Role of autophagy in the pathogenesis of inflammatory bowel disease

Tomoya Iida, Kei Onodera, Hiroshi Nakase

Inflammatory bowel disease (IBD) results from a complex series of interactions between susceptibility genes, the environment, and the immune system. Recently, some studies provided strong evidence that the process of autophagy affects several aspects of mucosal immune responses. Autophagy is a cellular stress response that plays key roles in physiological processes, such as innate and adaptive immunity, adaptation to starvation, degradation of aberrant proteins or organelles, antimicrobial defense, and protein secretion. Dysfunctional autophagy is recognized as a contributing factor in many chronic inflammatory diseases, including IBD. Autophagy plays multiple roles in IBD pathogenesis. Recent studies have identified susceptibility genes involved in autophagy, such as NOD2, ATG16L1, and IRGM, and active research is ongoing all over the world.

Key words: Autophagy; Inflammatory bowel disease; Genome-wide association study; Ulcerative colitis; Crohn’s disease

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IRGM, and active research is ongoing around the world. The aim of this review is a systematic appraisal of current literature to provide a better understanding of the role of autophagy in IBD pathogenesis.

Iida T, Onodera K, Nakase H. Role of autophagy in the pathogenesis of inflammatory bowel disease. World J Gastroenterol 2017; 23(11): 1944-1953 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i11/1944.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i11.1944

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease involving idiopathic inflammation, mainly in the gastrointestinal tract; defined more specifically, it comprises ulcerative colitis (UC) and Crohn's disease (CD). Both are characterized by onset at a young age, and the number of affected patients has risen sharply in recent years in Europe and the United States, as well as in Japan[1]. Thus, there is a pressing need to understand their pathologies and create effective treatments. Researchers, mainly in Europe and the United States, have been trying to identify disease-susceptibility genes for IBD. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) was the first susceptibility gene identified for CD[2,3], and in recent years genome-wide association studies (GWAS) have made it possible to perform comprehensive searches for susceptibility genes. In 2007, autophagy-related 16-like 1 (ATG16L1) was identified as an autophagy-related gene[4]. This was the first study to show a relationship between autophagy and a specific disease. Since then, the role of autophagy in the pathogenesis of IBD has been investigated all over the world.

This review will evaluate the current literature to provide a better understanding of the role of autophagy in the pathophysiology of IBD.

PATHOLOGY AND PATHOGENESIS OF IBD

The gastrointestinal tract not only absorbs fluid and nutrients, but is constantly involved in regulating and maintaining the gut flora, immune responses to food antigens and other substances, and homeostasis. IBD occurs when this homeostasis is impaired. Recent research has shown that IBD is caused by chronic intestinal inflammation, which occurs because of gene variations that can lead to disease susceptibility, changes in the structure of the intestinal flora needed to maintain intestinal homeostasis, and abnormal intestinal mucosal immune responses[5-7].

The role of genetic factors in IBD has been previously reported[8], and several researchers are seeking disease-susceptibility genes and trying to find customized treatments for individual patients[9]. To date, approximately 200 loci have been identified as being associated with both forms of IBD. Within these 200 loci, based upon single nucleotide polymorphism frequencies in IBD subjects versus controls, are approximately 1500 potential associated genes[10,11]. Representative autophagy-related genes are NOD2, ATG16L1, and immunity-related guanosine triphosphatase M (IRGM)[12-14,15]. Autophagy has been linked to a variety of diseases, but its link to IBD is currently the subject of much debate.

AUTOPHAGY

Autophagy (from the Greek “auto” oneself and “phag” to eat) refers to any cellular degradative pathway that involves the delivery of cytoplasmic cargo to the lysosome. During this process, the endoplasmic reticulum or other membranous cellular structures respond to stimuli by generating a double-membrane structure called a phagophore. On this phagophore, ATG16L1 forms a complex with an ATG5-ATG12 conjugate, which multimerizes and then lipitates LC3 (LC3-II). Simultaneously, the phagophore elongates to envelop the cytoplasm or organelle to be degraded, forming an autophagosome, a unique double-membrane organelle. The outer membrane of the autophagosome then fuses with a lysosome to form an autolysosome, and the inner membrane degrades and absorbs its contents (Figure 1)[13-15]. This process, along with the ubiquitin proteasome pathway (UPP) system, triggers the intracellular protein degradation mechanism. The process is also responsible for mechanisms such as adaptation to starvation, defense against infections, carcinogenesis, antigen presentation, and quality control of intracellular proteins. It maintains appropriate cellular homeostasis and provides the structural processes necessary for organ renewal[16]. Yet, unlike the UPP system, autophagy is also able to degrade mitochondria and other organelles.

A remarkable analysis of autophagy-related factor groups showed that, in addition to its role in metabolism, autophagy plays an important role in the innate immune response[13]. Innate immunity is a mechanism by which nearly all multicellular organisms protect themselves from pathogens. Innate immunity signaling pathways are activated when the structural patterns of a pathogen's components are recognized (i.e., the cell wall components of a bacterium or the genome of a virus). As noted above, autophagy was initially considered to be a nonspecific mechanism for degrading substances by incorporating them into a membrane structure, but recent research has shown that autophagosomes selectively isolate a variety of substrates[17]. However, besides autophagy of pathogens (xenophagy)[18,19] and autophagy of damaged mitochondria (mitophagy)[20,21], very little
is understood about which substrates autophagy degrades when it functions as part of innate immunity.

