Lessons Learned of Mice (and Men)

Developing the next-generation of AD mouse models

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<td><em>Money</em> magazine ranked UCI #1 best college in the nation</td>
<td><em>Forbes</em> magazine named UCI #1 in nation among public universities for “best value”</td>
<td>UCI named #1 college doing most for the American Dream in <em>NYT Upshot</em></td>
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<td>Sierra magazine recognized UCI as top green university</td>
<td>UCI was ranked the 9th best Public University in <em>U.S. News &amp; World Report</em></td>
<td>Research universities elected into the prestigious Association of American Universities</td>
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Another promising Alzheimer's drug trial ends in failure: "This one hurts" by Sanjay Gupta, CNN


An Alzheimer's Drug Trial Gave Me Hope, and Then It Ended by Emma Seith, New York Times
In shocking reversal, Biogen to submit experimental Alzheimer's drug for approval

By MATTHEW HERPER @matthewherper / OCTOBER 22, 2019
PARTICIPANT BURDEN

What is it to you?
Alzheimer’s Disease

In 2019
- 5.8 million afflicted
- Cost = $290 Billion/yr
- Medicare $: 1 out of 5 for AD Care

By 2050
- 14 million afflicted
- Cost = $1.1 Trillion/yr
- Medicare $: 1 out of 3 for AD care
NIH Alzheimer’s Disease Funding

...investing in a cure
NIH Centers of Excellence were established in 1984
- USC/UCI was part of the original five funded centers (Drs. Finch/Cotman)

Found at major medical institutions across the USA
- 31 centers across the network

Goal
- Translate research advances into improved diagnosis and care and prevent and treat AD

Each center has its own area of emphasis
- But the network shares new research ideas, approaches, and data (NACC)

NIH Alzheimer’s Disease Centers
ADRC | Core Leaders

Frank LaFerla
Administrative Core Director

Andrea Wasserman
Chief Administrative Officer

Claudia Kawas
Clinical Core Director

David Sultzer
Clinical Core Director

Ira Lott
Down Syndrome Core Director

Maria Corrada
90+ Core Director

Joshua Grill
Associate Director
ORE Core Director

Daniel Gillen
Data Management and Statistics Core Director

Edwin Monuki
Neuropathology Core Director

M Blurton-Jones
iPSC Core Director

Craig Stark
Biomarker Core Director

Elizabeth Head
Research Education Component Director
Ten new faculty hires since 2015

Special Populations Cores Established: 90+ and Down Syndrome

Biomarker Core

Research Education Component

Core Leadership

Recruitment Registry: Consent-to-Contact (C2C)
Neuroinflammation
Focus on role of microglia

Biomarkers
Imaging, Sleep, and CSF

Clinical Population
UDS, 90+, DS

Animal Models
Part of MODEL-AD Consortium

Registry
Consent-to-Contact

Stem Cells
First AD iPS cell bank as part of ADRC network

Clinical Trials
EXERT, NEAT, A4
Modeling Human Disease in Mice

**Practical Reasons**

- Mice breed quickly; age over 2-3 year lifespan
- Brain organization is comparable to humans
- Many genes/proteins/pathways are conserved between humans and mice
- Relatively “cheap” versus human studies

Insert and express human genes in mice, allowing them to develop human diseases/pathology, even in brain

Study disease processes, which are not possible in living humans

Evaluate new treatments and determine mechanism of action
Caveats

A mouse is not an accelerated human!

- Many AD-related biochemical and neuropathologic events may not develop in the normal lifespan of a mouse
- Need to be realistic, as not all findings will be translatable to humans
- Need to develop the next generation of animal models of the disease, particularly those mimic late-onset Alzheimer’s disease

Preclinical studies need to be conducted in several different models to better mimic the heterogeneity in the human population

Even in animal models, the sooner treatment begins the more likely the cognitive impairments are improved

Targeting Aβ after other pathologies set in, particularly phosphotau and NFT pathology, does not rescue cognitive impairments

Combination therapies are likely to be required
Genetics of Alzheimer’s

Causes of Alzheimer’s:
- **PS1**, **PS2**, **APP**: Autosomal dominant
- **Aβ deposition to 100%**

Risk of Alzheimer’s:
- **High Risk**
- **Medium Risk**
- **Low Risk**

Microglial function:
- **TREM2**: 3X
- **APOE 4/4**: 8X

Aβ deposition / clearance / microglial response?

