Alzheimer's disease: new mechanisms and early blood-based diagnosis

Robert Nagele, Ph.D.
Director of Translational Research
NJ Institute for Successful Aging
Department of Geriatrics and Gerontology
RowanSOM, New Jersey
DISCLOSURES

**Durin Technologies, Inc.**
Founder, Chief Scientific Officer, Stockholder

**Beren Technologies, Inc.**
Founder, Chief Scientific Officer, Stockholder

**Funding**
Osteopathic Heritage Foundation
Michael J Fox Foundation
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Foundation Venture Capital Group
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GlaxoSmithKline
Alzheimer’s Disease

Alzheimer’s disease is an irreversible, progressive brain disease that slowly destroys memory and thinking skills.

*The risk of developing AD increases with age
*In most cases, symptoms first appear after age 60
*Familial AD appears early – 4% of cases are inherited and nearly all of these are mutations in the presenilin gene
*AD is not a part of normal aging – it is a fatal brain disease.
AD is the most common cause of dementia among people age 65 and older.

- Estimate that around 4.5 million people now have AD.
- For every 5-year age group beyond 65, the percentage of people with AD doubles.

By 2050, 13.2 million older Americans are expected to have AD - if the current numbers hold and if no preventive treatments become available.
Prevalence of AD by Age

Alzheimer’s Disease Hits Home
Nagele Family

My Grandparents

My Mother’s Siblings

And now my father and mother-in-law
Pathology
Normal vs Alzheimer Brain
Gross Pathology

Brain shrinkage – thinning of gyral folds – broadening of fissures
Alzheimer’s Disease
Macroscopic Changes in the Brain
Alzheimer’s Disease
Amyloid Plaques and Neurofibrillar Tangles
Microscopic Pathological Hallmarks

Plaques

Alzheimer’s

Normal

Tangles

Courtesy of Harry Vinters, MD.
PET scans show much reduced glucose utilization in the AD brain compared to controls

**Reason:** Rampant cell death means less cells capable of metabolizing glucose
Why do our brain cells die?
One Reason:
Neurons die because of amyloid (Abeta42) is deposited in the brain.
Amyloid beta (Abeta42) deposits in the brain and arises from sequential cleavage of the amyloid precursor protein (APP).
How is amyloid deposited in the Alzheimer’s brain?

Extracellular vs Intraneuronal Amyloid (Abeta42) Deposition

Important to resolve for proper drug targeting
Neurons accumulate excessive Abeta42 intracellularly. Amyloid overburdened neurons die by lysis and release their fibrillized amyloid content to form plaques.

AD brain sections immunostained for Abeta42 (AB42)

Note abundant Abeta42-positive (presumably) endocytic vesicles
Brain regions with abundant plaques are generally devoid of pyramidal neurons.

No pyramidal neurons left – nearly all Abeta42 is found within plaques.
Brain regions with few plaques contain numerous Abeta42-burdened neurons.

Nearly all Abeta42 is contained within pyramidal neurons PiB- and Fluorbetapir-negative.
AP Density vs. PC Density in AD Brains

![Graph showing AP Density vs. PC Density in AD Brains. The x-axis represents different regions labeled from 1 to 11. The y-axis on the left represents Aps per sq. mm, ranging from 0 to 300. The y-axis on the right represents PCs per sq. mm, ranging from 0 to 300. The graph includes a line and bars indicating the density of Aps and PCs across different regions.](image-url)
Amyloid plaque size is strictly correlated to local neuron size.
Alzheimer’s is a synaptic loss disease
While Abeta accumulates, neurons lose synapses
Intraneuronal Abeta42 is not detectable via PiB or Fluorbetapir
Symptoms can emerge in “apparently” Abeta42-deficient brains?

Months to years – Synaptic loss is linked to onset of symptoms
How can we stop amyloid deposition in the brain?

Answer: Find the source

The **blood** is a major source.

In healthy brains, the **blood-brain barrier (BBB)** keeps the soluble amyloid in the vessel.

In AD brains, the **BBB is compromised** and plasma, including soluble amyloid, chronically leaks into the brain.
The Blood-brain Barrier

- Endothelial cell
- Vascular smooth muscle cell
- Blood flow

BVECs

Nature Reviews | Molecular Cell Biology
In **healthy people**, the BBB strictly regulates entry of plasma components into the brain.

In **Alzheimer’s disease patients**, the BBB leaks constantly, allowing all plasma components access to the brain tissue – disruption of brain homeostasis.

**The Blood-brain Barrier (BBB)**

**BBB** = Extensive tight junctions between vascular endothelial cells
Blood-brain barrier

- astrocyte end feet
- Brain Blood Vessel
- pericytes
In ALL AD brains, the BBB is chronically defective
Plasma components (including Abeta42) leak into the brain
Chronic leaks are revealed by telltale perivascular leak clouds

Abeta42 leak suggests that the blood is a major source of soluble and oligomeric amyloid beta (Abeta) in the brain.

