

Transcriptome analysis of apoE ^{-/-} mouse aortae

Abstract

Gene expression in aortas of 32 week old C57BL/6J apoE^{-/-} mice was compared to that in wild type C57BL/6J mice using the Affymetrix® GeneChip® Mouse Genome 430 2.0 array. Using filtering criteria of a 1.5 or greater fold-change in expression, all “present calls” in at least one group and a false discovery rate of less than 5%, 861 out of over 39,000 transcripts were differentially expressed. The expression of 742 transcripts was increased in the apoE^{-/-} aortas, while 119 showed decreased expression. The Gene Ontology and KEGG pathway terms associated with these genes were examined to identify biological themes associated with each set of genes. These analyses identified distinct biological themes associated with each group of genes. The ontology term analysis identified an over representation of genes involved in the immune response, cell adhesion and apoptosis among the up-regulated genes. The analysis of KEGG pathway terms identified a significant over representation of genes involved in the Jak-Stat signaling pathway and cytokine-cytokine receptor interactions among the up-regulated genes.

Introduction

In this study the GeneSifter® microarray data analysis system was used to analyze data generated from a comparison of aortas from 32 week old C57BL/6J wild type mice and C57BL/6J apoE^{-/-} mice. This analysis process used can be broken down into two discrete tasks: identification of differentially expressed genes and the determination of the biological significance of both individual genes and groups of genes. GeneSifter uses Gene Ontology (GO) Reports and z-scores to summarize the biological processes associated with a gene list. Z-scores can then be used to identify GO terms that are significantly over- or under-represented in a gene list. This study outlines the use of these methods to identify biological themes associated with genes up-regulated in the aortas of apoE^{-/-} mice.

The Data

Gene expression profiles were measured in the aortas of 32 week old apoE^{-/-} mice and wild type C57BL/6J mice using the Affymetrix® GeneChip® Mouse Genome 430 2.0 array. Three biological replicates were prepared for each mouse strain. Data for each sample was downloaded from GEO (GSE2372) and loaded into GeneSifter. The MAS 5 derived signal and detection calls were used for subsequent analysis.

Identification of differentially expressed genes

The Affymetrix® GeneChip® Mouse Genome 430 2.0 array contains probe sets representing over 39,000 transcripts. Using filtering criteria of a 1.5 or greater fold-change in expression, all “present calls” in at least one group and a p-value of < 0.05 from a unpaired, two sample t-test produced a list of 1025 genes that were differentially expressed. Correction for multiple testing was then performed using the method of Benjamini and Hochberg (Reiner, et al., 2003) to derive a false discovery rate estimate from the raw p-values. A false discovery rate of 5% was used as a cutoff for statistical significance giving a final list of 861 differentially expressed genes. 742 of these genes showed higher expression in the aortas of apoE^{-/-} mice, while 119 showed decreased expression. The parameters used for this filtering are summarized in figure 1.

Biological significance

The biological process ontologies and KEGG pathway terms associated with the differentially expressed genes were examined using a z-score report. The z-score report identifies ontologies or pathway terms that are significantly over-represented in a gene list (Doniger, et al., 2003). The most significantly enriched biological process ontology identified was “immune response” with a z-score of 15.7. Several other more specific terms related to “immune

A

Fold-change	1.5
Detection Filter	Present Only
Statistics	t-test
Correction	Benjamini and Hochberg
Confidence	5% False Discovery Rate

B

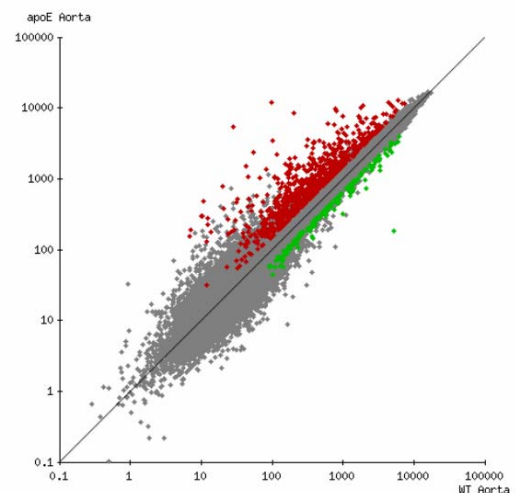


Figure 1: A) Parameters used to identify differentially expressed genes using GeneSifter. B) Scatterplot showing all genes. Red and green points indicate significantly up-regulated and down-regulated genes, respectively.

