

Analysis of Next Generation Sequencing Data Final Project Presentation

Differential Expression Analysis of RNA-seq
Data Derived from Alzheimer's Patient Microglia



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Presentation Structure

1. Introduction
2. Results
3. Methods
4. Discussion



Introduction

Alzheimer's, Microglia and Reference Study



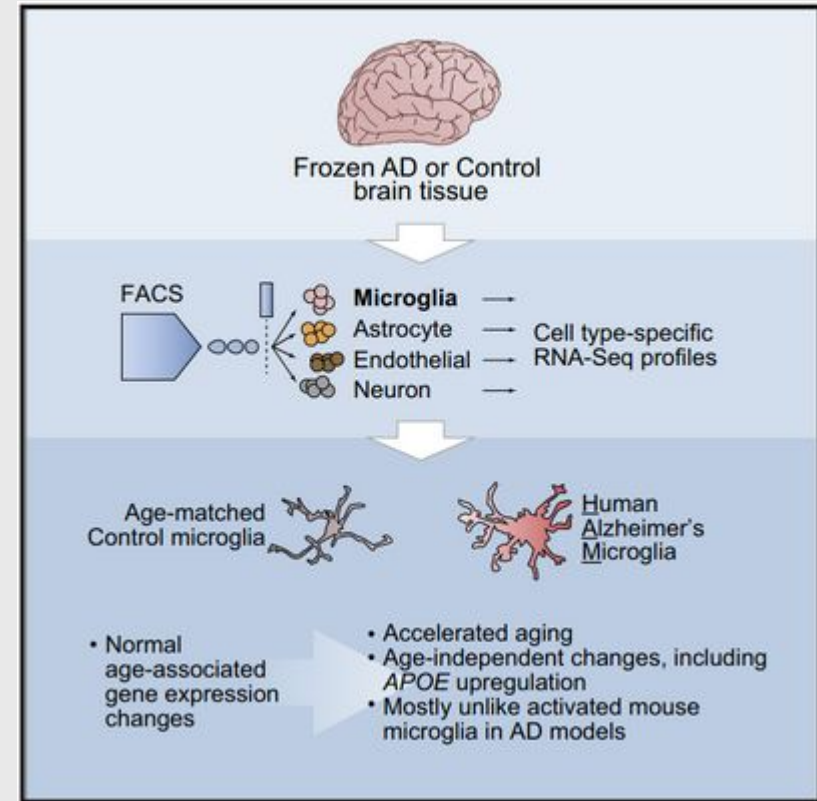
Alzheimer's Disease

- **Alzheimer's disease (AD) effects approximately 5.8 million people in the the United States ages 65 and older**
 - **Early sign and symptoms of the disease centering around forgetfulness**
 - **Impairment of memory follows, until loss of ability to carry out everyday tasks**
- **There is currently no treatment that cures AD or alters the disease process in the brain -- Mayo Clinic**
 - **Important to continue research in the area**
 - **Possibly NAD therapies:** Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor- γ coactivator 1 α regulated β -secretase 1 degradation and mitochondrial gene expression in Alzheimer's mouse models



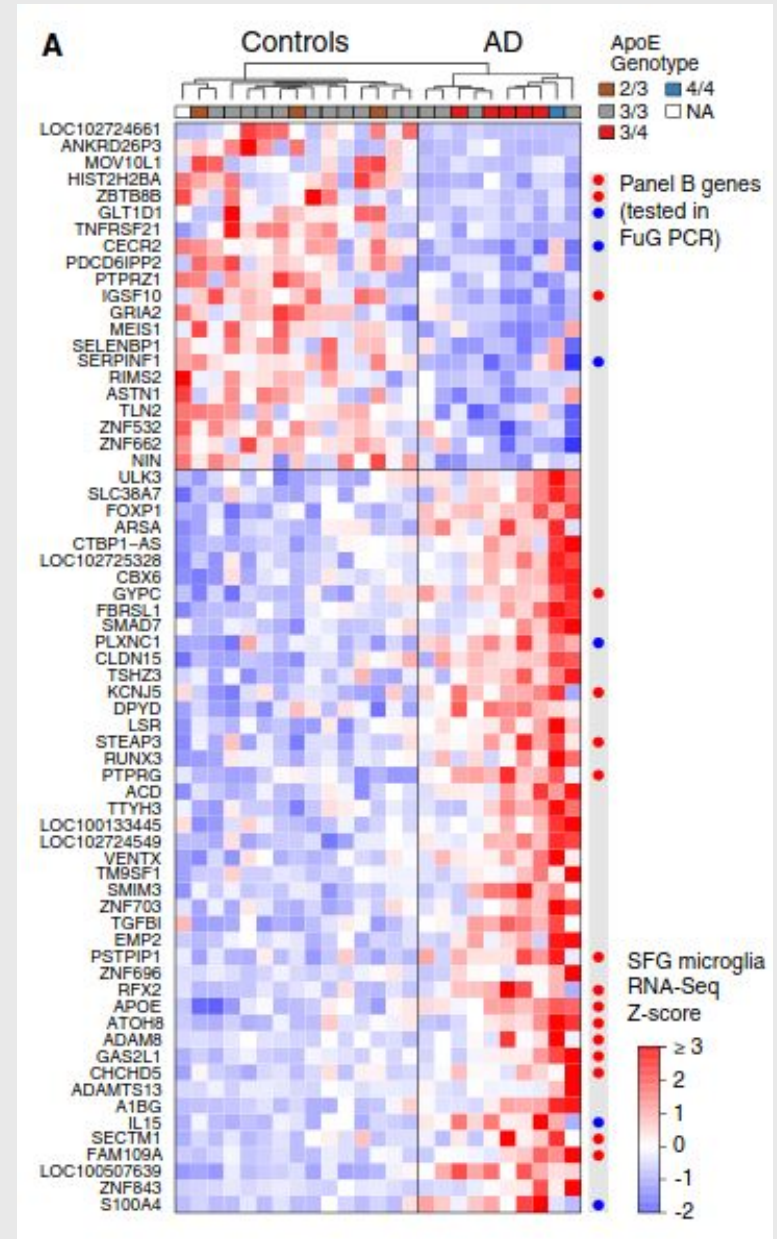
Alzheimer's Patient Microglia Exhibit Enhanced Aging and Unique Transcriptional Activation

