Plasma Phospholipids Identify Antecedent Memory Impairment in Older Adults

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So... what exactly is Alzheimer’s Disease?

- A progressive form of dementia that causes memory loss, disorientation, changes in mood/affect, increases in distrust or suspicion, and can cause physical impairments such as in talking, eating/swallowing, and walking

- Not a normal part of aging, worsens over time, and there is no cure (although some treatments are available)

- Currently the most common form of dementia (60-80% of dementia cases), with someone in the USA developing it every 66 seconds
So... what exactly is Alzheimer’s Disease?

- 1 in 9 adults in the USA over the age of 65 will develop Alzheimer’s Disease, with an average life expectancy of 2-10 years after diagnosis.

- In 2016: 5.4 million people with Alzheimer’s. 5.2 million are over age 65, but 200,000 people younger (early onset AD).

- Women tend to be diagnosed more often and have a worse prognosis than men.

- In women especially, increased risk of Alzheimer’s Disease can be linked to the E4 variation of the APOE gene (which works in fat and cholesterol distribution throughout the body).

  - This will come up again later! (APOE-ε4)
Inside a Healthy Neuron

Inside a Diseased Neuron

Neurotransmitter Vesicle

Normal Tau

Disintegrating Microtubules

Disrupted Transport

Hyperphosphorylated Tau

Microtubule

Tau Protein
AD Has HUGE and Lasting Implications for Both Patients and Caregivers

1 IN 3 SENIORS DIES WITH ALZHEIMER’S OR ANOTHER DEMENTIA

IN 2015, MORE THAN 15 MILLION CAREGIVERS PROVIDED AN ESTIMATED 18.1 BILLION HOURS OF UNPAID CARE

ALZHEIMER’S COSTS CAREGIVERS MORE THAN THEIR TIME

FAMILY CAREGIVERS SPEND MORE THAN $5,000 A YEAR CARING FOR SOMEONE WITH ALZHEIMER’S FOR SOME FAMILIES THIS MEANS MISSING A VACATION BUT FOR OTHERS, IT MAY MEAN GOING HUNGRY

MORE THAN 5 MILLION AMERICANS ARE LIVING WITH ALZHEIMER’S

ALZHEIMER’S DISEASE IS THE 6TH LEADING CAUSE OF DEATH IN THE UNITED STATES

EVERY 66 SECONDS SOMEONE IN THE UNITED STATES DEVELOPS THE DISEASE

IN 2016, ALZHEIMER’S AND OTHER DEMENTIAS WILL COST THE NATION $236 BILLION

IT KILLS MORE THAN BREAST AND PROSTATE CANCER COMBINED
What Can We Do to Help?

- The lack of a cure may be due, at least in part, to the current inability to diagnose AD prior to development of symptoms

- Current biomarkers include: CSF tau and amyloid beta levels, structural and functional MRIs, amyloid imaging, and inflammaging
  - Limited because invasive, expensive, and can take too much time
  - Blood biomarkers would be cheaper, easier, and safer, but none currently work well enough preclinically

- This study was designed to look at a set of 10 different lipid levels from peripheral blood (normal blood test) to detect future memory impairment

- Able to detect phenoconversion from normal cognition to either amnestic mild cognitive impairment (mild/early AD??) or full blown AD within a 2-3 year time frame with greater than 90% accuracy
At first glance...

In a 5 year observational study 525 community-dwelling participants 70 years and older enrolled

- 74 participants with aMCI or mild AD
- 46 participants incidental at entry
- 28 Converters (average 2.1 years)
How they broke it down

Three main groups

1. **aMCI/AD**
   a. Defined by primary memory impairment
   b. aMCI generally which reflects the earliest clinically detectable stage of AD
   c. Includes Converters after phenoconversion

2. **Converters**
   a. Includes prior phenoconversion (Converter Pre): memory not impaired
   b. Includes post phenoconversion (Converter Post): memory impaired, met criteria for aMCI/AD

3. **Normal Control (NC)**
   a. matched aMCI/AD group on basis of age, education and sex
In the third year...the Discovery phase

- **53 participants with aMCI/AD participants** selected for metabolomic and lipidomic biomarker discovery
  - 18 Converters
  - 53 matched with cognitively normal control (NC)
- **Internal Cross-Validation Procedure:** evaluate the accuracy of the discovered lipidomics profile
  - Classifying 41 additional subjects
    - 21 participants with aMCI/AD
    - 10 Converters
    - 20 matched NC participants
Discovery & Validation

