The Role of Lithium in Suicide Prevention

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Background

- 4.6% of Americans report attempting suicide at least once
- 1.2% of all American deaths are due to suicide
- Lithium used as treatment for bipolar disorder, depression, and schizophrenia
  - Listed on World Health Organization List of Essential Medicines
- Exact mechanism of action is unknown
- We worked with Dr. Willour's lab and did follow up to their genome-wide association study

Study Design

- Human embryonic kidney (HEK) cell lines
  - HEK cells have some neuronal properties
  - Cell line selected because it is easy to work with
- Cell lines were split into 4 groups based on treatment and time
- Lithium treatment groups received media supplemented with lithium
  - Constant exposure to lithium
  - Additional lithium added every 2-3 days
- RNA was extracted and sequenced; the number of each gene present was recorded
- RNA Sequencing
  - Allows researchers to see changes in gene expression between groups
  - Interested in how much of each gene is made

Study Design

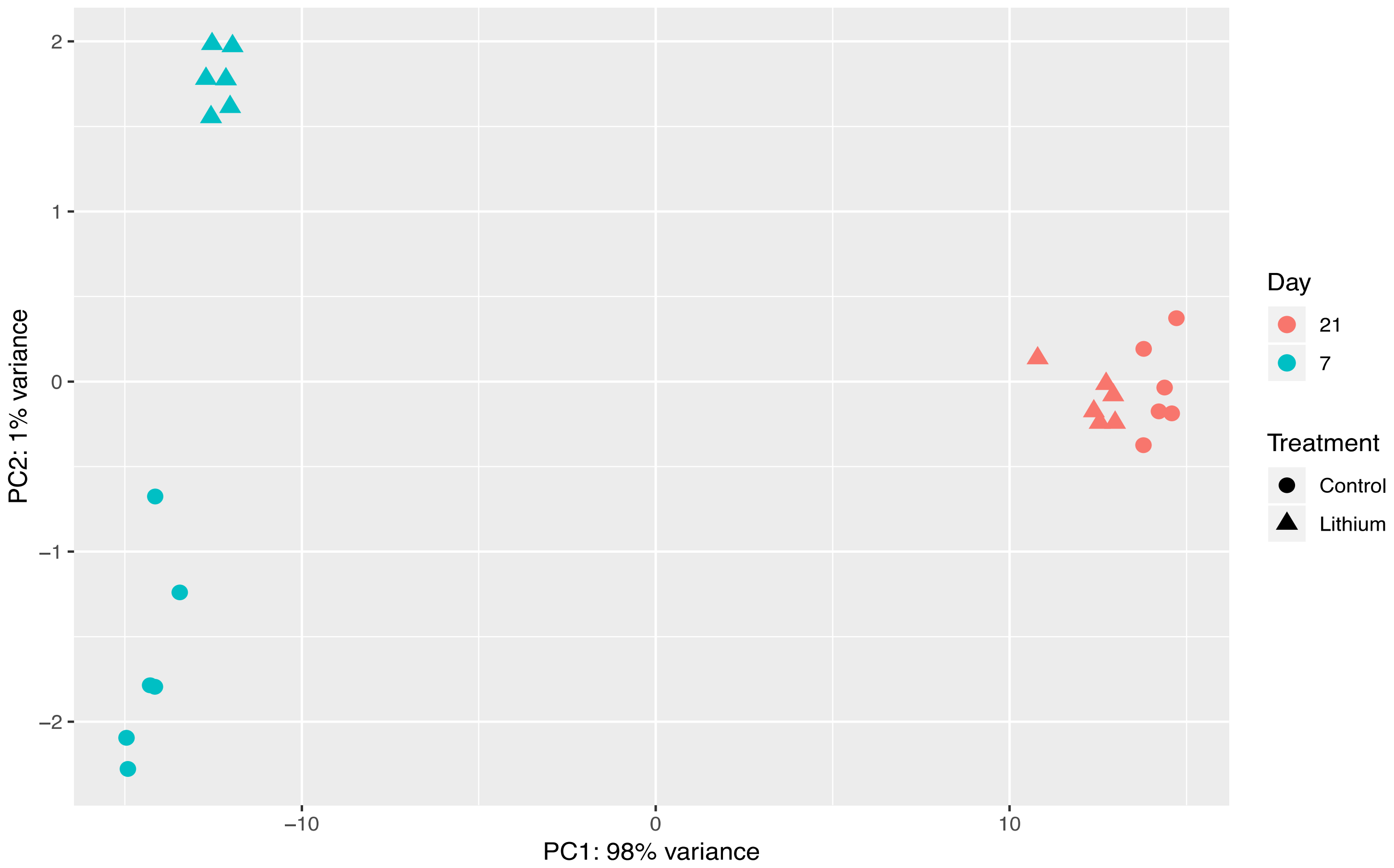
Group	Treatment Type	Treatment Length	Sample Size
1	Lithium	7 Days	6
2	Lithium	21 Days	6
3	Control	7 Days	6
4	Control	21 Days	6

