Syllabus

I. Introduction to Protein Aggregation Diseases
   - Quality controls of protein folding
   - The molecular basis of protein aggregation

II. Alzheimer’s Disease: Genes, Proteins and Therapy
   - The clinical features of AD
   - The Complex Neurotoxic Cascade of AD
   - Cell Biology of Amyloid-\beta Precursor Protein & Origin of Amyloid-\beta peptides
   - Genetics of Familial AD
   - Genotype-to-Phenotype Conversions in Familial AD
   - Function of Presenilins: A Central Role in Intramembranous Proteolysis
   - Treating and Preventing AD

III. Parkinson’s Disease & Prion Diseases

IV. Experimental and Biophysical Approaches to Investigate Protein Misfolding and Aggregation

V. Conclusion
Protein Aggregation Diseases

A summary of the main amyloidoses and the proteins or peptides involved

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Protein Aggregation Diseases

The Molecular basis of protein aggregation is Protein Misfolding: Quality controls of protein folding

1. Molecular intracellular machineries ensuring the quality control of protein folding:
   a. Molecular chaperones in the cytosol (heat-shock proteins, crystallins, prefolding, Hsc70) and in the ER (Bip, Grp94, calnexin)
   b. The ubiquitin-proteasome pathway.

2. Molecular extracellular (or membrane located) quality controls:
   a. Proteases such as nepriylsin and insulin degrading enzymes (IDE),
   b. Chaperones present in extracellular fluids such as clusterin.

The Molecular events leading to misfolded proteins

1. Specific inactivating mutations of any of the components of the quality control machineries (= chaperonepathies or ubiquitin protein catabolic disorders),

2. Harsh environmental conditions such as heat shock, oxidative stress, or chemical modification may impair the activity of the clearing machinery components and/or increase the number of misfolded or unfolded proteins the cells must face, resulting in the overwhelming of both the molecular chaperones and the proteasome,

3. An increase of the expression levels of the affected protein can cause a shift of the equilibrium between correctly folded and partially folded molecules.
The Molecular basis of protein aggregation

1. In non-native states the misfolded proteins become loosely packed and its **hydrophobic core becomes exposed to the solvent**, thus enhancing the tendency to nucleate the initial oligomeric assemblies where the **content of secondary beta structure is generally increased**.

2. **Net charge** affect the tendency to aggregate: α-Synuclein and Tau carrying specific mutations enhancing their mean hydrophobicity or reducing their mean net charge are prone to aggregate.

3. These “seeds” or “aggregation nuclei” provide a template where other misfolded or partially folded molecules (or natively folded molecules in the case of the infectious prion diseases) are recruited, thus increasing the size of the growing assemblies and eventually giving rise to fibrillar aggregates.
The Process of Amyloid Formation Can be divided into 4 stages

1. Misfolding
2. Oligomerization
3. Fibrillization
4. Inclusion formation and deposition

The species populated in each stage possess their own distinct physicochemical properties

1. Misfolding
   a. Changes in secondary and/or tertiary structure
   b. Possible changes in quaternary structure
   c. Loss of activity
The species populated in each stage possess their own distinct physicochemical properties

2. Oligomerization
   a. Changes in quaternary structure
   b. Additional rearrangements at the secondary/quaternary structure levels.
   c. Loss of activity
   d. Population of a unique structure with specific functionality

3. Fibrillization
   a. Changes in quaternary structure (filament formation)
   b. The proteins acquire distinct physical properties:
      i. Cross-beta structure
      ii. Decreased solubility
      iii. Increases stability towards proteases
   c. Loss of activity
The species populated in each stage possess their own distinct physicochemical properties

4. Inclusion formation

   a. Aggregation and sequestration of fibrils
   b. Incorporation of other proteins and small molecules
   c. Post-translational modifications
   d. Increased stability towards proteases

Different Biophysical Assembly States of the Alzheimer's disease \( \alpha \beta \) peptides

![Image of different biophysical assembly states]

What are the true toxic species causing cell impairment?

What are the true toxic species?

In most cases, the pre-fibrillar assemblies appear endowed with the highest toxicity (≠ true toxic species) whereas mature fibrils are much less toxic and can be considered as harmless reservoirs of the toxic assemblies (ex: AD).
The “channel hypothesis” of amyloid aggregate toxicity.

