Biomarkers of Alzheimer’s disease

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Taichung Veterans General Hospital
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Taiwan Life Expectancy at Birth

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>1960</td>
<td>62.3</td>
<td>66.4</td>
</tr>
<tr>
<td>1990</td>
<td>71.3</td>
<td>76.8</td>
</tr>
<tr>
<td>2010</td>
<td>76.1</td>
<td>82.6</td>
</tr>
</tbody>
</table>
Population Growth of Older Taiwanese (65 Years and Older)

YEAR

Number (thousands)

Percent of Population
Alzheimer’s disease (AD) is prevalent

- Alzheimer’s disease accounts for 60 to 80% of dementia
- An estimated 100,000 Taiwanese of all ages had Alzheimer’s disease in 2005
Mild cognitive impairment (MCI)

- 10 to 20% of people aged 65 and older have MCI.
  - An estimated 240,000 Taiwanese had MCI
- Impairment of memory or other essential cognitive ability
  - Severe enough to be noticeable to others
  - Demonstrated impairment on cognitive tests
  - Not severe enough to interfere with daily life.
- 15% of MCI progress to dementia each year.
• 10,432 people aged 65 years or older
  – Mean age 76.2±6.7
  – 52.3% women
• The age-adjusted prevalence of all-cause dementia was **8.04%** (95% CI 7.47–8.61)
  – Very mild dementia: **3.25%** (95% CI 2.89–3.61)
  – MCI: **18.76%** (95% CI 17.91–19.61)
• Women had a higher prevalence than men
  – All-cause dementia (9.71% vs. 6.36%)
  – MCI (21.63% vs. 15.57%)
Pathophysiology of AD
Amyloid pathology

Normal cleavage of amyloid precursor protein

- α-secretase
- APP

Abnormal cleavage of amyloid precursor protein leading to excess amyloid accumulation

- APP mutations increase
- β-secretase cleavage

- β-secretase
- γ-secretase
- PSEN1/PSEN2 mutations increase γ-secretase activity

- Aβ peptide
- Oligomer aggregate
- Extracellular space
- Cytoplasm
- Cell membrane
Tau pathology
Other mechanisms

• Synaptic dysfunction
• Mitochondrial dysfunction
• Cholinergic insufficiency
• Oxidative stress
• Insulin-signaling pathway
• Vascular effect
• Inflammation
• Calcium regulation
• Cholesterol metabolism
• Hormonal imbalance
Hypothetical model of AD pathophysiological cascade

- Age Genetics
- Cerebrovascular risk factors
  - Other age-related brain diseases

- Amyloid-β Accumulation

- Synaptic Dysfunction
  - Glial Activation
  - Tangle Formation
  - Neuronal Death

- Cognitive Decline

- Brain and cognitive reserve
  - ? Environmental factors
The current lifetime risk of AD dementia for a 65-year-old is estimated to be at **10.5%**.

A treatment slows down progression by 50% → reduce risk to **5.7%**.

http://www.alz.org/alzheimers_disease_trajectory.asp
Growth Forecasts

Americans age 65 and older expected to have Alzheimer’s disease, 2010-50

- Current estimate
- Estimate if onset delayed by five years

Source: Alzheimer’s Association
The Wall Street Journal
Medicare Costs, 5-Year Delayed Onset

<table>
<thead>
<tr>
<th>Year</th>
<th>Current Trajectory</th>
<th>Delayed Onset</th>
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<tr>
<td>2010</td>
<td>$88</td>
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<tr>
<td>2020</td>
<td>$128</td>
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<td>2030</td>
<td>$225</td>
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<td>2040</td>
<td>$406</td>
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<td>2050</td>
<td>$627</td>
<td>$344</td>
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http://www.alz.org/alzheimers_disease_trajectory.asp
Treat AD too late?

Early or pre-clinical diagnosis?