- Recent genetic studies of humans have identified brain specific myeloid cells (microglia) as a potential key cell type controlling an individual's risk of acquiring Alzheimer's Disease
- This study identified 45 differentially expressed genes between Control and AD Microglia (Myeloid) cells



Study Hypothesis

- Given scrupulous quality control and industry standard tools such as STAR and DESeq2, I will achieve a different set of differentially expressed genes than shown final in the reference publication
 - "Sorted cell and whole tissue RNA-Seq data were analyzed using the GSNAP aligner and HTSeqGenie"



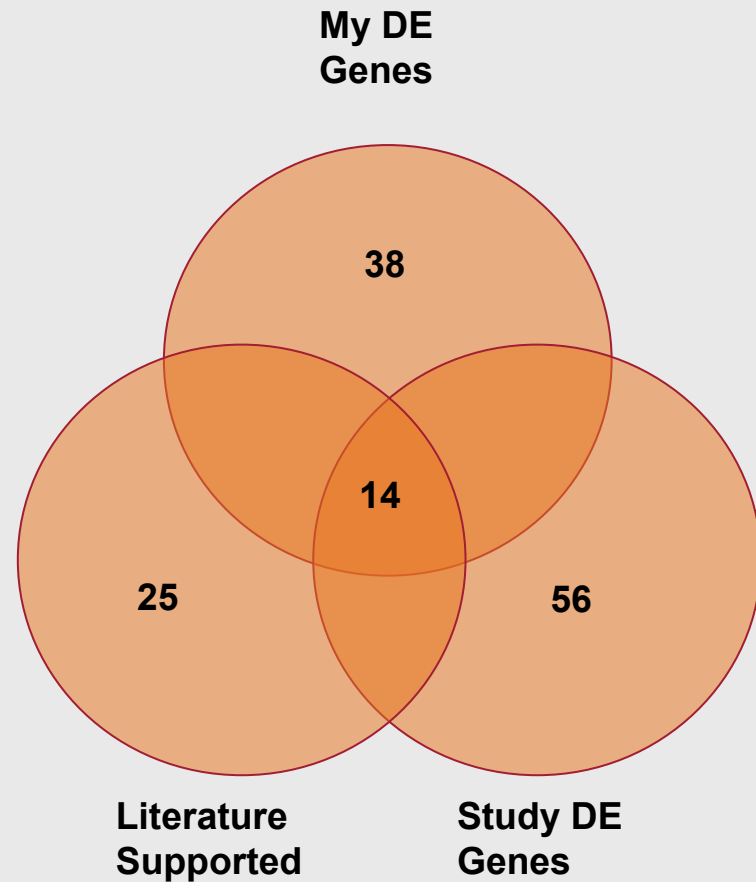
Results

DE Genes, Volcano Plot, Gene Ontology Treemap

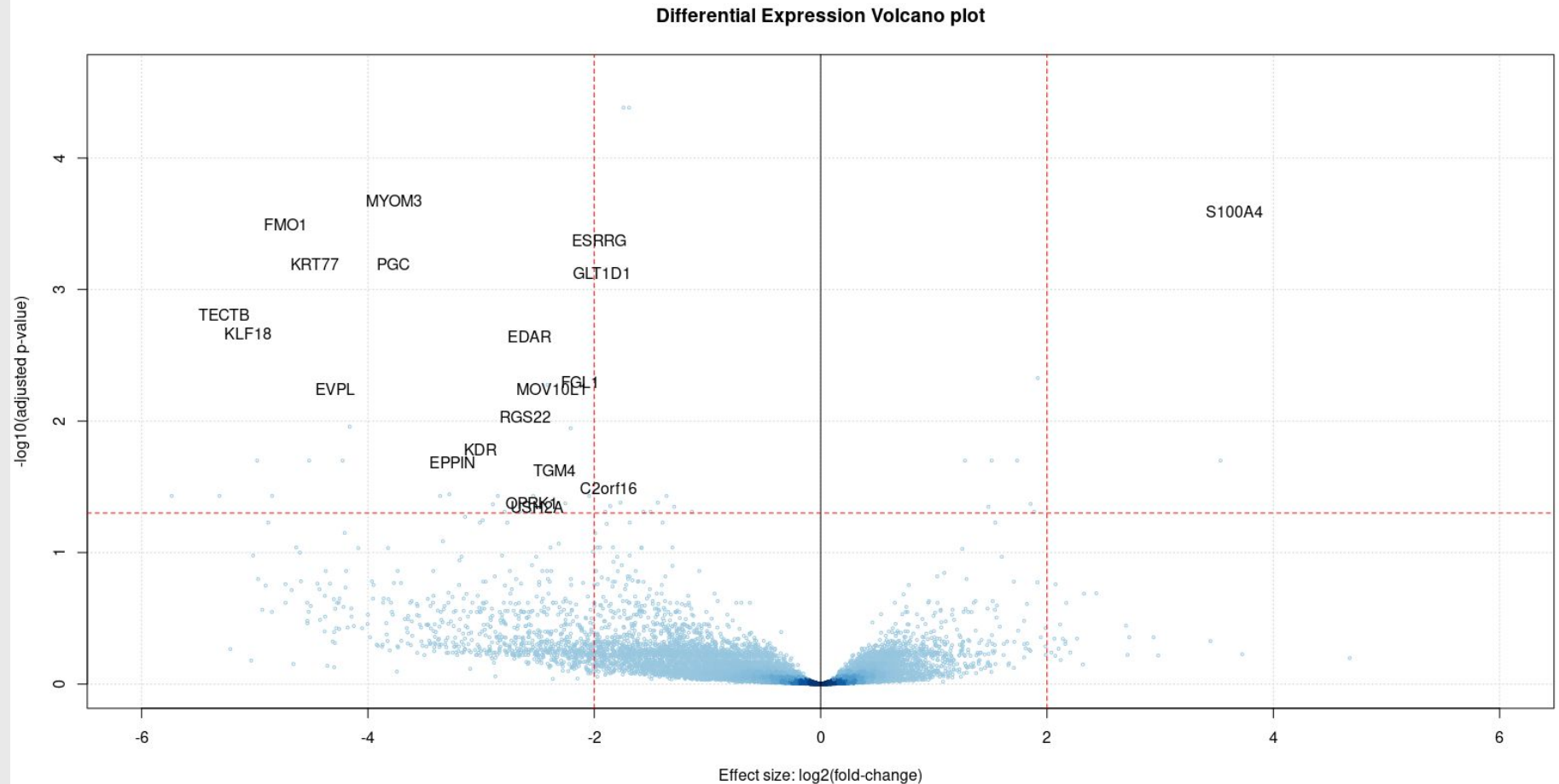


Differentially Expressed Genes

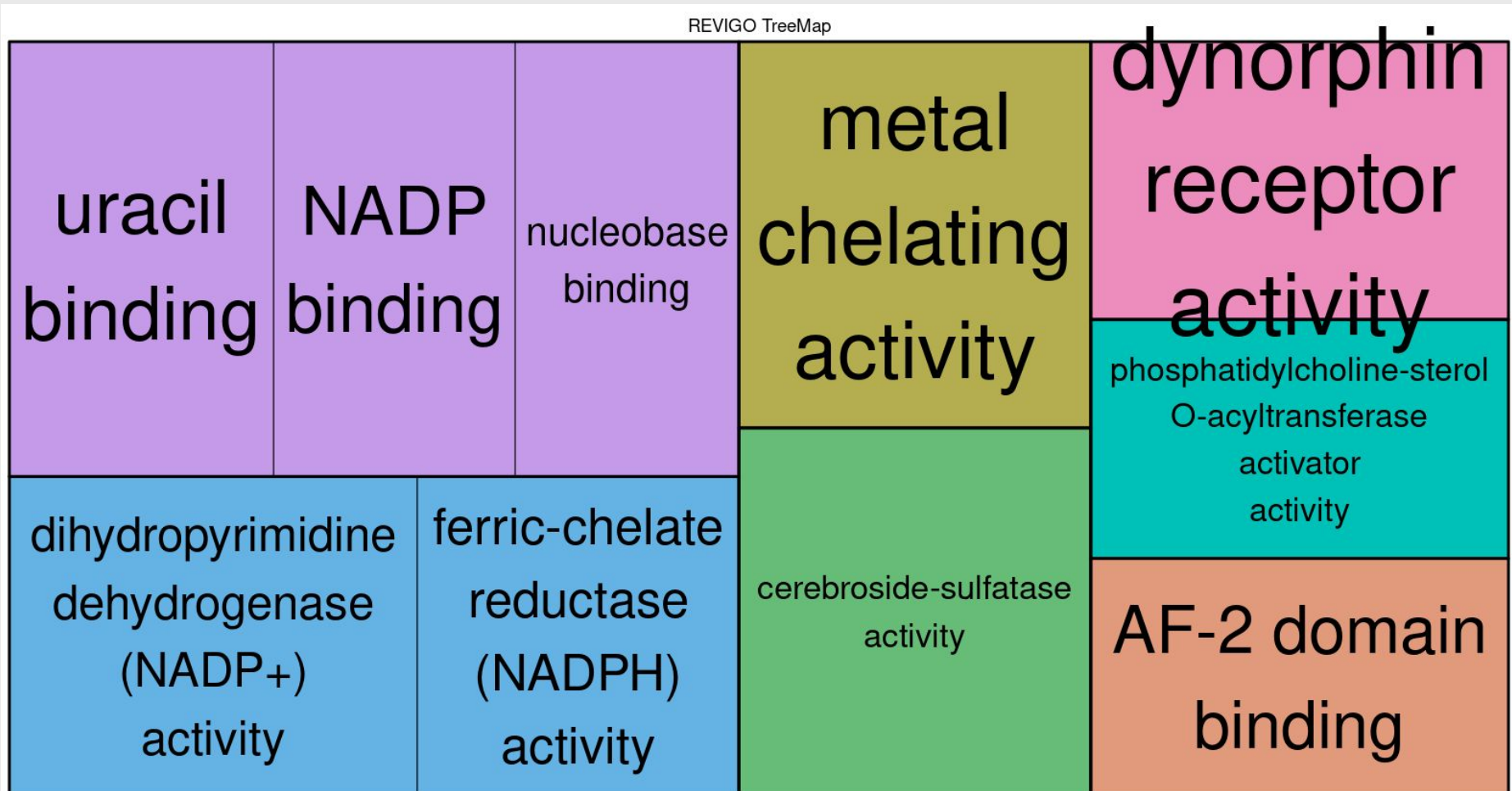
- Confirmed 38 genes to be differentially expressed between control and AD clinical groups
- Same 14 genes supported by my analyses, reference paper, and study-found literature-supported AD risk genes



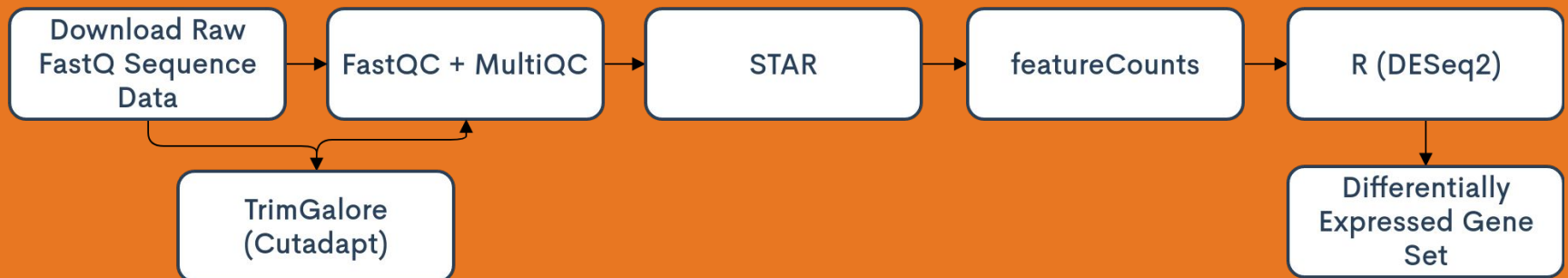
Differential Expression Volcano Plot



Gene Ontology Treemap -- Bio. Func.



