Published in final edited form as:

Nature. 2014 February 27; 506(7489): 441–442. doi:10.1038/nature13060.

Stressful genetics in Crohn's disease

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Abstract

A common variant of the autophagy protein ATG16L1 is a risk factor for Crohn's disease. But the genetic alteration is revealed only when the protein is cleaved by the enzyme caspase 3 during cellular stress.

A single variation within the approximately six billion base pairs of human DNA is sufficient to magnify our risk of developing common complex diseases. For example, a single nucleotide change in the gene *ATG16L1* is one of the strongest genetic risk factors for Crohn's disease¹, a chronic inflammatory disease of the digestive tract². On page 456 of this issue, Murthy *et al.*³ reveal a fascinating twist in the story of how this variant, which is present in more than 50% of the Caucasian population, may contribute to the disease. The authors report that the altered ATG16L1 protein, which contains the substitution of an alanine amino-acid residue for a threonine at position 300 (T300A), is susceptible to cleavage by the enzyme caspase 3, which is activated when a cell senses stress.

Like all complex disorders, Crohn's disease emerges on a background of many genetic⁴ and — mainly unknown — environmental factors. In Crohn's disease, these factors interact to disrupt the homeostasis between resident gut microorganisms, the epithelial layer lining the intestine and the immune system, resulting in chronic intestinal inflammation². The identification¹ of the T300A-encoding variant of *ATG16L1* as a risk factor for the disease hinted at a role for autophagy in this inflammatory response.

Autophagy (or 'self-eating') is a process whereby intracellular content, such as organelles or macromolecules, are engulfed by double-membrane structures called autophagosomes — ATG16L1 has a crucial role in the formation of autophagosomes⁵. These vesicles then fuse with other vesicles, lysosomes, which contain enzymes that degrade the contents⁶. Autophagy is a response to starvation that allows the cell to catabolize its contents to regenerate basic cellular building blocks, including amino acids and macromolecules. But the process has also been co-opted for other biological functions, such as the degradation of

intracellular pathogens, called xenophagy. Consequently, autophagy is involved in several diseases⁶, but the *ATG16L1*^{T300A} variant is associated only with Crohn's disease.

Caspases are endoproteases that hydrolyse peptide bonds at specific sequences⁷. Depending on the target protein, this can lead to the protein's destruction or to the generation of an active protein. Active caspase 3 is required for the cellular signalling pathway that leads to apoptotic cell death; this pathway is initiated by the activity of other caspases that respond to signals from cell-surface death receptors or irreparable organelle dysfunction. Caspase-3 activity during apoptosis irreversibly sets in motion a sequence of events that leads to the demise of the cell. By contrast, low-level caspase-3 activity that is insufficient to trigger apoptosis has homeostatic and protective functions, including guarding stressed organs against cell death⁸.

Through clever alignment of the ATG16L1 protein sequence from several species, Murthy *et al.* predicted and then directly demonstrated that the T300A variant protein (or the equivalent variant in mice, T316A) was highly sensitive to cleavage by caspase 3 (Fig. 1). The authors also show that, in human cells carrying the *ATG16L1*^{T300A} risk variant, signalling initiated by binding of the protein tumour-necrosis factor- α (TNF- α) to its cell-surface receptor (a death receptor), cellular stress caused by starvation, or infection with the pathogenic gut bacterium *Yersinia enterocolitica* all resulted in caspase-3-dependent degradation of ATG16L1^{T300A}, and consequently in impaired autophagy and xenophagy responses to these stresses. In the case of *Y. enterocolitica* infection, there was also an increased production of inflammatory cytokine proteins, such as interleukin-1 β and TNF- α , in mice engineered to express the ATG16L1^{T316A} variant.

In stark contrast, the authors show that autophagosome formation was not impaired by the presence of ATG16L1^{T300A} when autophagy was directly induced without caspase-3 activation. These findings suggest that the *ATG16L1*^{T300A} risk variant, through its sensitivity to caspase-3-mediated cleavage, disables a host's ability to properly respond to environmental challenges that require a compensatory autophagy response. Such challenges may include infections, inflammatory stimuli and metabolic disturbances, all of which induce autophagy to remove pathogens or inflammatory organelles (such as inflammasomes⁵) and to provide nutrients to overcome and survive cellular stress. Caspase 3 is commonly activated during these stress conditions.

The cellular state known as endoplasmic reticulum (ER) stress can also activate both caspase 3 and autophagy, and is commonly observed in intestinal epithelial cells of individuals with Crohn's disease⁹. Previous studies in mice¹⁰ showed that loss of compensatory autophagy in ER-stressed intestinal epithelium, owing to deletion of the *Atg16l1* gene, results in Crohn's-disease-like inflammation of the small intestine. The identification of the sensitivity of this protein variant to caspase-3-mediated destruction may provide insight into how specific environmental exposures convert genetic disease risk into clinical symptoms.

Murthy and colleagues' study also helps to integrate other observations from work on Crohn's disease. For example, caspase-3 activation can be regulated by inhibitor-of-apoptosis proteins such as XIAP, variants of which are the cause of a single-gene form of

Crohn's disease¹¹. XIAP, in turn, directly interacts with other proteins involved in microbial sensing that are associated with genetic risk for Crohn's disease^{4,11}, including RIPK2 and NOD2. The new data might also help to explain why agents targeting TNF- α are highly effective therapies for Crohn's disease² — in light of the authors' *Y. enterocolitica* infection studies, it seems plausible that TNF- α fuels an inflammatory feed-forward loop by inducing caspase-3-mediated degradation of ATG16L1^{T300A}, which in turn results in increased TNF- α secretion. Although further studies are necessary to test the authors' biochemical and cellular observations in animal models and patients with Crohn's disease, their observations provide an appealing and integrating hypothesis for how this common genetic element engenders disease risk.

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Figure 1. Fate determination by caspase 3

A primary genetic risk factor for Crohn's disease is a variant of the autophagy protein ATG16L1. Murthy *et al.*³ show that the single amino-acid change in the variant ATG16L1^{T300A} renders it sensitive to cleavage by the enzyme caspase 3. In the absence of caspase-3 activity, ATG16L1^{T300A} functions normally in the induction of autophagy. However, environmental stresses leading to the concomitant activation of autophagy and caspase 3, such as ligation of death receptors, bacterial infection or metabolic stress, result in destruction of ATG16L1^{T300A} and hence impaired autophagy. The authors also find that this impaired autophagy causes increased release of inflammatory proteins such as TNF- α and IL-1 β .