- Previous studies had treatment lengths of 1 and 7 days

Study Design

- We looked at the difference in gene counts between lithium and control treatments
  - Looked at difference at day 7, day 21, and the interaction
  - Interaction is difference between day 7 and day 21 treatments
    - Is the treatment effect at day 21 different than at day 7?
- Significant genes had a false discovery rate (FDR) < 0.05
- Created list of genes that are in the brain based on several definitions
  - Top 1000 most expressed genes in the brain
  - Genes that were highly expressed in the brain but not in any other tissue
  - Top 66% most highly expressed genes in the brain

Principal Components Analysis Plot



DESeq2: Overdispersion

- In a Poisson distribution, the variance is assumed to be equal to the mean
- Overdispersion occurs when the variance is greater than the mean, and is therefore greater than predicted

Poisson Distribution
$E(Y) = \lambda$
$Var(Y) = \lambda$

Negative Binomial Distribution
$E(Y) = \lambda$
$Var(Y) = \lambda + \omega\lambda^2$

- DESeq2 estimates the overdispersion factor (ω) for each gene

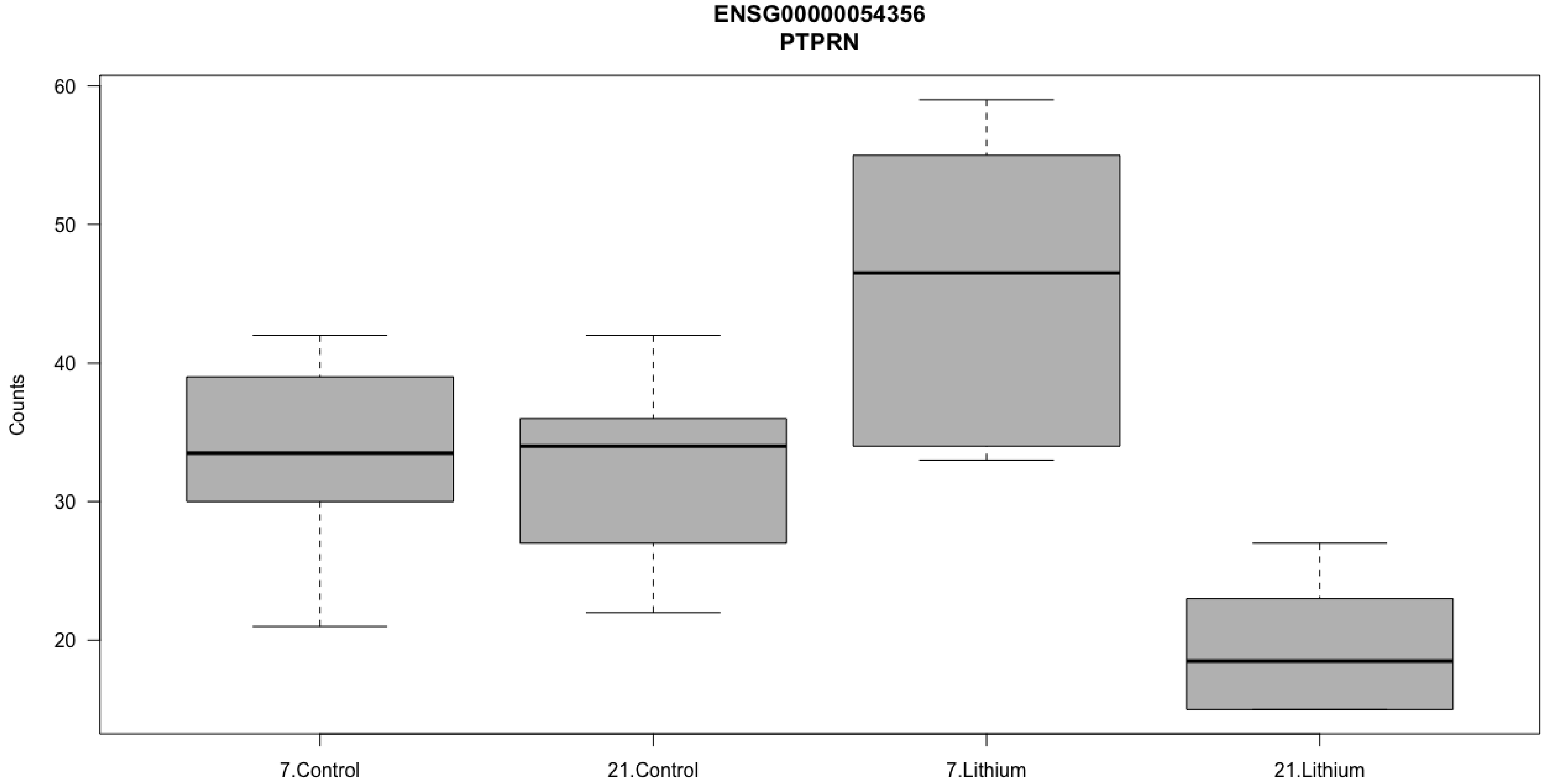
DESeq2: Shrinkage

- Log fold change (LFC) is a ratio used to represent change in expression levels of a gene at different treatments
- Genes that had low counts or were highly variable across samples must have their LFC adjusted through shrinkage
- Allows us to compare genes with actual differences to genes with high noise levels

Gene	Avg Count (C)	Avg Count (L)	Not Shrunk LFC	Shrunk LFC
ENSG00000187634 SAMD11	1423.08	1327.75	0.15	0.13
ENSG00000187642 PERM1	12.42	15.67	-0.70	-0.03

Results

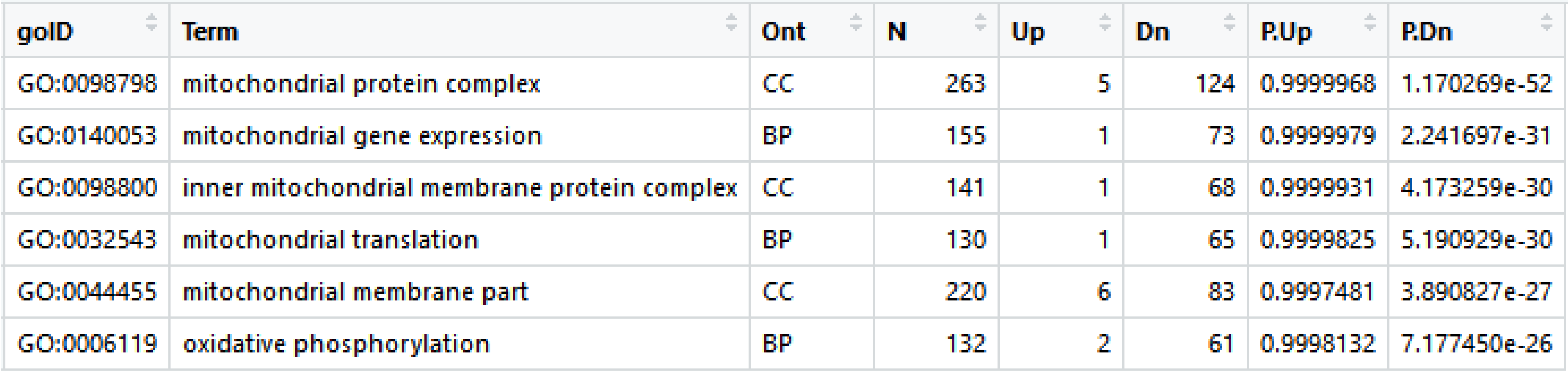
	Day 7	Day 21	Interaction
Significant Genes when FDR < 0.05	4872	2315	3660
Intersection with top 1000 brain genes	419	184	394
Intersection with 817 brain specific genes	225	105	183
Intersection with top 66% of genes expressed in brain	4061	2046	3161