Methods



Downloading Data

- **Data Available through NCBI GEO Accession Number**
 - **SRA Runs Selector**
 - **113 total isolated-cell samples**
 - **25 Myeloid samples**
 - **Age, APOE genotype, Sex, PMI metadata available**

Run ¹	BioSample ²	APOE ³	Bases ⁴	Bytes ⁵	Cell_type ⁶	Diagnosis ⁷
SRR8440443	SAMN10741236	3/3	2.27 G	694.72 Mb	myeloid	Control
SRR8440444	SAMN10741241	3/3	2.19 G	683.21 Mb	endothelial	Control
SRR8440445	SAMN10741240	3/3	2.17 G	700.85 Mb	neuron	Control
SRR8440446	SAMN10741239	3/4	2.44 G	791.87 Mb	neuron	AD
SRR8440447	SAMN10741238	NA	2.14 G	648.79 Mb	myeloid	Control
SRR8440448	SAMN10741237	3/4	2.16 G	664.66 Mb	myeloid	AD
SRR8440449	SAMN10741235	2/3	2.16 G	653.94 Mb	myeloid	Control
SRR8440450	SAMN10741234	2/3	2.99 G	940.45 Mb	endothelial	Control
SRR8440451	SAMN10741233	3/3	2.91 G	902.51 Mb	endothelial	AD
SRR8440452	SAMN10741232	3/3	2.46 G	793.50 Mb	neuron	Control
SRR8440453	SAMN10741231	3/3	2.34 G	766.49 Mb	neuron	AD
SRR8440454	SAMN10741230	3/3	2.47 G	789.37 Mb	neuron	AD
SRR8440455	SAMN10741229	3/3	2.24 G	829.08 Mb	endothelial	Control
SRR8440456	SAMN10741228	3/3	4.10 G	1.24 Gb	astrocyte	Control
SRR8440457	SAMN10741286	2/3	10.49 G	3.24 Gb	astrocyte	Control
SRR8440458	SAMN10741285	3/3	2.01 G	650.22 Mb	neuron	Control



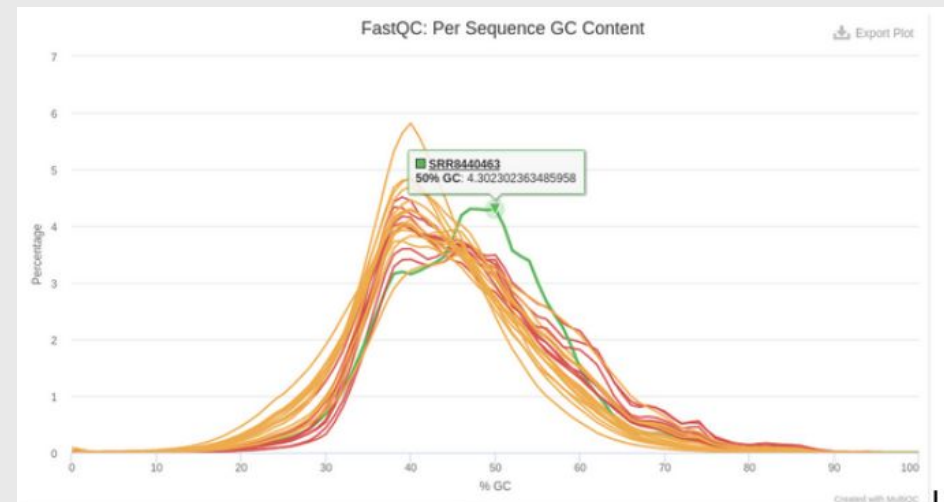
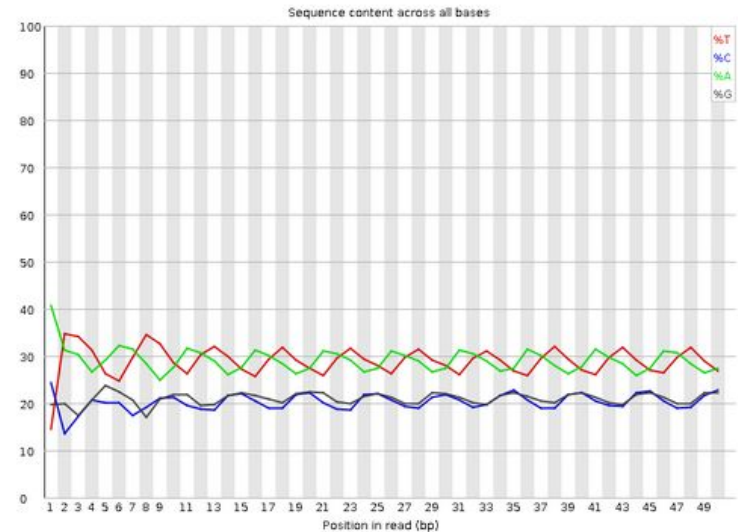
Raw Data FastQC

- At first detected Illumina adapters
 - TrimGalore
- 3 samples had cyclic GC content
 - No per-tile information available
- Skewed GC content found in 1 sample
 - 72 year old male with AD and 4/4 APOE genotype

Overrepresented sequences

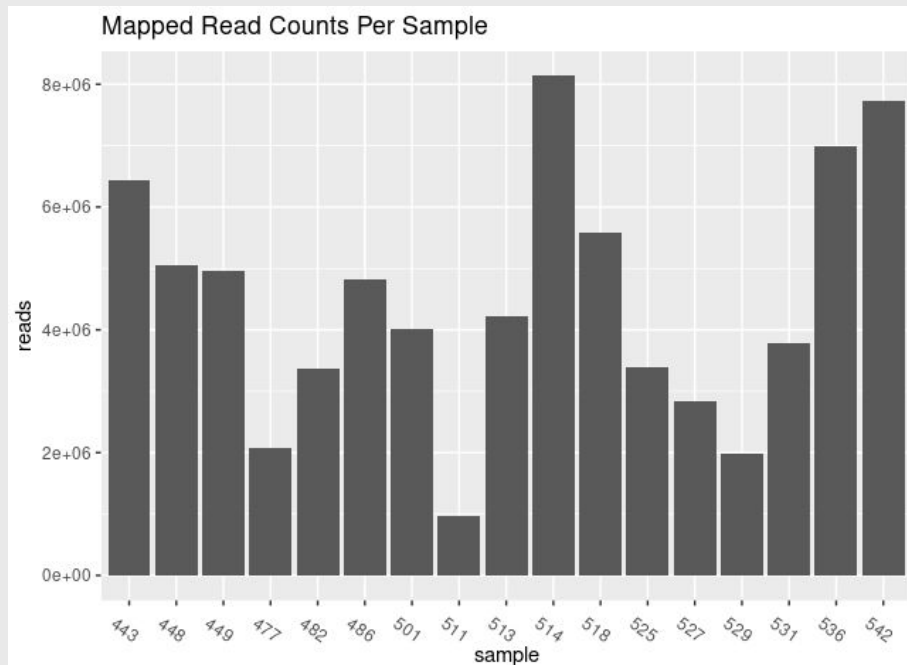
Sequence	Count	Percentage	Possible Source
GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGC	121984	0.34849652269629916	TruSeq Adapter, Index 5 (100% over 50bp)
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGC	43845	0.12534313917196513	TruSeq Adapter, Index 5 (100% over 49bp)

