Evaluating the Translational Validity of Mouse Models of Late-Onset AD (LOAD) through Deep-Phenotyping

Grant MacGregor PhD (Head)
UCI MODEL-AD Disease Modeling Project (DMP)

Gareth Howell PhD (Head)
IU/JAX MODEL-AD Disease Modeling Project (DMP)
Evaluating the Translational Validity of Mouse Models of Late-Onset AD (LOAD) through Deep-Phenotyping

MODEL-AD Consortium - Disease Modeling Project (DMP)

- General strategy for model development and phenotyping platforms.
- Examples of hAβ-KI (completed) and hTau-KI (in progress).
- Phenotyping of APOE4, Trem2\textsuperscript{R47H} models.
- Effect of mouse genetic background on development of pathology.
Concerns with Existing Animal Models of AD

Models Do Not Develop Robust Neurodegeneration

Largely focused on early-onset AD mechanisms

Appropriate species?

Reproducibility of findings in models and relation to human-relevant biomarkers

Difficulties in Relating Behavioral Deficits Observed in Mouse Models to Human AD

Many Models Generated/Maintained on Different Genetic Backgrounds

Toxic effects of overexpression of transgenes

Legal restrictions for some models
Using genome engineering to generate mouse models of Late-Onset Alzheimer’s Disease (LOAD)

- Use CRISPR/Cas9 to introduce coding and conserved non-coding LOAD GWAS risk-variants into cognate loci in mouse genome – e.g. $\text{Trem2}^{R47H}$

- Overcomes limitations associated with -
  - Random integration of transgenes.
  - Supra-physiologic expression.
  - Lack of availability of matched negative controls.

- Accelerated production compared with previous HR / ES-cell based strategies.

- Improve reproducibility and reduce experimental variability by using consistent genetic background (C57BL/6J, initially).
Using advanced genome engineering to generate mouse models of Late-Onset Alzheimer’s Disease (LOAD) – UCI DMP

- Use CRISPR/Cas9 with long (~ 2kb) ssDNA homology dependent repair (HDR) templates to introduce non-conserved LOAD GWAS risk-variants into cognate loci in mouse genome – e.g. humanizing non-conserved regions of mouse clusterin locus (Clu).

- Use of Recombinase Mediated Cassette Exchange (RMCE) to humanize entire loci – e.g. hTau-KI, hClu-KI.

- Generate LOAD mouse models on consistent genetic background (C57BL/6J, initially).

- Maximize researcher access to all models – available to both academics and pharma from Jackson Lab AD Mouse Model Resource, with minimal restrictions.
A humanized platform for introduction of GWAS AD-risk variants to generate mouse models of LOAD

Long-term goal

- Introduce different combinations of GWAS human LOAD risk alleles into hAβ-KI; APOEε4; hTau-KI via CRISPR/Cas9 or assisted reproduction.
- Perform initial screen, then deep-phenotyping on subset to analyze effects.

**B6J. hAβ-KI; APOEε4/ε4; hTau-KI**

- **Available now**
- **In development**

**Base platform**

- **Trem2** $^{R47H}$
- **Abca7** $^{A1527G}$
- **Plcg2** $^{M28L}$
- **Mthfr** $^{A222V}$
Goal - alignment of mouse models with clinical measures

clinical study cohorts

‘Omics

‘Omic data

Neuropathology

Brain Imaging

mouse models

MODEL-AD

INDIANA UNIVERSITY

UCI University of California, Irvine
Assessing reproducibility of findings at different sites

- Phenotype = Genes + Environment
- Harmonize environment and methodology to extent possible at each site.
- Assess reproducibility of data generated from deep-phenotyping.