Subject selection and outcome measures?
The diagnosis criteria of AD

• National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) criteria in 1984
  – the clinical diagnosis of AD could only be designated as “probable”
  – the clinical diagnosis of AD could be assigned only when the disease had advanced to the point of causing significant functional disability
Why we need AD biomarkers
ADNI has been launched since 2003

• The participants were recruited from over 50 sites across the U.S. and Canada
Goals of ADNI

• 2004~2009: ADNI-1
  – Develop CSF/blood and imaging biomarkers as outcome measures

• 2009~2011: ADNI-GO
  – Act as bridging grant between ADNI-1 and ADNI-2, examine biomarkers in earlier stage of disease progression

• 2011~2016: ADNI-2
  – Develop CSF/blood and imaging biomarkers as predictors of cognitive decline, and as outcome measures

• “Add-on” studies
  – Genetic or proteomic analysis
<table>
<thead>
<tr>
<th></th>
<th>Screening</th>
<th>Baseline</th>
<th>Month 6</th>
<th>Month 12</th>
<th>Month 18</th>
<th>Month 24</th>
<th>Month 36</th>
<th>Month 48</th>
<th>Ongoing 6-Month Interim (Phone)</th>
<th>Ongoing Annual Follow-up</th>
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<tr>
<td>Demographics</td>
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<td>Neurological Exam</td>
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<td>Vital Signs</td>
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<tr>
<td>Cognitive Assessments</td>
<td><img src="image" alt="Diagram" /></td>
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<td>Biospecimen Collection</td>
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<tr>
<td>Diagnostic Summary</td>
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<td>Lumbar Puncture</td>
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</table>

**Legend**

- **ADNI I**
  - CN
  - MCI
  - EMCI
  - AD

- **ADNI GO**
  - CN, EMCI

- **ADNI 2**
  - CN, EMCI, LMCI
  - AD
<table>
<thead>
<tr>
<th>Study characteristics</th>
<th>ADNI-1</th>
<th>ADNI-GO</th>
<th>ADNI-2</th>
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<tr>
<td><strong>Primary goal</strong></td>
<td>Develop CSF/blood and imaging biomarkers as outcome measures</td>
<td>Act as bridging grant between ADNI-1 and ADNI-2, examine biomarkers in earlier stage of disease progression</td>
<td>Develop CSF/blood and imaging biomarkers as predictors of cognitive decline, and as outcome measures</td>
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<tr>
<td><strong>Funding</strong></td>
<td>$40 million federal (NIA), $20 million industry and foundation, $7 million industry for supplemental studies</td>
<td>$24 million American Recovery Act funds (stimulus finds)</td>
<td>$40 million federal (NIA), $27 million expected industry and foundation</td>
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<td><strong>Duration/start date</strong></td>
<td>5 years/October 2004</td>
<td>2 years/September 2009</td>
<td>5 years/September 2011</td>
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<tr>
<td><strong>Cohort</strong></td>
<td>200 elderly control subjects 200 MCI 400 AD</td>
<td>Existing ADNI-1 cohort plus: 200 EMCI</td>
<td>Existing ADNI-1 and ADNI-GO cohort plus: 150 elderly control subjects 100 EMCI 150 MCI 150 AD</td>
</tr>
<tr>
<td><strong>Study techniques</strong></td>
<td></td>
<td></td>
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<tr>
<td>MRI</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>fMRI</td>
<td></td>
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<tr>
<td>FLAIR (microhemorrhage detection)</td>
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<td>X</td>
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<td>T2* GRE (microhemorrhage detection)</td>
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<td>X</td>
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<tr>
<td>Vendor-specific protocols (1) resting state fMRI to Phillips systems, (2) perfusion imaging (ASL) to Siemens, and (3) DTI to General Electric</td>
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<td>X</td>
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<td>FDG-PET</td>
<td>X</td>
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<td>AV45</td>
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<td>X</td>
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<tr>
<td>Biosamples</td>
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<tr>
<td>“Add-on” studies</td>
<td>GWAS, PiB-PET, lumbar puncture</td>
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</table>
Original Dynamic biomarkers of the AD pathological cascade model – 2010

Redefining the earliest stages of AD

- Underlying brain disease process
  - AD-pathophysiological process (AD-P)
- Clinical phases of the illness
  - AD-clinical (AD-C)
- AD begins with a long asymptomatic period during which the pathophysiological process is progressing
- Individuals with biomarker evidence of early AD-P
  - Increased risk for developing cognitive and behavioral impairment and progression to AD dementia
Current hypothesis