Per base sequence content



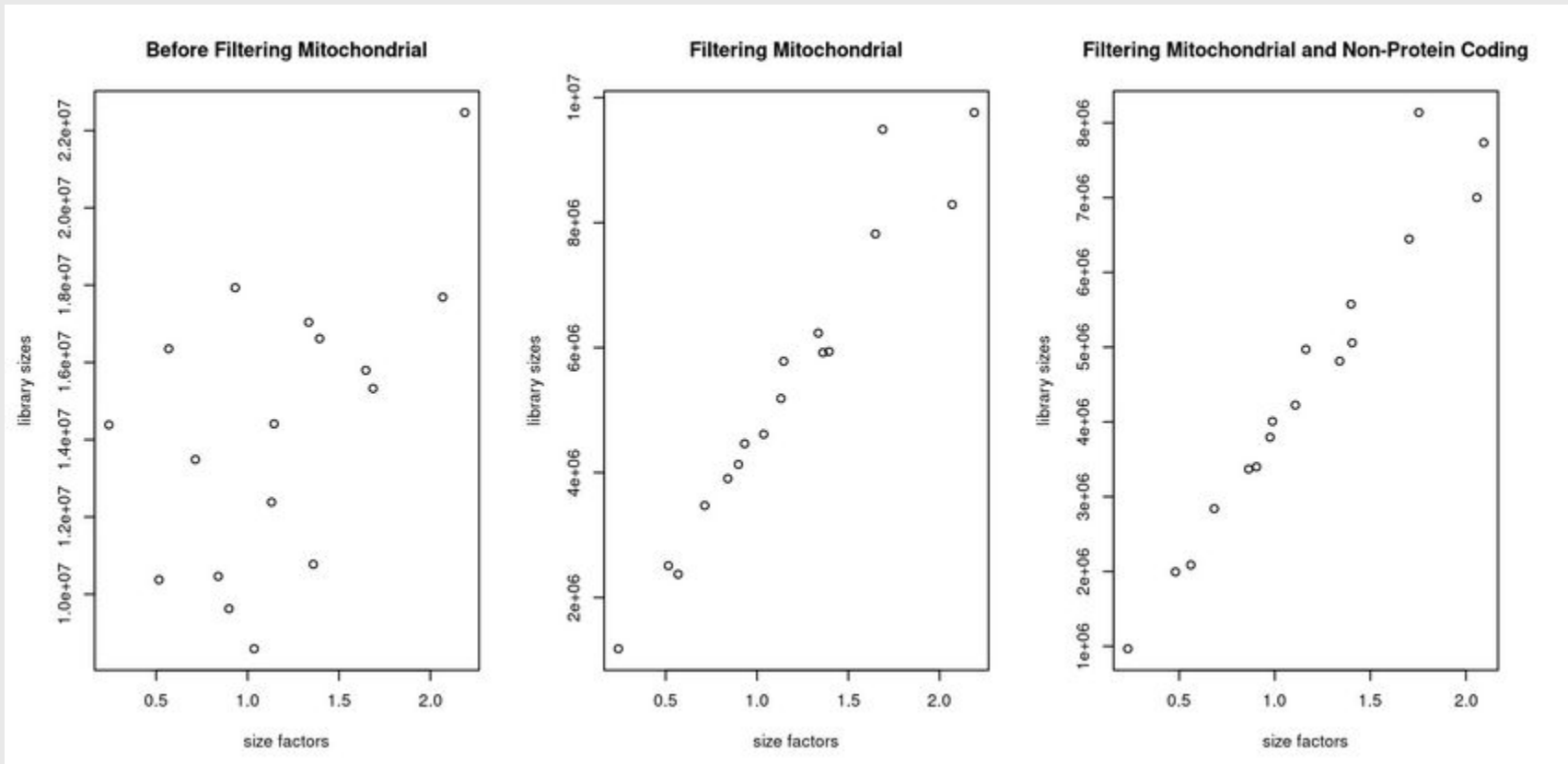
RNA-Seq Alignment with STAR

- Same 72 YO male sample seen with skewed GC content also has 33.79% of reads failing to map to human HG38 Genome
- Other samples look fairly uniform (5-10% of reads not mapping)



