Genetics of Alzheimer’s Disease

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What is Alzheimer’s disease?

Clinical Picture

- Gradual onset and progression of memory impairment combined with deficits in executive functioning, language, visuo-spatial abilities, personality, behavior and self-care.

Neuropathology
How common is Alzheimer’s disease?

- 5 million people in the U.S. with Alzheimer’s disease (AD)
- 100,000 deaths/yr attributed to AD
- Risk of developing AD increases with age
  - 5% of population over 65yrs develop AD
  - 30% of those over 85yrs develop AD
Genetics of dementia

- **Sporadic**
  - Usually most common form of the disease with a late age of onset. Genetic and environmental risk factors influence risk for disease.

- **Inherited**
  - Rare, early onset forms of the disease, autosomal dominant

- **Apart from age of onset sporadic and inherited forms of the disease are indistinguishable**
Genetic Epidemiology of AD

- Rare families with autosomal dominant inheritance

- In late onset AD recurrence risk to siblings ($\lambda_s$) is 4-5

- Twin studies
  - 0.49 in MZ, 0.18 in DZ twins
Inherited forms of Alzheimer’s disease

- **Mutations in 3 genes**
  - ß-amyloid precursor protein (APP) on chromosome 21
  - Presenilin 1 (PS1) on chromosome 14
  - Presenilin 2 (PS2) on chromosome 1
Most mutations causing FAD are in PSEN1

<table>
<thead>
<tr>
<th>Gene</th>
<th># Mutations</th>
<th># Families</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>32 (13.9%)</td>
<td>89 (17.2%)</td>
<td></td>
</tr>
<tr>
<td>PSEN1</td>
<td>185 (80.4%)</td>
<td>405 (78.5%)</td>
<td></td>
</tr>
<tr>
<td>PSEN2</td>
<td>13 (5.6%)</td>
<td>22 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>230</td>
<td>516</td>
<td></td>
</tr>
</tbody>
</table>

Duplications of APP also cause familial AD
Pathways of APP metabolism

**α-secretase pathway**
- sAPPα
- C83/CTFα
- γ-secretase
- p3
- CTFγ/AICD

**β-secretase pathway**
- sAPPβ
- C99/CTFβ
- γ-secretase
- PSEN1-2
- Aβ
- CTFγ/AICD

**γ-secretase**
- Aβ
- γ40/42
- S3-like
- TM

**TM**
- 1
- 672
- 713
- 770

** α-secretase pathway**

** β-secretase pathway**
Effect of early onset FAD mutations on Aß levels

Plasma Aß1-42/43 (pM)

Plasma Aß1-40(pM)

- Control
- βAPPK670N/M671L
- PS1-FAD
- βAPPV717
- PS-2 N141I
What effect do *APP* and *PSEN* mutations have on Aβ?

- Some mutations increase Aβ$_{42}$
- Some mutations decrease Aβ$_{40}$
- Some mutations affect both
- All mutations affect Aβ$_{40}$/Aβ$_{42}$ ratio by increasing the proportion of Aβ$_{42}$
- Aβ$_{42}$ is more fibrillogenic than Aβ$_{40}$ and leads to earlier oligomer/fibril formation
A mutation in **APP** protects against Alzheimer’s disease and age-related cognitive decline

Thorlakur Jonsson¹, Jasvinder K. Atwal², Stacy Steinberg¹, Jon Snaedal³, Palmi V. Jonsson³,⁸, Sigurbjorn Bjornsson³, Hreinn Stefansson¹, Patrick Sulem¹, Daniel Gudbjartsson¹, Janice Maloney², Kwame Hoyte², Amy Gustafson², Yichin Liu², Yannmei Lu², Tushar Bhangale², Robert R. Graham⁻¹, Johanna Huttenlocher¹,⁴, Gyda Bjornsdottir¹, Ole A. Andreassen⁵, Erik G. Jonsson⁶, Aarno Palotie⁷, Timothy W. Behrens², Olafur T. Magnusson¹, Augustine Kong¹, Unnur Thorsteinsdottir¹,⁸, Ryan J. Watts² & Kari Stefansson¹,⁸