**IBD- AND AUTOPHAGY-RELATED GENETIC VARIANTS**

Autophagic dysfunction causes several diseases, among which CD is being most extensively researched. The above mentioned GWAS found several genetic variants linked to CD onset, such as NOD2 and ATG16L1. A summary of these variants is given below (Table 1).

**NOD2, ATG16L1**

NOD2, located on chromosome 16q12.1, was the first disease-susceptibility gene discovered for CD. Its genetic variants are common in European and American patients, but have not been found in Asian patients. NOD2 is a pattern-recognition receptor that is involved in the homeostasis of intestinal immunity. It acts through mechanisms like autophagy, intracellular bacterial sensing, controlling the expression of the antibacterial peptide α-defensin in the Paneth cells of the small intestine, and improving immune tolerance by suppressing toll-like receptor (TLR) signals. NOD2 recruits the autophagy protein ATG16L1 to the plasma membrane at the bacterial entry site; mutant NOD2 failed to recruit ATG16L1 to the plasma membrane and wrapping of invading bacteria by autophagosomes was impaired. Therefore, patients with CD with NOD2 variants are considered to exhibit disorders of autophagy. When the mechanism of autophagy is impaired, lipopolysaccharides and damage-associated molecular patterns trigger signaling by stimulating TLR and NOD-like receptors, tumor necrosis factor (TNF), and other inflammatory cytokines. They also stimulate caspase-1 causing interleukin (IL)-1β and IL-18 cleavage from precursors, which promotes extracellular secretion (inflammasomes). In an experiment using mice knocked out for ATG16L1, which encodes ATG16L1, the protein necessary for the autophagic recruitment, TLR and TNF stimulation led to abnormal inflammasome activity in macrophages and other innate immunity cells.

ATG16L1 is a homolog of ATG16 that was first reported by Mizushima et al. Along with AT5 and ATG12, this molecule is required to form autophagosomes. Prescott et al. reported that the incidence of

**Table 1 Genetic variants related to inflammatory bowel disease and autophagy**

| Gene   | Chromosomal site | Relation to autophagy                          |
|--------|------------------|-----------------------------------------------|
| NOD2   | 16q12.1          | Intracellular bacterial sensing               |
| ATG16L1| 2p37.1           | Autophagosome formation                       |
| IRGM   | 5q33.1           | Phagosome maturation                          |
| IL-23R | 1p31.3           | Through effects on IL-1 secretion             |
| XIAP   | Xq25             | Physiological inhibitor of autophagy          |
| LRRK2  | 12q12            | Autophagosomal-lysosomal degradation          |
| ULK1   | 12q24.33         | Regulated by TORC1 and AMPK                   |
| VDR    | 12q13.11         | Regulate the expression of NOD2               |
| MTMR3  | 22q12.2          | Autophagosome formation                       |

**Figure 1 Autophagy mechanism.** The autophagy pathway. During this process, the endoplasmic reticulum or other membranous cellular structures respond to stimuli by generating a double-membrane structure called a phagophore. On this phagophore, ATG16L1 forms a complex with an ATG5-ATG12 conjugate, which multimerizes and then lipidates LC3 (LC3-II). Simultaneously, the phagophore elongates to envelop the cytoplasm or organelle to be degraded, forming an autophagosome, a unique double-membrane organelle. The outer membrane of the autophagosome then fuses with a lysosome to form an autolysosome, and the inner membrane degrades and absorbs its contents.
CD was likely to be two times higher in people with the T300A variant, an ATG16L1 variant with a threonine-to-alanine substitution at amino-acid position 300. Later, a meta-analysis of 25 studies showed that T300A caused disease susceptibility to CD\textsuperscript{[33]}. However, no significant difference was observed in an analysis of patients from Japan, South Korea, and China from 25 studies. This suggests that European and American patients exhibit different genetic factors compared to Asian patients, as is seen with NOD2. Moreover, a meta-analysis of 14 studies on UC reported an odds ratio of 1.06, or almost no difference\textsuperscript{[33]}.

The report that ATG16L1 is a CD-susceptibility gene was a groundbreaking discovery suggesting a role for autophagy in the onset of IBD. Since then, several researchers have published studies on the link between ATG16L1 and IBD.

Paneth cells are a specialized type of epithelial cell that are involved in innate immunity in the small intestine. When they come into contact with bacteria or other antigens, these cells release secretory granules containing antimicrobial peptides and a variety of proteins. In 2008, Cadwell et al\textsuperscript{[35]} engineered a mouse with low expression of ATG16L1 (Atg16L1\textsuperscript{HM} mouse). Tissue analysis did not find lysozymes that are normally seen in the ileal mucosa, but found abnormal Paneth cell granule secretion. Moreover, they analyzed Paneth cells in non-inflamed areas of the ileum in patients with CD homozygous for the ATG16L1 variant T300A, and found abnormal Paneth cells that strongly resembled those observed in Atg16L1\textsuperscript{HM} mice. This suggests that ATG16L1 may also play an important role by suppressing Paneth cells in humans. In a relatively recent study, Lassen et al\textsuperscript{[35]} generated a knock-in mouse model expressing ATG16L1\textsuperscript{T300A}. Such mice do not develop spontaneous inflammation, although they exhibit morphological defects in both Paneth cells and goblet cells. Furthermore, the presence of the T300A mutation in ATG16L1 leads to aberrant functionality of Paneth cells. These findings indicate the reason there is believed to be a close relationship between ATG16L1 variants and Paneth cells.

