhu WL, Mao C, Zhu LG, Zheng MH, Wang Y, Lei L, Shi J. ATG16L1 T300A polymorphism and Crohn's disease susceptibility: evidence from 13,022 cases and 17,532 controls. *Hum Genet* 2009; **125**: 627-631 [PMID: 19337756 DOI: 10.1007/s00439-009-0660-7]

124 **Cheng JF**, Ning YJ, Zhang W, Lu ZH, Lin L. T300A polymorphism of ATG16L1 and susceptibility to inflammatory bowel diseases: a meta-analysis. *World J Gastroenterol* 2010; **16**: 1258-1266 [PMID: 20222171]

125 **Okazaki T**, Wang MH, Rawsthorne P, Sargent M, Datta LW, Shugart YY, Bernstein CN, Brant SR. Contributions of IBD5, IL23R, ATG16L1, and NOD2 to Crohn's disease risk in a population-based case-control study: evidence of gene-gene interactions. *Inflamm Bowel Dis* 2008; **14**: 1528-1541 [PMID: 18521914 DOI: 10.1002/ibd.20512]

126 **Philpott DJ**, Sorbara MT, Robertson SJ, Croitoru K, Girardin SE. NOD proteins: regulators of inflammation in health and disease. *Nat Rev Immunol* 2014; **14**: 9-23 [PMID: 24336102 DOI: 10.1038/nri3565]

127 **Travassos LH**, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhães JG, Yuan L, Soares F, Chea E, Le Bourhis L, Boneca IG, Allaoui A, Jones NL, Nuñez G, Girardin SE, Philpott DJ. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* 2010; **11**: 55-62 [PMID: 19898471 DOI: 10.1038/ni.1823]

128 **Kuballa P**, Huett A, Rioux JD, Daly MJ, Xavier RJ. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. *PLoS One* 2008; **3**: e3391 [PMID: 18852889 DOI: 10.1371/journal.pone.0003391]

129 **Singh SB**, Ornatowski W, Vergne I, Naylor J, Delgado M, Roberts E, Ponpuak M, Master S, Pilli M, White E, Komatsu M, Deretic V. Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol* 2010; **12**: 1154-1165 [PMID: 21102437 DOI: 10.1038/ncb2119]

130 **Singh SB**, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006; **313**: 1438-1441 [PMID: 16888103]

131 **Cooney R**, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJ, Campbell BJ, Jewell D, Simmons A. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 2010; **16**: 90-97 [PMID: 19966812 DOI: 10.1038/nm.2069]

132 **Prescott NJ**, Dominy KM, Kubo M, Lewis CM, Fisher SA, Redon R, Huang N, Stranger BE, Blaszczyk K, Hudspith B, Parkes G, Hosono N, Yamazaki K, Onnie CM, Forbes A, Dermitzakis ET, Nakamura Y, Mansfield JC, Sanderson J, Hurles ME, Roberts RG, Mathew CG. Independent and population-specific association of risk variants at the IRGM locus with Crohn's disease. *Hum Mol Genet* 2010; **19**: 1828-1839 [PMID: 20106866 DOI: 10.1093/hmg/ddq041]

133 **Brest P**, Lapaquette P, Souidi M, Lebrigand K, Cesaro A, Vouret-Craviari V, Mari B, Barbry P, Mosnier JF, Hébuterne X, Harel-Bellan A, Mograbi B, Darfeuille-Michaud A, Hofman P. A synonymous variant in IRGM alters a binding site for miR-196 and causes deregulation of IRGM-dependent xenophagy in Crohn's disease. *Nat Genet* 2011; **43**: 242-245 [PMID: 21278745 DOI: 10.1038/ng.762]

134 **Lapaquette P**, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 2010; **12**: 99-113 [PMID: 19747213 DOI: 10.1111/j.1462-5822.2009.01381.x]

