Exposure to Environmental Triggers Results in Disease Signs in Rats Carrying a Human ATG16L1 Crohn's Disease Susceptibility Variant

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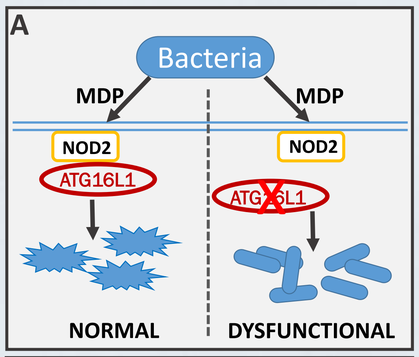
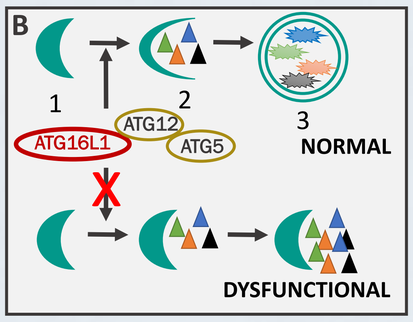
Abstract

Crohn's disease (CD) is one of two chronic inflammatory bowel diseases (IBD) that affect the lining of the gastrointestinal system. Several environmental factors, either through acute insult or chronic exposure, contribute in large part to the multifactorial etiology of this disease. Using CRISPR-Cas9 genome editing technology, our laboratory generated a rat strain carrying a human variant for CD in the ATG16L1 autophagy gene (F344-Atg16l1em8 – hereby referred to as em8). To determine whether CD onset could be triggered in this rat strain, we performed two studies, one acute and one chronic, in which we exposed heterozygous (HET) em8 rats and their wild type (WT) litter mates to known triggers of CD. For the acute study, rats were orally gavaged with 10 mg/kg of the NSAID diclofenac once per day for 7 days to simulate a short-term course of high-dose NSAID administration in humans. We found that HET em8 rats displayed overt signs of poor health as well as gross and histologic signs of NSAID toxicity and CD-like lesions at necropsy, with females showing more severe signs of disease than males. For the chronic study, rats were either orally gavaged with diclofenac at 1.25 mg/kg once per day on a 10 day on, 7 day off cycle for 12 weeks or provided ad libitum Western diet formulated rodent chow as their sole source of feed for 12 weeks. We found that HET em8 rats displayed changes in the gut microbiota composition when compared to WT litter mates. They also showed mild CD-like histologic signs in ileal and colonic tissues, with HET rats on the Western diet showing the most severe CD-like histologic lesions. Given that this rat strain carries the identical genetic susceptibility variant found in human CD patients and exhibits disease upon exposure to known environmental triggers of human disease, our findings support future use of this model to understand the underlying mechanism of ATG16L1 and autophagy in CD.

ATG16L1





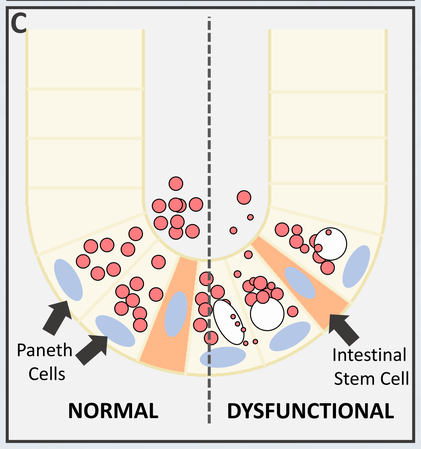


Figure 1. Functional and nonfunctional ATG16L1 in the cell. A. In all cells, muramyl dipeptide (MDP) from the membrane of intracellular bacterial stimulates NOD2, which normally recruits ATG16L1 causing autophagy and destruction of bacteria. Without ATG16L1, intracellular bacteria survive and multiply, causing cell death. **B.** During normal macroautophagy, ATG16L1 complexes with ATG12 and ATG5 for development of the autophagosome – a necessary component of autophagy and trafficking to the degradation center of the cell, called the lysosome. Dysfunctional ATG16L1 causes loss of the autophagosome and build-up of intracellular debris, causing cell stress and death. **C.** In ileal Paneth cells, ATG16L1 forms a complex with ATG5 and ATG7 to cause release of α-defensins, which preferentially bind to and disrupt bacterial cell membranes, causing lysis. Without functional ATG16L1, Paneth cell granules are not packaged correctly, causing abnormalities in shape, size and release into the lumen of the gastrointestinal tract.

