The Genetic Effects of Red Meat Intervention in Inflammatory Bowel Disease

By: The Gator Meat Team

Aaron Upchurch

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# Abstract

Inflammatory bowel disease is an umbrella diagnosis that encompasses several disorders that feature the persistent inflammation of the digestive track. The most prevalent types include Crohn’s disease, and ulcerative colitis [1]. Symptoms often appear in cyclical patterns and commonly include abdominal pain, weight loss, and fatigue. Although a significant amount of research has been conducted in the past decades, the exact cause of the disease remains unknown. Some researchers have speculated, however, that the food one eats may greatly affect the progression and symptoms of the disease [1]. The following paper outlines new research that suggests a genetically visible and causative association between the consumption of red meat and the disease does not exist. Both differential analysis and clustering methods were unable to produce results that supported the existence of the relationship. Despite the identification of many differentially expressed genes in samples from the red meat intervention, the identified pathways were only loosely related to inflammatory bowel disease. Furthermore, hierarchical clustering using genetic expression counts was unable to accurately group the samples based on the presence of red meat in their diets. The lack of a clear correlation is not particularly significant, though due to the relatively small size and homogeneity of the sample groups. Additional studies that utilize a larger sample size with more diverse membership may discover new information that supports the proposed connection between red meat and inflammatory bowel disease.

# Introduction

Genomic analysis was performed on array profiled RNA expression data gathered from the colon biopsies of humans with inflammatory bowel syndrome. Forty-four samples were taken from participants of varying genders and ages before and after participating in a red meat dietary intervention. Log2 transformed spot intensities were recorded for 41,094 genes in each sample. We sought to determine if an intervention of red meat could create genetically visible effects in patients with inflammatory bowel disease. To find evidence that supported our assumption, differentially expressed genes between samples before and after the red meat intervention were identified through differential analysis and examined through enrichment path analysis. Hierarchical clustering based on the gene expression counts was also utilized to attempt to accurately regroup the samples into the appropriate intervention groups.

Many published studies have found substantial evidence that a link between red meat consumption and inflammatory bowel disease exists. The effects of diets with high red meat consumption have been previously documented several in non-human trials. Studies on mice have identified a statistically significant correlation between high red meat diets and an increase in DSS induced colitis, a precursor to inflammatory bowel disease [2]. The chemical compounds within red meat have been identified as a possible explanation for the association. Arachidonic acid is normally abundant in red meats and has been identified as a possible etiologic factor in the development of many bowel diseases [3]. Excessive intake of margarine which, like red meat, contains great quantities of linoleic acid has also been significantly associated with a higher risk of developing bowel diseases [4]. The effects of red meat consumption may also be affected by the other foods found in a person’s diet. The heavy consumption of soft drinks along with red meat, for example, has been found to increase the risk of developing inflammatory bowel disease [5]. Certain types of red meat may also have different effects on the progression of inflammatory bowel disease. Processed red meat in particular has been found to trigger a significantly greater number of disease relapses in patients [6]. The animal in which the red meat comes from may also affect inflammatory bowel disease in varying ways. Subjects of a nutritional study reported that when separated, red meat by itself had little effect on their symptoms, but when combined into a single dish, meats such as beef, lamb, and steak increased the severity of their symptoms [7]. Qualitative measures have also suggested a link between red meat and bowel disease, with patients frequently reporting that meals that feature red meat often quickly trigger the disease’s affects [8]. A reduction in red meat consumption is often prescribed to patients suffering from inflammatory bowel disease, with 80% of published diets suggesting an avoidance of the food in daily meals [9]. The effects of a high intake of red meat, however, depends greatly on many other factors. For example, red meat consumption has been associated with a significant increase in rates of cancer in women, but not in men. [10] One’s genetic makeup has also been found to be a confounding variable in identifying the effects of a diet high in red meat. While the diets found in North America and Western Asia consists of approximately equal amounts of red meat, rates of inflammatory bowel disease are vastly lower in Asian populations [11].

# Methods

Gene count based differential expression analysis was first performed on the forty-four provided samples. The normalized count data for all 41,094 represented genes from samples taken before and after the dietary intervention were compared to isolate genes that may signify an effect on the sample groups. The expression analysis was conducted using function provided in the DESeq2 library of the R programming language. The differentially expressed genes found between the two sample groups were then analyzed using gene set enrichment analysis. A table of the differentially expressed genes was then passed to the gost function of the gprofiler2 library. The enriched processes determined from the differentially expressed genes were then recorded and analyzed to determine if any relationship existed with the consumption of red meat and the presence of inflammatory bowel disease.