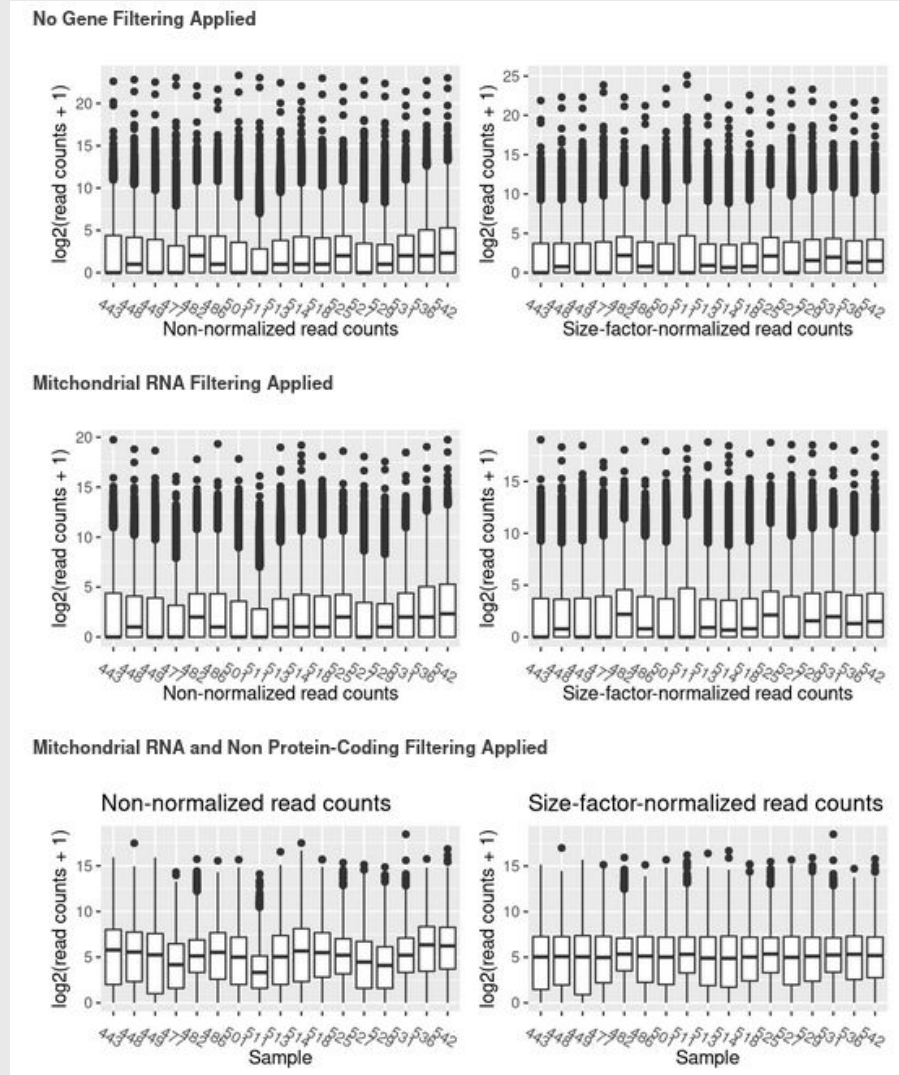
Percentage of unmapped reads	for	SRR8440443:	5.90%
Percentage of unmapped reads	for	SRR8440447:	4.17%
Percentage of unmapped reads	for	SRR8440448:	4.04%
Percentage of unmapped reads	for	SRR8440449:	8.38%
Percentage of unmapped reads	for	SRR8440463:	33.79%
Percentage of unmapped reads	for	SRR8440477:	5.14%
Percentage of unmapped reads	for	SRR8440482:	6.54%
Percentage of unmapped reads	for	SRR8440484:	3.65%
Percentage of unmapped reads	for	SRR8440486:	8.13%
Percentage of unmapped reads	for	SRR8440488:	5.19%
Percentage of unmapped reads	for	SRR8440501:	6.98%
Percentage of unmapped reads	for	SRR8440511:	5.47%
Percentage of unmapped reads	for	SRR8440513:	6.79%
Percentage of unmapped reads	for	SRR8440514:	4.97%
Percentage of unmapped reads	for	SRR8440517:	5.76%
Percentage of unmapped reads	for	SRR8440518:	5.02%
Percentage of unmapped reads	for	SRR8440524:	11.28%
Percentage of unmapped reads	for	SRR8440525:	9.51%
Percentage of unmapped reads	for	SRR8440527:	7.05%
Percentage of unmapped reads	for	SRR8440529:	6.67%
Percentage of unmapped reads	for	SRR8440531:	10.64%
Percentage of unmapped reads	for	SRR8440536:	7.61%
Percentage of unmapped reads	for	SRR8440538:	7.43%
Percentage of unmapped reads	for	SRR8440539:	12.34%
Percentage of unmapped reads	for	SRR8440542:	8.16%

Read Count Normalization -- Size Factors



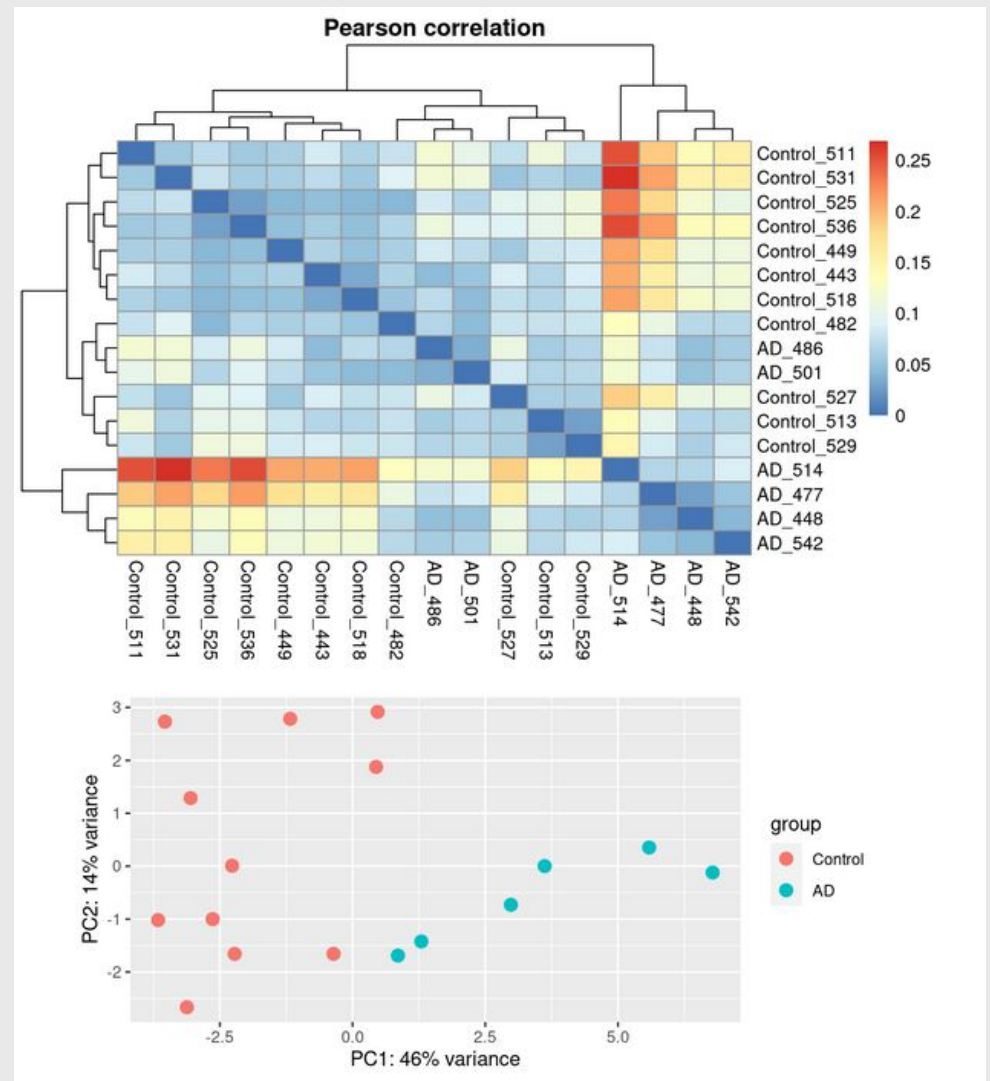
Gene Filtering

- Tissue from frozen samples
 - High lncRNA and Mitochondrial RNA content
- Filtered for only non-Mitochondrial protein-coding genes
 - Study does not, and identifies many mitochondrial associated differentially expressed genes