Further, Murthy et al\textsuperscript{[36]} reported that ATG16L1 amino-acid positions 296 to 299 form a caspase cleavage motif, which greatly increases ATG16L1 sensitivity when the cellular stress response activates caspase-3 in the presence of the T300A variant. This may result in impaired autophagy, leading to CD onset, and suggests that ATG16L1 plays a role at the molecular level in CD onset.

In 2010, Cadwell et al\textsuperscript{[37]} reported interesting data on role of ATG16L1 by using Atg16L1\textsuperscript{HM} mice infected with MNV CR6, a species of mouse norovirus. MNV CR6-infected Atg16L1\textsuperscript{HM} mice showed abnormal secretion of Paneth cell granules, similar to that described above. This was not observed in wild-type mice without an ATG16L1 variant, or in mice infected with a different MNV strain or with inactivated MNV. Administration of dextran sulfate sodium (DSS) to these infected mice led to pathology similar to that observed in human patients with CD: inflammation extending to the muscle layer and mesentery, and atrophy of the ileal villi, neither of which has been previously reported with DSS colitis. These symptoms were significantly suppressed by administering TNF-α antibodies or antibiotics. A recent report suggested that ATG16L1 polymorphisms promote disease through defects in “sensing” protective signals from the microbiome, defining a potentially critical gene-environment etiology for IBD\textsuperscript{[38]}.

These data suggest that in addition to ATG16L1 variants, CD onset is influenced by a complex variety of environmental factors, including viral infections and enterobacteria.

**IRGM**

In a 2007 GWAS, Parkes et al\textsuperscript{[39]} reported that the IRGM gene on chromosome 5q33.1 was a CD-susceptibility gene. In humans, IRGM is a 20 kDa protein formed from 181 amino acids that is expressed in the large intestine, small intestine, and lymphocytes. IRGM is related to bacterial killing, vascular trafficking and acidification, phagosome maturation, and virus-induced autophagy. Moreover, it is known to be involved in controlling intracellular *Mycobacterium tuberculosis* by autophagy in macrophages\textsuperscript{[40]}. A small nuclear polymorphism (SNP) with susceptibility is adjacent to IRGM, but detailed sequencing of IRGM did not reveal any CD-related variants with modified amino acids. This suggests the possibility that changes of IRGM expression, transcript splicing, or the ratio of translation of the protein are related to the development of CD.

In 2008, McCarroll et al\textsuperscript{[41]} discovered a 20 kb deletion polymorphism upstream from IRGM that was linked to an SNP correlating with CD. In addition, they reported that the expression of IRGM suppressed autophagy of intracellular bacteria, which has been linked to CD, suggesting a role in the pathology of CD.

Recently, Rufini et al\textsuperscript{[42]} reported that IRGM polymorphisms were important for CD susceptibility and phenotype modulation (fibrostricturing behavior, ileal disease, perianal disease, and intestinal resection).

**IL-23R**

IL-23 is a heterodimeric cytokine produced by activated macrophages and dendritic cells. It consists of two subunits, a p40 subunit, shared with IL-12, and a specific IL-23 subunit called p19\textsuperscript{[43,44]}. It has been shown that IL-23 is involved in the initiation of the innate and adaptive immune activation that characterizes IBD. It binds a complex of IL-23 receptor (IL-23R) and IL-12Rβ subunits. IL-23R is predominantly expressed on activated/memory T cells, T-cell clones, natural killer cells and, at low levels, in monocytes, macrophages, and dendritic cell populations\textsuperscript{[45,46]}.
Recent studies have shown association of the IL-23R gene with chronic inflammatory diseases, especially IBD\(^{[47,48]}\). It is also reported that autophagy regulates IL-23 secretion and innate T cell responses through effects on IL-1 secretion\(^{[49]}\).

