Understanding Crohn's disease through genetics

Aditya Murthy* and Menno van Lookeren Campagne*

Department of Immunology; Genentech Inc.; San Francisco, CA USA

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) with an eitologically complex pathology. Current evidence implicates dysregulation of the immune response to intestinal microflora and a parallel defect in maintenance of the epithelial barrier.[1] The host genetic makeup is a key variable that underlies risk of disease, as highlighted by genome-wide association studies (GWAS). This, along with positive evidence for an impact of industrialization on IBD incidence strongly suggests that, in addition to genetic factors, host – environment interactions need to be tightly controlled to prevent mucosal inflammation.[2]

GWAS have highlighted disease-associated susceptibility loci associated with regulators of innate immunity (NOD2/CARD15, RIPK2, TLR4, CARD9, ICOSLG, CD40, LGALS9), adaptive immunity (IL23R, IL18RAP, RORC, IL10, PTPN22, IFNAR1, STAT3), epithelial barrier function/stress response (MUC1, XBP1, IBD5), and autophagy (ATG16L1, LRRK2, IRGM).[1] Autophagy is a highly conserved and fundamental cytosolic recycling pathway that has the potential to impact the numerous cellular processes including cellular metabolism, turnover of cytosolic cargo (i.e. damaged organelles, cytotoxic aggregates), growth factor/cytokine secretion and intracellular pathogen clearance that are perturbed in Crohn's disease.[2]

A missense variant in the autophagy gene ATG16L1 (rs2241880) has been consistently associated with increase incidence of Crohn's disease. This variant results in an amino acid change of Thr-300-Ala (T300A) in exon 9 of human ATG16L1.[3] By performing multiple sequence alignments of ATG16L1 exon 9 across species, the presence of a highly conserved putative caspase cleavage substrate spanning amino acids 296-299 (296-DNVD-299) was observed.[4] Previous work has demonstrated that Glycine, Serine, or Alanine are preferred at the P1' site of a caspase cleavage substrate.[5] We hypothesized that the T300A variant would therefore be more sensitive to Caspase-mediated cleavage at D299. This was demonstrated first by cell-free assays of *in vitro* translated human ATG16L1β harboring the non-risk (T300), risk (A300) and non-cleavable (D299E) variants in exon 9. Next, using primary macrophages from healthy donors and a murine knock-in model of the T300A variant, Caspase 3 activation by death receptor stimulation was shown to result in enhanced ATG16L1 processing in the presence of the Crohn's disease risk variants. Knock down of individual effector caspases revealed Caspase 3 as the relevant protease needed for ATG16L1 processing, and this was confirmed in primary macrophages cultured from Caspase 3 knockout mice. Thus, the presence of a Crohn's disease risk variant and caspase-activating conditions results in enhanced degradation of ATG16L1, as also demonstrated by Lassen et al. (**Fig. 1**).[6] Compatible with the importance of this pathway in disease, loss-of-function mutations in X-linked Inhibitor of Apoptosis (XIAP), a negative regulator of Caspase 3 activation and NOD1/2 signaling, have been associated inflammatory bowel disease.[7]