The annular, “doughnut-shaped assemblies with a central pore are present among the heterogeneous population of pre-fibrillar aggregates of several different proteins and peptides.

These annular species are reminiscent of the pores formed by several bacterial pore-forming toxins (perfringolysin) as well as by some eukaryotic proteins (pro-apoptotic perforin), leading some authors to propose the “channel hypothesis”.

Protein Aggregation Diseases

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Affecting the central nervous system
### Global burden of disease in 2004

#### Year 2004

<table>
<thead>
<tr>
<th>Groups</th>
<th>World</th>
<th></th>
<th>EU25 (including Switzerland)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>191,660,641</td>
<td>30.8%</td>
<td>14,857,720</td>
<td>328</td>
</tr>
<tr>
<td>Mental</td>
<td>115,031,383</td>
<td>20.1%</td>
<td>10,088,093</td>
<td>222</td>
</tr>
<tr>
<td>CVD</td>
<td>181,536,258</td>
<td>31.2%</td>
<td>10,088,093</td>
<td>222</td>
</tr>
<tr>
<td>Cancer</td>
<td>77,152,633</td>
<td>12.8%</td>
<td>9,839,035</td>
<td>217</td>
</tr>
<tr>
<td>Injuries</td>
<td>182,590,897</td>
<td>31.6%</td>
<td>5,099,011</td>
<td>112</td>
</tr>
<tr>
<td>Respiratory</td>
<td>55,659,995</td>
<td>9.4%</td>
<td>15,523,243</td>
<td>7.8</td>
</tr>
<tr>
<td>Digestive</td>
<td>46,300,182</td>
<td>7.9%</td>
<td>2,925,351</td>
<td>6.5</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>25,349,766</td>
<td>4.4%</td>
<td>2,563,271</td>
<td>5.7</td>
</tr>
<tr>
<td>Infections</td>
<td>402,516,353</td>
<td>68.8%</td>
<td>2,282,694</td>
<td>50.8</td>
</tr>
<tr>
<td>Nutrition/End</td>
<td>61,520,078</td>
<td>10.5%</td>
<td>2,380,372</td>
<td>5.2</td>
</tr>
<tr>
<td>Sensory organs</td>
<td>69,379,818</td>
<td>11.8%</td>
<td>2,868,843</td>
<td>5.3</td>
</tr>
<tr>
<td>Maternal</td>
<td>128,804,829</td>
<td>22.0%</td>
<td>725,995</td>
<td>1.6</td>
</tr>
<tr>
<td>Oral</td>
<td>2,732,021</td>
<td>0.4%</td>
<td>434,767</td>
<td>0.9</td>
</tr>
<tr>
<td>Urinary</td>
<td>15,213,854</td>
<td>2.6%</td>
<td>601,288</td>
<td>1.3</td>
</tr>
<tr>
<td>Congenital</td>
<td>27,402,428</td>
<td>4.7%</td>
<td>698,394</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>1,491,416,317</td>
<td>100%</td>
<td>58,807,846</td>
<td>129</td>
</tr>
</tbody>
</table>


### Cases of brain diseases in 2004 (Europe)

![Cases of brain diseases in 2004 (Europe)](image-url)
**Cost per case of brain diseases in 2004 (Europe)**

Cost per patient (€ 2004)

- Tumour
- Multiple sclerosis
- Stroke
- Dementia
- Psychotic disorders
- Parkinson's disease
- Epilepsy
- Addictive disorders
- Trauma
- Anxiety disorders
- Migraine

**Neurodegenerative diseases**

Diseases characterized by the progressive loss of neurons in various brain structures

- Alzheimer’s disease
- Parkinson’s disease
- Huntington’s disease
- Amyotrophic lateral sclerosis

Main risk factor: age
Alzheimer’s Disease: Genes, Proteins, and Therapy.