GWAS:
- Lipid metabolism
- Microglial function
- Endocytic function

Frequency in the population:
- Very Rare
- Very Common

Genetics frequencies:
- Very Rare
- Very Common

SNPs:
- INPP5D
- MEF2C
- HLA-DRB5 & DRB1
- CR1
- CW2W1
- MS4A
- CASS4
- BIN1
- CELF1
- PICALM
- FERMT2
- CD33
- PTK2B
- SORL1
- SLC24A4
- CD2AP
- EPHA1
- CLU
- NME8
- ABCA7
Models of Human Disease (LaFerla)

- **Alzheimer’s Disease**
  - 3xTg-AD
  - Arctic-tau

- **Lewy body**
  - 3x-Tg-AD x alpha-synuclein

- **Hippocampal sclerosis**
  - CaKII-tTA x TRE-DTα

- **Inclusion body myositis**
  - MCK-APP

- **Tauopathy**
  - hTau mouse

- **Human Aβ Knockin**
  - Humanized *wildtype* Aβ (floxed)
3xTg-AD Model | Plaques and Tangles
Reasons for Clinical Trial Failure

- Stage of Disease
  Targeted (Current Phase III Clinical Trials)
- Mechanism of Delivery
- Suitability of the Patient Population
- Effective Target Engagement; Off-Target Effects; Suitability of the Target
- Face and Construct Validity of the Animal Models
Concerns with Existing Animal Models of AD

- Models Do Not Develop Robust Neurodegeneration
- Based 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
- Different Genetic Backgrounds
- Toxic effects of overexpression of transgenes
- Legal restrictions for some models
Recommendations from NIA AD Summit (2015)

1. Develop the next generation of in vivo models based on human data to explore ADRD
2. Standardize process to develop and characterize models; rapid availability to all researchers for preclinical drug development
3. Align pathophysiological features of AD models with corresponding stages of clinical disease using translatable biomarkers
4. Establish guidelines for rigorous preclinical testing in animal models and report positive and negative findings
MODEL-AD

Model
Organism
Development
Evaluation
Late-onset
Alzheimer’s Disease
Early versus Late-onset AD

Early-onset
- <65 years
- Autosomal Dominant (APP, PS1, PS2)
- <1-2%

Late-onset
- >65 years
- APOE; GWAS Genes
- Aging
- >98%
3xTg-AD Model | Plaques and Tangles
Rationale for Humanizing Aβ in Mice

- Human v. rodent Aβ
- Wildtype Aβ needed for modeling LOAD
- Physiological gene expression
- Cre/lox P – cell/time specific ablation
- Use of these mice as a platform
- Preclinical evaluation of potential therapies
Key Challenges in Modeling Late Onset Alzheimer’s

- **Humanize mouse genes**: Likely require the “humanization” of several key AD related genes.
- **Multiple Pathologies**: Not all human pathologies may occur in a single mouse model.
- **Aging/environment**: Pathology should ensue from aging/environmental factors vs. overexpression or FAD mutations.
- **Mouse Background**: Genetic background may have a profound impact on phenotype.
Gene/Variant Prioritization

Systematic assessment of LOAD loci

**Significance in multiple studies**

**Predicted effect on function**

**Human-mouse sequence conservation**

**Differential expression in AD**

**Noncoding variant effects?**

**Lipid homeostasis/vascular**
- APOE, APOC1, MTHFR
- CR1, CD33, TREM2, MYO1C, PSMA1, HMHA1, IL1RAP, TYROBP, STAT3, CSF1R, SPI1, STAT4, PLCG2

**Mitochondria**
- MTHFDL1, TOMM40, SPG7
- CR1, CD33, TREM2, MYO1C, PSMA1, HMHA1, IL1RAP, TYROBP, STAT3, CSF1R, SPI1, STAT4, PLCG2

**Immune**
- APOE, APOC1, MTHFR
- CR1, CD33, TREM2, MYO1C, PSMA1, HMHA1, IL1RAP, TYROBP, STAT3, CSF1R, SPI1, STAT4, PLCG2

**Membrane/ECM**
- CD2AP, INPP5D, MS4A4E, MS4A4A, PVRL2, ANK1, ASGR2, CDH23, GUCY2D, ITM2C, UNX1

**Synaptic Signaling**
- BIN1, SCI24A4, KCNN4, BCHE, SLC6A17, HTR4, CLASP2

- KIF21B, ERC2, PICALM

- CLU

- ABCA7

- FERMT2, SORL1

- NCR2, PLXNC1

- HLA-DRB5
Model Organism Development and Evaluation for Late-onset Alzheimer’s Disease