- 5xfAD
- APOE4
- APOE4;Trem2^{R47H}
- B6J

- 5xfAD
- hAβ-KI
- hAβ-KI; APOE4
- hAβ-KI; APOE4;Trem2^{R47H}
- B6J

E.g. IU v JAX mice nanoString Analysis
### Deep phenotyping pipeline for LOAD models – UCI DMP

#### Pathology
- Aβ/plaque load: Thio-S, 6E10
- Tau/NFT load: HT7, AT8, Gallyas
- Glial densities/activation: Microglia (Iba1, CD68) Astrocytes (Gfap, S100b)
- Neurodegeneration: Brain Volume, Neuronal Loss
- Vascular Damage: CD31/fibrin

#### Biochemistry
- Soluble and insoluble brain fractions (Aβ38, Aβ40, Aβ42) - MSD
- Tau, phospho-Tau
- Soluble brain fractions (Inflammatory cytokines) - MSD
- Plasma Biomarkers
- Colon / Fecal Sampling for Microbiome*

#### Functional Phenotyping
- Behavior / Cognition
- Long Term Potentiation (LTP)

#### Network Analysis
- Gene expression via RNA-seq

---

4, 8, 12, 18 month timepoints
18M / 18F available per timepoint
* proposed
Model Characterization at UCI – 4, 8, 12, 18 month timepoints

Neuropathology and Neurodegeneration

- Aβ-plaques
- Intracellular Tangles

Network analysis: Molecular Profiling (RNA-Seq)

ThioS-iba1
ThioS-GFAP

WT-22mo
hAβ-KI 22mo

Behavioral and Cognitive Phenotyping

Electrophysiology
Generation of mice expressing a *cre-loxP* conditional allele of humanized wild-type Aβ \(\text{hAβ-loxP-KI}\) model – UCI DMP

- No published allele of mouse *App* expresses normal human Aβ.
- Exon 16 humanized Aβ sequence is floxed, enabling *cre*-mediated cKO of humanized allele.
- IU/JAX has generated mice with complementary hAβ-KI allele without *loxP* sites.
- Important models to investigate inherent difference in Aβ biology, plus provide platform for LOAD modeling.
Mice expressing humanized wild-type Aβ display age-related altered cognition, electrophysiology and gene-expression
Timeline for Development of Pathology in Mouse AD MODELS

3xTg-AD
3 mo 6 mo 10 mo 14 mo 18 mo 22 mo+ Absent | No Data

5xFAD

hAβ-KI

Neuronal Loss
Plaques
Tangles
Gliosis
Cognitive Impairment
LTP/LTD Change
Synaptic Loss

Flurkey K, Currier JM, Harrison DE. 2007.
Strategy to humanize mouse *Mapt* (TAU), *Clu* and other loci using Recombinase Mediated Cassette Exchange (RMCE)

**PAC / BAC**
- Introduce *attB* sites via recombineering

**CHROMOSOME**
- Introduce *attP* sites via CRISPR

Microinjection of mouse embryos with BAC/PAC + integrase
hTau-KI mice - humanization of mouse Mapt via RMCE

Human H1c PAC RP1-61D06

Mouse Mapt locus

Mouse hMAPT locus

~ 80 kb

~ 66 kb

~ 50 kb

 integrase
IU/JAX Disease Modeling Project: 40 new models of LOAD

Early goals of IU/JAX DMP

- Characterize commonly used EOAD models
  - APP/PS1 (Borchelt)
  - 5xFAD (Vassar)
  - hTau (Davies)
- Characterize newly created B6J.hAT LOAD model
- Introduce known GWAS human variants into APOE/TREM ‘sensitizer’ strain.
- Characterize and stage F344-Tg(PrP-APP, PrP-PS1) – rat model of EOAD