A Portrait of an Alzheimer’s Patient

http://www.youtube.com/watch?v=tzFNT1HyTzo&mode=related&search=
I. A Historical Perspective

1864: Alois Alzheimer was born on the 14th of June in Marktbreit am Main (Germany).
1889: Alzheimers works as assistant than second physician in the municipal mental asylum in Frankfurt/Main.
1901: Alois Alzheimer first meets Auguste D. in Emil Sioli’s clinic in Frankfurt.
1903: Emil Kraepelin, head of the Psychiatry Department of the University of Munich calls Alzheimer to Munich.
1906: Auguste D. dies and her brain was send to Alzheimer from Frankfurt to Munich
1906: Alzheimer presented the case of his first patient at the 37th Meeting of the Southwest German Psychiatrists in Tübingen on November 3, 1906.
1910: The disease was named after Alzheimer by Emil Kraepelin.
Alzheimer gives a detailed description of the clinical history of a 56-year-old demented man (Johann Feigel).
1915: Alzheimer died in Breslau (now Poland) on December 19.

Alzheimer’s disease - epidemiology

- Alzheimer’s disease (AD), the most common dementia in elderly people, affects nearly 2% of the population in industrialized countries
- The AD prevalence equals 5.5 % above 60 years of age and increases for elderly people (up to 40% for > 85 years old)
Alzheimer's disease - epidemiology

II. The Progression and clinical features of AD
Alzheimer's disease - progression

Brain Aging  
MCI  
Clinical AD  
AAMI / ARCD

Cognitive Decline

Time (Years)

MCI: Mild Cognitive Impairment  
AAMI: Age-Associated Memory Impairment  
ARCD: Age-Related Cognitive Decline

Alzheimer’s disease - clinical features

Mild Cognitive Impairment  
Alzheimer's Disease Progression  
Death from pneumonia and/or other comorbidities

Mild
- Loss of recent memory
- Faulty judgment
- Personality changes

Moderate
- Verbal and physical aggression
- Agitation
- Wandering
- Sleep disturbances
- Delusions

Severe
- Loss of all reasoning
- Bedridden
- Incontinence

(Ferris, 4/03)
Alzheimer’s disease - clinical features

Preclinical AD

- Signs of AD are first noticed in the entorhinal cortex, then proceed to the hippocampus
- Affected regions begin to shrink as nerve cells die
- Changes can begin 10-20 years before symptoms appear
- Memory loss is the first sign of AD

Alzheimer’s disease - clinical features

Mild to Moderate AD

- AD spreads through the brain. The cerebral cortex begins to shrink as more and more neurons stop working and die
- **Mild AD signs** can include memory loss, confusion, trouble handling money, poor judgment, mood changes, and increased anxiety
- **Moderate AD signs** can include increased memory loss and confusion, problems recognizing people, difficulty with language and thoughts, restlessness, agitation, wandering, and repetitive statements
Alzheimer’s disease - clinical features

Severe AD

- In severe AD, extreme shrinkage occurs in the brain. Patients are completely dependent on others for care.
- Symptoms can include weight loss, seizures, skin infections, groaning, moaning, or grunting, increased sleeping, loss of bladder and bowel control.
- Death usually occurs from aspiration pneumonia or other infections.

AD changes the whole brain

AD leads to nerve cell death and tissue loss throughout the brain, impairs memory, reasoning and behavior and leads ultimately to complete social dependence and death. Over time, the brain shrinks dramatically, affecting nearly all its functions.

The cortex shrivels up, damaging areas involved in thinking, planning and remembering.

Ventricules (fluid-filled spaces within the brain) grow larger.
III. The Neuropathological Phenotype of AD

III.a Neuritic Plaques
Neuritic Plaques

Typical thioflavin stained plaques with amyloid core and dystrophic neurites. Plaques are prominent and frequent.

Abnormally enlarged synaptophysin-positive axon terminals are constantly observed around Aβ deposits. (Delatour et al., Neurobiol. of Disease 2004.).

Three-dimensional visualization of amyloid deposition in the brain of a person with mild Alzheimer's disease

PET images obtained after injecting the amyloid tracer PIB* were superimposed on the magnetic resonance image of the same patient. The movie shows extensive amyloid deposition in cortical and deep brain areas.

*PIB: 2-(4'-methylaminophenyl)benzothiazole, uncharged derivative of thioflavin-T that has high affinity for Aβ fibrils and shows very good brain entry and clearance.
III.b Neurofibrillary tangles

Tangles as drawn by Bonfiglio in 1908 with silver stain.
from F Bonfiglio, Organa della Societa Freniatrica Italiana, 1908,34: 670.