- Earliest detectable pathological change will be in the form of Aβ accumulation
  - Aβ accumulation is necessary but not sufficient to produce the clinical manifestations of AD
  - Some individuals with all diagnostic pathological features of AD at autopsy but never express dementia during their life

\[
\text{Aβ accumulation} + \text{synaptic dysfunction and/or neurodegeneration}
\]

- Brain reverse
- Protective genetic factors
- Environmental influences
- Cognitive decline
Biomarker model of the preclinical stage of AD
Amyloid accumulation

Pittsburgh compound B (PiB) amyloid PET

Decreased CSF β-amyloid(1-42) level
Synapse dysfunction

FDG PET

Functional MRI
Tau-mediated neuronal damage

Increased CSF Tau or p-Tau level
Brain structure atrophy

Structural brain MRI
### Staging categories for preclinical AD research in 2011

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Aβ (PET or CSF)</th>
<th>Markers of neuronal injury (tau, FDG, sMRI)</th>
<th>Evidence of subtle cognitive change</th>
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</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Asymptomatic cerebral amyloidosis</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Asymptomatic amyloidosis + “downstream” neurodegeneration</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Stage 3</td>
<td><strong>Stage 1</strong>&lt;br&gt;Asymptomatic amyloidosis&lt;br&gt;-High PET amyloid tracer retention&lt;br&gt;-Low CSF Aβ₁₋₄₂</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td><strong>Stage 2</strong>&lt;br&gt;Amyloidosis + Neurodegeneration&lt;br&gt;-Neuronal dysfunction on FDG-PET/fMRI&lt;br&gt;-High CSF tau/p-tau&lt;br&gt;-Cortical thinning/Hippocampal atrophy on sMRI</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><strong>Stage 3</strong>&lt;br&gt;Amyloidosis + Neurodegeneration + Subtle Cognitive Decline&lt;br&gt;-Evidence of subtle change from baseline level of cognition&lt;br&gt;-Poor performance on more challenging cognitive tests&lt;br&gt;-Does not yet meet criteria for MCI</td>
<td></td>
<td></td>
<td>MCI ➔ AD dementia</td>
</tr>
</tbody>
</table>
Stage 1: Aβ Plaque Pathology

Biomarkers (one or both)
Positive Amyloid PET scan
Low CSF Aβ42

Stage 2: Neurodegeneration
NFT Pathology

Stage 1 Biomarker plus one of the following:
- CSF tau or p-tau
- sMRI brain atrophy
- FDG-PET
- fMRI

Stage 3: Biomarkers + Subtle Cognitive Decline

→ MCI → AD
Clinical and biomarker changes in dominantly inherited AD

![Graph showing changes in standardized difference over estimated years from expected symptom onset.](N Engl J Med. 2012 Aug 30;367(9):795-804)
Aβ deposition in autosomal dominant AD years before expected clinical symptoms
an updated hypothetical model of dynamic biomarkers in 2013
an updated hypothetical model of dynamic biomarkers in 2013

Associations between these biomarkers
Cognitive decline and brain atrophy

• Cortical atrophy of the specific regions and ventricular enlargement have been correlated with measures of clinical severity
  – Early stage: medial temporal lobe, particularly the hippocampus
  – Later stages: parietal, occipital, and frontal lobes
  – Rates of atrophy are initially fastest in the temporal lobe, but accelerate in other regions as the disease progresses
Cognitive decline and glucose hypometabolism

- Patterns of glucose hypometabolism associated with AD on FDG-PET
  - most reduced cerebral metabolic rate for glucose on precuneus and posterior cingulate regions
  - reduced metabolism in these key areas were associated with lower scores on cognitive tests
CSF biomarker and amyloid PET

• Levels of CSF biomarkers, particularly Aβ and tau, have been associated with earlier stages of brain atrophy

• Decreased levels of CSF Aβ and increasing ¹¹C-PiB PET represent an early event in disease progression

• No association between amyloid imaging/CSF biomarkers and cognitive decline
Improved the accuracy of diagnostic classification using biomarkers?
• Single features are not as accurate as multiple features