**XIAP**

XIAP (X-linked inhibitor of apoptosis) is one of several inhibitor of apoptosis proteins (IAPs). IAPs were initially identified in baculoviruses, where they prevent defensive apoptosis of host cells\(^{[50]}\). Among the mammalian IAPs, XIAP is the most extensively studied and best characterized. XIAP has the most potent anti-apoptotic ability\(^{[51]}\), which is believed to be primarily related to direct binding and inhibiting of caspases, the apoptotic proteases that are responsible for the initiation and execution of apoptosis\(^{[52]}\). Huang et al\(^{[53]}\) showed that XIAP is a physiological inhibitor of autophagy, and has been associated with a variety of diseases that have been linked to autophagy. XIAP is related to X-linked lymphoproliferative syndrome type 2 (XLP2), a type of primary immunodeficiency. However, a genetic analysis performed by Zeissig et al\(^{[54]}\) found XIAP variants in only 4% of male patients with childhood-onset CD. Recently, Schwerd et al\(^{[55]}\) showed impaired antibacterial autophagy links XIAP with colonic biopsy specimens from inflamed CD. The CD-associated SNP is located upstream of the coding sequence of LRRK2\(^{[56,57]}\) coding sequence of LRRK2\(^{[58]}\). LRRK2 is known to be expressed only in mucosal lymphocytes in the colonic mucosa; whereas inflammation correlates negatively with colonic LRRK2 expression levels were found to be significantly upregulated in colonic biopsy specimens from inflamed tissues of patients with CD\(^{[59]}\). LRRK2 is known to be expressed only in mucosal lymphocytes in the colonic mucosa, but little else is known about it.

**MTMR3**

MTMR3 (myotubularin-related protein 3) plays a role in autophagosome formation\(^{[60]}\). The myotubularin family is a class of PI3-phosphatases that regulate several physiological and pathophysiological phenomena, including endosomal trafficking, apoptosis, autophagy, and muscle development. As a member of this family, MTMR3 has been considered to play a negative role in the initiation stage of autophagy. Recent reports indicate that MTMR3 has at least two opposite functions in the autophagy pathway, inhibition of mechanistic target of rapamycin complex 1 (mTORC1) and reduction of local PI3P levels\(^{[61,62]}\). In this regard, the function of MTMR3 in autophagy remains unclear.

**ROLE OF AUTOPHAGY IN IBD THERAPY AND FUTURE PROSPECTS**

Widely used therapeutic agents for IBD include...
steroids and 5-aminosalicylic acid (5-ASA), as well as immunomodulatory drugs such as azathioprine, and biologicals such as anti-TNF-α formulations. The process of autophagy is closely related to each of these existing therapeutic agents. The following sections summarize these relationships (Table 2).

### 5-ASA

The mechanism of action of 5-ASA has been described in several studies. The suppression of peroxisome proliferator-activated receptor gamma (PPARγ) due to the production of inflammatory cytokines is said to contribute to the intestinal inflammation seen in patients with IBD[69]. 5-ASA is considered to exert its anti-inflammatory action by acting on PPARγ in epithelial cells, and by regulating signal transmission from NF-κB and TLR[70]. Considering that NF-κB signaling is associated with autophagy[71], it might be that 5-ASA indirectly regulates autophagy.

### Corticosteroids

The first-line treatment to induce remission for CD and UC is often corticosteroids. Corticosteroids downregulate proinflammatory cytokines, including IL-1, IL-6, and TNFα. Furthermore, inflammatory signaling induced by NFκB is decreased by interaction with corticosteroid receptors[72], and, as noted above, NFκB signaling regulates autophagy[71]. It has also been shown that corticosteroid treatment affects mTORC1 signaling pathways[79]. It was reported that mTORC1 pathways and autophagy play an important role in the response to treatment with corticosteroids[74]. Corticosteroids are able to induce apoptosis in immature T lymphocytes, as these cells lack the inhibitor of apoptosis protein Bcl-2. It has been shown that overexpression of Bcl-2 in immature T lymphocytes can increase autophagy levels, presumably due to inhibition of apoptosis[75].

A relationship between corticosteroids and autophagy has been observed, not only for their therapeutic effects, but also for the adverse effects that accompany treatment. It has been shown, both in vitro and in vivo, that low doses of prednisolone and dexamethasone induce autophagy in osteocytes, and this is associated with osteocyte viability[76,77]. However, higher doses of corticosteroids induce apoptosis, suggesting that autophagy may act as a protective mechanism against the cytotoxic effects of corticosteroids[76].

### Thiopurines (azathioprine and 6-mercaptopurine)

Thiopurines, including azathioprine and 6-mercaptopurine, are immunosuppressant drugs used to maintain remission in patients with IBD[78]. Thiopurines and autophagy have also been shown to be correlated by the adverse effects of treatment. The thiopurine S-methyltransferase (TPMT) genetic polymorphism is important for thiopurine metabolism. Individuals with inherited decreases in TPMT activity, mainly as a result of the effects of the TPMT*3A allele (minor allele frequency in Caucasians of approximately 5%)[79], are at greatly increased risk for severe life-threatening myelosuppression when treated with “standard” doses of thiopurine drugs[80-83]. It was shown that autophagy might represent an important route for the clearance of TPMT*3A aggregates and/or aggregate precursors[84]. Due to the severe adverse effects of thiopurines, a potential protective role for autophagy in hepatocytes has been investigated; it has been shown that autophagy has a protective role in hepatocytes during thiopurine therapy[80].

### Immunomodulatory drugs (cyclosporine A, FK506, methotrexate)

Cyclosporine A (CsA), FK506, and methotrexate (MTX) are immunomodulatory drugs used mainly as second-line treatments to induce and maintain remission in severe, steroid-refractory CD[85], with more recent evidence suggesting a role for FK506 in UC[86]. Although some evidence suggests that CsA and FK506 are involved in autophagy, no relationship has been identified between MTX and autophagy.