Year 3 Discovery Phase

Year 5 Validation Phase
Participants

Discovery Phase
- Non-impaired Memory
  - Normal Control
    - N=53
  - Converter<sub>pre</sub>
    - N=18
- Impaired Memory
  - Incident aMCI/AD
    - N=35
  - Converter<sub>post</sub>
    - N=18
  - aMCI/AD
    - N=53

Validation Phase
- Non-impaired Memory
  - Normal Control
    - N=20
  - Converter<sub>pre</sub>
    - N=10
- Impaired Memory
  - Incident aMCI/AD
    - N=11
  - Converter<sub>post</sub>
    - N=10
  - aMCI/AD
    - N=21
Defining the aMCI/AD, Converter and NC groups

a. Quantified measurements of memory performance

b. Concentrations of the metabolites in each group
Cognitive composite Z-Scores for non-mnemonic domains

- Attention
- Executive
- Language
- Visioperceptual

Scores from the combined discovery and validation samples
<table>
<thead>
<tr>
<th>Attention ($Z_{\text{att}}$)</th>
<th>Executive ($Z_{\text{exec}}$)</th>
<th>Language ($Z_{\text{lan}}$)</th>
<th>Visuoperceptual ($Z_{\text{vis}}$)</th>
<th>Memory ($Z_{\text{mem}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wechsler Memory Scale-III Forward Digit Span (WMS-III FDS)</td>
<td>Wechsler Memory Scale-III Backward Digit Span (WMS-III BDS)</td>
<td>1-min Category fluency (Animals)</td>
<td>Hooper Visual Organization Test (HVOT)</td>
<td>Rey Auditory Verbal Learning Test Learning (RAVLT Learning)</td>
</tr>
<tr>
<td>Trail Making Test- Part A (TMT-A)</td>
<td>Trail Making Test- Part B (TMT-B)</td>
<td>Boston Naming Test 60- Item version (BNT-60)</td>
<td>Rey Auditory Verbal Learning Test Retrieval (RAVLT Retrieval)</td>
<td>Rey Auditory Verbal Learning Test Retention (RAVLT Recognition)</td>
</tr>
</tbody>
</table>
Methods:

Untargeted Metabolomic Analysis

- Metabolomic and lipidomic profiling yielded 2,700 positive-mode features and 1,900 negative-mode features
  - LASSO: least absolute shrinkage and selection operator penalty
    - Unambiguous class separation between the Converter Pre group and NC group which did not phenoconvert

- Metabolites
  - Lower phosphatidylinositol in Converter Pre group
  - Higher glycoursodeoxycholic acid in aMCI/AD group

- Tandem Mass Spectrometry
Results:

**LASSO** analysis

- amino acids and phospholipids to be potent discriminators of the NC and aMCI/AD groups

**Stable Isotopic Dilution-Multiple Reaction Monitoring (MRM) mass spectrometry (SID-MRM-MS)**

- May predict phenoconversion from NC to aMCI/AD
- Converter Pre: lower plasma levels serotonin, phenylalanine, proline, lysine, phosphatidylcholine (PC), and acylcarnitine (AC)
  - → later phenoconverted to aMCI/AD
Results in Tandem Mass Spectrometry
## Results in Metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Fold change</th>
<th>Comparison groups</th>
<th>Mode</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC ae C38:4</td>
<td>↓</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.00417</td>
</tr>
<tr>
<td>Proline</td>
<td>↓</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.00003</td>
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<tr>
<td>Lysine</td>
<td>↓</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.0020</td>
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<tr>
<td>Serotonin</td>
<td>↓</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.0160</td>
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<tr>
<td>Taurine</td>
<td>↓</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.0030</td>
</tr>
<tr>
<td>DOPA</td>
<td>↑</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.0001</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>↓</td>
<td>NC versus Converter_pre</td>
<td>POS</td>
<td>0.00001</td>
</tr>
<tr>
<td>Acylcarnitine C7-DC</td>
<td>↓</td>
<td>NC versus aMCI/AD</td>
<td>POS</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The arrows indicate upregulation or downregulation in the comparison group as compared to the NC participants. DOPA, dihydroxyphenylalanine; C7-DC, pimelyl-l-carnitine.
Performed an independent blind metabolomic and lipidomic analysis on blood plasma (b/c one person in the aMCI/AD group did not have a blood sample)

Found that the lipid analysis profiles are very similar in both the discovery and validation sets
Results:

