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ATG16L1 Crohn's disease risk stresses the endoplasmic reticulum of Paneth cells

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With 163 genetic loci identified to date, genome wide association studies have revealed the significant genetic complexity associated with risk for IBD.[1] Autophagy, pinpointed through the discovery of risk variants in *ATG16L1*[2] and other autophagy genes,[1] remains one of the most interesting disease-specific revelations of Crohn's disease genetics. Around a fifth of the overall genetic risk yet known for Crohn's disease may lie in genes that are directly involved in autophagy,[1] including *NOD2*, which has recently been exposed as an autophagy inducer,[3] a decade after it was reported as the first Crohn's disease risk gene.[4] Given this, chances are that further autophagy regulators might hide in the many currently unexplored genetic risk loci. As an ostensibly disease-specific, genetically affected biological process, autophagy stands out within all the genetic heterogeneity of IBD,[1] where the vast majority of risk loci (and computationally inferred genes and pathways) is shared between Crohn's disease and UC,[1] and with other immune-related diseases that have phenotypically little in common with Crohn's disease.[5] Understanding how autophagy risk genes and their variants contribute to Crohn's disease pathogenesis holds the promise of unravelling some of the secrets of this disease.

Autophagy (or `self-eating') represents a basic function of all cell types wherein it serves an essential homeostatic function or is induced by the needs associated with metabolic changes such as starvation or the removal of microbes (xenophagy) (reviewed in ref 6). *ATG16L1* or *NOD2* risk variants, which are functionally hypomorphic due to missense and frame-shift mutations, are associated with a secretory defect in Paneth cells in patients with Crohn's disease,[78] and this phenotype correlates well with that observed in murine genetic models.[89] Although genetic deletion of *Nod2* has been associated with changes in intestinal microbial ecology,[10] neither hypomorphic or absent ATG16L1 or NOD2 function leads to any form of spontaneous intestinal inflammation in murine model systems.[89] This has left the critical

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question unresolved whether these alterations in Paneth cell granule formation are mechanistically relevant or just an epiphenomenon.

The unfolded protein response (UPR) is another fundamental and essential cell biological process critical in allowing a cell to deal with its secretory responsibilities and in the resolution of endoplasmic reticulum (ER) stress (reviewed in ref 11). Recently, an important clue in understanding the role of autophagy in Crohn's disease came from a study which demonstrated that autophagy compensates for ER stress in Paneth cells.[12] In that study, ER stress was induced via deletion of the UPR transcription factor X box binding protein-1 (*Xbp1*) in the intestinal epithelium,[12] which resulted in autophagosome formation in Paneth cells via a mechanism that involved eukaryotic translation initiation factor 2α (eIF2α).[12] When this compensatory function of autophagy was lost via genetic deletion of *Atg16l1* or *Atg7* in the intestinal epithelium, mice developed discontinuous transmural ileitis, with knife-like inflammation to the muscularis propria and serosa, which was reminiscent of early fissuring ulcerations and fistulous tracts typically seen in Crohn's disease.[12] This was a consequence of massive overactivation of the ER stress sensor inositol requiring enzyme 1α (IRE1α) due to the inability of ATG16L1 and autophagy to remove inflamed ER membranes.[12] IRE1α in turn set in motion a cascade of events that involved nuclear factor κB (NFκB) signalling in intestinal crypts along with epithelial cell death downstream of a microbial signal, which converged on spontaneous inflammation that required tumour necrosis factor receptor type 1 signalling.[12] Importantly, genetic experiments unequivocally revealed that Paneth cells were the originators of this Crohn's disease-like inflammation in the small intestine of mice.[12]

Deuring *et al*[13] report an intriguing study which demonstrates that carriers of the *ATG16L1* risk variant exhibit ER stress in their Paneth cells. Specifically, the authors show that patients with quiescent Crohn's disease and healthy individuals homozygous or heterozygous for the *ATG16L1* risk allele exhibit staining for GRP78, an ER chaperone and specific marker of ER stress, specifically in Paneth cells.[13] Moreover, they demonstrate that in patients harbouring the *ATG16L1* risk variant, the phosphorylated form of eukaryotic translation initiation factor 2α (eIF2α) is present in Paneth cells, and elevated levels of NFκB p65 are detectable in crypts that exhibit evidence of ER stress.[13] This careful study uses genetic stratification to elegantly demonstrate that the hypomorphic *ATG16L1* risk allele is associated with ER stress in Paneth cells. These findings are in remarkable congruence with observations made in the aforementioned murine model,[12] where genetic deletion of *Atg16l1* in the intestinal epithelium resulted in GRP78 expression in Paneth cells along with increased splicing of *Xbp1*,[12] which is a consequence of IRE1α activation. The current study by Deuring *et al*[13] lends substantial weight to the validity of the murine model and the cause-effect relationships and pathophysiological insights derived through that model, including the identification of Paneth cells as originators of intestinal inflammation in small intestinal Crohn's disease.[12]