Several studies have shown that treatment with CsA can induce autophagy in response to toxicity (such as CsA-induced nephrotoxicity), either as a survival process or as part of a cell death mechanism[87-89]. FK506 inhibits calcineurin by forming a complex with the immunophilin FK506 binding protein 12 (FKBP12), which is involved in immunoregulation[90]. FKBP12 is also the direct target of rapamycin, an inhibitor of mTORC1. The molecular mechanism by which mTORC1 regulates autophagy in mammals is being investigated[91,92], while future research is expected to help understand the relationship between FK506 and autophagy.

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### Table 2  Therapeutic agents for inflammatory bowel disease related to autophagy

| Drug                        | Influence on autophagy | Mechanism related to autophagy |
|-----------------------------|------------------------|--------------------------------|
| 5-ASA                      | Promotion              | Through NFκB signaling pathway |
| Corticosteroid             | Promotion              | Through NFκB signaling pathway |
|                            |                        | Through mTORC1 signaling pathway |
| Thiopurine (AZA, 6-MP)     | Promotion              | Clearance of TPMT*3A aggregates and/or aggregate precursors |
| Immunomodulatory drugs    | Response to toxicity   | Protection role in hepatocytes |
| (CsA, FK506)               |                        |                                 |
| Biological drugs           | Inhibition             | Anti-TNF agents inhibit autophagy (not yet clear) |
| (IFX, ADA, etc.)           |                        |                                 |

5-ASA: 5-aminosalicylic acid; mTORC1: Mechanistic target of rapamycin complex 1; AZA: Azathioprine; 6-MP: 6-Mercaptopurine; TPMT: Thiopurine S-methyltransferase; CsA: Cyclosporine A; IFX: Infliximab; ADA: Adalimumab; TNF: Tumor necrosis factor.

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CONCLUSION

GWAS has identified several disease-susceptibility genes, and studies on the pathology and etiology of IBD are being regularly published; however, more aspects of IBD pathogenesis should be clarified. As seen in this review, autophagy plays an important role in controlling the immune system; hence drugs that regulate autophagy have received much attention as potential new therapeutic targets for IBD. Further investigation of the role of autophagy in existing IBD therapies, and development of new therapeutic agents regulating autophagy, are the needs of the hour.

REFERENCES

1. Moledoy NA, Soon IS, Rabbi DM, Ghali WA, Ferris M, Chernoff G, Benczemel EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of IBD; a systematic review. Gut 2017; 66: 1-8 [PMID: 27458280 DOI: 10.1136/gutjnl-2016-312498].
2. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Bertacchini L, Altermatt JP, Gieben I, Schade F, Duthie GW, Thomas F, Dayer JM, Seguela P, Gower-Rousseau C, Chamaillard M, Zouali H, Lesage S, Cezard JP, Bertacchini L, Altermatt JP, Gieben I, Schade F, Duthie GW, Thomas F, Dayer JM, Seguela P, Gower-Rousseau C. The role of Atg genes in inflammatory bowel disease. Gut 2017; 66: 1-8 [PMID: 27458280 DOI: 10.1136/gutjnl-2016-312498].
3. Ogura Y, Benes DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirshner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease. Nature 2001; 411: 603-606 [PMID: 11385577 DOI: 10.1038/35079107].
4. Hampe J, Franke A, Rosenberg P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Omnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzet M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 2007; 39: 207-211 [PMID: 17206669 DOI: 10.1038/ng1954].
5. Kaser A, Blumberg RS. Autophagy, microbial sensing, endoplasmic reticulum stress, and epithelial function in inflammatory bowel disease. Gastroenterology 2011; 140: 1738-1747 [PMID: 21530740 DOI: 10.1053/j.gastro.2011.02.048].
6. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010; 28: 573-621 [PMID: 20192811 DOI: 10.1146/annurev-immunol-030409-101225].
7. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011; 474: 307-317 [PMID: 21677747 DOI: 10.1038/nature10209].
8. Waterman IT, Peña AS. Familial incidence of Crohn’s disease in The Netherlands and a review of the literature. Gastroenterology 1994; 86: 449-452 [PMID: 8693011].
9. Fiocchi C. Tailoring Treatment to the Individual Patient - Will Inflammatory Bowel Disease Medicine Be Personalized? Dig Dis 2015; 33 Suppl 1: 82-89 [PMID: 26368553 DOI: 10.1159/000403708].
10. 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].
11. Ek WE, D’Amato M, Hallvarson J. The history of genetics in inflammatory bowel disease. Ann Gastroenterol 2014; 27: 294-303 [PMID: 25331623].
12. Goldstein DB. Common genetic variation and human traits. N Engl J Med 2009; 360: 1696-1698 [PMID: 19369660 DOI: 10.1056/NEJM200910151].
13. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schummm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Taylor KM, Savova K, Fidler JS, de Roos A, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Onnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzet M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 2007; 39: 207-211 [PMID: 17206669 DOI: 10.1038/ng1954].
14. Hampe J, Franke A, Rosenberg P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Omnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzet M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 2007; 39: 207-211 [PMID: 17206669 DOI: 10.1038/ng1954].
bowel diseases: a meta-analysis. *World J Gastroenterol* 2010; 16: 1258-1266 [PMID: 20222171 DOI: 10.3748/wjg.v16.i12.1258]

