Expression Profiling in Inflammatory Bowel Disease

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Introduction

Question

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestines. It is a complex disease which arises as a result of the interaction of environmental and genetic factors. There are two main forms of IBD: Crohn's disease (CD) and ulcerative colitis (UC). There is an overlap between the two forms in several areas including clinical criteria and therapy.

Objective: Our goal is to identify novel unknown genes involved in perpetuating inflammatory disease progression.

Data

We worked on a public dataset (GEO accession number: GSE1710). Our aim was to find Differentially regulated genes in High-density cDNA microarray data from the GPL284, Human UniGene Set RZPD 1, platform.

The dataset contains 34560 genes of 31 samples. Biopsies were taken from the sigmoid colon.

Group	Sex	Total
NC	Male	4
	Female	7
UC	Male	8
	Female	2
CD	Male	5
	Female	5

Table 1. Experimental design, number of samples for each group.

Data Exploration

	row.names	GSM29595	GSM29596	GSM29597	GSM29598	GSM29599	GSM29600	GSM29601
1	01A01	0.214269041	0.141720994	0.153143777	0.2013757224	0.128302990	0.137858628	0.179688616
2	01A02	0.116874023	0.079949199	0.068256213	0.0997897974	0.057652420	0.061531542	0.100846051
3	01A03	0.119945868	0.076125740	0.069136939	0.1136518264	0.088503479	0.058384946	0.098192795
4	01A04	0.124453886	0.071602313	0.069181426	0.1093685914	0.107247277	0.063042267	0.071402176
5	01A05	0.113459273	0.061464241	0.058253242	0.0837338854	0.082051321	0.050408623	0.063358425

Table 2. Data excerpt showing first 5 genes of first 7 samples.

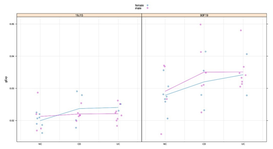


Fig 1. Stripplot of two random genes showing the effect of groups and sex.

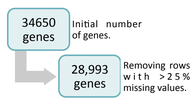


Fig 2. Number of genes after filtering.

- In the data exploration step, we found an outlier sample that has low correlation to other samples that we removed. The dataset also contained a large number of missing values.
- In the quality control steps, we removed rows with more than 25% missing values, and imputed the remaining missing values using k nearest neighbors and filled them with data from the 10 nearest neighbors.
- As a last step we performed quantile normalization to deal with technical variability.

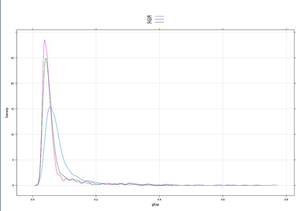


Fig 3. Distribution of first 100 genes for all samples in the three groups.

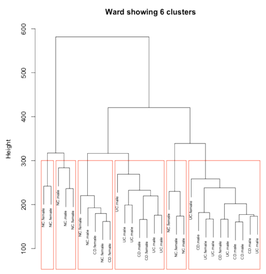


Fig 4. Hierarchical clustering using Ward method to explore the data where labels reflect groups and sex.

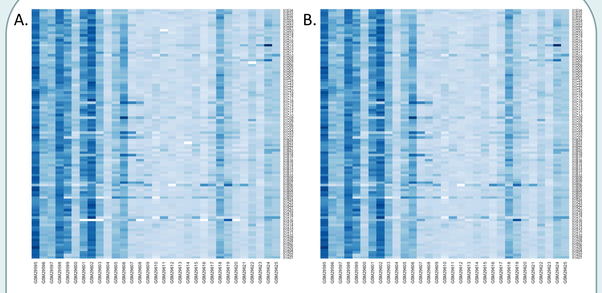


Fig 5. Imputation of missing values using k nearest neighbor. **A**. Heatmap of first 100 genes before imputation. **B**. Heatmap of first 100 genes after applying knn, using nearest neighbor averaging.

