Animal models of Disease
Risk of AD

Symptomatic Alzheimer's Risk

PERCENTAGE

AGE

Average Rate

50%

Lower Limit [95% confidence]
Prevalence of AD Through 2030

Alzheimer’s Disease Prevalence

Year


0 1,000,000 2,000,000 3,000,000 4,000,000 5,000,000 6,000,000 7,000,000 8,000,000 9,000,000 10,000,000

Affected Individuals

85+ 75-84 65-74

Alzheimer’s Disease

Early
- Insidious Memory Dysfunction
- Cognitive problems (finances, driving, following instructions)
- Lost during walking

Middle
- Can’t work
- Lost and confused
- Deficiencies in motor tasks (dressing, eating etc.)
- Needs services of a caregiver

Late
- Complete loss of cognitive abilities
- Wander aimlessly
- Hallucinations
- Delusions

Death
- Due to malnutrition, infections, heart disease
Alzheimer’s Disease and the Brain

Plaques and Tangles: The Hallmarks of AD

The brains of people with AD have an abundance of two abnormal structures:

- beta-amyloid plaques, which are dense deposits of protein and cellular material that accumulate outside and around nerve cells

- neurofibrillary tangles, which are twisted fibers that build up inside the nerve cell

An actual AD plaque

An actual AD tangle
AMYLOID HYPOTHESIS

- Aβ biosynthesis
  - γ-secretase
  - β-secretase

- Aβ degradation
  - α-secretase
  - IDE
  - Neprilysin
  - Plasmin

Expression

Biosynthesis

Assembly

Aggregation

Deposition

Neurotoxicity

Neuronal Death

Soluble Aβ

Aβ oligomers (acute)
  - Synaptic Impairment
  - Cognitive Deficits

Aβ Fibrils

Amyloid plaques (chronic)
  - Glial Activation
  - Inflammation
Microtubule Subunits Fall Apart

Disintegrating Microtubule

Tangled Clumps of Tau Proteins

The Alzheimer's Disease Education and Referral Center, NIA
Acetylcholine Pathways

- hippocampus
- frontal
- amygdala
- neocortex
- Nucleus basalis of Meynert


Proposed Chronology of Changes in AD

- OXIDATIVE STRESS
  - BACE
  - SAPKs
  - Phospho-Tau
  - HNE Modification

- Amyloid-β Deposition
  - Senile Plaque

- Tau Phosphorylation
  - Neurofibrillary Tangles

- Amyloid-β
Neuronal Exit from Quiescence in AD

- Mitosis
- G1
- 2n
- G2
- 4n
- S

E2F
Rb
Cyclin D
CDK4

Activates
Accumulates
Phosphorylates

E2F
Free to act as transcription factor

Proteins for S-phase

E2F
Rb
Alzheimer’s Disease & the Brain

Loss of nerve cells within brain “shrunken, shriveled”

Hippocampus affected first
Reduced activity of neurotransmitter acetylcholine
The Changing Brain in Alzheimer’s Disease

Normal Brain

Alzheimer’s Disease Brain
Animal/Not animals that we can use to Model disease

- **Vertebrates**
  - Primates
  - Mice
  - Rats
  - Zebrafish
  - Dogs/Cats

We chose models based on the phenotype that we are aiming to study

- Genetic/structural/behavioral
- Size
- Complexity of what we are trying to observe

- **Invertebrates**
  - Flies
  - Worms

- **Single Cell Organisms**
  - Phage
  - Bacteria
  - Yeast (Prion disease)
Types of animal Models

**Natural**
- Dogs/Primates to study AD

**Semi-natural (based on spontaneous mutations/Irradiation)**
- Accelerated aging mouse to study AD

**Lesion (Ablation of regions of interest)**
- Hippocampus to study AD

**Chemical (Neurotoxins)**
- Cholinergic toxins or Abeta to study AD

**Transgenic Models**
- The newest and most used – Models for everything... Are they good though?
What is a good Model?

- **Face validity** – Does the animal “look the same” as the species to be compared to? – If not – **NO/INCOMPLETE MODEL**

- **Reliability and Replicability** – Can the phenotype be reliably observed and replicated? – If not, Idiopathic– **NO/INCOMPLETE MODEL**

- **Construct Validity - Generalizablility** – Can you achieve the same results?
  - Across species
  - Environment (Different labs)
  - Behavioral tests that measure the same thing

- **Predictive Validity**– Can you predict outcomes after:
  - Pharmacological manipulations that are relevant to the model
  - Time
Development of an Animal Model of “Disease”

Induce non-specific Mutation (radiation) → Phenotyping, cover full behavioral repertoire for species → Phenotype changes found?

No: No animal model

Yes: Apply theory-driven tests, based on hypothesized function of gene structure/process → Hypothesized behavioral changes found?