Rat *Atg16l1* Susceptibility Model

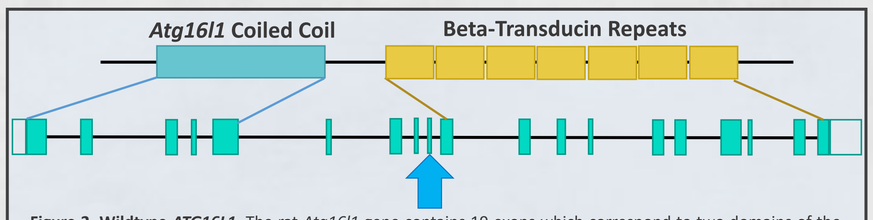


Figure 2. Wildtype *ATG16L1*. The rat *Atg16l1* gene contains 19 exons which correspond to two domains of the protein, the ATG16L1 coiled coil required for protein specificity and seven beta-transducin (WD40) repeats required for protein interaction. The knock-in mutation is a single, nonsynonymous adenine to guanine polymorphism in exon 9, resulting in a threonine to alanine substitution at amino acid position 300 (T300A).

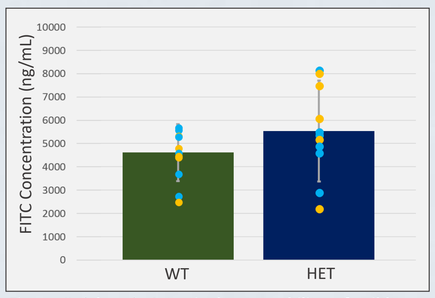


Figure 3 (above). Intestinal permeability of wildtype (WT) and heterozygous (HET) *Atg16l1* knock-in rats. No statistically significant difference exists in the intestinal permeability of wildtype and knock-in *Atg16l1* rats (ng/mL). Males = blue dots; Females = orange dots

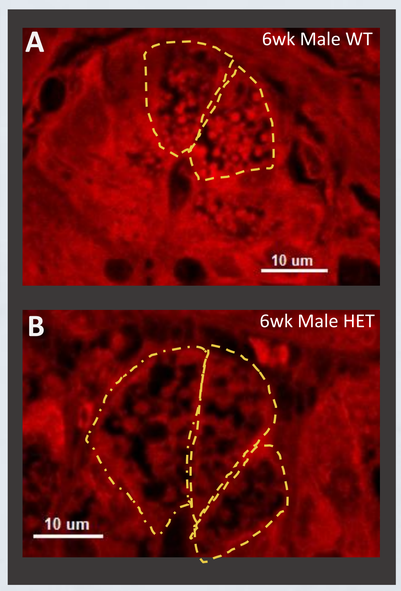


Figure 4 (right). Representative images of ileal immunohistofluorescence highlighting Paneth cell granulation. Yellow lines outline individual Paneth cells. **A)** Normal granulation pattern of Paneth cells from wildtype Fischer rats (n = 6). **B)** Abnormal granulation of Paneth cells from *Atg16l1* heterozygous knock-in rats (n = 6). Lysozyme is free-floating in the cytoplasm of the cell rather than packaged into uniform, circular granules.

Timeline of Exposure

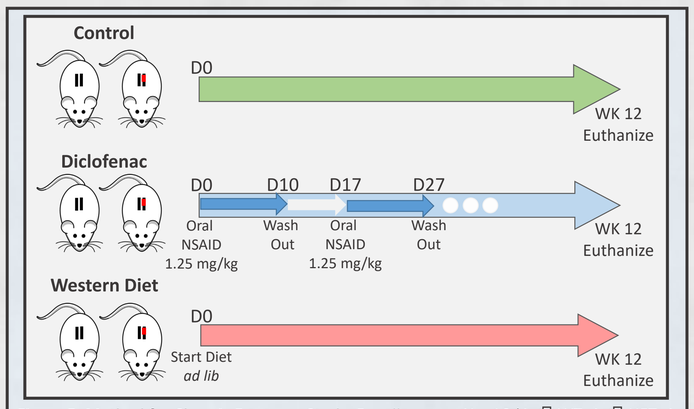


Figure 5. Method for Chronic Exposure Study. For all groups, N = 16 (4 ♂ WT; 4 ♂ HET; 4 ♀ WT; 4 ♀HET). D0 = 28 days of age. All animals were co-housed either in WT/HET pairs (males) or groups of 4 (both WT and HET females).