• The best classifiers combine optimum features from different modalities
  – CSF biomarkers
  – MRI
  – FDG-PET
  – Cognitive measures,
  – Others: age and APOE 4 allele status
• The most discriminative measures
  – hippocampal volume
  – entorhinal cortical thickness
  – entorhinal metabolism
  – CSF t-tau/Ab-42 ratio
  – ADAS-cog scores
• Currently, the best classifiers are able to discriminate between control and AD subjects with accuracies in the mid-90% range
• Lower accuracies when discriminating between control and MCI subjects or between MCI-non-converter and MCI-converter subjects
an updated hypothetical model of dynamic biomarkers in 2013
Other possible biomarkers to predict the risk?
Heritability of LOAD

• Studies of twins have demonstrated a significant role of genetics in LOAD, the heritability of LOAD was estimated to be 58% ~ 79%
• The ε4 allele of the apolipoprotein E (APOE) only accounts for 20 to 30% of LOAD risks
• A large part of its genetic contribution remained unclear
## Top 10 AlzGene

![ALZFORUM](https://www.alzforum.org)

Status: Updated 18 April 2011

<table>
<thead>
<tr>
<th>#</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>Ethnicity</th>
<th>OR (95% CI)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>1</td>
<td>APOE_e2/3/4</td>
<td>APOE_e2/3/4</td>
<td>All</td>
<td>3.685 (3.30-4.12)</td>
<td>&lt;1E-50</td>
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<tr>
<td>2</td>
<td>BIN1</td>
<td>rs744373</td>
<td>All</td>
<td>1.166 (1.13-1.20)</td>
<td>1.59E-26</td>
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<tr>
<td>3</td>
<td>CLU</td>
<td>rs11136000</td>
<td>Caucasian</td>
<td>0.879 (0.86-0.90)</td>
<td>3.37E-23</td>
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<tr>
<td>4</td>
<td>ABCA7</td>
<td>rs3764650</td>
<td>All</td>
<td>1.229 (1.18-1.28)</td>
<td>8.17E-22</td>
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<td>5</td>
<td>CR1</td>
<td>rs3818361</td>
<td>Caucasian</td>
<td>1.174 (1.14-1.21)</td>
<td>4.72E-21</td>
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<td>6</td>
<td>PICALM</td>
<td>rs3851179</td>
<td>Caucasian</td>
<td>0.879 (0.86-0.9)</td>
<td>2.85E-20</td>
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<td>7</td>
<td>MS4A6A</td>
<td>rs610932</td>
<td>All</td>
<td>0.904 (0.88-0.93)</td>
<td>1.81E-11</td>
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<tr>
<td>8</td>
<td>CD33</td>
<td>rs3865444</td>
<td>All</td>
<td>0.893 (0.86-0.93)</td>
<td>2.04E-10</td>
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<td>9</td>
<td>MS4A4E</td>
<td>rs670139</td>
<td>All</td>
<td>1.079 (1.05-1.11)</td>
<td>9.51E-10</td>
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<td>10</td>
<td>CD2AP</td>
<td>rs9349407</td>
<td>All</td>
<td>1.117 (1.08-1.16)</td>
<td>2.75E-09</td>
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</table>

[http://www.alzgene.org/TopResults.asp](http://www.alzgene.org/TopResults.asp)
Meta-analysis of GWAS studies of 74,046 individuals of AD
Based on pathophysiology

• Inflammation cytokine markers
  – Interleukin family, MCP-1, TNF-alpha...