34 Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Ke W, Carrero JA, Hunt S, Stone GD, Brunt EM, Xavier RJ, Sleekman 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]

35 Lassen KG, Kuballa P, Conway KL, Patel KK, Becker CE, Peloquin JM, Villablancja EJ, Norman JM, Liu TC, Heath RJ, Becker ML, Faghihari L, Horn H, Mercer J, Vilain OH, Jaffe ID, Shanfi AJ, Bhan AK, Carr SA, Daly MJ, Virgin HW, Schreiber SL, Stappenbeck TS, Xavier RJ. Atg16l1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. *Proc Natl Acad Sci USA* 2014; 111: 7741-7746 [PMID: 24821797 DOI: 10.1073/pnas.1407011111]

36 Murthy A, Li Y, Peng I, Reichelt M, Katakam AK, Noubade R, Rosie-Girrma M, DeVoss D, Diehl L, Graham RR, van Lookeren Campagne M, A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. *Nature* 2014; 506: 456-462 [PMID: 24553140 DOI: 10.1038/nature13044]

37 Cadwell K, Patel KL, Maloney NS, Liu TC, Ng AC, Storer CE, Head RD, Xavier R, Stappenbeck TS, Virgin HW. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16l1 phenotypes in intestine. *Cell* 2010; 141: 1131-1145 [PMID: 20602927 DOI: 10.1016/j.cell.2010.05.009]

38 Yano T, Katori S, Kubo OA, Parham C, Cunha LD, Park S, Yang M, Lu Q, Orchard Q, Li QZ, Yan M, Janke L, Guy C, Linkermann A, Virgin HW, Green DR. Intracellular recognition of pathogens and autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Autophagy* 2016; 12(12): 1679-1688 [PMID: 26537199 DOI: 10.1002/aut.20697]

39 Mizushima N, Kuma T, Ohsumi Y. Apg16p is required for the function of the Apg12-Apg5 conjugate in the yeast autophagy pathway. *EMBO J* 1999; 18: 3888-3896 [PMID: 10406794 DOI: 10.1093/emboj/18.14.3888]

40 Matsuoka Y, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Taka T, Natsume T, Ohsumi Y, Yonemori T. Mouse Apg16l1, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J Cell Sci* 2003; 116: 1679-1688 [PMID: 12665549]

41 Prescott NJ, Fisher SA, Franke A, Hampe J, Omnie CM, Soars D, Bagnall R, Mirza MM, Sanderson J, Forbes A, Mansfield JC, Lewis CM, Schreiber S, Mathew CG. A nonsynonymous SNP in Atg16l1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *J Hepatol* 2011; 55: 62-69 [PMID: 21586321 DOI: 10.1016/j.jhep.2010.05.009]

42 Cheng JF, Ning YJ, Zhang W, Lu ZH, Lin L. T300A polymorphism of ATG16L1 and susceptibility to inflammatory bowel disease: a meta-analysis. *World J Gastroenterol* 2010; 16: 1258-1266 [PMID: 20222171 DOI: 10.3748/wjg.v16.i12.1258]
D. Rennick DM, Kastelein RA, de Waal Malefyt R, Moore KW. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12beta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002; 168: 5699-5708 [PMID: 12023639 DOI: 10.4049/jimmunol.168.11.5699]

47 Bianco AM, Girardelli M, Tommussini A. Genetics of inflammatory bowel disease from multifactorial to monogenic forms. World J Gastroenterol 2015; 21: 12296-12310 [PMID: 26604638 DOI: 10.3748/wjg.v21.i43.12296]

48 Fujuyo Y, Yamasaki K, Takahashi A, Esaki M, Kawaguchi T, Takae T, Matsumoto T, Matsui T, Tsurumi Y, Kiyohara Y, Kitazato T, Kubo M. Genetic characteristics of inflammatory bowel disease in a Japanese population. J Gastroenterol 2016; 51: 672-681 [PMID: 26511940 DOI: 10.1007/s00535-015-1135-3]

49 Peral de Castro C, Jones SA, Ni Cheallaigh C, Heerden CA, Williams L, Winter J, Lavelle EC, Mills KH, Harris J. Autophagy regulates IL-23 secretion and innate T cell responses through effects on IL-1 secretion. J Immunol 2012; 189: 4144-4153 [PMID: 22972933 DOI: 10.4049/jimmunol.1201946]

50 Crook NE, Clem RJ, Miller LK. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. J Virol 1993; 67: 2168-2174 [PMID: 8445726]

51 Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. EMBO J 2001; 20: 988-994 [PMID: 11706456 DOI: 10.1038/sj.embr.7400795]

52 Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy to pathogens. LRRK2 is involved in the IFN-gamma response and host response to pathogens. J Immunol 2010; 185: 5577-5585 [PMID: 20921534 DOI: 10.4049/jimmunol.1000548]