- Used metabolic analysis from the untargeted LASSO analysis to further distinguish the NC group from the aMCI/AD/Converter groups
- Compared this to effects of the APOE gene on each of the groups (proportion of APOE-ε4 was the same in all groups)
  - Showed that the 10-metabolite biomarker panel is statistically unrelated to the APOE-ε4 mediated effects
Deficiencies in the Plasma Membrane in Alzheimer’s Disease (Past Studies Mentioned in the Paper)

Decreased lysoPC/PC ratios

- Amyloid-beta peptides may induce free radical oxidative stress
- Hypothesis: oxidation may lead to changed concentrations of choline containing phospholipids in cerebrospinal fluid (CSF) of AD patients.
- The lower lyso-PC/PC ratio in CSF of patients with AD may reflect alterations in the metabolism of choline-containing phospholipids in the brain in AD
Decreased plasma PC DHA levels

- DHA: abundant fatty acid in the brain, its concentration decreases with the patients with dementia.
- Plasma PC DHA content is determined by the degree of conversion of α-linolenic acid to DHA within the liver and by the consumption of foods rich in DHA
- In this cohort free of dementia at baseline, plasma PC DHA content predicted the occurrence of new dementia.

Interesting fact: The Framingham Heart Study is a long-term, ongoing cardiovascular study on residents of the town of Framingham, Massachusetts. The study began in 1948 with 5,209 adult subjects from Framingham, and is now on its third generation of participants.
Increased cerebrospinal fluid (CSF) PC metabolites in patients with AD

- Among cognitively normal patients the average CSF levels of GPCh, phosphocholine and choline (water-soluble metabolites of phosphatidylcholine (PtdCho)) did not change with increasing age in human cerebrospinal fluid.
- When compared with age-matched controls, patients with Alzheimer’s disease had elevated levels of all choline metabolites: GPCh was significantly increased by 76%, phosphocholine by 52% and free choline (Ch) by 39%.
- Takeaway: Alzheimer’s disease is accompanied by an increased PtdCho hydrolysis in the brain
- Water soluble metabolites of PtdCho: GPCh, PCh and choline
Amyloid-beta may directly disrupt bilayer integrity by interacting with phospholipids

- Sphingolipids role in regulating neuronal function (signal transduction in the membrane)
- In AD → increased membrane-associated oxidative stress and excessive production and accumulation of ceramides
- Perturbations in sphingolipid metabolism could contribute to the pathogenesis of neurodegenerative conditions such as Alzheimer’s disease
Sphingolipids

- Located in plasma membrane, particularly nerve cells and brain
- Part of lipid rafts
- Discovered in brain extracts in 1870
- Metabolites: ceramide and sphingosine-1-phosphate (role in variety of cellular signaling such as stress responses and apoptosis)
- Ample evidence shows that the accumulation of cellular ceramides is associated with obesity, diabetes, atherosclerosis, and cardiomyopathy.

The ability of ceramides to interfere with insulin receptor signaling is the result of blocking the receptors' ability to activate the downstream effector kinase, PKB/Akt.
PCs and ACs

-presented the data of plasma metabolite changes that distinguish Converters and NC group that remains cognitively normal in the near future using the discovery and validation groups

-10 metabolite profiles feature Phosphatidylcholines and Acylcarnitines

ACs

- useful in identifying inborn errors of metabolism
- most widely known function: involvement in beta oxidation of fatty acids
- acylcarnitine supplementation have resulted in beneficial effects in the treatment of various neurological diseases, even though fat is not the major fuel for brain
- other functions: brain acylcarnitines can function in synthesizing lipids, altering and stabilizing membrane composition, modulating genes and proteins, improving mitochondrial function, increasing antioxidant activity, and enhancing cholinergic neurotransmission
PCs

- most abundant phospholipid of the cell membrane and protects the liver
- easily obtained from a variety of readily available sources (egg yolk, soybeans)

Possible health implications in Alzheimer's

- Researchers used mutant mouse models with severe oxidative damage as a model of "accelerated aging"
  - investigate the possible role of phosphatidylcholine supplementation as a way of slowing down aging-related processes.
- No effect.
- Alzheimer’s disease: characterized by a decrease in cholinergic transmission
- BUT the basic defect in cholinergic transmission in Alzheimer’s disease relates to impaired activity of the enzyme acetylcholine transferase, not to a deficiency of choline
- Decreasing plasma AC levels in the Converter-pre participants in our study may indirectly signal an impending dementia cascade that features loss of these cholinergic neuronal populations.
- Ten phospholipid biomarkers, consisting of PC and AC species may indicate the individuals destined to phenoconvert from cognitive intactness to aMCI or AD
  - may mark the transition between preclinical states (where synaptic dysfunction and early neurodegeneration give rise) to subtle cognitive changes (clinical diagnosis)
AD Diagnosis