**Table 1 | APP A673T protects against Alzheimer’s disease**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>1/OR</th>
<th>OR</th>
<th>P-value</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>AD</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.13</td>
</tr>
<tr>
<td>AD versus population controls</td>
<td>4.24</td>
<td>0.236</td>
<td>4.19 × 10⁻⁵</td>
<td>0.45</td>
</tr>
<tr>
<td>AD versus population controls aged 85 or greater</td>
<td>5.29</td>
<td>0.189</td>
<td>4.78 × 10⁻⁷</td>
<td>0.62</td>
</tr>
<tr>
<td>AD versus cognitively intact controls at age 85</td>
<td>7.52</td>
<td>0.133</td>
<td>6.92 × 10⁻⁶</td>
<td>0.79</td>
</tr>
</tbody>
</table>

The table shows association results, comparing patients with Alzheimer’s disease (AD) to three different control groups (top line gives numbers for patients with Alzheimer’s disease only). Nchip, number of individuals with chip-based genotype information; Nin silico, number of individuals with genealogy-based genotype information.

Are there protective variants in these genes?

Figure 2 | A673T reduces BACE1 cleavage of APP. a, Western blot analysis of 293T cells transfected with wild-type (WT), A673T, A673V or K670N/M671L APP compared to GFP. Total cellular APP was compared to sAPPβ and sAPPα from cell supernatants. Note that sAPPβ is not detected from the K670N/M671L APP transfection as these mutations alter the epitope recognized by the anti-sAPPβ antibody. b, Immunoassay quantification of sAPPβ and sAPPα supernatants. c-d, ELISA quantification of Aβ42 (c) and Aβ40 (d) production.

Genetic Architecture of AD in 2014

Causes Alzheimer’s:
- PSEN 1
- PSEN 2
- APP

High risk
Med risk
Low risk

Risk of Alzheimer’s:

Frequency in the population

rare mutations in these genes cause AD in some families but may increase risk in other families.
How was \textit{APOE4} identified as a risk factor for AD?

- Linkage analysis in late onset AD families suggested an AD gene was located on chromosome 19
- \textit{APOE} gene maps to chromosome 19 in region of linkage
- \textit{APOE} is present in senile plaques
- \textit{APOE} is found bound to A\(\beta\) in cerebrospinal fluid
- Case-control studies show that individuals with 1 or 2 \textit{APOE4} alleles are at increased risk for AD
ApoE4 and Alzheimer’s Disease

Odds ratios for APOE alleles
- APOE2: 0.55 (0.36-0.84)
- APOE3: 1.00
- APOE4: 3.77 (3.29-4.32)

Allele frequency in most populations is ~15% - ~26% of population is heterozygous - ~2% of the population is homozygous
Effect of ethnicity and *APOE* genotype on odds ratios for developing AD

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>Caucasian</th>
<th>African American</th>
<th>Hispanics</th>
<th>Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>€3€3</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>€3€4</td>
<td>3.2 (2.8-3.8)</td>
<td>1.1 (0.7-1.8)</td>
<td>2.2 (1.3-3.4)</td>
<td>5.6 (3.9-8.0)</td>
</tr>
<tr>
<td>€4€4</td>
<td>14.9 (10.8-20.6)</td>
<td>5.7 (2.3-14.1)</td>
<td>2.2 (0.7-6.7)</td>
<td>33.1 (13.6-80.5)</td>
</tr>
</tbody>
</table>

From a meta-analysis Farrer, L., et al., JAMA 1997;
The β-amyloid cascade hypothesis

- All features of Alzheimer’s disease are caused by deposition of fibrillar β-amyloid (Aβ) in the brain.
  - Mutations in APP, PS1 and PS2 all lead to increased Aβ42 production and early Aβ deposition. APOE4 increases Aβ fibril formation and leads to increase Aβ deposition relative to other APOE alleles.
Genetic Architecture of AD in 2014

Causes of Alzheimer’s:
- PSEN 1
- PSEN 2
- APP

Risk of Alzheimer’s:
- High risk
- Med risk
- Low risk

Frequency in the population

Very rare to V Common

2% of the population have two copies of APOE4, making them 8 times more likely to develop AD.

