Assessing the effect of early-life tylosin antibiotic treatment and subsequent fecal microbial transplant on murine ileal gene expression

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Abstract

Antibiotics are prescribed excessively in the US and often to young children. The time between birth and 3 years of age is proven to be crucial for the development of gut microbiota. Disrupting the gut microbiota at such a young age can lead to obesity, asthma, and atopic diseases. This study asks the question what is the effect of early macrolide exposure and fecal microbial transplant after early-life antibiotic treatment on murine ileal gene expression? This project centers around the analysis of data from a previously conducted experiment. In the experiment, a group of mice was treated with antibiotics early in their life. Later, half of the mice were given a fecal microbial transplant. Our hypothesis was that macrolide exposure during the early stages of life can alter the healthy microbial community and result in the reduction of ileal gene expression and fecal microbial transplants can restore that change. Previously, C57BL/6 mice were exposed to tylosin (a macrolide antibiotic) from day 5 to day 10 of life. Mice were then given a fecal microbial transplant at weaning and followed for 5 weeks. At 7 weeks ileal tissue was collected and RNA was extracted and sequenced using Miseq platform. In this analysis, we used the green line of DNA subway to quantify the RNA-Seq results from the aforementioned experiment. While previous studies have examined the lasting impact of many courses of antibiotics and immunological development, this study aims to see if the relationship holds true at gene level for as little as one course of antibiotics.

Materials & Methods

The original experiment had two groups of mice (12 each), in which one group consumed solely water, and another had water with the addition of Tylosin (antibiotic) until a certain date. Then each group was divided into two more groups, with one group receiving media only (Sham), while another received media and healthy microbiota (mFMT). This amounted to 4 groups: Control Sham, Control mFMT, Pat Sham, and Pat mFMT.

Our work focused on using the green line of DNA subway to analyze our RNA-Seq data. First, the sample data was uploaded into Cyverse in the FASTQ file format. The data was then trimmed and cleaned using DNA Subway's built in system. Samples were pseudo aligned using Kallisto (differential analysis) and graphics were made using sleuth.