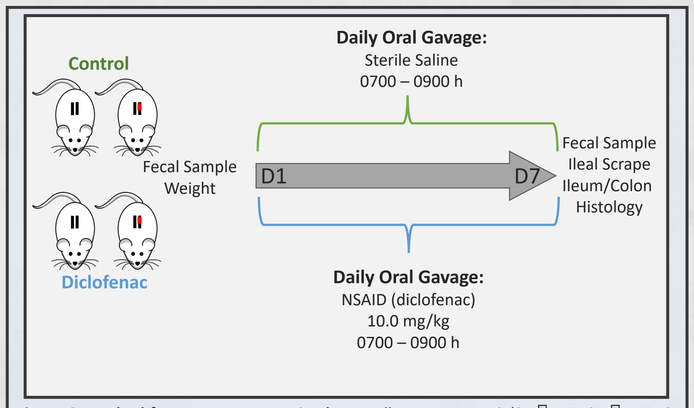


Figure 6. Method for Acute Exposure Study. For all groups, N = 12 (3 ♂ WT; 3 ♂ HET; 3 ♀ WT; 3 ♀HET). D1 = 10-11 weeks of age. All animals were co-housed either in WT/HET pairs (males) or groups of 4 (both WT and HET females).

Exposure to Environmental Triggers of Crohn's Disease

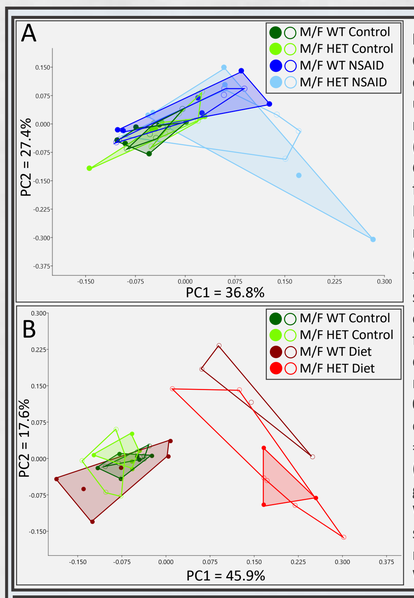


Figure 7. Microbiome Principal Component Analyses (PCA) PCAs demonstrate differences observed using a two-way permutational multivariate analysis of variance (PERMANOVA) of ranked Bray-Curtis similarity indices results from endpoint fecal samples. **A)** No statistical significance in overall microbiota exists between control (**green**) and NSAID-treated (**blue**) treatment groups. **B)** Statistical significance exists for the joint effect of genotype and treatment for control (**green**) and Western diet (**red**) groups on the gut microbiota ($F(3,34) = 3.2$, $p < 0.001$) as well as independent effects for both genotype ($F(3,34) = 3.1$, $p < 0.001$) and treatment ($F(1,34) = 13.2$, $p < 0.001$) on the gut microbiota. Interestingly, male WT rats in the diet group had no significant changes to their gut microbiota as compared to control WT and HET rats.

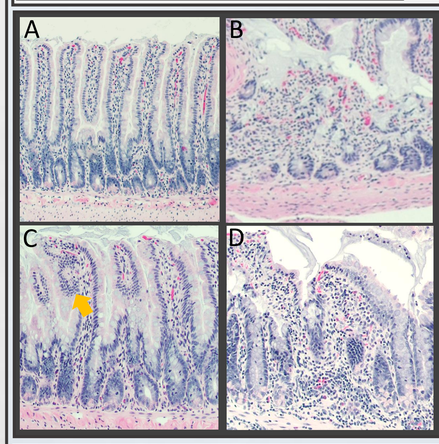


Figure 8. Representative examples of chronic ileal histology. HET animala exposed to NSAID and diet triggers trend to show mild clinical signs of inflammatory bowel disease. **A)** Male WT control (200x); normal ileum; **B)** Female HET diet treatment (200x); dysplastic epithelium (abnormal development of cells) ; **C)** Male HET diet (200x); arrow indicates area of mild epithelial hyperplasia (increase in reproductive rate of cells); **D)** Female HET NSAID (200x); inflammatory infiltrate within the lamina propria (plasma and mononuclear cells).