While autophagy is constitutively active as a cellular recycling program, it is strongly upregulated upon cellular stress conditions such as nutrient starvation or infection with intracellular pathogens. Consistently, defective autophagosome biogenesis in murine and human cells expressing the T300A variant of ATG16L1 was correlated with induction of Caspase 3 activation upon starvation.[4] Defective autophagosome biogenesis further impacts bacterial clearance by xenophagy, an evolutionarily conserved, cell autonomous anti-bacterial defense program. Host-microbe interactions in the intestinal microenviroment in turn strongly influence the onset and progression of IBD by controlling inflammation and dysbiosis, a state where pathogenic microbial strains bloom; these are usually suppressed under healthy conditions.[2] Defective clearance of intracellular bacteria, dysbiosis and inflammation act in concert to precipitate IBD. Genetics further contributes to disruption of intestinal homeostasis as the T300A variant of ATG16L1 impairs clearance of the enteric pathogen *Yersinia enterocolitica* in the ileum, a region of the small intestine commonly affected in Crohn's disease. In T316A knock-in mice, reduced clearance of *Y.enterocolitica* was evidenced by increased colonization in mesenteric lymph nodes following oral administration of the pathogen. Thus the Crohn's disease-associated variant in ATG16L1 results in defective autophagy following nutrient starvation or infection with an ileal pathogen, further emphasizing the importance of this pathway in disease.[4,6]
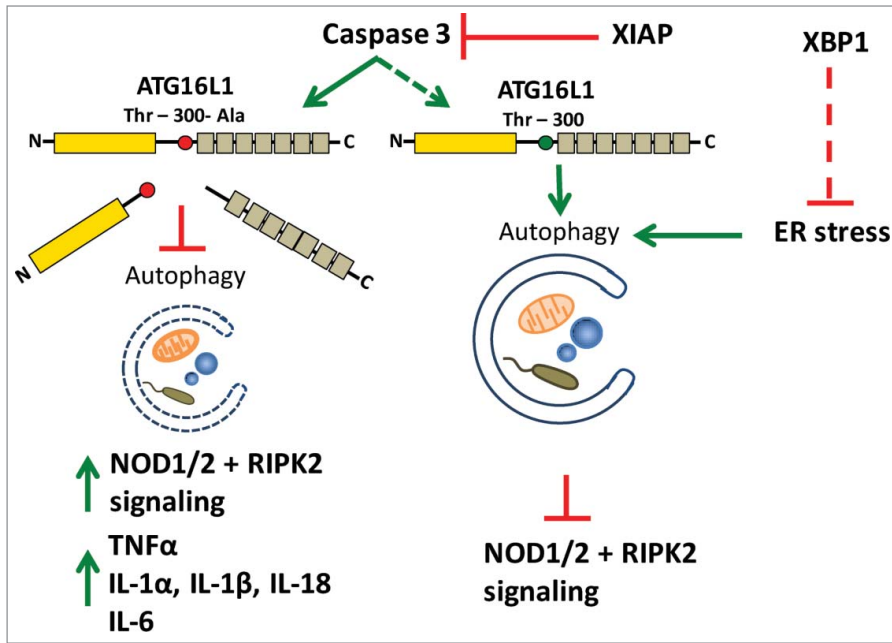
Figure 1. Interaction between IBD-associated genes coordinates gut inflammation. The T300A variant sensitizes ATG16L1 to processing by Caspase 3, which is inhibited by XIAP. Autophagy regulates processes such as pathogen sensing and ER-stress responses arising from misfolded proteins. Polymorphisms in NOD2 or loss of the ER-stress regulator XBP1 may coordinate with the T300A variant of ATG16L1 to sustain an inflammatory intestinal microenvironment .

macrophages produced elevated levels of IL-1β, TNF and IL-6 upon infection with live bacteria. Second, TNF levels correlated with intracellular bacterial survival, as no elevation in TNF was observed when T300A knock-in macrophages were infected with heat-killed bacteria. On the other hand, IL-6 and IL-1β levels were elevated even under comparable loads of killed bacteria.[5] These observations raise the intriguing possibility that innate inflammatory signaling downstream of specific pathogen stimuli may also be regulated by ATG16L1 genotype.

Recent studies have proposed functional cooperation between IBD associated genes (**Fig. 1**). Interactions between NOD2 and ATG16L1 seem to regulate cytokine production following recognition of bacterial peptidoglycan, whereas autophagy mitigates chronic ER-stress induced by loss of XBP1 in intestinal epithelial cells.[2] These studies reveal the molecular pathways that are regulated at multiple nodes by IBD associated genes and provide critical clues for new therapeutic targets and accompanying diagnostics.

The successes of adalimumab and etanercept, inhibitors of tumor necrosis factor α, in treating IBD, highlights a central role of this inflammatory cytokine in Crohn's disease.[2] Further upstream, understanding how autophagy proteins are implicated in sensing pathogens and regulating inflammatory responses is critical for understanding the impact of the genetic variants on disease. Using live and heat-killed *Y.enterocolitica*, we made 2 distinct observations: First, T300A knock-in

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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