**B6J.APOE^E4/E4 TREM2^R47H/R47H**
Common name: B6J.hAT
## Clinically-relevant deep phenotyping

<table>
<thead>
<tr>
<th>AMP-AD, ADNI etc.</th>
<th>MODEL-AD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay</strong></td>
<td><strong>Primary Screening</strong></td>
</tr>
<tr>
<td></td>
<td>2, 6, 12 months</td>
</tr>
<tr>
<td></td>
<td>24 models</td>
</tr>
<tr>
<td>Amyloid and tau pathology</td>
<td>•</td>
</tr>
<tr>
<td>Neuroinflammation</td>
<td>•</td>
</tr>
<tr>
<td>Neuronal cell loss</td>
<td>•</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td>•</td>
</tr>
<tr>
<td>Biomarkers (Quanterix)</td>
<td></td>
</tr>
<tr>
<td>Transcriptomes (NanoString)</td>
<td>•</td>
</tr>
<tr>
<td><strong>Transcriptomes (RNA-seq)</strong></td>
<td></td>
</tr>
<tr>
<td>Transcriptomes (scRNA-seq)</td>
<td></td>
</tr>
<tr>
<td>Proteomics</td>
<td>pilot study*</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>pilot study*</td>
</tr>
<tr>
<td><strong>Imaging (FDG, PET/MRI)</strong></td>
<td>•</td>
</tr>
<tr>
<td>Cognitive tests</td>
<td>•</td>
</tr>
</tbody>
</table>

### Pilot studies
- using B6J.5xFAD and B6J.hAT
- scRNA-seq: de Jager
- Proteomics: Seyfried
- Metabolomics: Kaddurah-Daouk
Clinically-relevant deep phenotyping

Aspects of deep phenotyping occurs at IU and JAX for reproducibility

12M/12F per genotype at each time point for scientific rigor

In vivo imaging by MR/PET:
Amyloid: 18F-AV45
Tau: 18F-1451
Glucose: 18F-FDG
Blood flow: 64Cu-PTSM

Biomarkers:
AB, Tau
Nfl
Neurogranin
sTREM2

Histology:
Gross morphology/white matter: Luxol fast blue and Cresol Violet
Neurons: NeuN and CTIP
Plaques, dystrophic neurites and myeloid cells: X34, LAMP1 and IBA1
TAU: AT8 and H&E
Neuroinflammation: IBA1 and GFAP
Vascular health: CD31 and IBA1

Pilots: Proteomic and metabolomics profiling
Considering: Microbiome
B6J.\textit{hAT}: No differences in hippocampal working memory between genotypes and ages

Spontaneous Alternation

Task validation
**B6J.hAT:** Differences in lipid profiles driven by $APOE^{E4}$

Blood collected at harvest (non-fasted)

**Assessed for:**
- Total Cholesterol
- LDL
- HDL
- Triglycerides
- Non-essential FA
- Glucose

*Similar trends in LDL*
**B6J.hAT: PET/MR imaging (3T) with 18F-FDG**

**Preliminary findings:** No significant changes across 27 brain regions comparing all genotypes at 4 and 8 mos
B6J.hAT: Summary and Future plans

• $APOE^{E4}$-dependent changes in lipid profiles
• No age-dependent decline in glucose uptake across all genotypes
• No evidence of cognitive decline (measured using spontaneous alternation) up to 12 months of age

• Analyses of RNA sequence data of half brains underway
• Histological and biochemical assessment of tissue underway
  • Neuroinflammation, cerebrovascular health, amyloid and Tau
Next step: Deep phenotyping $B6J.APOE^{4/4}$ $TREM2^{<R47H>}$ mice with humanized $APP$

$B6J.hATA$
$B6J.APOE^{E4/E4}TREM2^{R47H/R47H}App^{h/h}$
Primary screen of novel variants on sensitized genetic background

- New strains created and in the primary screening pipeline
  - $Abca7^{A1527G}$, $Il1rap^{KO}$, $Ceacam1^{KO}$, $Plcg2^{M28L}$, $Mthfr^{A222V}$
- Ten others in CRISPR pipeline using $B6J.APOE^{4/4}$ $TREM2^{<R47H>}$
- Up to 40 variants to be created with 24 to be screened
A primary screen to prioritize candidate variants for deep phenotyping

Promising strains prioritized for deep phenotyping
A nanoString panel to align mouse models to human data: AMP-AD panel

- Panel of 770+30 mouse gene probes
- Maximize coverage of 30 AMP-AD modules
- Include top AMP-AD candidates (Top 30, AGORA targets)
- Genes ranked by
  - representation of module PCs (gene score)
  - ortholog expressed in mouse brain at 6 months of age
  - 10 housekeeping genes

