Effects of p-glycoprotein deficiency on *Caenorhabditis elegans* intestinal development

Diamond Braxton, MaKenna DeYoung, Brittany Salciccioli and Scott Tanner
University of South Carolina Upstate, Division of Natural Sciences & Engineering

Introduction

Inflammatory Bowel Disease (IBD) is chronic inflammation of the digestive tract. IBD is an autoimmune reaction with an unknown cause. There is no known cure, only treatments to relieve symptoms. There are many genes linked to this disease, including *MDR1*.

The *MDR1* gene codes for a protein called P-glycoprotein (*pgp*), which is found in intestinal cells. The exact relationship between IBD and *pgp* is unknown, but it has been observed that some patients with IBD have a mutation that causes a loss of function of *pgp*. It is suggested that *pgp* affects the intestine through the formation and function of tight junctions, which forms a barrier between two intestinal cells by linking them together. Tight junctions act as protective barriers against toxins and other harmful chemicals, potentially damaging intestinal cells, and aid in nutrition by encouraging effective absorption of nutrients.

To examine the relationship between pgp and tight junctions, we used four strains of the model organism *C. elegans:* N2 (control) NL132 (pgp1$^{-/-}$), NL131 (pgp3$^{-/-}$), and NL130 (pgp1/3$^{-/-}$). Pgp1 and pgp3 are known to be expressed in the *C. elegans* intestine. We expect to see intestinal damage in pgp$^{-/-}$ worms because it is associated with intestinal damage in human disease. To test our hypothesis we measured growth, offspring production, and LET-413 tight junction expression.

Methods

Model Organism: C. elegans

C. elegans are simple, transparent organisms that have a short generation time and high offspring numbers and are frequently used in genetic and developmental biology studies. *C. elegans* were obtained from the Caenorhabditis Genetic Center and cultured on Nematode Growth Medium plates at 20-22°C.

LET-413 Staining

C. *elegans* were rinsed from plates and transferred to slides. Cover slips were placed, and the slides were froze in liquid nitrogen. After 5 minutes the cover slips were ripped off cracking open the C. *elegans.* The slides were submerged in Methanol then Acetone for 5 minutes each. After the slides were washed in PBS. The primary antibody was added and incubated overnight at 4 C. The next day slides were rinsed in PBS 3x and incubated (in the dark) for an hour at room temp. They were rinsed in PBS 3x and incubated 10 minutes (in the dark) at room temp. Washed again with PBS, a cover slip was added then glycerol and slides were viewed under the microscope.

Methods (Continued)

Growth Analysis

To complete a growth analysis, we started with new plates (OP50 seeded) of each strain with 3 adult/L4 worms, they were incubated at 20 C for 3 days. Worms were rinsed off plates with M9 and collected in microfuge tubes. The tubes were spun, and the buffer taken out, leaving worms at bottom. 50ul of M9 was resuspended in tube then transferred into a 96 well plate. 2ul of levamisole was added to anesthetize. Keyence scope was used to take images of each well. ImageJ/ Fiji program was used to measure worm lengths.

Offspring Count

To determine the number of offspring, new plates (OP50 seeded) of each strain with 3 adult/L4 stage worms were incubated at 20 C for 3 days. The plates were rinsed with M9 solution. Measuring and using the total volume number of worms; 50 ul was taken and added to new unseeded plates and the number of worms were counted.

Reduced offspring production in pgp$^{-/-}$ *C. elegans*

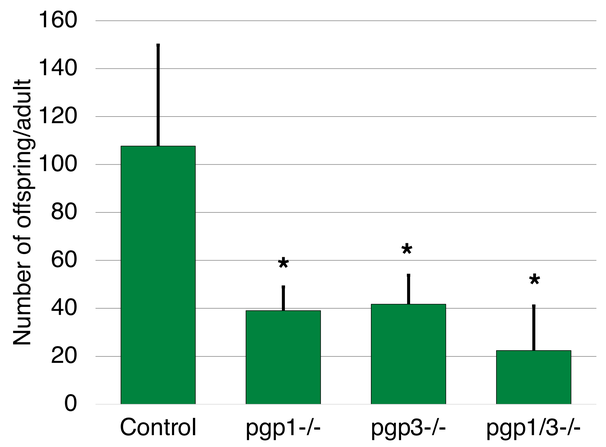

Figure 1: The average number of offspring produced from our N2 strain (control) displays a significant difference when compared to the three other strains of C. *elegans*. ANOVA, P value = 0.005. * Individual comparisons to Control by Tukey's HSD test ($P < 0.05$)