Rare mutations in these genes cause AD in some families but may increase risk in other families.
Genetics of Alzheimer’s disease

Sporadic AD:

- A complex *trait*
- Majority of cases have age of onset > 65 yrs
- 40% of cases have one or more affected first degree relatives
- Most important genetic risk factor
  - Apolipoprotein E4
- 50% of AD cases do not carry APOE4 alleles
1) Hundred of thousand to millions of common Single Nucleotide Polymorphisms (SNPs) are tested for association with disease status.

2) GWA studies are necessarily hypothesis-free.

3) Not all the Common variants in the genome are tested. The functional variant is in Linkage Disequilibrium (LD) with the tested variant.
Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer’s disease
GWAS for AD

- Aβ metabolism: CLU, SORL1
- Immune Response: CR1, EPHA1, CLU, MS4A6A, CD33, HLA, INPP5D
- Synapse Function: BIN1, PICALM, PTK2B, RIN3, MEF2C, CELF1
- Endocytosis: BIN1, RIN3
- Lipid metabolism: CLU, SORL1

- Odds ratios are modest compared to APOE
- It is more important the genes and the pathway involved those gene, than the SNPs itself
Genetic Architecture of AD in 2014

Causes of Alzheimer’s:
- PSEN 1
- PSEN 2
- APP

Risk of Alzheimer’s:
- Very rare
- V Common

Frequency in the population:

- Very rare
- V Common

- PSEN 1 and PSEN 2
- APP

Genes associated with high risk:
- APOE4
- 2% of the population have two copies of APOE4, making them 8 times more likely to develop AD.

Genes associated with moderate risk:
- MS4A, CR1
- PICALM, BIN1
- CD33, EPHA1
- CD2AP, ABCA7

Genes associated with low risk:
- Rare mutations in these genes cause AD in some families but may increase risk in other families.

Over half the population may carry these variants, but each one only has a small effect on risk.
Rare variants with large effect size: TREM2
**Table 1. Association between the rs75932628-T Variant and Alzheimer’s Disease in Comparisons with Three Control Groups.**

<table>
<thead>
<tr>
<th>Control Group</th>
<th>No. of Participants</th>
<th>Frequency</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All population controls</td>
<td>110,050</td>
<td>0.63</td>
<td>2.26 (1.71–2.98)</td>
<td>1.13x10⁻⁸</td>
</tr>
<tr>
<td>Population controls ≥85 yr of age</td>
<td>8,888</td>
<td>0.46</td>
<td>2.92 (2.09–4.09)</td>
<td>3.42x10⁻¹⁰</td>
</tr>
<tr>
<td>Cognitively intact controls ≥85 yr of age*</td>
<td>1,236</td>
<td>0.31</td>
<td>4.66 (2.38–9.14)</td>
<td>7.39x10⁻⁶</td>
</tr>
</tbody>
</table>

* Intact cognition was defined as a score of 0 on the Cognitive Performance Scale, which ranges from 0 to 6, with higher scores indicating more severe impairment. CI denotes confidence interval.

**Table 2. Replication Analysis of the Association between the rs75932628-T Variant and Alzheimer’s Disease.**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>No. of Controls</th>
<th>Frequency*</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emory</td>
<td>399</td>
<td>402</td>
<td>0.12</td>
<td>3.03 (0.33–78.35)</td>
<td>0.37</td>
</tr>
<tr>
<td>Munich</td>
<td>517</td>
<td>1891</td>
<td>0.19</td>
<td>3.15 (1.06–10.40)</td>
<td>0.04</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>944</td>
<td>4950</td>
<td>0.15</td>
<td>2.45 (0.94–6.35)</td>
<td>0.07</td>
</tr>
<tr>
<td>Norway</td>
<td>177</td>
<td>2484</td>
<td>0.16</td>
<td>3.52 (0.54–17.21)</td>
<td>0.14</td>
</tr>
<tr>
<td>Combined</td>
<td>2037</td>
<td>9727</td>
<td>0.16</td>
<td>2.83 (1.45–5.40)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* The reported frequency is for the presence of the rs75932628-T variant in controls.
Genetic Architecture of AD in 2014

Causes of Alzheimer’s:

- PSEN 1
- PSEN 2
- APP

High risk:

- 0.3% of Northern European ancestry have risk variant in TREM2 which makes them 3 times more likely to develop AD.