<table>
<thead>
<tr>
<th>AMP-AD Module</th>
<th>Nanostring probes per module</th>
<th>AMP-AD Module Size</th>
<th>% of Module Covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>aggregateCBEblue</td>
<td>177</td>
<td>4505</td>
<td>3.93</td>
</tr>
<tr>
<td>aggregateCBEbrown</td>
<td>95</td>
<td>504</td>
<td>18.85</td>
</tr>
<tr>
<td>aggregateCBEturquoise</td>
<td>200</td>
<td>1977</td>
<td>10.12</td>
</tr>
<tr>
<td>aggregateCBEyellow</td>
<td>157</td>
<td>1738</td>
<td>9.03</td>
</tr>
<tr>
<td>aggregateDLPFCblue</td>
<td>183</td>
<td>1751</td>
<td>10.45</td>
</tr>
<tr>
<td>aggregateDLPFCbrown</td>
<td>139</td>
<td>882</td>
<td>15.76</td>
</tr>
<tr>
<td>aggregateDLPFCTurquoise</td>
<td>144</td>
<td>2489</td>
<td>5.79</td>
</tr>
<tr>
<td>aggregateDLPFCyellow</td>
<td>192</td>
<td>3016</td>
<td>6.37</td>
</tr>
<tr>
<td>aggregateFPblue</td>
<td>278</td>
<td>1991</td>
<td>13.96</td>
</tr>
<tr>
<td>aggregateFPbrown</td>
<td>76</td>
<td>1287</td>
<td>5.91</td>
</tr>
<tr>
<td>aggregateFPturquoise</td>
<td>107</td>
<td>1001</td>
<td>10.69</td>
</tr>
<tr>
<td>aggregateFPyellow</td>
<td>188</td>
<td>4420</td>
<td>4.25</td>
</tr>
</tbody>
</table>

Coverage ranges from 76-278 genes per module
Genetic context is important

APP/PS1 on B6J and wild-derived strains

Male Cortical Neuron Counts

Female Cortical Neuron Counts

Figure 15. The eight founder strains for the DO strain capture the vast majority of genetic diversity available in the DO strain. Incorporation of wild derived strains (e.g. CAST and PWK) greatly enhanced the genetic variability in the DO strain.
Transcriptome analyses by WGCNA shows variation in amyloid response between strains

Genes in module

<table>
<thead>
<tr>
<th>B6</th>
<th>WSB</th>
<th>PWK</th>
<th>CAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itgb2</td>
<td>Tbxas1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd52</td>
<td>Tyrobp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spi1</td>
<td>Tgfb2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ptpn6</td>
<td>Arpp21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctsd</td>
<td>Vav1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctsz</td>
<td>Cd84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abi3</td>
<td>Ctss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd68</td>
<td>Gpr34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd180</td>
<td>Cd53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fyb</td>
<td>Irf8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>App</td>
<td>Fam46c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pros1</td>
<td>Tlr7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trem2</td>
<td>Mpeg1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Csf1r</td>
<td>Gpr84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cndp2</td>
<td>Csf2rb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ptprc</td>
<td>Prnp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slamf9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laptm5</td>
<td>2900079G21Rik</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Strain-specific transcriptome analyses shows variation in genetic ‘drivers’ of AD
Summary: Evaluating the Translational Validity of Mouse Models of LOAD by clinically relevant deep phenotyping

• Creating up to 50 mouse models relevant to Alzheimer’s disease
  • Includes creating a humanized platform (APP, TAU, APOE) for testing novel variants
  • Approximately 20 models created or in progress including AB-KI, hAT

• Perform clinically-relevant deep phenotyping of key (>10) models
  • Including in vivo imaging (MR/PET) and RNA-seq
  • Data available for 4 existing (5xFAD, 3xTG, APP/PS1, hTau) and 2 new models (hAB-KI, APOE4/TREM2<R47H>)
  • Pilots for proteomics and metabolomics underway