• Circulating amyloid related protein
  – Amyloid Aβ
  – Aβ-degrading Enzymes

• Neuroprotection ?
  – Vitamin D

Brain Pathol. 2008 Apr;18(2):240-52
Plasma $\text{A}\beta$-40 & $\text{A}\beta$-42

• Variable results from previous studies
  – Meta-analysis in 2012
    • Lower $\text{A}\beta$(42):$\text{A}\beta$(40) ratios were significantly associated with development of AD (summary risk ratio, 1.60; 95% CI, 1.04-2.46; $P=0.03$)
  – A longitudinal study in 2014
    • A decrease in $\text{A}\beta$(42)/$\text{A}\beta$(40)
      – in patients with AD ($p=0.04$)
      – inversely correlated with neocortical amyloid burden ($P=0.002$ in base-line and $P=0.018$ in 18 months follow-up)
    • Controls transitioning to the MCI group showed a decrease in $\text{A}\beta$(42) levels ($P=0.003$)
  – $\text{A}\beta$(42):$\text{A}\beta$(40) ratios may be the surrogate of amyloid burden and amyloid clearance

Arch Neurol. 2012 Jul;69(7):824-31
25-hydroxy vitamin D (25-OH-D)

- The prevalence of vitamin D deficiency in AD patients was 70-90%
- Meta-analysis in 2012
  - Lower vitamin D concentrations are associated with poorer cognitive function and a higher risk of AD

[Graphs showing data on AD risk and cognition]
Angiotensin-converting enzyme (ACE)

• One of the Aβ-degrading enzymes?
• Patients diagnosed with AD
  – Higher ACE activity in the hippocampal, para-hippocampal and temporal cortex
  – Decreased serum ACE protein level
  – ACE gene polymorphism is associated with AD risk, serum ACE protein level, and CSF ACE protein level
Taiwan Biobank

Recruiting/Following Participants
Collecting Information/Specimen

Banking Information/Specimen

Profiling Genomic and Epigenomic Features

Making All Information/Specimen Publicly Available

General Population
200,000
(aged 30-70 yrs)

Patients, 100,000
Breast CA, Lung CA
Colon CA, Liver CA
Gastric CA, Head/Neck CA
CVD, Stroke,
DM, Alzheimer's disease,
Chronic Kidney Dis, Asthma, Endometriosis
Biosignature study of AD

Clinical diagnosis (aMCI or AD)
Cognitive test scores
Genotyping of APOE and selected SNPs
Circulating biomarkers
(plasma Aβ, serum ACE, Vitamin D)
Base-line brain MRI

T0
1st visit (2012.Aug)

T1\textsubscript{year} 2nd visit

T2\textsubscript{year} 3rd visit

Clinical diagnosis (sMCI, MCI conversion AD, AD)
Cognitive test scores
Circulating biomarkers
Follow-up brain MRI (selected cases)

Hospital controls

Clinical diagnosis (aMCI=amnestic MCI
sMCI=stable MCI

Taiwan Biobank
642,832台灣漢人基因體變異位置
進行1802位民眾基因定型

DATABASE INFORMATION
Subject: 1802
Source: healthy controls
Update: 2012/12/17
Patients

• From VGHTPE and VGHTC
• The patients who are diagnosed as
  – Probable AD according to NIA and Alzheimer Association criteria in 2011
  – Amnestic MCI according to revised consensus criteria in 2004
    • Wechsler Memory Scale-3rd ed. (WMS-III) Logical Memory (LM) subtest
Methods

• Life style questionnaires

• Cognitive tests
  – Clinical dementia rating (CDR)
  – MMSE, 12-item memory test, WMS-III logical memory test, digit span, category fluency, trail making A, and modified Boston naming
Candidate circulating biomarkers

- Doublet test, CV less than 20%
- ELISA  
  - Serum ACE protein level
- Radioimmunoassay  
  - 25-OH Vitamin D
- xMAP® assay  
  - Plasma Aβ(42), Aβ(40)
Genotyping

• Genotyping of APOE and selected GWAS-identified SNPs
  • The ε2, ε3, and ε4 alleles of APOE were determined by two SNPs (rs429358 and rs7412)
  • Top 10 AlzGene SNPs
Follow-up progress

T0
1st visit (n=653)

2012: 260
2013: 296
2014: 97

T1year
2nd visit (n=220)

T2year
3rd visit
# Demographic data (2014/5)

