AddNeuroMed Update
WWADNI : July 2011

Andy Simmons
for the AddNeuroMed Group

European Federation of Pharmaceutical Industries and Associations, Pharmidex Pharmaceutical Services, Capsant Neurotechnologies LTD, Università degli Studi di Perugia, Aristotle University of Thessaloniki, Roskilde University, AstraZeneca, Kungl Tekniska Högskolan, Karolinska Institutet, King's College, London, Centre Hospitalier Universitaire de Toulouse, GlaxoSmithKline Research & Development Ltd, Proteome Sciences PLC, University College London, University of Southampton, Hunter Fleming Limited, BioWisdom, Cerebricon Ltd.

Institute of Psychiatry and South London and Maudsley Neuroimaging
Overview

- Study design and recruitment
- Imaging update
- Blood plasma proteomics update
- Combining imaging and omics
AddNeuroMed Study

- Six European sites
- 385 subjects with MRI (of total > 700 subjects)
  » 133 AD, 134 MCI, 118 Controls
- All subjects
  » Clinical / cognitive assessments
  » Blood / plasma / RNA
  » 1.5 T structural MRI
- Imaging time points
  » Baseline, 3 months, 1 year, 2 year, 3 year
Imaging-omics-clinical database

385 AddNeuroMed
- 0, 3, 12m

821 ADNI
- 0, 6, 12, 18, 24, 36m

200 London cohort
- 0, 12, 24, 36m

130 Memory clinic
- 0m

2000 Young controls
Multivariate Analysis

- Orthogonal partial least squares (OPLS)
- Regional cortical thickness measures
- Regional MRI volumes
- Total of 75 MRI measures
Multivariate Analysis

- Orthogonal partial least squares (OPLS)
- Regional cortical thickness measures
- Regional MRI volumes
- Total of 75 MRI measures
Visual Assessment Scales and Multivariate Analysis

Visual assessment of hippocampus proper, dentate gyrus, subiculum, parahippocampal gyrus, entorhinal cortex and surrounding CSF spaces such as temporal horns and choroid fissure.

MTA 0-4 Increasing atrophy

Westman et al
PlosOne 2011

Institute of Psychiatry and South London and Maudsley Neuroimaging
Multivariate Analysis Comparison - ADNI and AddNeuroMed

Table 2
Sensitivity/specificity and likelihood ratio for the different cohort models

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AddNeuroMed (cross-validation)</td>
<td>79.0</td>
<td>90.0</td>
</tr>
<tr>
<td>ADNI (cross-validation)</td>
<td>86.9</td>
<td>86.7</td>
</tr>
<tr>
<td>Combined (cross-validation)</td>
<td>83.4</td>
<td>87.8</td>
</tr>
</tbody>
</table>

Table 3
MCI predictions subjects characteristics

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>AD-like</th>
<th>CTL-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>AddNeuroMed MCI converters</td>
<td>22</td>
<td>14 (64%)</td>
<td>8 (36%)</td>
</tr>
<tr>
<td>ADNI MCI converters</td>
<td>62</td>
<td>46 (74%)</td>
<td>16 (26%)</td>
</tr>
</tbody>
</table>
MCI Conversion & Hippocampal Shape

Costafreda et al, Neuroimage 2011
Cortical Thickness and Neuropsych

Paajanen et al, submitted

CTI v MCI cortical thickness differences

Correlation of word list recall with cortical thickness in CTL+MCI group
AddNeuroMed Proteomics Studies

- **Approach I**  "diagnostics discovery"
  - Case - control study
  - 2DGE & LC-MS/MS; immuno-validation

- **Approach II**  "severity markers discovery"
  - Imaging correlation study
  - Gel and MS based methods; immuno-validation

- **Approach III**  "progression markers discovery"
  - Longitudinal study; serial time points
  - Proteomics and genomics
Approach I: Diagnostics Discovery

- Exploratory multivariate analyses and class prediction
  - Parametric statistics, test and replication sets
  - Sensitivity 56% ; Specificity 80%
- 15 spots prioritised by FDR and identified by mass spectrometry
  - Fold change 1.5 – 13.8; P<0.04 to <0.0005
  - Two most significant ; CFH and α2M

**Approach 1 - CFH and $\alpha_2$M correlate with MRS Markers of Disease**

Approach 2: Plasma Biomarker Panel Correlation with Imaging Markers

- 2DGE correlation with hippocampal volume
- Multivariate analyses (partial least squares)
- Cross validation analysis of model prediction of ‘large’ / ‘small’ hippocampi
- N~250
  - Mean C3a
  - Mean \( \beta\)-FIB
  - R Sq Linear
  - R
  - P

- PLS model accounts for 30% of atrophy variance
- Protein x gene interaction
Clusterin Association with Severity, Pathology and Progression in AD

Experiment 1: Discovery Phase Proteomics
- 2D-Dig electrophoresis is used to identify plasma proteins associated with hippocampal volume in MO and AD mice and correlated rate of cognitive decline in AD patients.
- Search against databases and plasma proteome using bioinformatics software.