Decreased LET-413 tight junctional protein in pgp1$^{-/-}$ *C. elegans*

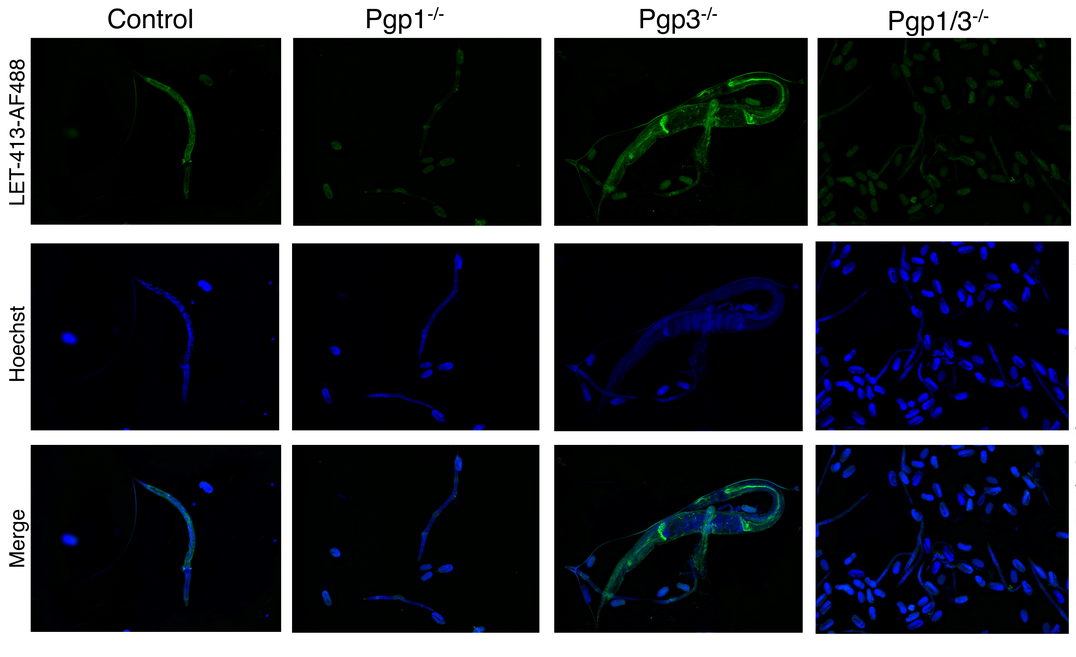

Figure 2: LET-413 staining of *C. elegans*. LET-413 is a tight junctional protein in *C. elegans*. *C. elegans* were stained using an anti-LET-413 and an AlexaFluor 488 conjugated anti-mouse IgM antibody. Worms were then counterstained with Hoescht. Images captured at 200x magnification. All exposures identical.

Slower growth observed in pgp$^{-/-}$ *C. elegans*

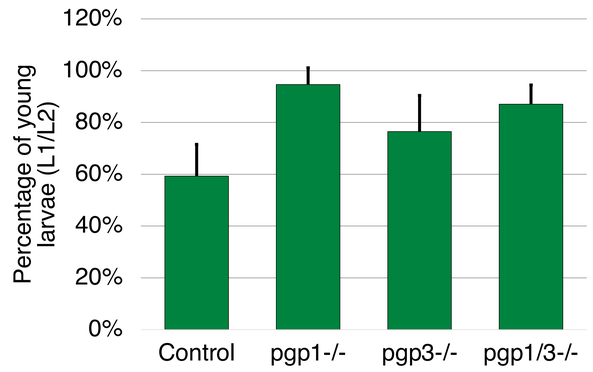

Figure 3: The percent L1/L2 larvae in knockout strains was significantly higher after 3 days growth. ANOVA, P value = 0.01. No difference between individual groups.

Summary/Conclusions

- The pgp1-/-, pgp3-/-, and pgp1/3-/- produced fewer offspring than control. Decreased offspring production suggests poor absorption of nutrients.
- The pgp1$^{-/-}$ and pgp1/3$^{-/-}$ worms developed slower than the control and pgp3$^{-/-}$ worms. Slower growth suggests poor nutritional uptake.
- Staining of LET-413 indicates there is less tight junction protein expression in pgp1$^{-/-}$ and the pgp1/3$^{-/-}$ worms. This suggests a more permeable intestinal barrier.
- Although pgp3$^{-/-}$ displays signs of intestinal damage (decreased offspring and growth), the pgp1 and pgp1/3$^{-/-}$ worms also show less LET-413 tight junction expression, indicating higher levels of intestinal damage.
- **Our data suggest pgp1 is essential for the expression of intestinal tight junctions in *C. elegans*.**

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