<table>
<thead>
<tr>
<th></th>
<th>AD (N=525)</th>
<th>MCI (n=128)</th>
<th>Controls (n=160)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at study (y)</strong></td>
<td>79.6 ± 7.5*</td>
<td>74.8 ± 8.8</td>
<td>74.6 ± 8.1</td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>52.2 %</td>
<td>53.1 %</td>
<td>54.4 %</td>
</tr>
<tr>
<td><strong>Education (y)</strong></td>
<td>9.8 ± 4.7**</td>
<td>10.5 ± 4.7</td>
<td>11.0 ± 5.0</td>
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<tr>
<td><strong>MMSE</strong></td>
<td>18 ± 6.0*</td>
<td>25.4 ± 3.2*</td>
<td>27.6 ± 2.4</td>
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<tr>
<td><strong>CDR</strong></td>
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<tr>
<td>CDR=0.5</td>
<td>56 (10.7%)</td>
<td>128 (100%)</td>
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<tr>
<td>CDR=1.0</td>
<td>315 (60%)</td>
<td></td>
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<tr>
<td>CDR=2.0</td>
<td>122 (23.3%)</td>
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<tr>
<td>CDR=3.0</td>
<td>23 (4.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE ε4 carrier (%)</td>
<td>40.8%*</td>
<td>23.4%*</td>
<td>18.8%</td>
</tr>
</tbody>
</table>

CDR missing in 9 AD patients; *p<0.01 compared with controls; **p=0.03 compared with controls;
## Genetic analysis of SNPs between AD patients and VGH controls

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Model</th>
<th>Allele</th>
<th>VGH controls</th>
<th>AD cases</th>
<th>HWE</th>
<th>OR, p value</th>
<th>OR, Adj p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA7</td>
<td>rs3764650</td>
<td>Rec</td>
<td>T/G</td>
<td>139/120/48 (45.3%/39.1%/15.6%)</td>
<td>242/247/47 (45.1%/46.1%/8.8%)</td>
<td>0.64*</td>
<td>0.52, p = 0.0027</td>
<td>0.46, p = 0.0013</td>
</tr>
<tr>
<td>CD33</td>
<td>rs3865444</td>
<td>Rec</td>
<td>G/T</td>
<td>196/95/15 (64.1%/31.0%/4.9%)</td>
<td>336/182/18 (62.7%/34.0%/3.4%)</td>
<td>0.88</td>
<td>0.67, p = 0.27</td>
<td>0.87, p = 0.71</td>
</tr>
<tr>
<td>CR1</td>
<td>rs3818361</td>
<td>Rec</td>
<td>G/A</td>
<td>128/141/36 (42.0%/46.2%/11.8%)</td>
<td>216/241/78 (40.4%/45.0%/14.6%)</td>
<td>0.63</td>
<td>1.28, p = 0.26</td>
<td>1.28, p = 0.30</td>
</tr>
<tr>
<td>CLU</td>
<td>rs11136000</td>
<td>Dom</td>
<td>C/T</td>
<td>188/103/16 (61.2%/33.6%/5.2%)</td>
<td>349/164/23 (65.1%/30.6%/4.3%)</td>
<td>0.43</td>
<td>0.85, p = 0.26</td>
<td>0.83, p = 0.24</td>
</tr>
<tr>
<td>PICALM</td>
<td>rs3851179</td>
<td>Rec</td>
<td>C/T</td>
<td>117/141/47 (38.4%/46.2%/15.4%)</td>
<td>214/250/69 (40.2%/46.9%/12.9%)</td>
<td>0.98</td>
<td>0.82, p = 0.32</td>
<td>0.65, p = 0.06</td>
</tr>
<tr>
<td>BIN1</td>
<td>rs744373</td>
<td>Rec</td>
<td>A/G</td>
<td>116/144/44 (38.2%/47.4%/14.5%)</td>
<td>202/244/89 (37.8%/45.6%/16.6%)</td>
<td>0.42</td>
<td>1.18, p = 0.41</td>
<td>1.13, p = 0.57</td>
</tr>
<tr>
<td>MS4A6A</td>
<td>rs610932</td>
<td>Rec</td>
<td>G/T</td>
<td>127/147/32 (41.5%/48.0%/10.5%)</td>
<td>221/247/66 (41.4%/46.3%/12.4%)</td>
<td>0.40</td>
<td>1.21, p = 0.41</td>
<td>1.15, p = 0.57</td>
</tr>
<tr>
<td>SORL1</td>
<td>rs1784933</td>
<td>Add</td>
<td>A/G</td>
<td>130/137/39 (42.5%/44.8%/12.7%)</td>
<td>266/222/48 (49.6%/41.4%/9.0%)</td>
<td>0.67</td>
<td>0.78, p = 0.0216</td>
<td>0.72, p = 0.0049</td>
</tr>
<tr>
<td>SORL1</td>
<td>rs3737529</td>
<td>Add</td>
<td>C/T</td>
<td>179/111/17 (58.3%/36.2%/5.5%)</td>
<td>335/176/25 (62.5%/32.8%/4.7%)</td>
<td>0.81</td>
<td>0.87, p = 0.23</td>
<td>0.78, p = 0.06</td>
</tr>
<tr>
<td>GAB2</td>
<td>rs2373115</td>
<td>Dom</td>
<td>C/A</td>
<td>118/140/46 (38.8%/46.1%/15.1%)</td>
<td>220/236/79 (41.1%/44.1%/14.8%)</td>
<td>0.22</td>
<td>0.91, p = 0.51</td>
<td>0.95, p = 0.74</td>
</tr>
<tr>
<td>EPHA1</td>
<td>rs11767557</td>
<td>Rec</td>
<td>T/C</td>
<td>232/70/4 (75.8%/22.9%/1.3%)</td>
<td>396/127/13 (73.9%/23.7%/2.4%)</td>
<td>0.74</td>
<td>1.88, p = 0.28</td>
<td>1.35, p = 0.62</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; AD, Alzheimer’s dementia; VGH, Veteran General Hospital; M, major allele; m, minor allele