No: BIAS

Yes: Assess role of genetics, development factors, environment → Fully characterized and validated model
“Natural Models”

- **Good** - Most related to the natural disease, most likely to portray all aspects of the disease

- **Bad** - Restrictive in terms of time and types of animals used, also in terms of animals developing illnesses (in aging studies).

- 1) Expensive 2) Long studies 3) Restrictive at an experimental level
“Semi-Natural Models”

- **Good** – Usually good models to study disease because they are based on phenotypical presentations of the disease.

- **Bad** – Often lack appropriate controls because they are bred for a phenotype and the genetics are unknown.
Lesion Models

- **Good** to study the function of a structure and how the loss of that structure affects other neuronal populations

- **Bad** because most diseases are not specific to a structure or a neuronal population – High mortality & variability
  - HM – Hippocampus removal does not lead to AD, it leads to retrograde amnesia!
Chemical Models

• **Good** to study selective depletion of specific neuronal populations and examine the role of neurotransmitters in disease, more specific than lesions and sometimes leads to full phenotype expression (MPTP in drug addicts)

• **Bad** because it is artificially induced and is incomplete with regards to phenotype (pathology/not pathology), it does not always translate to the real population (rotenone and farmers)
Transgenic Models

- **Good** excellent to study the role of genes and genetic mutations in disease

- **Bad** because diseases like all biological events are a mix of environment and genetics so a lot of times the genetic mutations do not recapitulate the disease.

- Also, while some of the genetics are highly conserved a mouse/fish/fly/worm do not respond the same way than humans do nor do animals of the same species!!! (BACKGROUNDS!!!)
Animal disease models:

Humans

Genetic Mutation

Mutant or missing Protein

Mutant Phenotype (disease)

Animal models

Genetic Mutation

Mutant or missing Protein

Mutant Phenotype (disease model)
Transgenic models of neurodegeneration

• Alzheimer’s disease:
• APOE null mice
• APP transgenic
• Tau transgenic
• GSK3beta transgenic
The endless list of AD transgenics

- PDAPP
- Tg2576
- APP23
- CRND8
- PSAPP
- JNPL3
- tauP301L
- 3xTg-AD

Legend:
- Red arrows: Plaques
- Yellow arrows: NFTs
- Green arrows: OS
- Blue arrows: Behavior
- Orange arrows: Cell cycle
- Gray arrows: Degeneration

Timeline:
- 0
- 12M
- 24M
What does a good model of AD need to show?

• Face validity – Have plaques and tangles, cognitive deficits and neurodegeneration.

• Reliability/Reproducibility – Needs to be able to be observed consistently in the same animals.

• Construct validity – Observed in different environments, in different tests measuring the same thing AND across species.

• Predictive validity – It needs to show temporal relevance to the disease and it needs to show that treatment aimed at the insult will make it better.
Transgenic mice recapitulate part of Alzheimer pathology

- Presence of amyloid plaques
- Presence of hyper-phosphorylated tau
- OR BOTH

ALL BASED ON OVER-EXPRESSION OF MUTATIONS ASSOCIATED WITH EARLY ONSET AD. Therefore excellent to study the biology of the peptide but less relevant to disease dynamics.
• How CAN we study late-onset AD?

• Aged animals – Expensive, low availability

• Mice do not develop AD so we have to study higher level animals like dogs and primates

  • More expensive/ethically complicated

  • Life span of dogs/primates is much longer than 3 years so even less available!
• The SAM strain of mice is derived from AKR/J strain.

• Littermates which became senile at an early age in life and had a shorter life span were selected as the progenitors of the SAMP.

• Littermates in which the aging process seemed normal were also selected as the progenitors of SAMR.

• Retrospective pedigree selection and inbreeding were applied based on the degree of senescence, the lifespan and the age-associated pathologic phenotypes.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mice</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl cholinesterase Inhibitors (Aricept)</td>
<td>Cognitive benefits</td>
<td>Marginal - up to 1 year stabilization in 30-50%</td>
</tr>
<tr>
<td>NMDA Inhibitors (Mementine)</td>
<td>Cognitive benefits</td>
<td>Marginal - up to 6 months stabilization</td>
</tr>
<tr>
<td>Vitamin E trial</td>
<td>Cognitive benefits and reduction of plaques</td>
<td>Failed</td>
</tr>
<tr>
<td>Ibuprophen trial - Failed</td>
<td>Cognitive benefits and reduction of plaques</td>
<td>Failed</td>
</tr>
<tr>
<td>Estrogen replacement</td>
<td>Cognitive benefits and reduction of plaques</td>
<td>Failed</td>
</tr>
<tr>
<td>Amyloid beta Vaccine</td>
<td>Cognitive benefits and reduction of plaques</td>
<td>Reduction of plaques but no cognitive benefits + inflammation</td>
</tr>
<tr>
<td>GSK3 beta inhibition</td>
<td>Cognitive benefits and reduction of tangles</td>
<td>Marginal doing larger trial</td>
</tr>
</tbody>
</table>
Phenotyping of Complex Behavioral Traits to Assess Nervous System Function and Dysfunction
How do we go about this?