The induction of ER stress as a consequence of the hypomorphic function of the *ATG16L1* risk gene product does fortunately not suffice to induce Crohn's disease, as revealed from the data by Deuring *et al*[13] as well as from the murine model system of $Atg16l1^{\Delta IEC}$ or $Atg16l1^{HM}$ mice.[812] This is important as ~50% of the normal population carries the

ATG16L1 risk allele.[1] However, when environmentally induced[14] and/or genetically determined ER stress in the intestinal epithelium, such as due to (rare) *XBP1* or (common) *ORMDL3* risk variants,[14] affects a carrier of the *ATG16L1* risk variant that lacks the capacity to mount an effective compensatory autophagic response and/or disrupts the compensation provided by the UPR in the setting of hypomorphic autophagy function, small intestinal Crohn's disease may commence out of Paneth cells.[12]

Interestingly indeed, the intestinal epithelium of Crohn's disease (and also UC), even in the non-inflamed state, exhibits evidence of ER stress,[1415] and the preva lence of this observation makes it unlikely that this is solely, or even primarily, caused by genetic risk factors that map to the UPR. Rather, the common occurrence of ER stress in IBD may reflect the effects of a variety of environmental factors that are a consequence of the disease such as inflammation per se or possibly even causative factors involved in risk for disease development.[14] In accordance with this observation is also the common induction of autophagy, again specifically in Paneth cells, irrespective of the presence of genetic risk factors in autophagy genes.[16] Autophagy and the UPR may therefore constitute a highly integrated `superpathway', operative within Paneth cells and that may be at the basis of a significant subset of patients with small intestinal Crohn's disease.

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REFERENCES

1. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012; 491:119–24. [PubMed: 23128233]
2. Hampe J, Franke A, Rosenstiel P, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. 2007; 39:207–11. [PubMed: 17200669]
3. Cooney R, Baker J, Brain O, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat Med. 2010; 16:90–7. [PubMed: 19966812]
4. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature. 2001; 411:599–603. [PubMed: 11385576]
5. Parkes M, Cortes A, van Heel DA, et al. Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nat Rev Genet. 2013; 14:661–73. [PubMed: 23917628]
6. Ravikumar B, Sarkar S, Davies JE, et al. Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev. 2010; 90:1383–435. [PubMed: 20959619]
7. Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci USA. 2005; 102:18129–34. [PubMed: 16330776]
8. Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature. 2008; 456:259–63. [PubMed: 18849966]
9. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science. 2005; 307:731–4. [PubMed: 15692051]

10. Couturier-Maillard A, Secher T, Rehman A, et al. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. J Clin Invest. 2013; 123:700–11. [PubMed: 23281400]
11. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011; 334:1081–6. [PubMed: 22116877]
12. Adolph TE, Tomczak MF, Niederreiter L, et al. Paneth cells as a site of origin for intestinal inflammation. Nature. 2013; 503:272–6. [PubMed: 24089213]
13. Deuring JJ, Fuhler GM, Konstantinov SR, et al. Genomic ATG16L1 risk allele-restricted Paneth cell ER stress in quiescent Crohn's disease. Gut. Published Online First: 20 Aug 2013. doi: 10.1136/gutjnl-2012-303527.
14. Kaser A, Zeissig S, Blumberg RS. Inflammatory Bowel Disease. Annu Rev Immunol. 2010; 28:573–621. [PubMed: 20192811]
15. Treton X, Pedruzzi E, Cazals-Hatem D, et al. Altered endoplasmic reticulum stress affects translation in inactive colon tissue from patients with ulcerative colitis. Gastroenterology. 2011; 141:1024–35. [PubMed: 21699776]
16. Thachil E, Hugot JP, Arbeille B, et al. Abnormal activation of autophagy-induced crinophagy in Paneth cells from patients with Crohn's disease. Gastroenterology. 2012; 142:1097–9e4. [PubMed: 22285936]