Med risk:

- 2% of the population have two copies of APOE4, making them 8 times more likely to develop AD.

Low risk:

- Very rare

V Common

Frequency in the population

- MS4A
- CR1
- PICALM
- BIN1
- CLU
- CD33
- EPHA1
- ABCA7

Over half the population may carry these variants, but each one only has a small effect on risk.
Study Design

- Sequence FAD and FTD genes to remove families with pathogenic mutations in known genes
  - *APP*, *PSEN1*, *PSEN2*
  - *MAPT*, *GRN* and **C9ORF72** cause FTD but can present with a clinical phenotype indistinguishable from AD

- Use exome-sequencing in the most promising families without coding variants in the screened genes to identify novel genes
  - Validate promising variants in large case-control series

*Cruchaga et al., PLoS One 2012
**Harms et al., JAMA Neurol, 2013
Exome-sequencing in LOAD families

• Whole-exome sequencing was performed in 14 LOAD families
  • No coding variant in FAD or FTD genes
  • At least 4 affected individuals, 3 with DNA
  • At least two cases and one control per family were sequenced
  • Exclude families if APOE4 segregates with disease
• Identified coding variants present in cases but absent in the control
  • Variants with a MAF<0.5% were tested for segregation
  • Only variants showing perfect segregation were selected for follow-up

• Examine whether the same variant or gene was detected in more than one family
Exome-sequencing filter

**Variant/Gene Discovery**

**NIA-LOAD Families**
- 14 families represented by 2 cases and 1 control

**Exome-sequencing individual samples**
- 947 non-synonymous variant/sample

**Present in Cases within the family**
- 250 non-synonymous variant/family

**Not present in the unaffected within the family**
- 171 non-synonymous variant/family

**MAF<0.5%**
- 75 non-synonymous variant/family

All the selected variants were genotyped in the rest of the family members (4 affected and 10 unaffected/family)

**Perfect segregation**
- 8 non-synonymous variant/family

**Present in >1 family**
- 1 non-synonymous variant: V232M PLD3

Cruchaga et al., Nature
PLD3 V232M association with AD

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Cases</th>
<th>Control</th>
<th>OR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIA-LOAD</td>
<td>29/1077</td>
<td>8/920</td>
<td>3.09 (1.41-6.81)</td>
<td>4.00×10^-3</td>
</tr>
<tr>
<td>Knight-ADRC</td>
<td>16/1098</td>
<td>2/911</td>
<td>6.63 (1.52-28.9)</td>
<td>3.40×10^-3</td>
</tr>
<tr>
<td>NIA-UK</td>
<td>1/142</td>
<td>0/183</td>
<td>2.30 (0.88-6.01)</td>
<td>0.384</td>
</tr>
<tr>
<td>Cache-County</td>
<td>6/249</td>
<td>29/2442</td>
<td>2.03 (0.83-4.93)</td>
<td>0.131</td>
</tr>
<tr>
<td>U. Toronto</td>
<td>5/260</td>
<td>1/245</td>
<td>4.71 (0.54-40.7)</td>
<td>0.212</td>
</tr>
<tr>
<td>U. Nottingham</td>
<td>6/519</td>
<td>3/271</td>
<td>1.05 (0.26-4.25)</td>
<td>0.627</td>
</tr>
<tr>
<td>U. Pittsburg</td>
<td>15/1253</td>
<td>6/958</td>
<td>1.82 (0.74-5.00)*</td>
<td>0.191</td>
</tr>
<tr>
<td>NIMH</td>
<td>4/318</td>
<td>-</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Wellderly</td>
<td>-</td>
<td>1/376</td>
<td>- N/A</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>82/4916</strong></td>
<td><strong>50/6306</strong></td>
<td><strong>2.02 (1.41-2.89)</strong></td>
<td><strong>2.93×10^-5</strong></td>
</tr>
</tbody>
</table>

The table shows the counts for Carriers and non-carriers. P-values were calculated by Fisher exact-test.