Tangles as seen with Thioflavin S.
© Robert D. Terry
III.c Amyloid Microangiopathy

Amyloid infiltrates the wall of small vessel.

Thioflavin shows segmental amyloid in small artery.
The amyloid is exclusively in small arteries – not in veins.

IV. The complex Amyloid β-Peptides
Neurotoxic Cascade of Alzheimer’s disease

--

DOMINANTLY INHERITED FORMS OF AD

Missense mutations in the APP or Presenilin 1 or 2 genes
Increased Aβ42 production throughout life
Accumulation and oligomerization of Aβ42 in limbic and association cortices
Subtle effects of Aβ oligomers on synaptic efficacy
Gradual deposition of Aβ42 oligomers as diffuse plaques
Microglial and astrocytic activation and attendant inflammatory responses
Altered neuronal ionic homeostasis; oxidative injury
Altered kinase/phosphatase activities lead to tangles
Widespread neuronal/synaptic dysfunction and selective neuronal loss, with attendant neurotransmitter deficits

DEMENTIA

NON-DOMINANT FORMS OF AD
(including “sporadic” AD)

Failure of Aβ clearance mechanisms (e.g., inheritance of ApoE4, faulty Aβ degradation, etc.)
Gradually rising Aβ42 levels in brain
Accumulation and oligomerization of Aβ42 in limbic and association cortices
Subtle effects of Aβ oligomers on synaptic efficacy
Gradual deposition of Aβ42 oligomers as diffuse plaques
Microglial and astrocytic activation and attendant inflammatory responses
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DEMENTIA
DOMINANTLY INHERITED FORMS OF AD

Missense mutations in the APP or Presenilin 1 or 2 genes

NON-DOMINANT FORMS OF AD (including “sporadic” AD)

Failure of Aβ clearance mechanisms (e.g., inheritance of ApoE4; faulty Aβ degradation, etc.)

According to the amyloid-β hypothesis, Aβ peptides are the primary causative agents of AD

Aβ peptides have remained the focus of the vast majority of studies in the AD field

V. Origin of Amyloid-β Peptides: Cell Biology of Amyloid-β Precursor Protein (APP)
APP Processing and Aβ Accumulation

Toxic (Aβ producing)

Non-Toxic

V.a Expression and Heterogeneity of APP

677-770 amino acids type I trans-membrane protein

Domain functions: partly identified

The APP - Splicing

The APP - Splicing

The APP - Splicing
# Differential Expression of APP mRNAs in Peripheral and Brain Tissues

The table below shows the differential expression of APP mRNAs in various tissues. APP751, predominantly found in peripheral tissues, lacks exon 8. Neuronal APP, on the other hand, predominantly contains exon 15 and lacks exons 7 (protease inhibitor domain) and exon 8.

### APP in Peripheral tissues: Predominantly APP751, lacks exon 8

<table>
<thead>
<tr>
<th>Tissue</th>
<th>APP695</th>
<th>APP751</th>
<th>APP852</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Testis</td>
<td></td>
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<td></td>
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<tr>
<td>Spleen</td>
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<td></td>
<td></td>
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<tr>
<td>Muscle</td>
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<td>Blood</td>
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<td></td>
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<tr>
<td>Brain</td>
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### Neuronal APP: Predominantly APP695, lacks exons 7 and exon 8, always contains exon 15.

### APP - the Gene Family

- **APP**
  - Expression of alternatively spliced exons is high in non-neuronal tissues, low in neuronal tissues.
  - **KPI**
    - Expression of alternatively spliced exons is high in non-neuronal tissues, low in neuronal tissues.

- **APLP2/APPH**
  - Expression of alternatively spliced exons is high in non-neuronal tissues, high in neuronal tissues.

- **APLP1**
  - Expression of alternatively spliced exons is high in non-neuronal tissues, high in neuronal tissues.
V.b Trafficking and proteolytic processing of APP

APP - sorting

Three categories of protein distribution

1. Axonal    2. Somatodendritic    3. Mixed (i.e. axonal and dendritic)
Trafficking of APP

APP is first sorted to the axon and then into dendrites.