135 **Latiano A**, Palmieri O, Cucchiara S, Castro M, D'Incà R, Guariso G, Dallapiccola B, Valvano MR, Latiano T, Andriulli A, Annese V. Polymorphism of the IRGM gene might predispose to fistulizing behavior in Crohn's disease. *Am J Gastroenterol* 2009; **104**: 110-116 [PMID: 19098858 DOI: 10.1038/ajg.2008.3]

136 **Moon CM**, Shin DJ, Kim SW, Son NH, Park A, Park B, Jung ES, Kim ES, Hong SP, Kim TI, Kim WH, Cheon JH. Associations between genetic variants in the IRGM gene and inflammatory bowel diseases in the Korean population. *Inflamm Bowel Dis* 2013; **19**: 106-114 [PMID: 22508677 DOI: 10.1002/ibd.22972]

137 **Sim WH**, Wagner J, Cameron DJ, Catto-Smith AG, Bishop RF, Kirkwood CD. Expression profile of genes involved in pathogenesis of pediatric Crohn's disease. *J Gastroenterol Hepatol* 2012; **27**: 1083-1093 [PMID: 22098497 DOI: 10.1111/j.1440-1746.2011.06973.x]

138 **Seidelin JB**, Bjerrum JT, Coskun M, Widjaya B, Vainer B, Nielsen OH. IL-33 is upregulated in colonocytes of ulcerative colitis. *Immunol Lett* 2010; **128**: 80-85 [PMID: 19913053 DOI: 10.1016/j.imlet.2009.11.001]

139 **Sponheim J**, Pollheimer J, Olsen T, Balogh J, Hammarström C, Loos T, Kasprzycka M, Sørensen DR, Nilsen HR, Küchler AM, Vatn MH, Haraldsen G. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am J Pathol* 2010; **177**: 2804-2815 [PMID: 21037074 DOI: 10.2353/ajpath.2010.100378]

140 **Beltrán CJ**, Núñez LE, Díaz-Jiménez D, Farfan N, Candia E, Heine C, López F, González MJ, Quera R, Hermoso MA. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 1097-1107 [PMID: 20014018 DOI: 10.1002/ibd.21175]

141 **Kugathasan S**, Baldassano RN, Bradfield JP, Sleiman PM, Imielinski M, Guthery SL, Cucchiara S, Kim CE, Frackelton EC, Annaiah K, Glessner JT, Santa E, Willson T, Eckert AW, Bonkowski E, Shaner JL, Smith RM, Otieno FG, Peterson N, Abrams DJ, Chiavacci RM, Grundmeier R, Mamula P, Tomer G, Piccoli DA, Monos DS, Annese V, Denson LA, Grant SF, Hakonarson H. Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease. *Nat Genet* 2008; **40**: 1211-1215 [PMID: 18758464 DOI: 10.1038/ng.203]

142 **Funke B**, Autschbach F, Kim S, Lasitschka F, Strauch U, Rogler G, Gdynia G, Li L, Gretz N, Macher-Goeppinger S, Sido B, Schirmacher P, Meuer SC, Roth W. Functional characterisation of decoy receptor 3 in Crohn's disease. *Gut* 2009; **58**: 483-491 [PMID: 19039087 DOI: 10.1136/gut.2008.148908]

143 **Amre DK**, Mack DR, Morgan K, Fujiwara M, Israel D, Deslandres C, Seidman EG, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Grimard G, Levy E. Investigation of reported associations between the 20q13 and 21q22 loci and pediatric-onset Crohn's disease in Canadian children. *Am J Gastroenterol* 2009; **104**: 2824-2828 [PMID: 19623168 DOI: 10.1038/ajg.2009.430]

144 **Amre DK**, Mack DR, Morgan K, Israel D, Deslandres C, Seidman EG, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Xhu Z, Grimard G, Levy E. Association between genome-wide association studies reported SNPs and pediatric-onset Crohn's disease in Canadian children. *Hum Genet* 2010; **128**: 131-135 [PMID: 20473688 DOI: 10.1007/s00439-010-0835-2]