- **The pathological hallmarks of AD**: neuritic plaques composed of aggregated extracellular Aβ fibrils and intraneuronal neurofibrillary tangles of hyperphosphorylated tau.
- **Computed tomography (CT) and magnetic resonance imaging (MRI)**: Brain volume and structure investigated
- **Imaging techniques (such as PET)**: Study the functional activity of the brain, used to measure cerebral brain glucose metabolism and cerebral blood flow, both of which correlate with cognitive function.
  - Disadvantage: expensive and only accessible in highly specialized centers
- **Fluid biomarker (CSF)**: easily obtained by lumbar puncture, look at total tau (T-tau), hyperphosphorylated tau (P-tau) and the 42 amino acid isoform of amyloid β (Aβ42)
**Diagnostic biomarkers:** developed to detect AD neuropathology even in individuals at preclinical stages of the disease. Diagnostic biomarkers are present at all stages of the disease, and they can therefore be used to detect AD pathological changes even in the asymptomatic state.

**Progression marker:** have poor disease specificity and might not be present at early stages, indicates clinical severity (ie, changes as the disease progresses).

1. Increased T-tau concentration: axonal degeneration
2. Increased P-tau concentration: presence of neurofibrillary tangles
3. Decreased 42-aminoacid form of Aβ (Aβ42): senile plaque pathology
Fibrillar amyloid plaques

Measured with PiB (radioactive molecule that binds to amyloid beta peptide)

PET scans

MCI + low PiB retention

MCI + high PiB retention

MMMSE: Mini Mental State Examination

SUVR: Standard uptake value
Fluid-based Biomarkers

- focused on amyloid-1–42 (A42), total tau and phosphorylated tau-181 obtained from CSF
- **ADVANTAGE:** Classification of symptomatic patients vs normal controls or other dementias or conversion from MCI to AD is high
- **DISADVANTAGE:** predictive value of these CSF biomarkers in preclinical patients is not as strong, suggesting that these markers may be useful only for confirmation of clinical diagnosis
Blood-based Biomarkers

Not routinely used in clinical practice but may be more useful because they are easily obtained with less risk of complication in older adults.

Recent study: using plasma, identified 18 proteins that discriminated subjects with symptomatic AD from normal control subjects with nearly 90% accuracy and predicted conversion from symptomatic MCI to AD with 91% accuracy.
Alzheimer’s early indicator

Scientists have been unsure whether the immune system goes on the attack in Alzheimer’s. The Kodek team’s discovery suggests that it does, and could lead Alzheimer’s research in new directions. The team’s research method (below) could potentially be used in any disease that involves an immune response.

Finding Alzheimer’s signature in the body:

1) The body responds to disease by making large numbers of unique antibodies.

2) The Kodek team created a system for reading antibodies. Blood from a diseased person and from a well person is applied to two identical slides, each covered with thousands of artificial ‘peptoid’ beads.

3) The antibodies are treated so that they emit tiny bits of light. The brightest spots on the slides represent antibodies specific to the disease.

Source: Cell Journal, Jan. 7

BRENNAN KING/Staff Artist
Alzheimer's drug sneaks through blood–brain barrier

http://www.nature.com/news/alzheimer-s-drug-sneaks-through-blood-brain-barrier-1.16291
**Intro:** Antibodies abundant in human sera and capable of accurately diagnosing mild-moderate stages of AD and PD

**Methods:** 236 subjects, 50 w/ MCI screened with human protein microarrays to identify biomarkers

**Results:** Autoantibody biomarkers can differentiate MCI patients from age-matched and gender-matched controls with an overall accuracy, sensitivity, and specificity of 100.0%. They were also capable of differentiating MCI patients from those with mild-moderate AD and other neurologic and non-neurologic controls with high accuracy.
Significance of This Study

First published report of a blood-based biomarker panel with very high accuracy for detecting preclinical AD

It identifies (with accuracy above 90%) cognitively normal individuals who, on average, will phenoconvert to aMCI or AD within 2–3 years.

The accuracy for detection is equal to or greater than that obtained from most published CSF studies.

Why blood? Easier to obtain and costs less to acquire, making it more useful for screening in large-scale clinical trials and for future clinical use.
Looking into the future

This biomarker panel requires external validation using similar rigorous clinical classification before further development for clinical use (e.g. more diverse demographic group than the initial cohort)

Results of this experiment are a major step towards finding biomarkers of preclinical diagnosis.
Alzheimer's Awareness

Samuel Cohen

October 16th, 2015