Biomarkers for AD: Towards a Systems Understanding of Risk

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Disclosures

• Founder and consultant to MedGenesis Therapeutix
• Advisor to Perthera
• Founder of Socratech
• Inventor on blood based biomarker patent applications
Overarching Thesis

• Neurodegeneration, while anatomically circumscribed, is associated with systemic characteristics

• Preclinical states have systemic features that can be recognized
What is Motivating Change?

- Demographics
- Containing costs
- Changing reimbursement structure
- Shift from symptomatic therapy to targeted prevention
- Gene-environment interactions drive disease
- Need for new drugs that modify natural history
- Next generation healthcare providers must be equipped with newer diagnostics, knowledge, therapeutics
Systems NeuroMedicine

Well

At Risk
• Drugs 2\textdegree prevention
• Other interventions
• Dynamic risk assessment
• Stabilization vs reversal?

Disease
• Pathogenic nodal directed medicine
• Dynamic assessments
• Individualized?
• Multimodal therapy

What is the single greatest risk factor for Alzheimer disease?

Age
We are an aging society

Age 65+ = 40 million

Age 65+ = 87 million
The Silver Tsunami vs The Golden Wave
Longitudinal Study Overview

Initial Cohort
N=525

Baseline

Discovery group
35 MCI/AD
18 Converters
53 Matched Controls

Validation group
11 MCI/AD
10 Converters
20 Matched Controls
Continuum of Alzheimer’s Disease

- Normal Aging
- MCI
- Dementia

Preclinical Biomarkers?

Asymptomatic

Receptive neural substrate?

Symptomatic

Unresponsive neural substrate?
Operationalizing Research Diagnosis

- Conservative approach to identifying subjects for biomarker discovery in order to increase signal/reduce noise
- Developed cohort specific norms for each cognitive measure
- Computed Z-scores for each test for each subject at each visit, then averaged the Z-scores of relevant tests to create a composite score for each cognitive domain
- Z-scores used to define groups for biomarker profiling

<table>
<thead>
<tr>
<th>Attention (Zatt)</th>
<th>Executive (Zexe)</th>
<th>Language (Zlan)</th>
<th>Memory (Zmem)</th>
<th>Visuoperceptual (Zvis)</th>
</tr>
</thead>
</table>
Memory Impairment Defines Groups

- AD - memory Z-score < -1.3 and other cognitive domain Z-score < -1.3
- Amnestic MCI – memory Z-score < -1.3 and normal in all other domains
- Normal – Memory Z-scores -0.7 – 0.7 and all others > -1.3
- Super Normal – memory Z-score > 1.3 and all other domains > -0.7
Normal and Impaired Memory Z-scores

- **Symptomatic**
  - aMCI/AD

- **Asymptomatic**
  - Normal Control

- **Not Classified**

Z-scores:
- 25th%ile
- 50th%ile
- 75th%ile

-1.3 SD
- 0.7 SD
Mean
+ 0.7 SD

10th%ile
25th%ile
50th%ile
25th%ile
Peripheral Blood Biomarkers for AD

Subjects:
- Independent
- Community-dwelling
- Age ≥75 years
- No neurological probs

AGING COHORT
N=525

Defining:
- Cognitively Normal
- MCI
- AD
- Undefined

Processed and stored for:
- Metabolomics
- Proteomics
- Transcriptomics
- Epigenomics
- Genomics
- Exosomics

Longitudinal Study

Phenoconverters (MCI/AD)
N=28

No change
N=119
Metabolomic Biomarkers
Metabolomics Approach

- Untargeted by UPLC-ESI-QTOF-MS
- Data acquired from 50 to 1200 m/z mass range
- Data log transformed and quantile normalized
- Metabolites selected from 4600 features by ROC regularized learning based on least absolute shrinkage and selection operator (LASSO)
- SID MRM MS to quantitate and confirm identity of ten selected metabolites
- Unambiguous classification of aMCI/AD, Con\textsubscript{pre} and NC
- Discovery phase
- Validation phase
Definitions

• Converter pre ($\text{Con}_{\text{pre}}$) - Analyses of blood from cognitively normal subjects who on follow-up are impaired

• Converter post ($\text{Con}_{\text{post}}$) - Analyses of blood from previously cognitively normal subjects now with aMCI or AD