145 **Barber LJ**, Youds JL, Ward JD, McIlwraith MJ, O'Neil NJ, Petalcorin MI, Martin JS, Collis SJ, Cantor SB, Auclair M, Tissenbaum H, West SC, Rose AM, Boulton SJ. RTEL1 maintains genomic stability by suppressing homologous recombination. *Cell* 2008; **135**: 261-271 [PMID: 18957201 DOI: 10.1016/j.cell.2008.08.016]

146 **Bai C**, Connolly B, Metzker ML, Hilliard CA, Liu X, Sandig V, Soderman A, Galloway SM, Liu Q, Austin CP, Caskey CT. Overexpression of M68/DcR3 in human gastrointestinal tract tumors independent of gene amplification and its location in a four-gene cluster. *Proc Natl Acad Sci USA* 2000; **97**: 1230-1235 [PMID: 10655513]

147 **Imielinski M**, Baldassano RN, Griffiths A, Russell RK, Annese V, Dubinsky M, Kugathasan S, Bradfield JP, Walters TD, Sleiman P, Kim CE, Muise A, Wang K, Glessner JT, Saeed S, Zhang H, Frackelton EC, Hou C, Flory JH, Otieno G, Chiavacci RM, Grundmeier R, Castro M, Latiano A, Dallapiccola B, Stempak J, Abrams DJ, Taylor K, McGovern D; Western Regional Alliance for Pediatric IBD, Silber G, Wrobel I, Quiros A; International IBD Genetics Consortium, Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmuda MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ; NIDDK IBD Genetics Consortium, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E; Belgian-French IBD Consortium; Wellcome Trust Case Control Consortium, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwillam R, Tremelling M, Delukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ, Heyman MB, Ferry GD, Kirschner B, Lee J, Essers J, Grand R, Stephens M, Levine A, Piccoli D, Van Limbergen J, Cucchiara S, Monos DS, Guthery SL, Denson L, Wilson DC, Grant SF, Daly M, Silverberg MS, Satsangi J, Hakonarson H. Common variants at five new loci associated with early-onset inflammatory bowel disease. *Nat Genet* 2009; **41**: 1335-1340 [PMID: 19915574 DOI: 10.1038/ng.489]

148 **Steinman L**. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; **13**: 139-145 [PMID: 17290272]

149 **Latiano A**, Palmieri O, Latiano T, Corritore G, Bossa F, Martino G, Biscaglia G, Scimeca D, Valvano MR, Pastore M, Marseglia 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]

150 **Latiano A**, Palmieri O, Pastorelli L, Vecchi M, Pizarro TT, Bossa F, Merla G, Augello B, Latiano T, Corritore G, Settesoldi A, Valvano MR, D'Incà R, Stronati L, Annese V, Andriulli A. Associations between genetic polymorphisms in IL-33, IL1R1 and risk for inflammatory bowel disease. *PLoS One* 2013; **8**: e62144 [PMID: 23634226 DOI: 10.1371/journal.pone.0062144]

151 **Zhao C**, Wang I, Lehrer RI. Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. *FEBS Lett* 1996; **396**: 319-322 [PMID: 8915011]

152 **Frye M**, Bargon J, Lembcke B, Wagner TO, Gropp R. Differential expression of human alpha- and beta-defensins mRNA in gastrointestinal epithelia. *Eur J Clin Invest* 2000; **30**: 695-701 [PMID: 10964161]

153 **Gersemann M**, Wehkamp J, Fellermann K, Stange EF. Crohn 's disease--defect in innate defence. *World J Gastroenterol* 2008; **14**: 5499-5503 [PMID: 18810765]

