Blood-Brain molecular alterations in depressed suicides

Adolfo Sequeira PhD
Associate Research Professor
Psychiatry and Human Behavior
Functional Genomics Laboratory

UNIVERSITY of CALIFORNIA - IRVINE
The suicide epidemic

- 3rd cause of death in men between 18-44 in the US
- 1.4 million attempts and 48,000 deaths per year in the US
- Men die by suicide four times more often than women
- 1 week after patients leave psychiatric care suicide death rate is increased by 300%
- ~45% of those who die by suicide see a clinician in the month prior to death and a third see a healthcare professional within the week of dying by suicide
Risk factors for suicide

- Previous attempt
- Family history of suicide
- History of depression or other mental illness
- History of alcohol or drug abuse
- Stressful life event or loss
- Easy access to lethal methods
- Aggression and impulsivity
- Hopelessness
- High cortisol levels
- Psychological pain

- Currently no risk prediction algorithm
Suicide specific biomarkers in MDDs

- A high proportion of patients visit a health-care provider in the month prior to dying by suicide but there is currently no accurate way to predict who is at risk for suicide
- GWAS and functional studies (methylation, gene expression) often compared MDD-Suicides to Controls or studied suicidal behaviors
- Most studies to date have looked at Brain or Blood independently
- Post-mortem Blood
  - Difficult to collect when PMI is high
  - High levels of coagulation and cell lysis
  - Not always collected in blood tubes with a preservation buffer
  - RNA often too degraded for qPCR, microarrays or RNA-Seq
- AFSP: Can we combine brain and blood expression signatures to develop a transcriptional (mRNA) blood test to assess suicide risk?
Novel target identification and validation

- Postmortem brain gene expression data:
  - Proprietary (McGill, Pritzker) and public (PsychENCODE, Allen Institute, GTEx, etc.)

- Analysis of data using commercial and proprietary Bioinformatics tools

- Integration of proprietary/public genetic and other relevant biological data
  - (UK Biobank, GWAS database extraction of nominally significant associated SNPs)

- Classification on GO, Ingenuity pathway analysis, KEGG

- Selection of targets based on:
  - Differential expression in tissue or circuit of interest
  - Genetic association
  - Drug-Target specificity

- Translational biomarkers of response (SNPs, expression, methylation) tested in humans
The vast majority of gene expression (from 16 brain regions) changes associated with depression and suicide involve GABA and glutamate receptors and transporters.
Post-mortem psychological autopsy

- Interviews with family and friends
- Medical files
- Coroner notes/investigation
- Toxicological reports
- Hospital files
- 144 item questionnaire based on the DSM-IV and the SCID
Coronal brain slicing

- Brain is encased in alginate to keep the shape/integrity and to prevent deformation/warping while slicing
- Coronal slicing (1 cm) is done using a precise guide with the ventral surface up to avoid damage to ventral areas
RNA degradation (<1000 bp) is a problem for cDNA production (qPCR, microarrays) and for RNA-Seq.

Some degradation in post-mortem blood still allows the extraction of enough RNA (>100 bp) to reliably detect changes in gene expression using NanoString.

QC was improved by loading more RNA for blood samples compared to brain samples.

NanoString Platform

The NanoString platform allows for the direct counting of mRNA molecules (no cDNA) using a barcode-probe system.
Field of view count (FOV) or total number of FOVs imaged per lane is used to control for possible RNA quality differences.

<table>
<thead>
<tr>
<th></th>
<th>Average FOV</th>
<th>FOV Stdev</th>
<th>% FOV</th>
<th>Min % FOV</th>
<th>Max % FOV</th>
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</thead>
<tbody>
<tr>
<td>Brain</td>
<td>533.94</td>
<td>10.84</td>
<td>96.21</td>
<td>85.23</td>
<td>98.74</td>
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<tr>
<td>Blood</td>
<td>540.06</td>
<td>8.27</td>
<td>97.31</td>
<td>92.43</td>
<td>99.64</td>
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</table>
Biomarker Signature for Suicide

- Pilot study in post-mortem **BRAIN and BLOOD** using NanoString technology to study 117 genes relevant for suicide:

  - Highly significant differences in peripheral gene activity were identified in **MDD-Suicides versus MDD Non-Suicides** for eleven genes ($Q \leq 0.1$, FDR corrected)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>N</th>
<th>Gender</th>
<th>Age</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Brain</strong></td>
<td>Control</td>
<td>21</td>
<td>17</td>
<td>45.71</td>
</tr>
<tr>
<td></td>
<td>MDD-NS</td>
<td>24</td>
<td>11</td>
<td>50.73</td>
</tr>
<tr>
<td></td>
<td>MDD-S</td>
<td>24</td>
<td>11</td>
<td>42.82</td>
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<td><strong>Blood</strong></td>
<td>Control</td>
<td>16</td>
<td>12</td>
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<tr>
<td></td>
<td>MDD-NS</td>
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<td>11</td>
<td>51.45</td>
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<tr>
<td></td>
<td>MDD-S</td>
<td>19</td>
<td>12</td>
<td>42.83</td>
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</tbody>
</table>

Mamdani et al, Translational Psychiatry, 2022
Gene expression changes in suicide - DLPFC

Mamdani et al, Translational Psychiatry, 2022
Gene expression changes in suicide - Blood
Biomarker Signature for Suicide