53 Henckaerts L, Cleynen I, Brinar M, John JM, Van Steen K, Rutgeerts P, Vermeire S. Genetic variation in the autophagy gene ULK1 and risk of Crohn’s disease. Inflamm Bowel Dis 2011; 17: 1392-1397 [PMID: 21560199 DOI: 10.1002/ibd.21486]

54 Kim J, Kundu M, Violett B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011; 13: 132-141 [PMID: 21258367 DOI: 10.1038/ncb2152]

55 Lee JW, Park S, Takahashi Y, Wang HG. The association of AMPK with ULK1 regulates autophagy. PLoS One 2010; 5: e13594 [PMID: 2072212 DOI: 10.1371/journal.pone.0013594]

56 Pei FH, Wang YJ, Gao SL, Liu BR, DU YJ, Liu W, Hu YH, Zhao LX, Chi BR. Vitamin D receptor gene polymorphism and ulcerative colitis susceptibility in Han Chinese. J Dig Dis 2011; 12: 90-98 [PMID: 21401893 DOI: 10.1111/j.1751-2980.2011.00483.x]

57 Simmons JD, Mullighan C, Welsh KJ, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn’s disease susceptibility. Gut 2006; 47: 211-214 [PMID: 15986192]

58 Wu Z, Zhang YG, Lu R, Xia Y, Zhou D, Petrof EO, Clau C, Chen D, Chang EB, Carole M, Sun J. Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis. Gut 2015; 64: 1082-1094 [PMID: 25080448 DOI: 10.1136/gutjnl-2014-307436]

59 Abreu-Delgado Y, Isidro RA, Torres EA, Gonzalez A, Cruz ML, Isidro AA, Gonzalez-Kedan CI, Medero P, Appleyard CB. Serum vitamin D and colonic vitamin D receptor gene expression in inflammatory bowel disease. World J Gastroenterol 2016; 22: 3581-3591 [PMID: 27053850 DOI: 10.3748/wjg.v22.i13.3581]

60 Henderson P, van Lierenberg J, Wilson DC, Satsangi J, Russell RK. Genetics of childhood-onset inflammatory bowel disease. Inflamm Bowel Dis 2011; 17: 346-361 [PMID: 20839313 DOI: 10.1002/ibd.21283]

61 Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lee CW, Balschun T, Lee J, Roberts R, Anderson CA, Bis JC, Bumpstead S, Ellingshaus D, Festen EM, Georges M, Green T, Haritunians T, Jostins L, Latino 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, Marie LA, Bayless TM, Brand S, Coven C, Cohen AM, Colombel JF, Cottone M, Stronati L, Denson T, De Vos M, D’Inca R, Dubinsky M, Edwards C, Florin T, Franckonit D, Geary R, Glaz J, van Gossuin A, Guthery SL, Halfvarson J, Verspaget HW, Hugot JP, Karban A, Lankau D, Lawrance I, Lemann M, Levine A, Liabolle C, Louis E, Mortaw C, Newman W, Panés J, Phillips A, Proctor DD, Regueiro M, Russell R, Rutgeerts P, Sanderson J, Sans M, Seibold F, Steinheit AR, Stokkers PC, Torkvist L, Kullak-Ublick G, Wilson DC, Wernemann B, Wilms M, Yarduna A, Yuce A, Zinman S, Zinsmeister AR, Satsangi J, Sandor P, Russell DC, Anthony D, D’Amato M, Weersma RK, Kugathasan S, Griffiths AM, Mansfield JC, Vermeire S, Duerr RH, Silverberg MS, Satsangi J, Schreiber S, Cho JH, Annesse 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.1177]

62 Lahiri A, Hedl M, Abraham C. MDR3 risk allele enhances innate receptor-induced signaling and cytokine production by decreases autophagy and increasing caspase-1 activation. Proc Natl Acad Sci USA 2015; 112: 10461-10466 [PMID: 26240347 DOI: 10.1073/pnas.1501752112]

63 Yamamoto-Furusho JK, Peñaloza-Coronel A, Sánchez-Muñoz F, Barreto-Zuñiga R, Dominguez-Lopez A. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression is downregulated in patients with active colitis. Inflamm Bowel Dis 2011; 17: 680-681 [PMID: 20848495 DOI: 10.1002/ibd.21322]

64 Rousseaux C, Lefebvre B, Dubuquoy L, Lefebvre F, Romano O, Auwerx J, Metzger D, Wahli W, Naccari GC, Chapatte P, Farce A, Bulois P, Cortot A, Colombel JF, Desreumaux P. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. J Exp Med 2005; 201: 1205-1215 [PMID: 15824083 DOI: 10.1084/jem.20041948]

65 Chacon-Cabrer a A, Fernoselle C, Uttegje AJ, Mateu-Jimenez M, Diament MJ, de Kieroff ED, Sandri M, Barreiro E.
Pharmacological strategies in lung cancer-induced cachexia: effects on muscle protein synthesis, structure, and weakness. J Cell Physiol 2014; 229: 1660-1672 [PMID: 24615622 DOI: 10.1002/jcp.24611]

Kue nzig ME, Rezaie A, Seow CH, Otley AR, Steinhardt AH, Griffiths AM, Kaplan GG, Benchimol EL. Budesonide for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev 2014; (8): CD002913 [PMID: 25141071 DOI: 10.1002/14651858.CD002913.pub3]