VI. Genetics of AD
Dementia as a result of an ageing population

![Graph showing prevalence of dementia by age and gender.](Prevalence of dementia in Europe: pooled analyses of 11 studies from the EURODEM group (Lobo et al., 2000))

Alzheimer’s disease - etiology

Risk factors
- Age, female sex
- Presence of the apolipoprotein ε4 (APOE ε4) allele
  - Lifetime risk of AD for an individual
    - ° without the ε4 allele is approximately 9%
    - ° carrying at least one ε4 allele is 29%
- Head injury
- Low serum levels of folate and vitamin B12
- Elevated plasma and total homocysteine levels
- Family history of AD or dementia
- Fewer years of formal education
- Lower income
- Lower occupational status
Alzheimer's disease - etiology

Genetic factors

- Chromosome 21 (APP): Early Onset FAD
- Chromosome 14 (PS1): Early Onset FAD
- Chromosome 1 (PS2): Volga German FAD

1991

APP

1995

Presenilin 1

Presenilin 2

1995

VI.a Missense mutations in APP: a very rare cause of Familial Autosomal Dominant AD (FAD)

VI.b Missense mutations in Presenilins:
the most common cause of Familial
Autosomal Dominant AD to date
VII. Genotype-to-Phenotype Conversions in Familial Alzheimer’s disease

VII.a APP mutations increase the production of Aβ_{42} peptides

- Cleavage by β-secretase
  - Aβ_{40} & Aβ_{42}

- Cleavage by α-secretase
  - Aβ_{40} & Aβ_{42}

- Cleavage by γ-secretase
  - Aβ_{42} (selective)
VII.b Presenilin clinical mutations increase the $A\beta_{42}/A\beta_{40}$ ratio

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Mean age at onset (years)</th>
<th>Mean age at death (years)</th>
<th>In proteins</th>
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</thead>
<tbody>
<tr>
<td>PS1-19</td>
<td>45.5</td>
<td>51.2</td>
<td>H/L-VI</td>
</tr>
<tr>
<td>T91-S116I; S39A/C</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PS1-L166P</td>
<td>34.9</td>
<td>42.2</td>
<td>TM-VII</td>
</tr>
<tr>
<td>PS1-D382A</td>
<td>30.9</td>
<td>36.0</td>
<td>TM-VI</td>
</tr>
<tr>
<td>PS1-A246E</td>
<td>52.6</td>
<td>ND</td>
<td>TM-VI</td>
</tr>
<tr>
<td>PS2-N141I</td>
<td>50.9</td>
<td>ND</td>
<td>TM-VI</td>
</tr>
</tbody>
</table>

In PS1-19, even if it is delayed which is accompanied by the substitution of Ser 200 by a cysteine (Braun et al., 1996). H/L, hydrophobic loop. TM, Transmembrane domain. ND, not determined.

PS clinical mutations induce loss of function in APP & Notch processing
PS clinical mutations cause variable loss in $A\beta_{40}$ production
PS clinical mutations do not affect the $A\beta_{42}$ production
PS clinical mutations cause a relative increase in $A\beta_{42}/A\beta_{40}$ ratio
Genotype-to-Phenotype Conversions in Familial AD: APP & PS1 mutations affect the $A\beta_{42}/A\beta_{40}$ ratio

APP23
B6-TgN(mThy1-hAPP<sup>K670N/M671L</sup>)
23 months

APP/PS1
B6-TgN(mThy1-hAPP<sup>K670N/M671L</sup>; PS1<sup>L166P</sup>)
5 months-old

Ab2 (1:100): overnight, 4C, in PBS diluted 1:1 with blocking buffer (horse serum: 1.5%, Triton x-100: 0.1%)

Genotype-to-Phenotype Conversions in Familial AD: APP & PS1 mutations affect the $A\beta_{42}/A\beta_{40}$ ratio

VIIc. Inheritance of ApoE4 alleles increases steady-state levels of $A\beta$ peptides in the brain

ApoE has critical functions in:

- Redistributing lipids among CNS cells for normal lipid homeostasis
- Repairing injured neurons
- Maintaining synapto-dendritic connections
- Scavenging toxins

ApoE4 allele is associated with early onset, progression, or severity of:

- Head trauma
- Stroke
- Complications after coronary artery bypass surgery
- Parkinson’s disease
- Amyotrophic lateral sclerosis (ALS)
- Multiple sclerosis (MS)
- Diabetic neuropathy
Plaques and Tangles Contain ApoE


The Apolipoprotein-E Molecule

Predicted secondary structure of human apoE. The α helices in C domain are amphipathic and thus most suitable for interactions with lipids.