Transgenic Phenotyping

Observational battery

Anxiety  Motor  Cognition  Sensory

Phenotype?

No  Yes

Expand testing for phenotype

Hypothesis Driven

Targeted behavioral battery

Phenotype?

No  Yes

Expand test number  Rule out confounds
## Strain differences on cognitive performance

### Morris Water Maze

<table>
<thead>
<tr>
<th>Great</th>
<th>Adequate</th>
<th>Impaired</th>
<th>Blindness</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB129F1</td>
<td>C57BL/6J</td>
<td>129/SvJ</td>
<td>A/J</td>
</tr>
<tr>
<td>FVBB6F1</td>
<td>C57BL/10J</td>
<td>DBA/2</td>
<td>SJL/J</td>
</tr>
<tr>
<td>129B6F1</td>
<td>BALB129F1</td>
<td>BALB/cByJ</td>
<td>C3H/lbg</td>
</tr>
<tr>
<td>129/Svev</td>
<td>B6SJLF1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LP/J</td>
<td></td>
<td>BuB/BnJ</td>
</tr>
</tbody>
</table>

### Fear Conditioning

<table>
<thead>
<tr>
<th>Great</th>
<th>Adequate</th>
<th>Impaired</th>
<th>Freezers</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB129F1</td>
<td>C57BL/6J</td>
<td>FVB/NJ</td>
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## Behavior tests

<table>
<thead>
<tr>
<th>Motor Function</th>
<th>Cognition</th>
<th>Emotion</th>
<th>Sensory</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Rota-rod</td>
<td>• Morris Water Maze</td>
<td>• Plus maze</td>
<td>• Hot-plate</td>
</tr>
<tr>
<td>• CatWalk</td>
<td>• Fear Conditioning</td>
<td>• Light-dark box</td>
<td>• Tail flick</td>
</tr>
<tr>
<td>• BMS scale</td>
<td>• Passive avoidance</td>
<td>• Open-field</td>
<td>• PPI</td>
</tr>
<tr>
<td>• Muscle strength</td>
<td>• Object recognition</td>
<td>• Startle response</td>
<td>• Visual Cliff</td>
</tr>
<tr>
<td>• Inclined screen</td>
<td>• T-maze</td>
<td>• Fear - startle</td>
<td>• Visual placing</td>
</tr>
<tr>
<td>• Beam Walk</td>
<td>• Y-maze</td>
<td>• PPI</td>
<td>• Visual WM</td>
</tr>
<tr>
<td>• Sticky tape test</td>
<td></td>
<td>• Aggression Beh</td>
<td>• Sticky-tape</td>
</tr>
<tr>
<td>• Open-field</td>
<td></td>
<td>• Maternal Behav</td>
<td></td>
</tr>
<tr>
<td>• Home-cage</td>
<td></td>
<td>• Social Behav.</td>
<td></td>
</tr>
<tr>
<td>• Treadmill Exercise</td>
<td></td>
<td>• Feeding Behav.</td>
<td></td>
</tr>
<tr>
<td>• Pole test</td>
<td></td>
<td>• Forced swim test</td>
<td></td>
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</table>
Phenotyping of coordination impairment in a mouse model of Ataxia

• Ataxia is a disorder that presents with lack of coordination associated with dysfunction in CNS regions that control movement and balance, such as the cerebellum.

• Determine the face validity of a novel transgenic mouse model of Ataxia

• Transgenic manipulation lead to specific changes in motor coordination
Motor Tests

- Coordination tests progressively declines

Rotarod

Inclined screen

Pole Test

Bar graphs showing the performance of Tg and WT mice in the Rotarod, Inclined screen, and Pole tests. The graphs indicate significantly lower performance in Tg mice compared to WT mice.
Importance of the Findings

• The genetic manipulation has an effect that is consistent with the disease studied.

• Face validity from an animal model standpoint
  – Allows to test treatments
  – Dissect molecular mechanisms further
Role of PET-1 on Maternal Behavior

• The Pet-1 transcription factor was found to be essential for serotonergic differentiation

• Pet-1 mutant mice exhibited anxiety and aggression. They also had very low survival of litters
Maternal behavior Observations

• Is the poor survival of the pups due to physiological problems of the mother or poor maternal behavior?
Retrieval Test

Pup Retrieval

- Number of pups
- Bar graph comparing the number of pups retrieved by different groups

*** Significant difference
Importance of Findings

• The serotonergic nervous system is important for:
  • Anxiety and aggression

• Enables normal maternal behavior that ensures the survival of offspring

• Published in Nature Neurosciences (2008)