• Normal Controls - Subjects who remained cognitively normal

• aMCI/AD - Subjects who met clinical criteria for amnestic MCI or probable Alzheimer’s disease
Plasma Lipidomics: Discovery (SID MRM MS)

Ten Metabolite Signature in Discovery
ROC Curve Discovery Phase
(SID MRM MS)

AUC = 0.96 (95% CI 0.93 - 0.99)
Plasma Lipidomics: Validation
(SID MRM MS)

Ten Metabolite Signature in Validation
ROC Curve Validation Phase
(SID MRM MS)

AUC = 0.92 (95% CI 0.87 - 0.96)
Metabolomics I: Ten Lipid Panel

- Defines “at risk” Neuropsychologically Normal Individuals that will Phenoconvert to aMCI/AD within 2-3 years
- The discovered and validated classifier model provides up to 90% specificity with a 90% sensitivity in our subjects, and in assessing individual risk
- Plasma lipidomics - offers significant advantages in screening for preclinical AD, compared to neuroimaging or cerebrospinal fluid
  - Familiar procedure, well tolerated by elderly
  - Minimally-invasive
  - Not time-consuming
  - Less expensive than other current methods
- Requires additional corroboration and assessments in larger populations
New Plasma Biomarker Panels

• Expansion of the Plasma metabolite panel
  • 17 plasma metabolites
  • Share only one lipid species from the Ten Lipid panel

• Plasma Exosome Cargo
  • Four analytes tested (so far)
Expansion of the Plasma Metabolite Panel

- Methods as described in Nature Medicine paper
- Lowest number of metabolites that provided highly significant results
- 17 metabolites defined (IDs blinded)
- Assessed in Discovery and Validation Groups
- ROC AUCs are 1.00 and 0.995, respectively
- Plasma Metabolite Risk Index with confidence limits
# Blinded 17 Metabolite Panel

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<thead>
<tr>
<th>Metabolite Name</th>
<th>p value</th>
<th>Discovery Set</th>
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<th></th>
<th>Validation Set</th>
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<td></td>
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<td>log ratio (mean)</td>
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<td>log ratio (median)</td>
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<td>log ratio (median)</td>
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ROC AUC = 1.00  
ROC AUC = 0.995
Blinded 17 Metabolite Panel

**Targeted discovery**

**Targeted validation**

ROC AUC = 1.00

ROC AUC = 0.99
Plasma Metabolite Classifier

ROC Curve

PLASMA 17 METABOLITE INDEX

AUC: 100.0%
New Plasma Biomarker Panels

- Expansion of the Plasma metabolite panel
  - 17 plasma metabolites
  - Increased predictive accuracy for Converter\textsubscript{pre} from NC (sensitivity and specificity \(\sim100\%\)) in Discovery and Validation
  - Combined Discovery and Validation Set ROC AUC = 1.00
- Plasma metabolite Risk Index
- PPV and NPV \(\sim100\\%)
Peripheral Blood Biomarkers for AD

Subjects:
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Baseline

Longitudinal Study

1 2 3 4 5

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Phenoconverters (MCI/AD)
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Exosomes

• Exosomes formed in all viable cells, including neurons and glia
• Formed via complex process involving late endosomes and multivesicular bodies (MVBs)
• Complex sorting of associated membrane proteins
• Enclosed cytoplasmic cargo, including proteins, metabolites, and nucleic acids
• Nucleic acids include mRNA and miRNA species
• Released into ECF when MVBs fuse with plasma membrane
Exosomes
Exosome Methods

METHODS

• Plasma rather than serum
• Isolate Exosome pellet
• Resuspend and immuno-isolate with antibody against a CNS preferred
• Assay with ELISA for CD81 protein marker
• Assay protein analytes associated with AD pathogenesis
Plasma Exosome Cargos

![Box plot graph showing the levels of cargos in different conditions.](image)
Plasma Exosome Cargo ROC

Converter_{pre} versus NC

Cargo 1
Cargo 2
Cargo 3
Cargo 4

ROC AUC = 0.985
ROC AUC = 0.974
ROC AUC = 1.00
ROC AUC = 1.00
Plasma Exosome Cargos Collapsed ROC and Exosome Index