**Structure of a Lipoprotein**

- Triacylglycerols
- Phospholipids
- ApoB48
- ApoC
- ApoE
- Cholesterol
- Free Cholesterol
- Triglycerides
- Phospholipids


**Brain and Plasma ApoE Lipoproteins**

**Plasma - LP**

**CSF - LP**

**Astrocyte – LP**

- Triglycerides
- Cholesterol Ester

**Serum (left panels) and CSF (right panels) lipoproteins.**

1. ApoE is the major CSF lipoprotein. ApoA is second in abundance. The CSF lipoproteins also contain apoJ.

2. Astrocytes secrete lipoprotein discs which are similar to immature HDL. Presumably, and like in the serum, these particles can pick up cholesterol and mature into HDL-like particles. Indeed these particles contain the appropriate enzymes (e.g. Lecitin:cholesterol acetyltransferase (LCAT)) which catalyze the esterification of cholesterol to cholesterol ester by transferring fatty acids from phosphatidylcholine.

5. Serum and CSF apoE pools do not mix. Liver transplantation operations in which the apoE genotype of the donor and recipient happened to differ revealed that the serum apoE of the recipient became that of the donor whereas the brain apoE genotype was unaltered.

From:

The apoE binding domain is the complement-like, cysteine-rich domain which exists in varying copies in the different receptors. The megalin and LRP receptors are unique in that they contain several clusters of these repeat domains.

Receptor Mediated Protein and Lipoprotein Uptake

Ligand uptake mechanisms mediated by members of the LDL receptor family.

1. The uptake of circulating proteins and lipoproteins is by endocytosis for all receptors with the specificity determined by ligand-receptor interaction.

3. The first example (i.e. lipoprotein uptake) is mediated by LDL-receptor and LRP.

5. The uptake by LRP of circulating proteins such as protease inhibitors or bacterial Exotoxin A (PEA) (second and third panel) is illustrated in the absence of lipoproteins, which of course could compete with the proteins for binding and internalization.

4. The megalin example can refer to Vitamin D complexed to its binding proteins.


Isoform Specific Biochemical Features of ApoE
ApoE Genotype in Control and AD Populations

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>Control</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>15%</td>
<td>5%</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>58%</td>
<td>33%</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>24%</td>
<td>44%</td>
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<tr>
<td>ε4/ε4</td>
<td>2%</td>
<td>18%</td>
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<tr>
<td>APOE alleles</td>
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<td></td>
</tr>
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<td>ε2</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>ε3</td>
<td>0.77</td>
<td>0.56</td>
</tr>
<tr>
<td>ε4</td>
<td>0.14</td>
<td>0.39</td>
</tr>
</tbody>
</table>

The frequency of apoE genotype and apoE alleles in AD.
Results shown are of a European population. Note that an allele frequency of 39% translates to 61% of the cases carrying either one or two alleles.


The Effects of ApoE Genotype on the Age of Onset of AD

ApoE4 Decreases the Age of Onset of AD in a Gene Dosage Manner

0, 1, and 2 in the figure correspond to the number of apoE alleles. Consequently, 50% of the apoE4 homozygous subjects who are going to get the disease do so at age ~65 years whereas of the non-apoE4 subjects who are going to get the disease, 50% do so at the age of ~85 years.

ApoE Isoform-Specific Regulation of Aβ Plaque Burden

Plaque load is highest in the hippocampi of mice expressing murine ApoE (A) and lowest in APOE knockout animals (C).

Plaque load in the hippocampi of human ApoE4-expressing mice (E) is greater than plaque load in the hippocampi from human ApoE3-expressing mice (G).