Differential Analysis

The used linear model is the ANOVA style, 'reference + treatment effects' parameterization.

$$Y_{ij} = \theta + \tau_i + \varepsilon_{ij} \text{ where } \tau_1 = 0.$$

$$\mu_{NC} = \theta, \mu_{CD} = \theta + \tau_2, \text{ and } \mu_{UC} = \theta + \tau_3.$$

i = 1, 2, 3.

j = 1, 2, 3, ..., 28933 genes.

Equation 1. ANOVA style linear model for group effect.

The statistical test performed is the limma F-test to compare models where the following null hypothesis is tested:

$$\mu_{NC} = \mu_{CD} = \mu_{UC}$$

Equation 2. Null hypothesis to test if all the means of the three groups are equal.

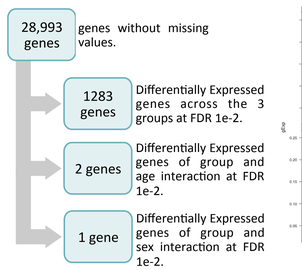


Fig 6. Number of genes after DEA.

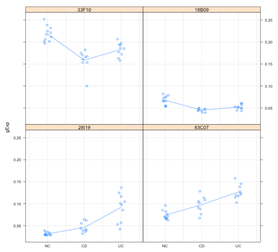


Fig 7. Four random hits where the means of the three different groups are NOT equal.

Thus, testing for differentially expressed genes between normal and IBD samples resulted in 1283 significant genes. While, testing for DE genes between Crohn's disease and ulcerative colitis samples did not show any significant results.

Further tests were done to explore the interaction effect between group and sex, and group and age. However, age and sex proved to be nuisance factors at best.

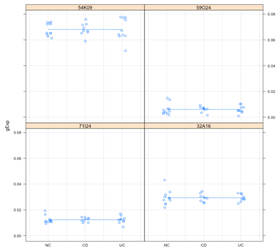
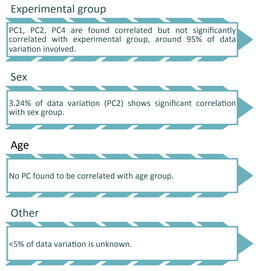


Fig 8. Four random non-hits where the means of the three different groups are equal.

Principle Component Analysis



Principal Component Analysis shows that from PC1, PC2 and PC4, we can tell the difference between the normal control group (NC) and the IBD patient group (CD and UC), but we cannot tell the difference between the Crohn's disease (CD) group and the ulcerative colitis (UC) group.

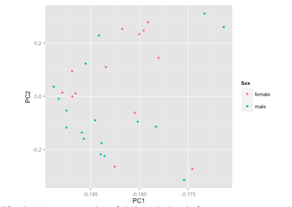


Fig 9. Unsupervised PCA. Principle component 1 and 2 were plotted against each other to show the difference between samples. PC2 shows correlation with sex group.

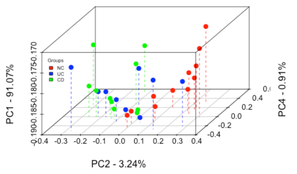


Fig 10. Unsupervised PCA. Principle component 1, 2, and 4 were plotted against each other to show the difference between samples.

We believe that due to the limited sample size, the PCA results above are not significant and could be wrong.

Gene clustering

In gene clustering analysis, 1283 genes which were found to be differentially expressed were analyzed. Setting k as 5, we performed k-means clustering to get the results.

In k-means gene clustering results, with the differentially expressed genes partitioned into 5 clusters, there is one cluster that shows significantly higher differential expression levels than the other clusters.

- This cluster only contains 7 genes.
- The differential expression level of this cluster is 10 times higher than of other clusters.
- Genes in this cluster have much higher expression levels in the IBD patients group than in the control group.
- Differences between UC and CD in this cluster are still not significant.

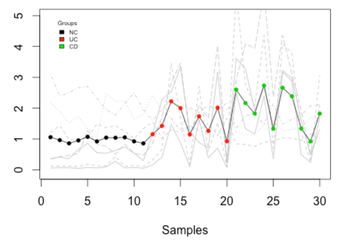


Fig 11. Gene clustering. The different genes and cluster centers were plotted against each other to show the difference between samples.

