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

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Type</th>
<th>Treatment Length</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lithium</td>
<td>7 Days</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Lithium</td>
<td>21 Days</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>7 Days</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>21 Days</td>
<td>6</td>
</tr>
</tbody>
</table>

- 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

PC1: 98% variance

PC2: 1% variance

Day
- 21
- 7

Treatment
- Control
- Lithium
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.

<table>
<thead>
<tr>
<th>Poisson Distribution</th>
<th>Negative Binomial Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E(Y) = \lambda$</td>
<td>$E(Y) = \lambda$</td>
</tr>
<tr>
<td>$Var(Y) = \lambda$</td>
<td>$Var(Y) = \lambda + \omega \lambda^2$</td>
</tr>
</tbody>
</table>

- DESeq2 estimates the overdispersion factor ($\omega$) 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.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Avg Count (C)</th>
<th>Avg Count (L)</th>
<th>Not Shrunk LFC</th>
<th>Shrunk LFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSG00000187634</td>
<td>1423.08</td>
<td>1327.75</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>SAMD11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENSG00000187642</td>
<td>12.42</td>
<td>15.67</td>
<td>-0.70</td>
<td>-0.03</td>
</tr>
<tr>
<td>PERM1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Results

<table>
<thead>
<tr>
<th></th>
<th>Day 7</th>
<th>Day 21</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant Genes when FDR &lt; 0.05</td>
<td>4872</td>
<td>2315</td>
<td>3660</td>
</tr>
<tr>
<td>Intersection with top 1000 brain genes</td>
<td>419</td>
<td>184</td>
<td>394</td>
</tr>
<tr>
<td>Intersection with 817 brain specific genes</td>
<td>225</td>
<td>105</td>
<td>183</td>
</tr>
<tr>
<td>Intersection with top 66% of genes expressed in brain</td>
<td>4061</td>
<td>2046</td>
<td>3161</td>
</tr>
</tbody>
</table>
ENSG00000054356
PTPRN

Counts

Gene Ontology Analysis

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

Interaction, down regulated

<table>
<thead>
<tr>
<th>goID</th>
<th>Term</th>
<th>Ont</th>
<th>N</th>
<th>Up</th>
<th>Dn</th>
<th>P.Up</th>
<th>P.Dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0098798</td>
<td>mitochondrial protein complex</td>
<td>CC</td>
<td>263</td>
<td>5</td>
<td>124</td>
<td>0.9999968</td>
<td>1.170269e-52</td>
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<tr>
<td>GO:0140053</td>
<td>mitochondrial gene expression</td>
<td>BP</td>
<td>155</td>
<td>1</td>
<td>73</td>
<td>0.9999979</td>
<td>2.241697e-31</td>
</tr>
<tr>
<td>GO:0098800</td>
<td>inner mitochondrial membrane protein complex</td>
<td>CC</td>
<td>141</td>
<td>1</td>
<td>68</td>
<td>0.9999931</td>
<td>4.173259e-30</td>
</tr>
<tr>
<td>GO:0032543</td>
<td>mitochondrial translation</td>
<td>BP</td>
<td>130</td>
<td>1</td>
<td>65</td>
<td>0.9999825</td>
<td>5.190929e-30</td>
</tr>
<tr>
<td>GO:0044455</td>
<td>mitochondrial membrane part</td>
<td>CC</td>
<td>220</td>
<td>6</td>
<td>83</td>
<td>0.997481</td>
<td>3.890827e-27</td>
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<tr>
<td>GO:0006119</td>
<td>oxidative phosphorylation</td>
<td>BP</td>
<td>132</td>
<td>2</td>
<td>61</td>
<td>0.9998132</td>
<td>7.177450e-26</td>
</tr>
</tbody>
</table>
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
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
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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Questions?