154 **Gupta N**, Bostrom AG, Kirschner BS, Ferry GD, Gold BD, Cohen SA, Winter HS, Baldassano RN, Abramson O, Smith T, Heyman MB. Incidence of stricturing and penetrating complications of Crohn's disease diagnosed in pediatric patients. *Inflamm Bowel Dis* 2010; **16**: 638-644 [PMID: 19760783 DOI: 10.1002/ibd.21099]

155 **Shaoul R**, Karban A, Reif S, Weiss B, Shamir R, Tamir A, Davidovich O, Halevi J, Silver EL, Levine A. Disease behavior in children with Crohn's disease: the effect of disease duration, ethnicity, genotype, and phenotype. *Dig Dis Sci* 2009; **54**: 142-150 [PMID: 18594982 DOI: 10.1007/s10620-008-0326-7]

156 **de Zoeten EF**, Pasternak BA, Mattei P, Kramer RE, Kader HA. Diagnosis and treatment of perianal Crohn disease: NASPGHAN clinical report and consensus statement. *J Pediatr Gastroenterol Nutr* 2013; **57**: 401-412 [PMID: 23974063 DOI: 10.1097/MPG.0b013e3182a025ee]

157 **Eglinton TW**, Roberts R, Pearson J, Barclay M, Merriman TR, Frizelle FA, Gearry RB. Clinical and genetic risk factors for perianal Crohn's disease in a population-based cohort. *Am J Gastroenterol* 2012; **107**: 589-596 [PMID: 22158027 DOI: 10.1038/ajg.2011.437]

158 **Stange EF**, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, Feakins R, Fléjou JF, Herfarth H, Hommes

DW, Kupcinskas L, Lakatos PL, Mantzaris GJ, Schreiber S, Villanacci V, Warren BF. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. *J Crohns Colitis* 2008; **2**: 1-23 [PMID: 21172194 DOI: 10.1016/j.crohns.2007.11.001]

159 **Levine A**, de Bie CI, Turner D, Cucchiara S, Sladek M, Murphy MS, Escher JC. Atypical disease phenotypes in pediatric ulcerative colitis: 5-year analyses of the EUROKIDS Registry. *Inflamm Bowel Dis* 2013; **19**: 370-377 [PMID: 22570259 DOI: 10.1002/ibd.23013]

160 **Aloi M**, D'Arcangelo G, Pofi F, Vassallo F, Rizzo V, Nuti F, Di Nardo G, Pierdomenico M, Viola F, Cucchiara S. Presenting features and disease course of pediatric ulcerative colitis. *J Crohns Colitis* 2013; **7**: e509-e515 [PMID: 23583691 DOI: 10.1016/j.crohns.2013.03.007]

161 **Aloi M**, Nuti F, Stronati L, Cucchiara S. Advances in the medical management of paediatric IBD. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 99-108 [PMID: 23958601 DOI: 10.1038/nrgastro.2013.158]

162 **Joo M**, Abreu-e-Lima P, Farraye F, Smith T, Swaroop P, Gardner L, Lauwers GY, Odze RD. Pathologic features of ulcerative colitis in patients with primary sclerosing cholangitis: a case-control study. *Am J Surg Pathol* 2009; **33**: 854-862 [PMID: 19295408 DOI: 10.1097/PAS.0b013e318196d018]

163 **Loftus EV**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511]

164 **Ordonez F**, Lacaille F, Canioni D, Talbotec C, Fournet JC, Cerf-Bensussan N, Goulet O, Schmitz J, Ruemmele FM. Pediatric ulcerative colitis associated with autoimmune diseases: a distinct form of inflammatory bowel disease? *Inflamm Bowel Dis* 2012; **18**: 1809-1817 [PMID: 22238154 DOI: 10.1002/ibd.22864]

165 **Johansson ME**, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci USA* 2008; **105**: 15064-15069 [PMID: 18806221 DOI: 10.1073/pnas.0803124105]

166 **Maloy KJ**, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* 2011; **474**: 298-306 [PMID: 21677746 DOI: 10.1038/nature10208]