Three models of inheritance: Dom (dominant); Rec (recessive); Add (additive)

Adj p, adjustment of age, sex and ApoE4 allele

* Controls failed in HWE (control, HWE p = 0.012)
Genetic analysis of SNPs between AD patients and biobank controls

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Model</th>
<th>Allele</th>
<th>Biobank controls (M/m)</th>
<th>AD cases (M/M/Mm/mm)</th>
<th>OR, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA7</td>
<td>rs3764650</td>
<td>Rec</td>
<td>T/G</td>
<td>740/841/219 (41.1%/46.7%/12.2%)</td>
<td>242/247/47 (45.1%/46.1%/8.8%)</td>
<td>0.69, p = 0.0297</td>
</tr>
<tr>
<td>CD33</td>
<td>rs3865444</td>
<td>Dom</td>
<td>G/T</td>
<td>1237/515/50 (68.7%/28.6%/2.8%)</td>
<td>336/182/18 (62.7%/34.0%/3.4%)</td>
<td>1.30, p = 0.0098</td>
</tr>
<tr>
<td>CR1</td>
<td>rs3818361</td>
<td>Rec</td>
<td>G/A</td>
<td>744/847/210 (41.3%/47.0%/11.7%)</td>
<td>216/241/78 (40.4%/45.0%/14.6%)</td>
<td>1.29, p = 0.07</td>
</tr>
<tr>
<td>CLU</td>
<td>rs11136000</td>
<td>Dom</td>
<td>C/T</td>
<td>1156/577/69 (64.2%/32.0%/3.8%)</td>
<td>349/164/23 (65.1%/30.6%/4.3%)</td>
<td>0.96, p = 0.68</td>
</tr>
<tr>
<td>PICALM</td>
<td>rs3851179</td>
<td>Rec</td>
<td>C/T</td>
<td>673/856/273 (37.3%/47.5%/15.1%)</td>
<td>214/250/69 (40.2%/46.9%/12.9%)</td>
<td>0.89, p = 0.24</td>
</tr>
<tr>
<td>BIN1</td>
<td>rs744373</td>
<td>Rec</td>
<td>A/G</td>
<td>724/833/244 (40.2%/46.3%/13.5%)</td>
<td>202/244/89 (37.8%/45.6%/16.6%)</td>
<td>1.27, p = 0.07</td>
</tr>
<tr>
<td>MS4A6A</td>
<td>rs610932</td>
<td>Dom</td>
<td>G/T</td>
<td>681/879/220 (38.3%/49.4%/12.4%)</td>
<td>221/247/66 (41.4%/46.3%/12.4%)</td>
<td>0.88, p = 0.19</td>
</tr>
<tr>
<td>SORL1</td>
<td>rs1784933</td>
<td>Add</td>
<td>A/G</td>
<td>884/767/150 (49.1%/42.6%/8.3%)</td>
<td>266/222/48 (49.6%/41.4%/9.0%)</td>
<td>1.00, p = 0.98</td>
</tr>
<tr>
<td>SORL1</td>
<td>rs3737529</td>
<td>Add</td>
<td>C/T</td>
<td>1132/607/63 (62.8%/33.7%/3.5%)</td>
<td>335/176/25 (62.5%/32.8%/4.7%)</td>
<td>1.05, p = 0.60</td>
</tr>
<tr>
<td>GAB2</td>
<td>rs2373115</td>
<td>Dom</td>
<td>C/A</td>
<td>681/881/240 (37.8%/48.9%/13.3%)</td>
<td>220/236/79 (41.1%/44.1%/14.8%)</td>
<td>0.87, p = 0.16</td>
</tr>
<tr>
<td>EPHA1</td>
<td>rs11767557</td>
<td>Rec</td>
<td>T/C</td>
<td>1340/430/32 (74.4%/23.9%/1.8%)</td>
<td>396/127/13 (73.9%/23.7%/2.4%)</td>
<td>1.36, p = 0.34</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; AD, Alzheimer’s dementia; M, major allele; m, minor allele
Three models of inheritance: Dom (dominant); Rec (recessive); Add (additive)
ABCA7 gene and the risk of Alzheimer’s disease in Han Chinese in Taiwan

