SOMATIC MUTATION PROFILING AND PATHOGENESIS IN ALZHEIMER’S DISEASE
BY SINGLE-NEURON GENOME SEQUENCING
Somatic Mutation in Non-Neoplastic Tissues

Blood

- One mutation
- Two mutations

Participants with Multiple Candidate Drivers

Genovese et al. (2014)
NEJM PMID 25426838

Jaiswal et al. (2014)
NEJM PMID 25426837

Skin

- NOTCH1-3
- TP53
- FGFR3
- FAT1
- RBM10
- Other

Martincorena et al. (2015)
Science PMID 25999502

Esophagus

50-55yr male (non-smoker)

Martincorena et al. (2018)
Science PMID 30337457
Somatic Mutations in Neurons

SNV in Human Neurons

Clonal SNV Show Developmental History

Lodato et al. (2015) *Science* PMID 26430121
Hazen et al. (2016) *Neuron* PMID 26948891
Neuronal Somatic SNV Increase with Age

Prefrontal Cortex (PFC)

Somatic SNV per neuronal genome

~20 somatic SNVs / year increase

Lodato et al. (2018) Science 29217584
Luquette, Miller, Zhou et al. (2022) Nature Genetics
Xing et al. (2021) PNAS PMID 33593904
Abascal et al. (2021) Nature PMID 33911282
Alzheimer’s Disease

Amyloid Plaques

Neurofibrillary Tangles

Single-Cell Whole-Genome Sequencing

- Dissection of Brain Tissue
- Nuclear isolation
- Fluorescence Activated Nuclear Sorting (FANS)
- Single Nucleus Whole Genome Amplification
- Single Cell Whole Genome Sequencing (scWGS)

Bioinformatic analysis
- Detection of phased somatic single nucleotide variants using linked-read analysis (LiRA)
- Burden analysis
- Signature analysis
- Transcriptional analyses

Alzheimer's disease
- Prefrontal cortex (PFC)
- Hippocampus (HC) – CA1

Control
Somatic SNV in Alzheimer’s Disease Neurons

Control
AD
PFC
HC

Somatic SNVs / neuron

$P = 6.5 \times 10^{-5}$
(linear mixed-effects regression)

$P = 7.1 \times 10^{-5}$

Miller, Huang et al. (2022) Nature PMID 35444284
Somatic SNV in Alzheimer’s Disease: Distribution Across the Genome

Miller, Huang et al. (2022) Nature PMID 35444284
Mutational Signatures and Mutagens

Mutational signature analysis of human cancers

Mutations observed following exposure of cultured cells

Simulated solar radiation

Benzo-a-pyrene (PAH in tobacco smoke)

Alexandrov et al. (2013) Nature 23945592

Kucab et al. (2019) Cell 30982602
Mutational Signature Analysis in AD Neurons

**Signature A**

<table>
<thead>
<tr>
<th>Trinucleotide context</th>
<th>C&gt;A</th>
<th>C&gt;G</th>
<th>C&gt;T</th>
<th>T&gt;A</th>
<th>T&gt;C</th>
<th>T&gt;G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Signature C**

<table>
<thead>
<tr>
<th>Trinucleotide context</th>
<th>C&gt;A</th>
<th>C&gt;G</th>
<th>C&gt;T</th>
<th>T&gt;A</th>
<th>T&gt;C</th>
<th>T&gt;G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation %</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Age vs. Signature A contribution (sSNVs / neuron)**

- Control
- AD
- PFC
- HC

**Age vs. Signature C contribution (sSNVs / neuron)**

**Reference:** Miller, Huang *et al.* (2022) *Nature* PMID 35444284
DNA Oxidation in Individual Neurons in AD

Miller, Huang et al. (2022) Nature PMID 35444284
**AD Somatic SNV Distribution by Gene Expression**

(a) Total mutations

- **Gene Expression Decile**
- **Relative sSNV density / Mb**
- **R² = 0.39**
- **P = 3.1 x 10⁻³**

(b) Signature A and Signature C

- **Gene Expression Decile**
- **Signature A**
- **R² = 0.61**
- **P = 5.0 x 10⁻⁵**
- **Signature C**
- **R² = 0.35**
- **P = 6.5 x 10⁻³**

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Miller, Huang et al. (2022) *Nature* PMID 35444284
Potential Effect of Somatic SNVs on Neurons: Gene Inactivation

Miller, Huang et al. (2022) *Nature* PMID 35444284
Somatic SNV in AD: Potential Neoantigens

**Article**

Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer’s disease

**Figure a**

Somatic SNVs in gene coding regions

- **nonsynonymous**
- **synonymous**
- **stopgain**
- **stoploss**

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>AD</th>
<th>control</th>
<th>AD</th>
<th>control</th>
<th>AD</th>
<th>control</th>
<th>AD</th>
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</thead>
<tbody>
<tr>
<td><strong>nonsynonymous</strong></td>
<td>15</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td><strong>synonymous</strong></td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7</td>
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<tr>
<td><strong>stopgain</strong></td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>stoploss</strong></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
</tbody>
</table>

*P = 1.6 x 10^-4 (nonsynonymous), P = 2.6 x 10^-1 (synonymous), P = 1.3 x 10^-4 (stopgain, stoploss)*

**Figure b**

CD3+CD8+ T cells per mm²

- **Control (n = 7)**
- **AD (n = 7)**

**P = 0.027**

*8d) Clonally expanded CD8 T cells represent a potential source of neoantigens in Alzheimer’s disease, with increased expression of cytotoxic effectors in the CSF of patients with AD.*

**Table**

<table>
<thead>
<tr>
<th></th>
<th>CD8 T cells</th>
<th>TCR clones</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td><strong>AD</strong></td>
<td>700</td>
<td>300</td>
</tr>
</tbody>
</table>

**Figure 3b**

- **Brain 1**
  - CAA in AD-affected hippocampi
  - CD8+ T cells
  - GZMA+ expression

**Figure 3c**

- **Brain 2**
  - CAA in AD-affected hippocampi
  - CD8+ T cells
  - GZMA+ expression

**Figure 3d**

- **Brain 3**
  - CAA in AD-affected hippocampi
  - CD8+ T cells
  - GZMA+ expression

**Legend**

- **CCA**
- **DNA**
- **CD8**
- **GZMA**

**References**

- Miller, Huang et al. (2022) *Nature* PMID 35444284
- Gate et al. (2020) *Nature* PMID 31915375
Somatic Mutations in Alzheimer’s Disease Pathogenesis

Miller, Huang et al. (2022) Nature PMID 35444284
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**BWH**
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Pilot Award

**NIH**
National Institute of Mental Health

**F·PRIME**
Howard Hughes Medical Institute

**Startup funding**
Seeking **talented scientists** to work with us on single-cell and somatic mutation biology in neurodegenerative diseases:

- Postdoctoral Fellows
- Graduate Students

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