Polman JA, Hunter RG, Speksnijder N, van den Oever JM, Kero MG, Ot B, Munteanu FS, de Kleot ER. Datson NA. Glucocorticoids modulate the mTOR pathway in the hippocampus: differential effects depending on stress history. Endocrinology 2012; 153: 4317-4327 [PMID: 22778218 DOI: 10.1210/en.2012-1255]

Wang H, Kubica N, Ellisen LW, Jefferson LS, Kimball SR. Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of REDD1. J Biol Chem 2006; 281: 39128-39134 [PMID: 1704751 DOI: 10.1074/jbc.M610023200]

Swerdlov S, McColl K, Rong Y, Lam M, Gupta A, Distelhorst W, Horner M, Chu YQ, Kalwinsky D, Roberts WM, Wang L, Burgess RJ, Weinshilboum RM. Thiopurine S-methyltransferase pharmacogenetics: autophagy as a mechanism for variant allozyme degradation. Pharmacogenet Genomics 2008; 18: 1083-1094 [PMID: 18820593 DOI: 10.1097/ FPC.0b013e328333e03f]

Markowitz J, Grancher K, Kohn N, Daum F. Immunosupulatory therapy for pediatric inflammatory bowel disease: changing patterns of use, 1990-2000. Am J Gastroenterol 2002; 97: 928-932 [PMID: 12003428 DOI: 10.1111/j.1572-0241.2002.00611.x]

Nuki Y, Esaki M, Asano K, Maehata Y, Umeno J, Moriyama T, Nakamura S, Matsumoto T, Kitazono T. Comparison of the therapeutic efficacy and safety between tacrolimus and infliximab for moderate-to-severe ulcerative colitis: a single center experience. Scand J Gastroenterol 2014; 49: 1037-1045 [PMID: 26814866 DOI: 10.3109/03005652.2016.1138239]

Pallet N, Bouvier N, Legendre C, Gillieron J, Codogno P, Beauce P, Thervet E, Anglicheau D. Autophagy protects renal tubular cells against cyclosporine toxicity. Autophagy 2008; 4: 733-781 [PMID: 1862650]

Kimura T, Takahashi A, Takabatake Y, Namba T, Yamamoto T, Kaimori JY, Matsui I, Kitamura H, Niumura F, Matsuoka T, Soga T, Rakugi H, Isaka Y. Autophagy protects kidney proximal tubule epithelial cells from mitochondrial metabolic stress. Autophagy 2013; 9: 1876-1886 [PMID: 24128672 DOI: 10.4161/auto.24148]

Kim HS, Choi SI, Jeong EB, Yoo YM. Cyclosporine A induces apoptotic and autophagic cell death in rat pithitary GH3 cells. PlaS One 2014; 9: 1-108981 [PMID: 25292910 DOI: 10.1371/journal. pone.0108981]

Liu J, Albers MW, Wandel LS, Luu S, Alberg DG, Belshaw PJ, Cohen P, MacKintosh C, Kee CB, Schreiber SL. Inhibition of T cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. Biochemistry 1992; 31: 3896-3901 [PMID: 1373650]

Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, Kim DH. ULK-AgI3-FIP200 complexes mediate mTOR signaling for autophagy induction. Autophagy 2010; 4: 1229-1234 [PMID: 20980264 DOI: 10.1038/jbc.M109057320]

de Riddler L, Waterman M, Turner D, Bronsky J, Hauer AC, Dias JA, Strisciuglio C, Ruemmele FM, Levine A, Lionetti P. Use of Biosimilars in Paediatric I nflammatory Bowel Disease: A Position Statement of the ESPGHAN Paediatric IBD Porto Group. J Pediatr Gastroenterol Nutr 2015; 61: 503-508 [PMID: 26154301 DOI: 10.1097/MPG.0000000000000903]

Connor AM, Mahomed N, Gandhi R, Keithen EC, Berger SA. TNFα modulates protein degradation pathways in rheumatoid arthritis synovial fibroblasts. Arthritis Res Ther 2012; 14: R62 [PMID: 22417670 DOI: 10.1186/ar3778]

Keller CW, Fukken C, Turville SG, Lünemann A, Schmidt J, Münz C, Lünemann JD. TNFα-Alpha induces macroautophagy and regulates MHC class II expression in human skeletal muscle cells. J Biol Chem 2011; 286: 3970-3980 [PMID: 20980264 DOI: 10.1074/jbc.M110.159329]

Cha IH, Hwang JR, Kim HY, Choi SJ, Oh SY, Roh CR. Autophagy induced by tumor necrosis factor α mediates intrinsic apoptosis in trophoblastic cells. Reprod Sci 2014; 21: 612-622 [PMID: 24198074 DOI: 10.1177/193719113508816]

Nys K, Agostinis P, Vermeire S. Autophagy: a new target or an old strategy for the treatment of Crohn’s disease? Nat Rev Gastroenterol Hepatol 2013; 10: 395-401 [PMID: 23591407 DOI: 10.1038/nrgastro.2013.66]

Reviewers: Bezmín Abadi AF, de Almeida Araujo EJ, Patial V
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