167 **Gersemann M**, Stange EF, Wehkamp J. From intestinal stem cells to inflammatory bowel diseases. *World J Gastroenterol* 2011; **17**: 3198-3203 [PMID: 21912468 DOI: 10.3748/wjg.v17.i27.3198]

168 **Gersemann M**, Becker S, Kübler I, Koslowski M, Wang G, Herrlinger KR, Griger J, Fritz P, Fellermann K, Schwab M, Wehkamp J, Stange EF. Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. *Differentiation* 2009; **77**: 84-94 [PMID: 19281767 DOI: 10.1016/j.diff.2008.09.008]

169 **Strugala V**, Dettmar PW, Pearson JP. Thickness and continuity of the adherent colonic mucus barrier in active and quiescent ulcerative colitis and Crohn's disease. *Int J Clin Pract* 2008; **62**: 762-769 [PMID: 18194279 DOI: 10.1111/j.1742-1241.2007.01665.x]

170 **Doecke JD**, Simms LA, Zhao ZZ, Huang N, Hanigan K, Krishnaprasad K, Roberts RL, Andrews JM, Mahy G, Bampton P, Lewindon P, Florin T, Lawrance IC, Gearry RB, Montgomery GW, Radford-Smith GL. Genetic susceptibility in IBD: overlap between ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis* 2013; **19**: 240-245 [PMID: 23348120 DOI: 10.1097/MIB.0b013e3182810041]

171 **Barrett JC**, Lee JC, Lees CW, Prescott NJ, Anderson CA, Phillips A, Wesley E, Parnell K, Zhang H, Drummond H, Nimmo ER, Massey D, Blaszczyk K, Elliott T, Cotterill L, Dallal H, Lobo AJ, Mowat C, Sanderson JD, Jewell DP, Newman WG, Edwards C, Ahmad T, Mansfield JC, Satsangi J, Parkes M, Mathew CG, Donnelly P, Peltonen L, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, McCarthy MI, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Samani N, Trembath RC, Viswanathan AC, Wood N, Spencer CC, Barrett JC, Bellenguez C, Davison D, Freeman C, Strange A, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Perez ML, Potter SC, Ravindrarajah R, Ricketts M, Waller M, Weston P, Widaa S, Whittaker P, Deloukas P, Peltonen L, Mathew CG, Blackwell JM, Brown MA, Corvin A, McCarthy MI, Spencer CC, Attwood AP, Stephens J, Sambrook J, Ouwehand WH, McArdle WL, Ring SM, Strachan DP. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet* 2009; **41**: 1330-1334 [PMID: 19915572 DOI: 10.1038/ng.483]

172 **Battle MA**, Konopka G, Parviz F, Gaggl AL, Yang C, Sladek FM, Duncan SA. Hepatocyte nuclear factor 4alpha orchestrates expression of cell adhesion proteins during the epithelial transformation of the developing liver. *Proc Natl Acad Sci USA* 2006; **103**: 8419-8424 [PMID: 16714383]

173 **Garrison WD**, Battle MA, Yang C, Kaestner KH, Sladek FM, Duncan SA. Hepatocyte nuclear factor 4alpha is essential for embryonic development of the mouse colon. *Gastroenterology* 2006; **130**: 1207-1220 [PMID: 16618389]

174 **Karayiannakis AJ**, Syrigos KN, Efstathiou J, Valizadeh A, Noda M, Playford RJ, Kmiot W, Pignatelli M. Expression of catenins and E-cadherin during epithelial restitution in inflammatory bowel disease. *J Pathol* 1998; **185**: 413-418 [PMID: 9828841]