A

Biological Process Ontology	Up regulated	Array	z-score
immune response	74	398	15.7
inflammatory response	26	103	11.4
chemotaxis	22	94	9.95
tumor necrosis factor-alpha biosynthesis	4	7	7.36
humoral immune response	13	60	7.23
immune cell activation	16	91	6.88
prostaglandin biosynthesis	3	6	5.9
regulation of cytokine biosynthesis	7	28	5.86
signal transduction	98	1623	5.1
cell adhesion	31	431	3.74
lymphocyte proliferation	5	31	3.58
proteolysis and peptidolysis	32	465	3.53
positive regulation of apoptosis	9	82	3.4
integrin-mediated signaling pathway	6	56	2.7

B

Molecular Function Ontology	Up regulated	Array	z-score
chemoattractant activity	12	35	9.57
signal transducer activity	124	1805	7.75
receptor activity	92	1234	7.37
cytokine activity	20	187	5.09
actin binding	18	188	4.28
interleukin receptor activity	5	30	3.75
caspace activity	3	13	3.69
scavenger receptor activity	4	23	3.47
cysteine-type peptidase activity	12	136	3.17
metallopeptidase activity	12	147	2.87
hydrolase activity	78	1654	2.33
calcium ion binding	25	458	2.01

Table 1: Z-score reports identified distinct biological themes associated with genes up-regulated in apoE ^{-/-} aortas. A) Biological process ontologies. B) Molecular function ontologies.

response” such as “inflammatory response” and “chemotaxis” were also identified. Molecular function ontologies identified included “chemoattractant activity”, “cytokine activity” and “peptidase activity”. See table 1 for

a list of selected ontology terms over-represented among the genes up-regulated in the apoE ^{-/-} aortas. Z-score analysis of the KEGG pathway terms found that genes involved in the Jak-Stat signaling pathway were up-regulated in the apoE ^{-/-} aortas (figure 2). See supplemental material to view a comprehensive list of ontologies and pathway terms identified as being significantly over-represented among the genes up-regulated in the apoE ^{-/-} aortas.

Summary

861 differentially expressed genes were identified in this comparison of gene expression in aortas from apoE ^{-/-} mice with that in wild type mice. Analysis of the Gene Ontology and KEGG pathway terms associated with these genes showed enrichment of gene families associated with immune response, signal transduction, apoptosis and proteolysis among the 742 up-regulated genes. This analysis has identified a set of genes that may contribute to the phenotype observed in apoE ^{-/-} mice.

Supplemental Material

Raw data is available from the Gene Expression Omnibus (GSE2372).

Data can be viewed and analyzed using the GeneSifter Data Center (www.genesifter.net/web/DC).

References

Reiner, et al. 2003. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics* 19(3):368-375

Doniger, et al. 2003. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biology* 4:

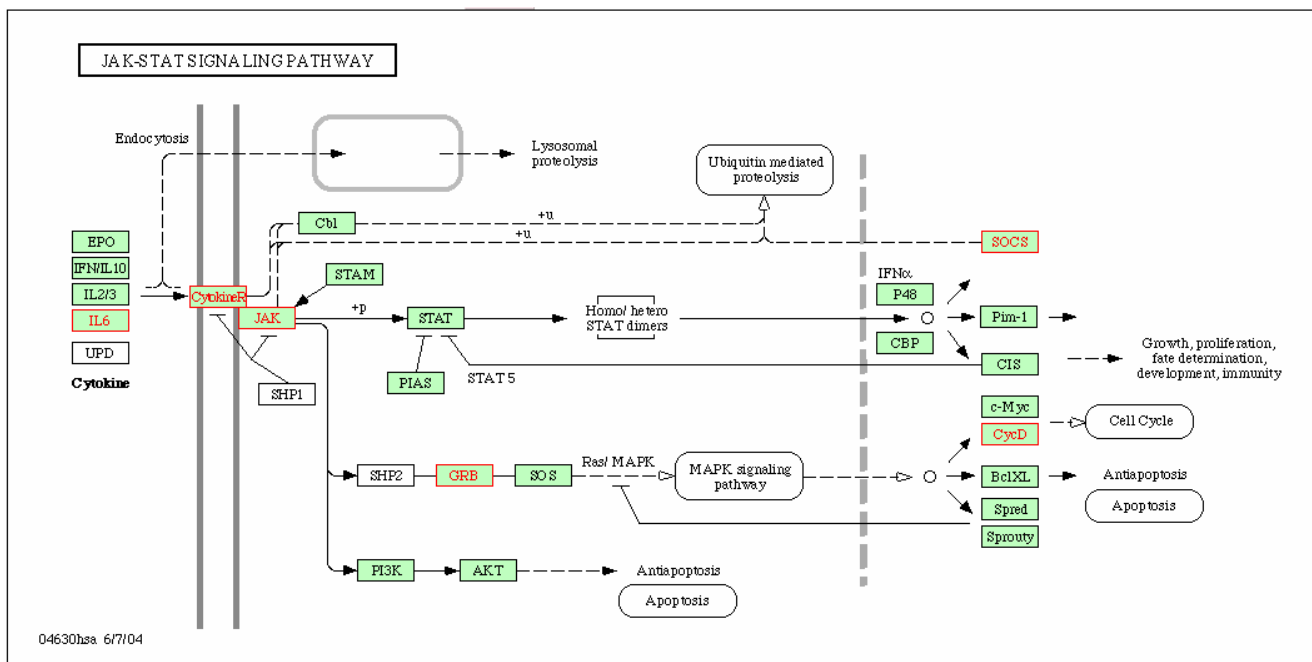


Figure 2: KEGG pathway diagram showing genes involved in the Jak-Stat Signaling Pathway up-regulated in apoE ^{-/-} aortas. The genes that were up-regulated are highlighted in red.