• All data and mouse strains made available through Synapse and JAX mouse repository (as well as other sources)
  • 23 models either available to order, available for preorder, or in preparation
  • ~265 RNA-seq data files submitted/being submitted to Synapse (many more to come!)
Strains and data available from model-ad.org

### Strain Table

<table>
<thead>
<tr>
<th>Common name</th>
<th>Late-onset AD-related models</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE4/Trem2*R47H</td>
<td>B6j</td>
</tr>
<tr>
<td>Familial AD models</td>
<td>B6j</td>
</tr>
<tr>
<td>APOE4.KI</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.KO</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.1</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.2</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.3</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.4</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.5</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.6</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.7</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.8</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.9</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.10</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.11</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.12</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.13</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.14</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.15</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.16</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.17</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.18</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.19</td>
<td>B6j</td>
</tr>
<tr>
<td>APPPS1.20</td>
<td>B6j</td>
</tr>
</tbody>
</table>

**MOUSE STRAIN DATASHEET - 028709**

**B6(SJL)-Apoe<sup>tm1.1(APOE4)Adej</sup> Trem2<sup>em1(Adej)</sup> J**

Stock No: 028709 | APOE4/Trem2*R47H

- New
- Repository Live

3–6 week average lead time depending on quantity and age requests are not accepted

**Trem2.KO**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Late-onset AD-related models</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6j</td>
<td>B6(SJL)-Apoe&lt;sup&gt;tm1.1(APOE4)Adej&lt;/sup&gt; Trem2&lt;sup&gt;em1(Adej)&lt;/sup&gt; J</td>
</tr>
<tr>
<td>C57BL/6J-Trem2&lt;sup&gt;em1(Adej)&lt;/sup&gt; J</td>
<td>Live</td>
</tr>
</tbody>
</table>

**Place Order**

---

**Strains and Data Available from**

- [model-ad.org](http://model-ad.org)
- Various models with specific genetic backgrounds and modifications for Alzheimer's disease research.

**Resources**

- ANIMAL MODELS
  - All mouse models will be made available from the JAX.
  - For strains currently available, please see our Strain List.

- DATA SETS
  - All data describing novel models will be made available through the Sage Bionetworks Synapse portal.
  - Additional resources include appended RNA-Seq data release.
The MODEL-AD Consortium

Indiana University
Bruce Lamb, Program Director
Paul Territo, PTC Head
Andrew Saykin, BDMC Co-Head
Adrian Oblak, Project Manager
Kwangsik Nho
Li Shen
Tatiana Foroud
Dino Ghetti
David Jones
Sarah Quinney
Deborah DeBusk, Administrator

The Jackson Laboratory
Gareth Howell, DMP Head
Greg Carter, BDMC Head
Mike Sasner, DMP Co-Head
Stacey Rizzo, PTC Co-Head
Harriet Williams, Project Manager
Christoph Preuss
Asli Uyar
Yi Li
Ravi Pandey
Cai John
Nikhil Milind
Kristen Onos
Martha Abbott, Administrator

UC Irvine
Frank LaFerla, Program Director
Andrea Tenner, Program Director
Grant MacGregor, DMP Head
Ali Mortazavi, BDMC Head
Kim Green, DMP Co-Head
Marcelo Wood, DMP Co-Head
Stefania Forner, Project Manager
David Baglietto-Vargas
Shan Jiang
Shimako Kawauchi
Sherilyn Collins
Jonathan Neumann
Eniko Kramar
Edna Hingco
Dina Matheos
Maria Fonseca
Andrea Wasserman, Administrator

Sage Bionetworks
Lara Mangravite, BDMC Co-Head
Larsson Omberg
Ben Logsdon
Mette Peters
Solveig Sieberts
Yooree Chae

Contact
www.model-ad.org
modelad@iupui.edu
@Model_ad_alz

National Institute on Aging
Suzana Petanceska
Lorenzo Refolo
U54 AG054345, U54 AG054349