175 **Houlston RS**, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, Chandler I, Vijayakrishnan J, Sullivan K, Penegar S, Carvajal-Carmona L, Howarth K, Jaeger E, Spain SL, Walther A, Barclay E, Martin L, Gorman M, Domingo E, Teixeira AS, Kerr D, Cazier JB, Niittymäki I, Tuupanen S, Karhu A, Aaltonen LA, Tomlinson IP, Farrington SM, Tenesa A, Prendergast JG, Barnetson RA, Cetnarskyj R, Porteous ME, Pharoah PD, Koessler T, Hampe J, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Chang-Claude J, Hoffmeister M, Brenner H, Zanke BW, Montpetit A, Hudson TJ, Gallinger S, Campbell H, Dunlop MG. Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. *Nat Genet* 2008; **40**: 1426-1435 [PMID: 19011631 DOI: 10.1038/ng.262]

176 **Anderson CA**, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A, Lagacé C, Scott R, Amininejad L, Bumpstead S, Baidoo L, Baldassano RN, Barclay M, Bayless TM, Brand S, Büning C, Colombel JF, Denson LA, De Vos M, Dubinsky M, Edwards C, Ellinghaus D, Fehrmann RS, Floyd JA, Florin T, Franchimont D, Franke L, Georges M, Glas J, Glazer NL, Guthery SL, Haritunians T, Hayward NK, Hugot JP, Jobin G, Laukens D, Lawrance I, Lémann M, Levine A, Libioulle C, Louis E, McGovern DP, Milla M, Montgomery GW, Morley KI, Mowat C, Ng A, Newman W, Ophoff RA, Papi L, Palmieri O, Peyrin-Biroulet L, Panés J, Phillips A, Prescott NJ, Proctor DD, Roberts R, Russell R, Rutgeerts P, Sanderson J, Sans M, Schumm P, Seibold F, Sharma Y, Simms LA, Seielstad M, Steinhart AH, Targan SR, van den Berg LH, Vatn M, Verspaget H, Walters T, Wijmenga C, Wilson DC, Westra HJ, Xavier RJ, Zhao ZZ, Ponsioen CY, Andersen V, Torkvist L, Gazouli M, Anagnou NP, Karlsen TH, Kupcinskas L, Sventoraityte J, Mansfield JC, Kugathasan S, Silverberg MS, Halfvarson J, Rotter JI, Mathew CG, Griffiths AM, Gearry R, Ahmad T, Brant SR, Chamaillard M, Satsangi J, Cho JH, Schreiber S, Daly MJ, Barrett JC, Parkes M, Annese

V, Hakonarson H, Radford-Smith G, Duerr RH, Vermeire S, Weersma RK, Rioux JD. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011; **43**: 246-252 [PMID: 21297633 DOI: 10.1038/ng.764]

177 **Kuester D**, Guenther T, Biesold S, Hartmann A, Bataille F, Ruemmele P, Peters B, Meyer F, Schubert D, Bohr UR, Malfertheiner P, Lippert H, Silver AR, Roessner A, Schneider-Stock R. Aberrant methylation of DAPK in long-standing ulcerative colitis and ulcerative colitis-associated carcinoma. *Pathol Res Pract* 2010; **206**: 616-624 [PMID: 20630662 DOI: 10.1016/j.prp.2010.05.004]

178 **Koren I**, Reem E, Kimchi A. DAP1, a novel substrate of mTOR, negatively regulates autophagy. *Curr Biol* 2010; **20**: 1093-1098 [PMID: 20537536 DOI: 10.1016/j.cub.2010.04.041]

179 **Sabath E**, Negoro H, Beaudry S, Paniagua M, Angelow S, Shah J, Grammatikakis N, Yu AS, Denker BM. Galpha12 regulates protein interactions within the MDCK cell tight junction and inhibits tight-junction assembly. *J Cell Sci* 2008; **121**: 814-824 [PMID: 18285450 DOI: 10.1242/jcs.014878]

P- Reviewer: Singhal S, van Langenberg DR
S- Editor: Zhai HH **L- Editor**: A **E- Editor**: Liu XM

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327