ENSG00000054356
PTPRN
Counts
60
50
40
30
20
7.Control
21.Control
7.Lithium
21.Lithium

Gene Ontology Analysis

- Way of annotating genes based on gene function
  - BP – biological process, CC – cellular component, MF – molecular function

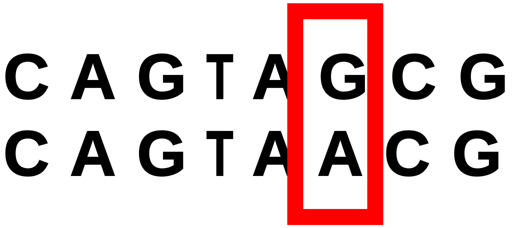
Interaction, down regulated



goID	Term	Ont	N	Up	Dn	P.Up	P.Dn
GO:0098798	mitochondrial protein complex	CC	263	5	124	0.9999968	1.170269e-52
GO:0140053	mitochondrial gene expression	BP	155	1	73	0.9999979	2.241697e-31
GO:0098800	inner mitochondrial membrane protein complex	CC	141	1	68	0.9999931	4.173259e-30
GO:0032543	mitochondrial translation	BP	130	1	65	0.9999825	5.190929e-30
GO:0044455	mitochondrial membrane part	CC	220	6	83	0.9997481	3.890827e-27
GO:0006119	oxidative phosphorylation	BP	132	2	61	0.9998132	7.177450e-26

GWAS

- Genome-Wide Association Study
- Searches whole genome for single nucleotide polymorphisms (SNPs)
  - Differences in bases between 2 samples of DNA
- Prior to our study, bipolar disorder patients were split into 2 groups
  - One group had attempted suicide, other group had not attempted suicide
  - Study finds association between SNPs and suicide risk