Yi-Chu Liao a,b, Wei-Ju Lee b,c,d, Jeng-Ping Hwang b,e, Yen-Feng Wang a,b,c,f, Chia-Fen Tsai b,e,g, Pei-Ning Wang a,b,f, Shuu-Jiun Wang b,a,f,g,*,1, Jong-Ling Fuh a,b,f,*,1
Plasma Aβ
AD vs. MCI vs. Controls

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>MCI</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42 pg/ml</td>
<td>35.3</td>
<td>32.7</td>
<td>33.4</td>
</tr>
<tr>
<td>Aβ40 pg/ml</td>
<td>158.4</td>
<td>156.8</td>
<td>145.1</td>
</tr>
<tr>
<td>AB42/AB40</td>
<td>0.23</td>
<td>0.21</td>
<td>0.24</td>
</tr>
</tbody>
</table>

P = 0.02
Subgroup analysis of AD

Mild AD: CDR=0.5 and 1
Moderate: CDR=2
Severe: CDR=3

Aβ42/Aβ40

Mild AD: 0.22
Moderate AD: 0.22
Severe AD: 0.27

P<0.01
P=0.01
Vitamin D and ACE
AD vs. MCI vs. Controls

**25OH-D**
- **AD**: 20.5 ng/ml
- **MCI**: 21.2 ng/ml
- **Control**: 23.3 ng/ml

**ACE**
- **AD**: 142.6 ng/ml
- **MCI**: 144.7 ng/ml
- **Control**: 161.7 ng/ml

- P<0.01
- P<0.01
- P=0.03
Subgroup analysis of AD

No statistical difference

25OH-D

Mild AD: 20.7
Moderate AD: 20.3
Severe AD: 20.7

ACE

Mild AD: 143.2
Moderate AD: 144.8
Severe AD: 128.4
Summary of AD biosignature study

• Plasma Aβ40
  – Significant difference between AD and controls
  – Significant difference between different severities of AD
  – **Markers of amyloid clearance**
    • Many covariates
    • Renal function, platelets, total protein, and glucose level

• ACE and vitamin D
  – Significant difference between AD/MCI and controls
  – No difference between different severities of AD
  – **May have some influence on early AD and MCI**
Take home messages

- Alzheimer disease is a multifactorial disorder
- Preclinical AD might be diagnosed by biomarkers
Fight against Alzheimer disease!!