McKnight
Inter-Institutional
Meeting
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Epigenomics Core – What the Core can do for you. Translational potential

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Summary of last year’s MBI presentation

• Our preliminary studies indicate that in mice, parental (F0) methamphetamine exposure and maternal care induce unique behavioral phenotypes in F1, F2 and F3 progeny and alter hippocampal DNA methylation (F1).

• These findings support the hypothesis that METH causes transgenerational effects through epigenetic mechanisms.
Exaggerated claims of clinical relevance and unrealistic translational timelines

The public and funding agencies prize the rapid translation of basic biological science and animal studies into human health benefits.

It remains unclear yet whether similar epigenetic dynamics operate in rodents and humans.

Important to complement animal-based epigenetic research with human studies.
Translational potential

A comparative encyclopedia of DNA elements in the mouse genome

- Chromatin state landscape in a cell lineage is relatively stable in both human and mouse.
- Chromatin domains, are developmentally stable and evolutionarily conserved.
Translational potential

- Validated molecular biomarkers of human aging
- Novel targets
- Could be used to test effects of interventions
  - Circulating RNA
  - DNA methylation
Biomarker potential of RNA transcripts in CSF

Peripheral biofluids such as cerebrospinal fluid and serum might contain markers of central nervous system disorders.

Typical CSF RNA profile

CSF RNA sequencing library
Modified library prep yields directional mRNAseq data

- Similar levels of coverage to standard RNAseq
- Able to resolve Sense-antisense pairings
- More accurate quantitation – counts attributed to one transcript only
Biomarker potential of RNA transcripts in CSF

• We processed 57 CSF samples received from VA Puget Sound Health Care system. (68-89 y.o.)
• We performed extracellular RNA extraction and directional RNA-seq library preparation.
• we utilized an optimized NEB-based directional mRNA-Seq sample preparation kit to prepare all libraries.
• The directional approach generated in average 67 million pair-end reads using the Illumina HiSeq 2000 platform
• Bioinformatics Analysis of the data
Transcriptome of the human CSF

• We sought to ascertain the defining transcriptome of the human CSF by identifying a set of transcript present in all CSF samples analyzed (irrespective of disease status) at similar levels.

• We have found 454 gene transcripts present in all samples with no significant difference between sample groups.

• Interestingly, the most enriched GO molecular function is voltage-gated channel activity as identified by EnrichR (http://amp.pharm.mssm.edu/Enrichr/enrich).

<table>
<thead>
<tr>
<th># of genes</th>
<th>Known %</th>
<th>Novel %</th>
<th>Protein coding %</th>
<th>ncRNA %</th>
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<tbody>
<tr>
<td>454</td>
<td>89.2</td>
<td>10.8</td>
<td>80.4</td>
<td>19.6</td>
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</tbody>
</table>
miRNA full genome

MIR3201
IncRNAs – chromosome 6

RP11-524K22.1
Biomarker potential of RNA transcripts in CSF

**GO Molecular Function**
Hover each row to see the overlapping genes.

<table>
<thead>
<tr>
<th>Index</th>
<th>Name</th>
<th>P-value</th>
<th>Z-score</th>
<th>Combined Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>voltage-gated channel activity (GO:002832)</td>
<td>0.0001267</td>
<td>-2.45</td>
<td>13.09</td>
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<td>2</td>
<td>voltage-gated ion channel activity (GO:0005244)</td>
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<tr>
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<td>8</td>
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<td>0.0002852</td>
<td>-2.41</td>
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**KEGG 2015**
Hover each row to see the overlapping genes.

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<thead>
<tr>
<th>Index</th>
<th>Name</th>
<th>P-value</th>
<th>Z-score</th>
<th>Combined Score</th>
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<tbody>
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<td>0.04684</td>
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</table>
Genome wide profiling of CpG methylation in brain samples.

- Dorsal motor nucleus of the vagus
- Substantia nigra
- Cingulate cortex

**Brain Samples**

- Tissue collection

**RNA**

- RNA-seq analysis
- Validation by real-time PCR
- Biological validation
- Blood correlate?

**DNA**

- Methylation profiling
- Validation by bisulfite sequencing
- Biological validation

**Validation by**

- real-time PCR
- bisulfite sequencing

**63-86 y.o.**
Methylation was recorded as a fraction between zero and one, representing the frequency of methylation of a given CpG marker.
Changes in the methylome associate with age of the donor

• 1.05% of the markers had significant associations between methylation value and age ( \([FDR] < 0.05\)).
Summary

- Through CSF-RNA transcriptome profiling and brain tissue methylation profiling, we have identified potential biomarkers with reproducible recovery and reliable measurements.
Acknowledgements

• The Morris K. Udall Parkinson Disease Research Center of Excellence
• Jeff Vance
• Claes Wahlestedt
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