Literature-Supported Gene Set

- Did not find clinical group separation with rlog normalized gene expression, or top 300-1000 variable genes
- Study identifies 25 literature-supported AD-risk genes
 - Used in exploratory data analysis phase



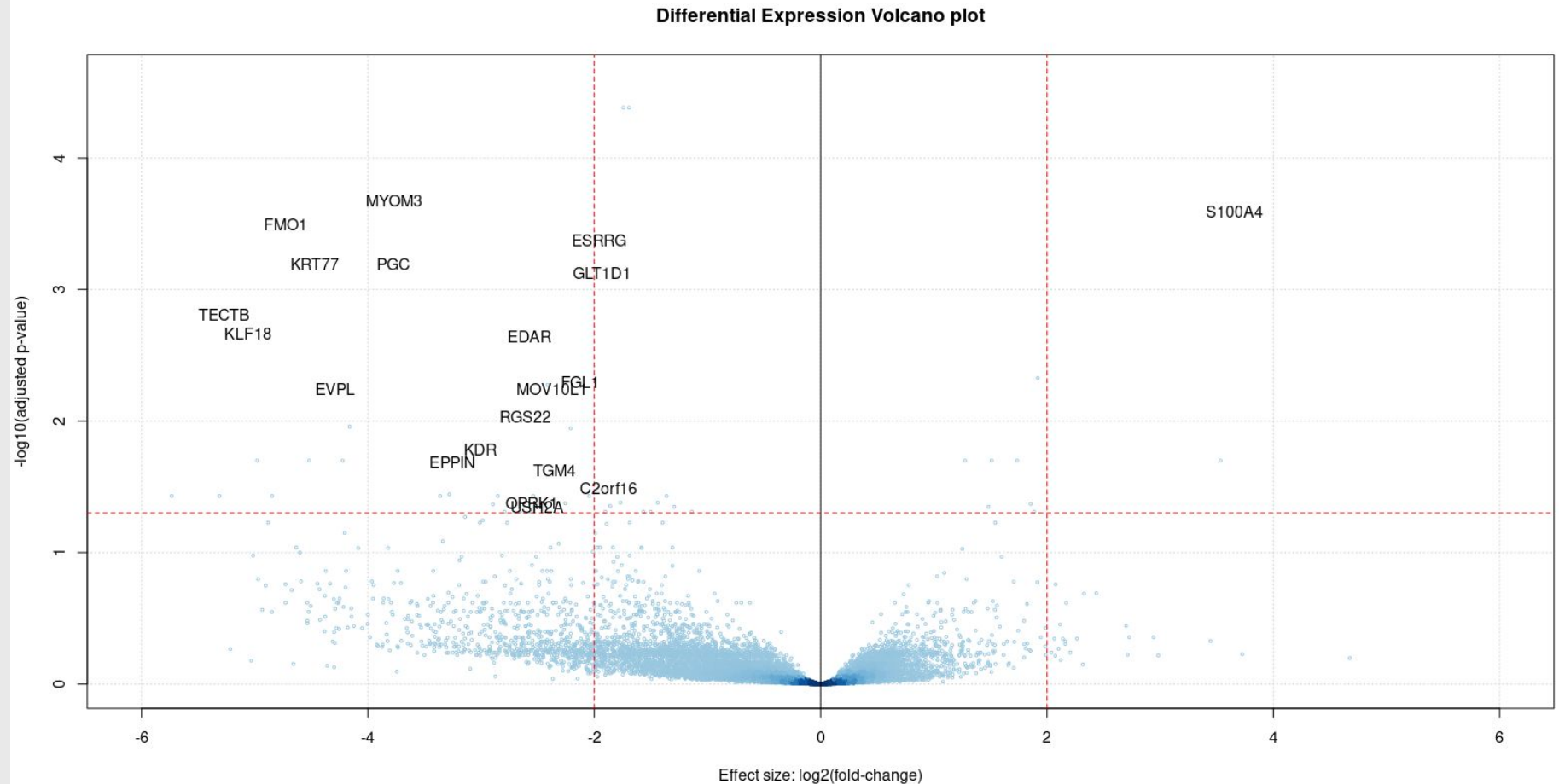
Differentially Expressed Genes

- **Confirmed 14 genes to be differentially expressed between control and AD clinical groups**
- **Same 14 genes appear in paper as gathered literature-supported AD risk genes, and appear in final DE gene list of paper**

	padj	log2FoldChange
SERPINF1	0.000041	1.7407
CECR2	0.000041	1.6923
TGFB1	0.004718	-1.9188
GLT1D1	0.005319	2.4133
ARSA	0.019995	-1.2752
PTPRG	0.019995	-1.7361
S100A4	0.019995	-3.5340
APOE	0.019995	-1.5115
MOV10L1	0.037131	2.5397
ASTN1	0.041758	1.4381
DPYD	0.042672	-1.8551
STEAP3	0.044855	-1.4818
IGSF10	0.048952	1.5660
PSTPIP1	0.048952	-1.8852