Combined Classifier with 4 Cargo Analytes

Converter\textsubscript{pre} versus NC

Plasma Exosome Index
New Plasma Biomarker Panels

• Plasma Exosome Cargo
  • Four analytes tested (so far)
  • Individual analytes increase predictive accuracy for defining Converter_{pre} from NC (ROC AUC >0.97)
  • Four combined analytes provide ROC AUC of 1.00
  • Define a Plasma Exosome Risk Index
  • Provide PPV and NPV of 100%
Case Control Study: Exosomal Cargo

- Elevated levels of P-tau species and Aβ are known to be present within the brain and CSF during stages of AD progression.
- The presence of P-tau species in association within the NCAM positive exosomes rules out their origin from either NK or muscle* cells.

*Only rare cases of sporadic inclusion body myositis express phosphorylated tau*
Case Control Study: Exosominal Cargo

Peripheral Blood Biomarkers for AD

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Phenoconverters (MCI/AD) N=28

No change N=119
Longitudinal Cohort: Exosomal Cargo
Longitudinal Cohort: Exosomal Cargo

Converter$_{\text{pre}}$ versus NC

Cargo 1

ROC AUC = 0.985

Cargo 2

ROC AUC = 0.974

Cargo 3

ROC AUC = 1.00

Cargo 4

ROC AUC = 1.00

[Graphs showing ROC curves for each cargo]
Longitudinal Cohort: Exosomal Cargo

Combined Classifier with 4 Cargo Analytes

Converter$_{\text{pre}}$ versus NC

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Longitudinal Study

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Phenoconverters (MCI/AD)
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DEGs from RNAseq Analysis
## Differentially Expressed Genes in Peripheral Leukocytes (RNAseq)

<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Number of DEGs via RNA-seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Converter}<em>{\text{post}}$ vs. $\text{Converter}</em>{\text{pre}}$</td>
<td>1215</td>
</tr>
<tr>
<td>$\text{Converter}_{\text{pre}}$ vs. Normal</td>
<td>1175</td>
</tr>
<tr>
<td>MCI/AD vs. Normal</td>
<td>23</td>
</tr>
<tr>
<td>MCI/AD(all) vs. Normal(all)</td>
<td>6</td>
</tr>
</tbody>
</table>
Accuracy of Classification $\text{Con}_{\text{pre}}$ vs NC
ROC Curve $\text{Con}_{\text{pre}}$ vs NC
25 genes

ROC AUC = 0.9966

AUC: 0.9966
95% CI: 0.9890 -- 1.0000
Summary

- A longitudinal cohort of community dwelling >75 year subjects were enrolled
- Baseline and serial neuropsychological tests were undertaken and bloods drawn
- Prospective ascertainment of amnestic MCI/AD defined a subset of phenoconverters
- Plasma lipidomic analysis of Con_pre disclosed a set of lipids that predicted the emergence of cognitive decline within a mean of 2.1 years
- Prognostic lipids representing PC and AC were decreased in Con_pre subjects.
  - We speculate that these changes reflect altered clearance of the lipids rather than diminished production
  - The ten lipid biosignature provided greater than 90% specificity with a 90% sensitivity
- The expanded 17 metabolomic panel increased sensitivity and specificity to >99%
- Plasma exosomal analytes produced a sensitivity and specificity >99%
- 25 DEGs produced a highly sensitive and specific classifier
- All measures can be done inexpensively and with low risk
- These results must be corroborated in other cohorts
Aging Study Collaborative Group

- **University of Rochester**
  - Mark Mapstone
  - William J. Hall
  - Susan G. Fisher
  - Derick R. Peterson
  - James M. Haley
  - Michael D. Nazar
  - Steven A. Rich
  - Anthony Almudevar
  - Eileen Johnson
  - Pamela Bailie

- **UC Irvine**
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  - Dan J. Berlau
  - Carrie B. Peltz
  - Dana Greenia
  - Mukti Patel
  - Archana Balasubramanian

- **UCSF**
  - Ed Goetzl

- **Georgetown University**
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  - Massimo S. Fiandaca
  - Xiaogang Zhong
  - Timothy R. Mhyre
  - Linda H. MacArthur
  - Ming T. Tan
  - Robert M. Padilla
  - Ishmeal Conteh
  - Rajbir Singh
  - P. Kaur
  - Howard J. Federoff