Classification

- In the classification analysis, we performed and compared 6 different classification methods with different numbers of features.
- The 30 samples were separated into a training set (18 samples) and a testing set (12 samples), 2-fold cross validation was performed on dividing training data and test data to get the error rates to compare those methods.
- Features were chosen from the 1283 differentially expressed genes identified in the differential analysis stage, the number of features was set as 100, 300, 500, and 1000.

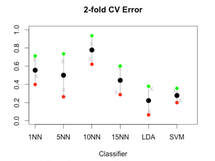


Fig 12. 2-fold CV error rate results of 6 methods with the top 300 hits as features. Plot shows we should choose LDA or SVM. Final results shows SVM method had the lowest error rate of 25. 0%.

Gene Enrichment Analysis

#Term	ID	Input number	Background number	P-Value
multi-multicellular organism process	GO:0044706	10	109	0.000389
multi-organism reproductive process	GO:0044703	10	113	0.000506
positive regulation of apoptotic signaling pathway	GO:2001235	6	41	0.000641
positive regulation of kinase activity	GO:0033674	16	267	0.000825
response to wounding	GO:0009611	30	678	0.000955
regulation of response to stress	GO:0080134	25	529	0.001031
positive regulation of transferase activity	GO:0051347	16	277	0.001185
AMP-activated protein kinase complex	GO:0031588	3	7	0.001224

Table 3. results of gene enrichment from the gene ontology (GO).

#Term	ID	Input number	Background number	P-Value
HTLV-I infection	hsa05166	20	237	0.003266
Circadian rhythm	hsa04710	5	28	0.009123
Inflammatory bowel disease (IBD)	hsa05321	5	55	0.088759
Asthma	hsa05310	3	24	0.089762
Lysosome	hsa04142	8	112	0.104265

Table 4. results of gene enrichment from KEGG pathway.

- The gene enrichment analyses above indicate differentially expressed genes in IBD using GO Ontology and KEGG pathways (KOBAS 2.0).

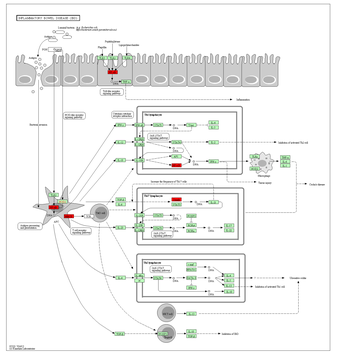


Fig 13. IBD gene network (KEGG). The DE genes are in red.

Conclusions

- A number of genes were found to have statistically significant expression values across all three groups.
- Age and sex had no apparent effect in our experiments even though age could be a factor for Crohn's disease and ulcerative colitis.
- In the overall analysis, significant differences between the genes of normal people and IBD patients' genes were found, while any differences between the Crohn's disease group and the ulcerative colitis group still need to be confirmed.
- Classification showed promising results, the classifier we built could be used for prediction, but we should not rely on it.
- Enrichment in IBD pathways (KEGG) is meaningful for our study. Though we only found five DE genes, they are all located in key positions on the pathway.

Improvements

- Increased sample size would increase the reliability of the conclusion, especially for PCA analysis and classification analysis. Results from those analyses need to be further validated.

References

[1] Expression profiling in inflammatory bowel disease data set.

[2] Costello, Christine M., et al. "Dissection of the inflammatory bowel disease transcriptome using genome-wide cDNA microarrays." PLoS medicine 2.8 (2005): e199.

[3] Chen, Chao, et al. "Removing batch effects in analysis of expression microarray data: an evaluation of six batch adjustment methods." PloS one 6.2 (2011): e17238.

[4] Eickhoff, H. et al. Tissue gene expression analysis using arrayed normalized cDNA libraries. Genome Res 10, 1230-40. (2000).