**SOX9:**
- Activated B cells (Blood)
- Astrocytes (Brain)

**CD19:**
- B-Cell (Blood)
- Mural cell (Brain)

**PER3:**
- Delayed sleep phase syndrome gene

**TERF1:**
- Telomere Repeat-Binding Factor 1

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**Immune**

**Circadian**

**Telomeres**
Telomeres

Telomeres in brain cells

Stan Watson (University of Michigan)
Telomere

DLPFC

Blood

TERC

Control  MDD-NS  MDD-S

TERF1

Control  MDD-NS  MDD-S

POT1

Control  MDD-NS  MDD-S

0.104  0.124  0.001

0.178  0.331  0.023

0.0009  0.272  0.034
Depression specific shortening of telomeres in the hippocampus

![Graph showing telomere length in different brain regions (DLPFC, Amygdala, Nacc, Hippocampus, SN) with C, BD, SZ, MD groups. The significance level is marked as P=0.005.](Mamdani et al, Translational Psychiatry, 2015)
Hippocampal telomere shortening in depression

- Protective caps at the end of chromosomes formed by a repetitive sequence (TTAGGG).

- MDDs have shorter telomeres in the hippocampus.

- In future studies suicide specific effects and cellular differences will be investigated.
Circadian changes
## Top three genes significantly affected by adjusted* time of death in the DLPFC

<table>
<thead>
<tr>
<th>Column ID</th>
<th>p (Sex)</th>
<th>p (Age)</th>
<th>p (Status)</th>
<th>( p (\text{adjdec}^*) )</th>
<th>( p (\text{MDD-S vs. MDD-NS}) )</th>
<th>FC(\text{MDD-S vs. MDD-NS})</th>
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<tbody>
<tr>
<td>PER2</td>
<td>0.00030212</td>
<td>0.0572825</td>
<td>0.204982</td>
<td>\textbf{0.00251788}†</td>
<td>0.158159</td>
<td>1.19292</td>
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<tr>
<td>CRY1</td>
<td>0.0126436</td>
<td>0.0338407</td>
<td>0.503564</td>
<td>\textbf{0.0155696}†</td>
<td>0.880263</td>
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<tr>
<td>CRH</td>
<td>0.00149369</td>
<td>0.0148091</td>
<td>0.0644279</td>
<td>0.0298922</td>
<td>0.0216211</td>
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</table>

* adjusted time of death from sunrise corrected by daylight savings time
† significant after FDR correction
PER2 gene expression correlated with “time of death” in the brain (DLPFC)

\[ y = 4.6071x + 150.06 \]

\[ R^2 = 0.28 \]
Inflammation-stress response
Stress - FKBP5

Blood-P=0.014
DLPFC-P=0.035
Blood specific inflammation in suicide

**A**

<table>
<thead>
<tr>
<th></th>
<th>IFNG</th>
<th>TNF</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>MDD-NS</td>
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<td>MDD-S</td>
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**B**

<table>
<thead>
<tr>
<th></th>
<th>CD6</th>
<th>CD19</th>
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<td></td>
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<tr>
<td>MDD-S</td>
<td></td>
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</tr>
</tbody>
</table>

Brain | Blood
- Suicide biomarker signature that can be evaluated in non-preserved blood

- We think some of these **clinical manifestations** and **molecular changes** are involved in the transition from thinking about suicide to attempting and dying by suicide
Future directions

- **Brain circuits** involved in stress, depression and suicide: DLPFC-Hippocampus-NAcc-Amygdala.

- Single nuclei/cell RNA-Seq

- Telomere changes in depression, association with the stress hormone cortisol and suicide risk

- Test a suicidal behaviors “blood test” (expression/metabolomics) to identify at-risk patients for suicide in a clinical sample followed over time (baseline, 1 and 2 year follow-ups)
BrainGENIE using paired blood–brain transcriptome data from the GTEx dataset

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  - Mark Vawter
  - William Bunney

- Pritzker Consortium
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  - Huda Akil (Michigan)
  - Stanley Watson (Michigan)
  - Rick Myers (Hudson Alpha)
  - Francis Lee (Cornell)
  - Jack Barchas (Cornell)
Depression
### Blood

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mean(Control)</th>
<th>Mean(MDD-NS)</th>
<th>Mean(MDD-S)</th>
<th>P (MDD vs. Control)</th>
<th>Q Value (MDD vs. Control)</th>
<th>FC (MDD vs. Control)</th>
<th>FC Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC1</td>
<td>7.747</td>
<td>8.723</td>
<td>8.643</td>
<td>0.007</td>
<td>0.070</td>
<td>1.878</td>
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<tr>
<td>ACD</td>
<td>8.306</td>
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<td>7.724</td>
<td>0.013</td>
<td>0.103</td>
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<tr>
<td>POT1</td>
<td>6.624</td>
<td>6.886</td>
<td>7.403</td>
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<td>EIF5A</td>
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<td>9.873</td>
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<td>TERC</td>
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<td>HTR2A</td>
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<td>MT-ND6</td>
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<td>PMFBP1</td>
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<td>SRM</td>
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<td>NOP10</td>
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<td>9.439</td>
<td>9.482</td>
<td>0.042</td>
<td>0.103</td>
<td>1.412</td>
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### Brain

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mean(Control)</th>
<th>Mean(MDD-NS)</th>
<th>Mean(MDD-S)</th>
<th>P (MDD vs. Control)</th>
<th>Q Value (MDD vs. Control)</th>
<th>FC (MDD vs. Control)</th>
<th>FC Direction</th>
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<tbody>
<tr>
<td>CRY2</td>
<td>10.478</td>
<td>10.382</td>
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<td>0.548</td>
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<td>SMOX</td>
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<td>FKBPS</td>
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