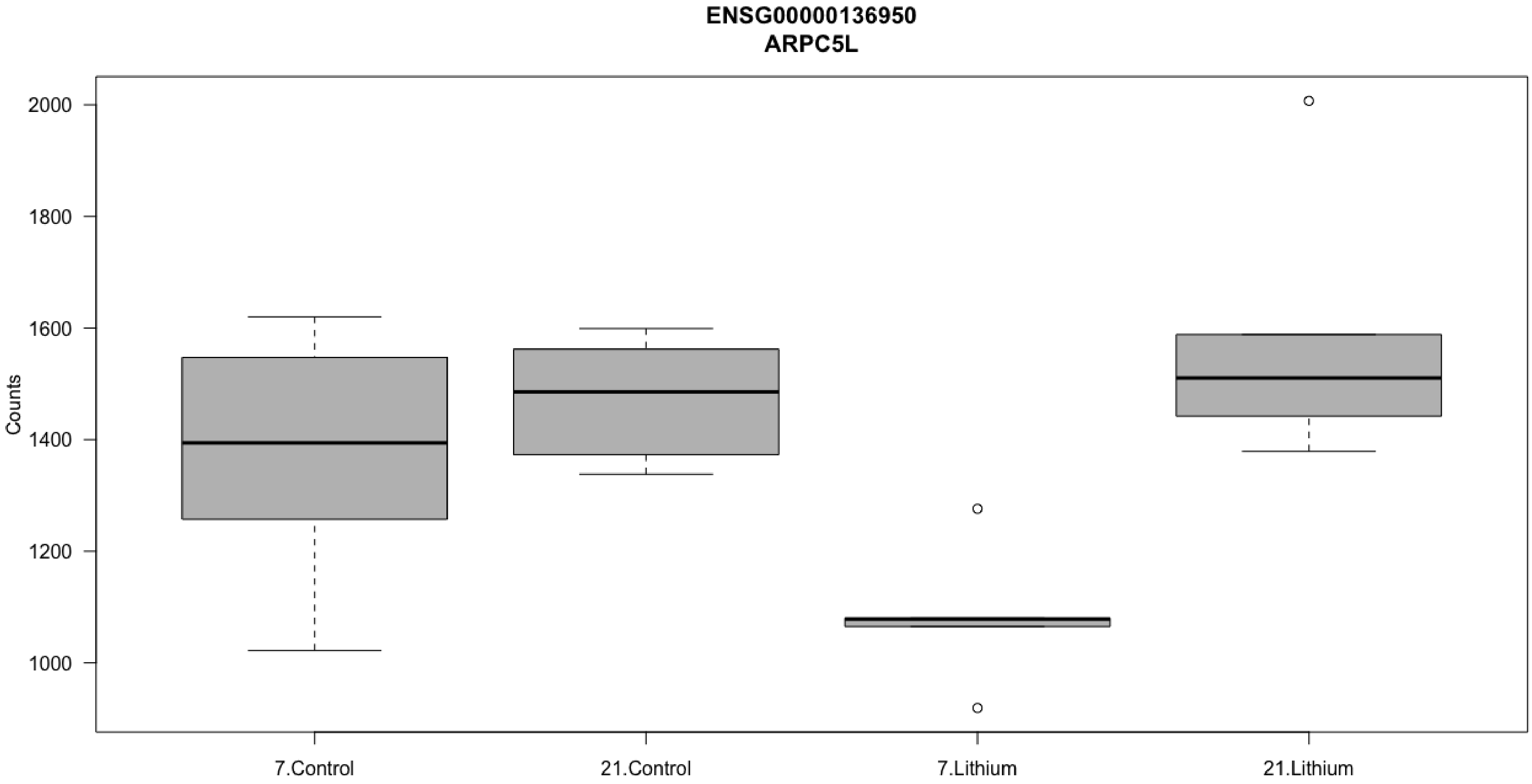


C A G T A G C G

C A G T A A C G

Whole Exome Sequencing

- Technique to sequence all protein coding regions of genome to identify variants
- **Exons** are the protein coding regions
- Whole collection of exons is called an **exome**
- Cheaper and faster method than sequencing whole genome


ENSG00000136950
ARPC5L
Counts
2000
1800
1600
1400
1200
1000
7.Control
21.Control
7.Lithium
21.Lithium

Discussion

- Our results show that many of the genes altered by lithium treatment are located in the brain
- Future work
  - Replicate experiment using neural cells from embryotic rats
  - Compare results to other genome-wide association studies
  - Identify genes that have multiple types of evidence pointing to role in suicidal behavior

References

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- Monson ET, Pirooznia M, Parla J, Kramer M, Goes FS, Gaine ME, Gaynor SC, de Klerk K, Jancic D, Karchin R, McCombie WR, Zandi PP, Potash JB, Willour VL. 2017. Assessment of Whole-Exome Sequence Data in Attempted Suicide within a Bipolar Disorder Cohort

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NIH National Heart, Lung, and Blood Institute

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Questions?