Genotype specific Effects of ApoE on Aβ Metabolism

APP V717 +/- TG mice

APP → Aβ → Diffuse Aβ → Fibrillar Aβ

Removal from Brain
Degradation → Dissolution

Stimulated specifically by ApoE4
Stimulated by all ApoE forms
ANNEX 1: Alzheimer’s disease – clinical stages

Stage 1: No impairment (normal function)
Unimpaired individuals experience no memory problems and none are evident to a health care professional during a medical interview.

Stage 2: Very mild cognitive decline (may be normal age-related changes or earliest signs of Alzheimer’s disease)
Individuals may feel as if they have memory lapses, especially in forgetting familiar words or names or the location of keys, eyeglasses or other everyday objects. But these problems are not evident during a medical examination or apparent to friends, family or co-workers.

Stage 3: Mild cognitive decline
Early-stage Alzheimer’s can be diagnosed in some, but not all, individuals with these symptoms
Friends, family or co-workers begin to notice deficiencies. Problems with memory or concentration may be measurable in clinical testing or discernible during a detailed medical interview. Common difficulties include:
° Word- or name-finding problems noticeable to family or close associates
° Decreased ability to remember names when introduced to new people
° Performance issues in social or work settings noticeable to family, friends or co-workers
° Reading a passage and retaining little material
° Losing or misplacing a valuable object
° Decline in ability to plan or organize
Stage 4: Moderate cognitive decline (Mild or early-stage Alzheimer's disease)

At this stage, a careful medical interview detects clear-cut deficiencies in the following areas:
° Decreased knowledge of recent occasions or current events
° Impaired ability to perform challenging mental arithmetic—for example, to count backward from 75 by 7s
° Decreased capacity to perform complex tasks, such as planning dinner for guests, paying bills and managing finances
° Reduced memory of personal history
° The affected individual may seem subdued and withdrawn, especially in socially or mentally challenging situations

Stage 5: Moderately severe cognitive decline (Moderate or mid-stage Alzheimer's disease)

Major gaps in memory and deficits in cognitive function emerge. Some assistance with day-to-day activities becomes essential. At this stage, individuals may:
° Be unable during a medical interview to recall such important details as their current address, their telephone number or the name of the college or high school from which they graduated
° Become confused about where they are or about the date, day of the week or season
° Have trouble with less challenging mental arithmetic; for example, counting backward from 40 by 4s or from 20 by 2s
° Need help choosing proper clothing for the season or the occasion
° Usually retain substantial knowledge about themselves and know their own name and the names of their spouse or children
° Usually require no assistance with eating or using the toilet

Stage 6: Severe cognitive decline (Moderately severe or mid-stage Alzheimer's disease)

Memory difficulties continue to worsen, significant personality changes may emerge and affected individuals need extensive help with customary daily activities. At this stage, individuals may:
° Lose most awareness of recent experiences and events as well as of their surroundings
° Recollect their personal history imperfectly, although they generally recall their own name
° Occasionally forget the name of their spouse or primary caregiver but generally can distinguish familiar from unfamiliar faces
° Need help getting dressed properly; without supervision, may make such errors as putting pajamas over daytime clothes or shoes on wrong feet
° Experience disruption of their normal sleep/waking cycle
° Need help with handling details of toileting (flushing toilet, wiping and disposing of tissue properly)
° Have increasing episodes of urinary or fecal incontinence
° Experience significant personality changes and behavioral symptoms, including suspiciousness and delusions (for example, believing that their caregiver is an impostor); hallucinations (seeing or hearing things that are not really there); or compulsive, repetitive behaviors such as hand-wringing or tissue shredding
° Tend to wander and become lost
Stage 7: Very severe cognitive decline (Severe or late-stage Alzheimer's disease)

This is the final stage of the disease when individuals lose the ability to respond to their environment, the ability to speak and, ultimately, the ability to control movement.

° Frequently individuals lose their capacity for recognizable speech, although words or phrases may occasionally be uttered
° Individuals need help with eating and toileting and there is general incontinence of urine
° Individuals lose the ability to walk without assistance, then the ability to sit without support, the ability to smile, and the ability to hold their head up. Reflexes become abnormal and muscles grow rigid. Swallowing is impaired.