Experiment 2: Validation Phase ELISA
- Plasma concentration of candidate biomarkers is measured by immunocytoassay to test associations with hippocampal volume in AD mice and rate of cognitive decline in AD patients.
- A priori criteria for validation of candidate AD biomarker(s):
  1. Amyloid on MRI
  2. MMSE score at baseline
  3. Accelerated rate of cognitive decline

Experiment 3: Testing Association of candidate biomarker(s) With Brain Amyloid Burden
- In older humans using "CMB PET".
- In APP/PS1 transgenic mice.

Institute of Psychiatry and South London and Maudsley Neuroimaging
Plasma Clusterin is Associated with

- Volume of ERC in AD (N=113, R=-0.31, p=0.001)
- MMSE at Baseline in AD+MCI (N=576, R=-0.22, p<0.001)
- Rapid Clinical Progression ie decline >2 MMSE points per year (N=344, p=0.0007)
- Higher antecedent Clusterin concentration is associated with greater PIB retention in the Entorhinal Cortex
Approach 3: In silico Identification of a Potential Marker for AD

- Sofia™ (BioWisdom), used to generate an Intelligence Network, from public domain sources, for the discovery of AD biomarkers

- The intelligence consisted of assertions describing proteins expressed and upregulated in AD tissue, and proteins involved in AD pathology, for e.g.

  » AD hippocampus has increased Nerve Growth Factor
  » AD is associated with Cerebral Atrophy
Candidate AD Progression Biomarker

- No significant association between baseline measure and MMSE (controlling for age)

- Highly significant correlation between baseline measure and rate of brain atrophy in AD
  
  » Spearman $r = -0.79$, $p=0.001$
AddNeuroMed–ADNI GWAS Imaging

- Data acquisition used the ADNI acquisition protocol on > sixty 1.5 T MR systems
- WBV, ventricular volume, hippocampal volume, entorhinal cortical volume and thickness selected compared to SNP data (1121 subjects)
- 1118 subjects run on Illumina 610 Quadcore array.
- Exclusion of related individuals, individuals with SNP missingness >2%, MAF < 5%, SNP gender different to clinical gender.
- Generalised linear model run in PLINK.

\[ Y = \beta_0 + \beta_1{\text{ADD}} + \beta_2 \text{DS} + \beta_3{\text{ADD}}*{\text{DS}} + \varepsilon \]

Y is the quantitative trait (QT), DS is the disease status, ADD is a term for the additive effects of minor allele dosage on the QT in the model, ADD*DS is a term assessing the interactive effects of diagnosis and the model, \( \beta_1 \ldots \beta_3 \) the regression coefficients of the model terms and \( \varepsilon \), the random error.
AddNeuroMed-ADNI GWAS Imaging

• One SNP with a disease-specific effect associated with entorhinal cortical volume in an intron of the **ZNF292** gene
  • rs1925690; p-value = 2.6 x 10^{-8}; corrected p-value for equivalent number of independent quantitative traits = 7.7 x 10^{-8}

• One intergenic SNP, flanking the **ARPP-21** gene, with an overall effect on entorhinal cortical thickness
  • rs11129640; p-value = 5.6 x 10^{-8}; corrected p-value=1.7 x 10^{-7}

• Gene-wide scoring highlighted **PICALM** as the most significant gene associated with entorhinal cortical thickness
  • p-value = 6.7 x 10^{-6}
Combining Imaging and Omics

- Gene expression and imaging
- Vitamin E forms and imaging
- Proteomics and imaging
- Genetics and imaging

Institute of Psychiatry and South London and Maudsley Neuroimaging
Next Steps

- RNA analysis studies
  - Differential expression analysis of disease status
  - Genetic / network analysis of peripheral blood expression
- Proteomic studies
- Vitamin E forms
- Combined imaging-omics MCI conversion studies
- AddNeuroMed 2
KI
Christian Spenger
Lars-Olof Wahlund
Eric Westman
Johan Bengtsson
Tony Segerdahl

King’s College
Simon Lovestone
Andy Simmons
Catherine Tunnard

University of Kuopio
Hilkka Soininen
Yawu Liu, Teemu Paajanen
Mervi Kononen
Ritva Vanninen

Aristotle University of Thessaloniki
Magda Tsolaki
Eleni Kantoglou
Penelope Mauredaki

University of Perugia
Patrizia Mecocci
Roberto Tarducci
Emanuela Costanzi

McGill University
Louis Collins
Alan Evans
Sebastian Muehlboeck

University of Toulouse
Bruno Vellas
Celine Caillaud
Pierre Payoux

University of Lodz
Iwona Kłoszewska
Tadeusz Biegański
Radoslaw Magierski