*For the U. Pittsburg, age, gender, APOE genotype and principal component factors for population stratification were available. Association of the V232M with AD risk was performed by logistic regression including age, sex, APOE genotype and the first four principal component factors as covariate.
PLD3: gene-based analysis

- Twenty (20) non-synonymous variants were found.
- Three variants are nominally significant ($p=0.02$, $1.05 \times 10^{-5}$, and $1.08 \times 10^{-5}$, and OR=2.31-7.73).
- Nine (9) variants were present only in cases ($n=16$ cases)
- Two variants were present only in control ($n=2$ controls)

<table>
<thead>
<tr>
<th>PLD3</th>
<th>P value</th>
<th>Odd Ratio (OR)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All variants</td>
<td>$1.44 \times 10^{-11}$</td>
<td>2.75</td>
<td>2.05-3.68</td>
</tr>
<tr>
<td>Removing V232M</td>
<td>$1.58 \times 10^{-8}$</td>
<td>2.48</td>
<td>1.79-3.42</td>
</tr>
</tbody>
</table>

Based on sequencing data from 2,363 cases and 2,024 controls (European-descent)
NIA-LOAD Families

14 families represented by 2 cases and 1 control

- Exome-sequencing individual samples
  - 947 non-synonymous variant/sample

- Present in Cases within the family
  - 250 non-synonymous variant/family

- Not present in the unaffected within the family
  - 171 non-synonymous variant/family

- MAF<0.5%
  - 75 non-synonymous variant/family

All the selected variants were genotyped in the rest of the family members (4 affected and 10 unaffected/family)

- Perfect segregation
  - 8 non-synonymous variant/family

- Present in >1 family
  - 1 non-synonymous variant: V232M PLD3

Replication Single variant analysis (V232M)

European-descendent: (4, 998CA/6,356 CO)
- All cases and controls (unrelated)
  - $2.93 \times 10^{-5}; \text{OR}=2.02, 95\%\text{CI}=1.41-2.89$

- Familial cases vs. controls
  - $1.18 \times 10^{-6}; \text{OR}=3.39, 95\%\text{CI}=2.13-5.83$

PLD3 gene-based analysis

Resequeencing 2363 cases and 2024 controls (European)
- Exome-sequencing individual samples
  - $1.44 \times 10^{-11}; \text{OR}=2.75, 95\%\text{CI}=2.05-3.68$

- Removing V232M
  - $1.58 \times 10^{-8}; \text{OR}=2.48, 95\%\text{CI}=1.79-3.42$

Resequeencing 130 cases and 172 controls (African-American)
- Exome-sequencing individual samples
  - $1.40 \times 10^{-3}; \text{OR}=5.48, 95\%\text{CI}=1.77-16.92$

Cruchaga et al., under review
Genetic Architecture of AD in 2014

**Causes Alzheimer’s**

- PSEN1
- PSEN2
- APP

**High risk**

- TREM2

**Med risk**

- PLD3

**Low risk**

- APOE4

**Risk of Alzheimer’s**

- **Very rare**
  - 0.4% of Northern European ancestry have risk variant in TREM2 which makes them 3 times more likely to develop AD.

- **Very rare**
  - 0.5% of European have V232M risk variant in PLD3 doubling risk for AD

- **V Common**

  - 2% of the population have two copies of APOE4, making them 8 times more likely to develop AD.
  - Over half the population may carry these variants, but each one only has a small effect on risk
  - 0.5% of European have V232M risk variant in PLD3 doubling risk for AD

**Frequency in the population**

- Very rare
- V Common

**Genes and Variants**

- MS4A
- CR1
- B1N
- CLU
- CD2AP
- CD33
- ABCA7
- EPHA1
- PICALM
- BIN1
- PLD3
- TREM2
- APOE4
- APP
- PSEN1
- PSEN2