01 Prioritize LOAD variants for animal modeling
02 Create new mouse models with CRISPR (piloting rats)
03 High-capacity screen of all models | Deep phenotyping of promising ones
04 Align mouse & human phenotypes (neuropath, imaging, omics)
05 Preclinical testing of the most promising models and therapeutics
06 Broad, unrestricted distribution of all data and models
Human Data + Other Variants
Maximize human data to identify relevant variants such as tau and others

Knockin mice
Use hAβ mice as platform

Environment & Diet
Introduce risk factors

Share

Funded
Not Funded
Deep Phenotyping Pipeline

TIMELINE PHENOTYPING PIPELINE (months)

2 4 6 8 10 12 14 18 22

hAβ-KI

Crosses hAβ-KI

x

variants

5xFAD 3x-TgAD

Pathology
Biochemistry
Functional Phenotyping (LTP)
Network analysis (RNAseq)
DNA sequence analysis shows that hAβ-KI mice encode human wt Aβ.

hAβ-KI and wt mice have similar APP levels.

hAβ-KI mice display significant synaptic and cognitive impairments (CX and HC).

Important gene expression in metabolic, neuroplasticity and transcriptional regulation pathways are altered in the hAβ-KI mice.

hAβ-KI mice show age-dependent amyloid accumulation.

hAβ-KI mice contain seeds that facilitate Aβ aggregation.

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Summary | hAβ-KI Mice
Construction Plan for Model Development

Platform Mouse: Humanized Aβ and Tau

Crosses
- hAβ-KI x ApoE4
- hAβ-KI x ApoE4 x variants
- hAβ-KI x hTau
- hAβ-KI x hTau x variants

GWAS variants
1. ABI3 S209F
2. ABCA7 V1599M
3. BIN1 K358R and rs2279590
4. EPHA1 P460L
5. PICALM H458R
6. CLU rs9331888
Resource Sharing

Enabling researchers to find the right model

Data
- Mouse genetic information: variant(s), strain background
- Mouse phenotype data: RNA-seq, imaging, etc.
- Preclinical data: standards, protocols, results
- Preclinical results searchable on AlzPED

Mice
Available from JAX mouse repository without restrictions
Approaches to Study Complex Disorders

such as Alzheimer’s disease

- Clinical
  - Neuropsychology
  - Epidemiology
  - Observational

- Imaging
  - MRI
  - PET
  - Others

- Tissues
  - Postmortem Brain
  - Blood
  - CSF

- Cell Models
  - Primary
  - Cell lines
  - iPS

- Animal Models
  - Natural
  - Pharmacological
  - Viral
  - Genetic
Value of AD Mouse Models

- Mechanistic aspects with high relevance to the clinical scenario
- Identify factors that exacerbate or attenuate phenotype
- Pharmacodynamic vs. pharmacokinetic considerations
- May inform clinical trial design
- Direct comparison between new treatment vs. competitor drugs
- Potential liabilities
Preclinical Studies:

Considerations for Moving from Bench to Bedside

- Multiple appropriate models (including sexes)
- Studies across age spectrum
- Multiple doses/reversal studies
- Multiple sites
- Mechanism of Action
What does a successful model of late-onset AD look like?

**FACE**
- Age-related, region-specific
- Plaques
- Tau 4/3; NFTs
- Synaptic Loss
- Neuronal Loss
- Neuroinflammation
- Cognitive Decline
- Behavior (Anxiety)
- Sleep

**CONSTRUCT**
- No mutations
- Physiological expression
- Humanize all genes?

**PREDICTIVE**
- Translatability
- Insights into the human disease
- Identification of novel targets
- Biomarkers
- Imaging
- ‘Omics
MODEL-AD.org

MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

MODEL-AD consortium consisting of a Center at Indiana University, The Jackson Laboratory, and
Honeywell and a Center at the University of California Irvine has been established by the
Institute on Aging to:

- Develop the next generation of in vivo AD models based on human data
- Create a standardized and rigorous process for characterization of animal models
- Characterize pathophysiological features of AD models with corresponding stages of clinical disease
- Incorporate durable biomarkers
- Establish guidelines for rigorous preclinical testing in animal models
- Increase availability of animal models, protocols and validation data to all researchers for
  drug development

RECENT POSTS
- MODEL-AD presentations at AAN 2018
- MODEL-AD presentations at ICMN
- Indiana U. Alzheimer’s symposium
- New method for identifying candidate loci for late-onset Alzheimer’s disease published
- Workshop on the use of mouse models to study neurodegenerative disease

QUICK LINKS
- AMP-AD Knowledge Portal
- JAX AD Models
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