Results

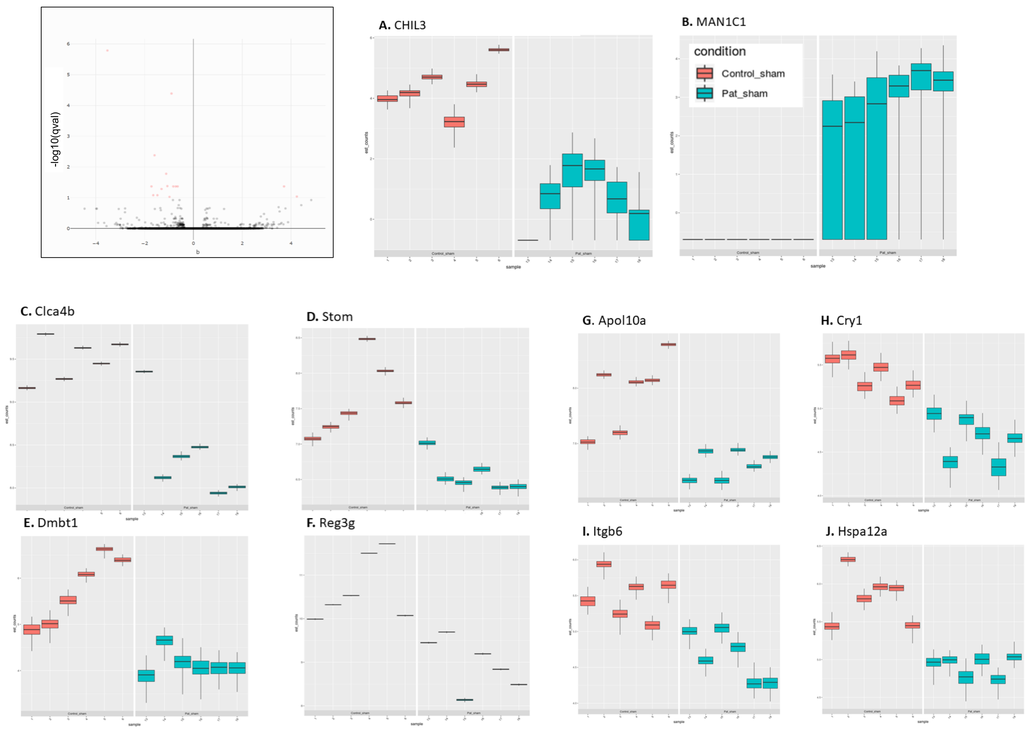

Figure 1. Differential expression between control and antibiotic treatment in mice. Volcano plot showing significantly different expressed genes. A total of nine genes were down regulated in antibiotic treated mice while only one gene was up regulated.

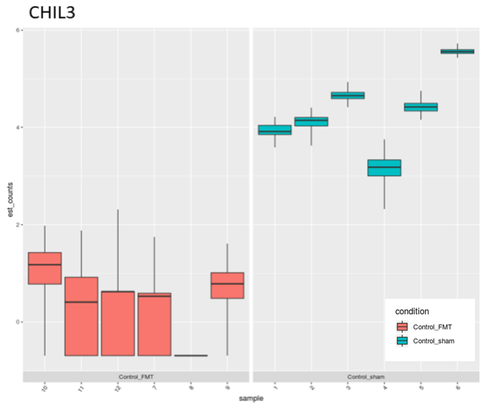

Figure 2. After a fecal matter transplant in control mice, one gene was significantly altered (in terms of gene expression).

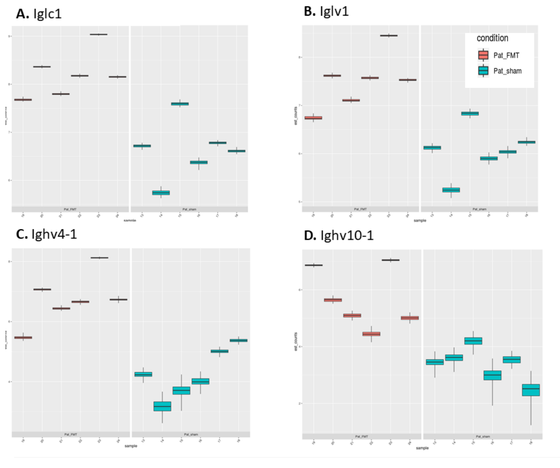

Figure 3: Differential expression between antibiotic treated mice with and without FMT in mice treated with antibiotics, those who also received fecal matter transplants showed upregulation in the genes Iglv1, Iglc1, Ighv10-1, Ighv4-1 all of which are associated with immunoglobulin receptor binding

Conclusion

- Ileal gene expression is altered after tylosin treatment
- Fecal microbial transplant altered ileal gene expression in both antibiotic treated mice and control mice
- The addition of a fecal microbial transplant to mice not treated with tylosin only results in significant downregulation of one gene
- Tylosin's effects of downregulation for several genes can be partially restored to its original state with the use of fecal microbial transplants

Discussion

We analyzed data from a previously completed experiment regarding the use of antibiotic treatment and fecal microbial transplant on ileal gene expression. After a group of mice were given solely antibiotic treatment, we observed a decrease in the gene expression of nine genes and an increase in one compared to the Control Sham group. For example, in mice that received tylosin without any fmts, two genes that were downregulated, Reg3g and Dmbt1, play a role in cellular immune defense, which may mean host immunity may be reduced due to the tylsoin. As seen in Figure 1A, in antibiotic-treated mice Chil3 was downregulated. Since Chil3 is linked to the inflammatory response, it is plausible that host immunity was decreased. Furthermore Man1c1, a gene that enables calcium binding, was upregulated with mice that were treated with antibiotics, which may result in impacts on immune-related calcium signaling. Since the genes Iglv1, Iglc1, Ighv10-1, Ighv4-1 were upregulated in mice who were treated with antibiotics and FMT (compared to mice that received only tylosin), an increase in antibody variability in those mice can be postulated. This study has implications on the use of antibiotics in young children as well as the benefits of probiotics or other microbiome restoring treatments.

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