After the results of the differential analysis were recorded and processed with gene set enrichment analysis, clustering methods were applied to the samples. Sample clustering was performed to attempt to regroup the samples into their original intervention groups based solely on the gene expression counts. Hierarchical based clustering was first implemented using the 5,000 most variable genes found between samples. The clustering was then repeated using the 10, 100, 1000, and 10,000 most variable genes. The five produced hierarchical dendrograms were then compared to the original intervention groups and analyzed. The hclust function was utilized to perform all hierarchical clustering methods. The sample sizes of the two largest groups produced by each clustering method were then compared with the original intervention group populations. Chi-squared tests of independence were performed to identify any clustering methods that produced similar group sizes with the original groups. The code utilized to perform all mentioned analyses can be found at: https://github.com/AaronUpchurch/CIS-4930-BioInformatics

# Results

Based on the analysis performed on the gene expression levels of the samples, there is not sufficient information to identify a clear genetic effect of red meat intervention diets in patients with inflammatory bowel disease. Of the original 41,094 genes recorded in each sample, 550 were determined to be differentially expressed between the intervention groups. Gene set enrichment analysis revealed a connection between the differentially expressed genes and both ligand-receptor interactions and G alpha signaling events. Ligand-receptor interactions have been identified as an important part of the metabolism process and the development of colon cancer but has not been found to play a role in the development of inflammatory bowel disease. G alpha signaling events are similarly associated with cell communication and not bowel disease.

The gene expression level based hierarchical clustering methods were similarly unable to produce strong evidence for the existence of an effect of the intervention. Hierarchical clustering with the five different counts of variable genes were all unable to create clusters that reflected the original intervention groups. As shown in figure one, the produced dendrograms only slightly reflected the original groupings.

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Figure 1

Chart, bar chart

Description automatically generated The alluvial diagram as shown in figure 2 also displays the populations of the two largest clusters produced by each hierarchical clustering methods. As shown by the lack of separation of before and after intervention samples, the performed hierarchical clustering does not support the existence of an effect on inflammatory bowel disease brought on by the red meat intervention.

Figure 2

The meaningfulness of the analysis performed in the project was, however, diminished by the lack of quality samples. With only forty-four samples presented by the study, conclusions drawn by the analysis were difficult to generalize to larger populations. The use of samples solely from citizens of the Netherlands who lived near Maastricht University also reduced the total sample diversity and constricted the generalizability of the conclusion. The lack of ethnic diversity prohibits the drawn conclusion from being accurately generalized to patients of non-Norwegian nationality. The lack of any samples taken from patients under the age of 24 also prohibits any meaningful conclusions from being applied to younger patients with inflammatory bowel disease.

A frequently experienced bioethical issue encountered in the analysis of biological data is the often-unintended discrimination of results based on the sex of each patient. Extensive analysis of previously conducted research studies have shown that researchers may draw unjustified conclusions about data solely based upon the sample’s sex [12]. The concluded effectiveness of experimental drugs in many studies, for example was found to be incorrectly based upon the sex of the participant, even if the results were equivalent [12]. To avoid this phenomena, new studies have often opted to conceal the sex or race of participants. Instead of labeling samples with either male or female, the data could be altered to display only gender A and gender B. This choice was, however, not performed during the described experiment. While the possibility of an incorrect analysis is minimal as the sex of the samples was never considered, the study should have been conducted in a more cautious manner. Future analysis of the data should follow the outlined suggestions to reduce the probability of accidental discrimination.

If the project were to be continued, further analysis would be performed to identify any confounding variables that may have clouded hypothesis supporting evidence. Differential expression analysis and hierarchical clustering could be performed with the samples segregated by particular values. Of the information recorded on each sample, the age and sex of each patient possesses the greatest ability to act as a confounding variable. To prevent the effects of a sample’s age and sex from interfering with the results, the samples could be subdivided by intervention status as well as age or sex. Differential expression analysis and hierarchical clustering could then be performed on the new groups and may produce new information about the effects of the red meat intervention. To prevent possible researcher bias as described above, the samples could be labeled with gender A and gender B instead of male or female. The patient’s level of progression of the disease could also be analyzed by reconducting the study with two separate groups. Samples with the less severe irritable bowel syndrome diagnosis would be separated from patients suffering from the more advanced inflammatory bowel disease. The results produced by the two new studies may suggest that the red meat intervention had an effect on only one of the diagnoses, and not the other.

# Conclusion

Despite previous predictions, the analysis of data from this study was unable to discover a genetically significant effect of red meat interventions on inflammatory bowel disease. Differential expression analysis revealed many differentially expressed genes between the two sample groups. Despite this, enrichment path analysis suggested the intervention may affect only neuroactive ligand receptor interaction and G alpha signaling events, which are only loosely associated with inflammatory bowel disease. Hierarchical clustering methods based on the gene expression data were also unable to accurately cluster the samples into the before and after intervention groups. It is difficult to assume, therefore, that the red meat intervention had a significant effect on the bowel disease within the patients. The conclusion of the study is, however, limited in its generalizability due to the homogeneity of the samples. Further analysis performed with samples separated by age or sex may lead to a new conclusion. Despite the abundance of research studies, the true cause of inflammatory bowel disease still, however, remains a mystery.

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