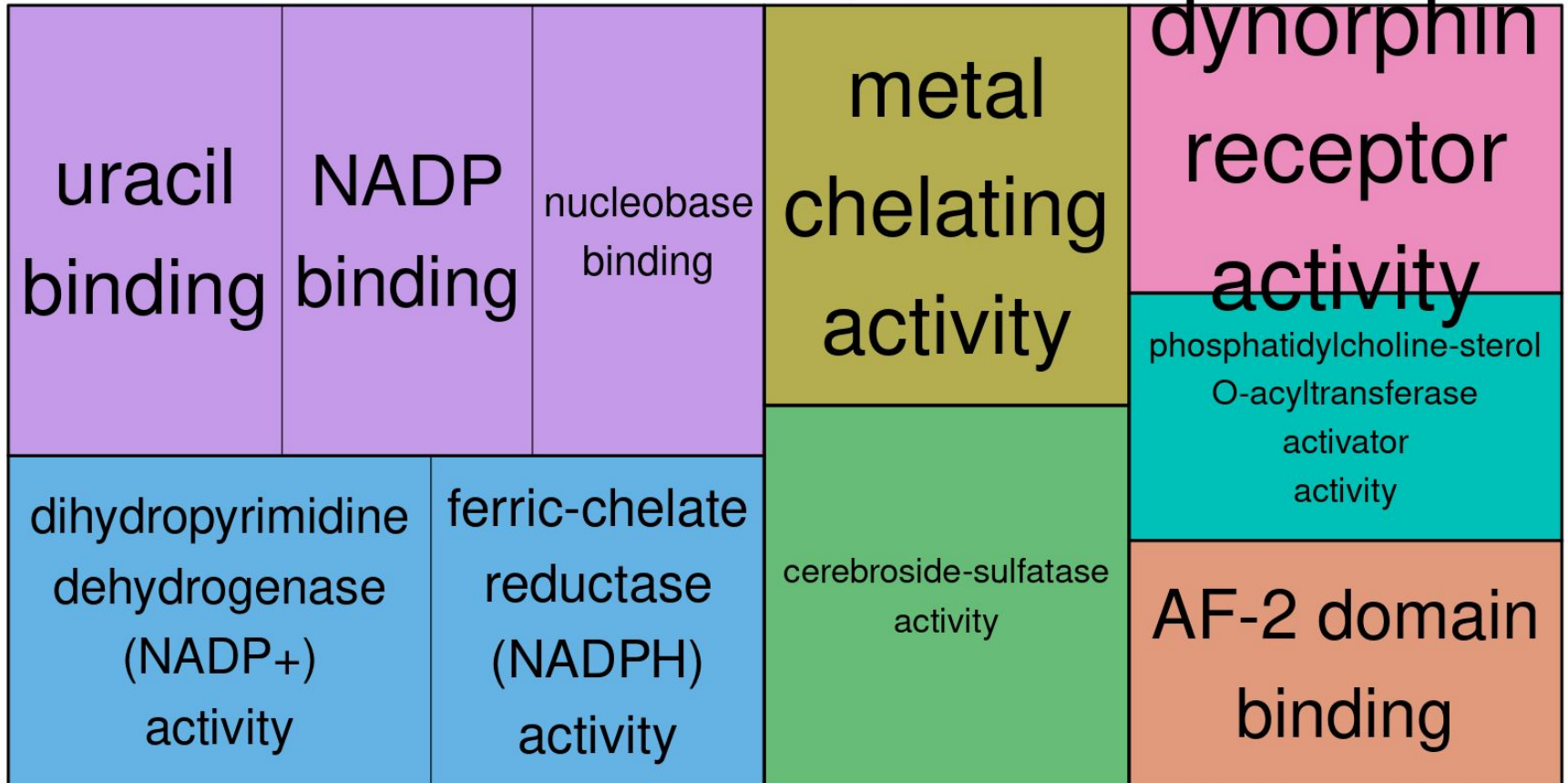


Differential Expression Volcano Plot



Gene Ontology Treemap -- Bio. Func.

REVIGO Treemap



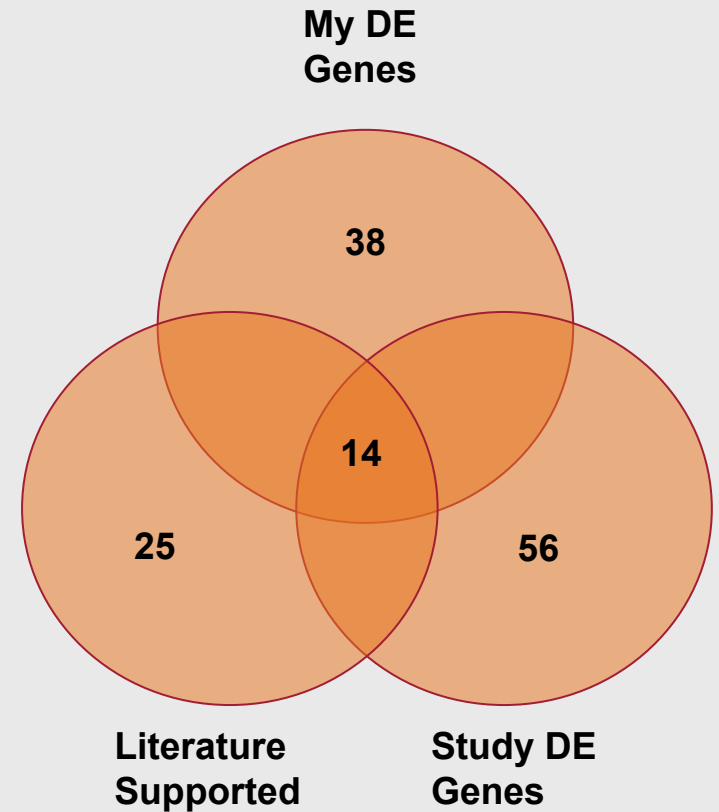
Discussion

Limitation of Scope, Interesting Identified Genes



Limitations and Findings

- **Scope was limited due to high lncRNA and Mitochondrial RNA content**
 - Could only confirm protein-coding genes
 - lncRNAs in DE list of study
- **However, genes that were confirmed are interesting**
 - Confirmation from literature, reference study and my own DE gene list



Interesting Identified Genes

SERPINF1 (serpin family F member 1) – The encoded protein is secreted and strongly **inhibits angiogenesis**. In addition, this protein is a **neurotrophic factor** involved in neuronal differentiation in retinoblastoma cells. Mutations in this gene were found in individuals with osteogenesis imperfecta.

CECR2 (CECR2 histone acetyl-lysine reader) – Involved in chromatin remodeling, and may additionally play a role in **DNA damage response**. The encoded protein functions as part of an ATP-dependent complex that is involved in neurulation.

ARSA (arylsulfatase A) – Defects in this gene lead to metachromatic leucodystrophy (MLD), a progressive demyelination disease which results in a **variety of neurological symptoms** and ultimately death.

S100A4 (S100 calcium binding protein A4) – S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the **regulation of a number of cellular processes such as cell cycle progression and differentiation**.

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MOV10L1	0.037131	2.5397
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DPYD	0.042672	-1.8551
STEAP3	0.044855	-1.4818
IGSF10	0.048952	1.5660
PSTPIP1	0.048952	-1.8852



Weill Cornell Medicine