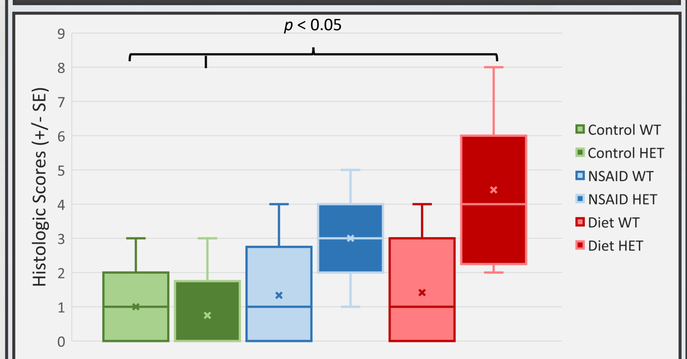


Figure 9. Chronic histologic scores. Tissue scores were based on extent and severity of epithelial and inflammatory changes. Samples were scored by two blinded laboratory animal veterinarians. Scoring range: 0 (no disease) to 48 (severe disease). Combined ileal and colon scores seen in WT and HET *Atg16l1* rats (+/- SE). Differences were determined by two-way ANOVA followed by Tukey's post-hoc testing.

Figure 10. Acute study gross necropsy. A) Female HET control; *in situ* abdominal organs; no signs of disease; **B)** Female HET NSAID treatment; jaundiced/fibrotic omentum, mild ascites, multifocal, hepatic necrosis. Findings consistent in 3/5 HET females on NSAID treatment. Male HET NSAID treatment rats (3/5) displayed mild fibrosis of the omentum but no jaundice or hepatic necrosis.

Figure 11. Acute study ileal histology. A) Female WT control; H&E stain; normal sample (200x); **B)** Female HET NSAID treatment; gram stain; massive bacterial infiltration of the mucosal and muscular layers and ulceration (100x)

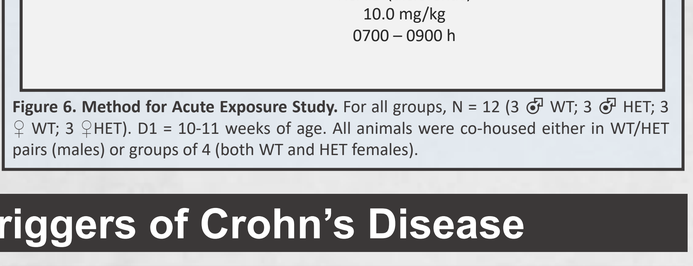


Figure 12. Acute study colonic histology. A) Female WT control; H&E stain; normal sample (100x); **B)** Female HET NSAID; gram stain; bacterial infiltration of the mucosal and muscular layers, sloughed mucosa and ulceration (100x); **C)** Image B at 630x; bacterial infiltration into muscularis (dark blue infiltrate; circled) and loss of mucosa.

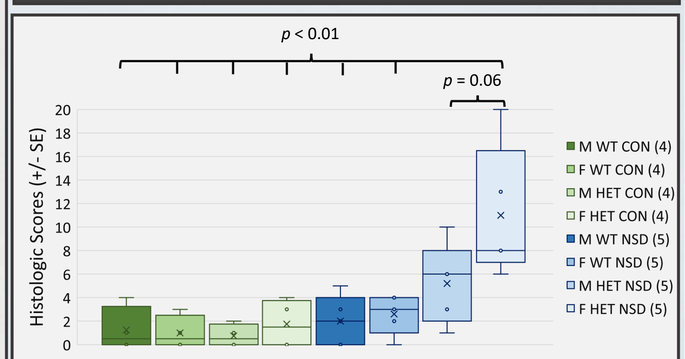


Figure 13. Acute histologic scores. Scores were based on extent and severity of epithelial and inflammatory changes. Samples were scored by a blinded laboratory animal veterinarian. Scoring range: 0 (no disease) to 48 (severe disease). Combined ileal and colon scores seen in WT and HET rats (+/- SE). Differences were determined by two-way ANOVA followed by Tukey's post-hoc testing. Numbers in legend indicated n per group.

Conclusions

- Chronic Western diet feed causes a sex-genotype X treatment interaction effect of the gut microbiota.
- HET rats on chronic Western diet feed show very mild histologic signs of inflammatory bowel disease
- Acute high-dose NSAID administration causes sick rodent signs, such as weight loss, scruffy hair coat, and hunched posture; as well as severe gross changes and severe histologic signs of inflammatory bowel disease in female HET rats

Future Directions

- Gut microbiota analysis of acute study rats
- Additional blinded scoring of acute histology
- Acute serum sample testing for inflammatory markers

Acknowledgements

This project was supported by NIH P40 OD11062, NIH P40 OD011062-14S1 and Phi Zeta. K.C. is supported by NIH T32 OD011126. Thank you to Dr. Marcia Hart for use of her mucosal scoring system and to Drs. Susheel Busi, Marcia Hart and Willie Bidot for their training in microbiome statistical analyses.