IgG leak includes brain-reactive autoantibodies
What makes the Blood-Brain Barrier (BBB) leak?
Atherosclerosis, diabetes, hypertension and vascular inflammation
Anything that damages the endothelial lining of the vessel
Anesthesia causes catastrophic, short-term BBB breakdown
- Probable mechanism of post-surgical delirium and trigger of dementia

Results

1. Anesthesia (sevoflurane and isoflurane) induces immediate changes in the surfaces of brain vascular endothelial cells (BVECs), including a profound smoothing of surface membranes and visible holes in the BBB.

2. Old rats showed much more anesthetic-induced BBB breakdown

Collaboration with Dept. of Anesthesiology at Johns Hopkins
Acharya et al., 2015 Brain Research
Rat leptomeningeal artery

BVECs

20 μm
The BBB can be visualized directly using SEM - leptomeningeal artery wall -

BBB tight junctions

Arrows point to BBB – looks like rows of dots
BBB Tight Junctions and Holes in the BBB
Anesthesia causes short-term BBB breakdown
- Probable mechanism of post-surgical delirium and trigger of dementia

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Results

Collaboration with Dept. of Anesthesiology at Johns Hopkins
Manuscript in preparation
Sevoflurane and Isoflurane Induce Blood-brain Barrier Leak in Aged Rats

Acharya et al., 2015 Brain Research
Holes in the BBB
BBB breakdown caused by endothelial cell contraction

Relaxed BBB cells

BBB intact (closed)

Long-term contraction due to chronic inflammation
AD, PD, MS, athero

or

Short-term contraction due to irritating drugs and anesthetics
Delirium from drugs or anesthetics

Contracted BBB cells

BBB breakdown (open)

Solution: Block short- and long-term BBB cell contraction
Evidence that the blood can be a main source of the soluble Abeta42 that deposits in the brain?

**Experiment:** Directly demonstrated that Abeta42 can leak from the blood into the brain in the mouse.

FITC-Abeta42 accumulates in the brain

Inject fluorescent FITC-Abeta42 into tail vein of mouse with *Pertussis* toxin-induced BBB breakdown

Clifford et al., 2007  Brain Research
**Intraneuronal accumulation of exogenous FITC-Abeta42**

FITC-Abeta42 accumulates within the same neurons that are affected in human AD brains.

Mouse brain hippocampus

Note selective accumulation of FITC-Abeta42 in hippocampal hilar neurons.
Brain-reactive antibodies leak from blood vessels and bind selectively to pyramidal neurons in AD brains.
BBB breakdown, autoantibody influx and Alzheimer’s disease

Neurons with autoantibodies (brown color) bound to their surfaces are abundant in all AD brains.

Western blot analysis reveals numerous brain-reactive autoantibodies in the blood.

Brain-reactive autoantibodies are nearly ubiquitous in human sera and may be linked to pathology in the context of blood-brain barrier breakdown.

Eli C. Levin\textsuperscript{a, b}, Nimish K. Acharya\textsuperscript{b}, Min Han\textsuperscript{b}, Semah B. Zavareh\textsuperscript{b}, Jonathan C. Sedeyn\textsuperscript{b}, Venkateswar Venkataraman\textsuperscript{c}, Robert G. Nagele\textsuperscript{a,*}

\textsuperscript{a}New Jersey Institute for Successful Aging, University of Medicine and Dentistry of New Jersey, 2 Medical Center Drive, Stratford, New Jersey 08084, USA.
\textsuperscript{b}Graduate School of Biomedical Sciences, University of Medicine and Dentistry of New Jersey, 2 Medical Center Drive, Stratford, New Jersey 08084, USA.
\textsuperscript{c}Department of Cell Biology, University of Medicine and Dentistry of New Jersey, 2 Medical Center Drive, Stratford, New Jersey 08084, USA.

Levin et al., 2010 Brain Research, 1345(23):221-232.
Brain-reactive autoantibodies bind to the same pyramidal neurons that accumulate Abeta42 in AD brains.

**Conclusion:** Antibody binding to neurons may play a role in the accumulation of Abeta42 in these cells.
How do neurons respond to autoantibody binding?

**Answer**

Surface-bound IgGs are internalized via endocytosis and degraded in lysosomes.

The pathological significance of this event

Abeta42 bound to neuronal cell surfaces is also internalized. Within the lysosomal compartment, Abeta42 self-assembles into fibrils that cannot be degraded.

This drives chronic amyloid accumulation within neurons.
Brain-reactive autoautoantibodies in human serum can drive amyloid deposition in adult mouse neurons in vitro

Nagele et al. 2011  
*J. Alzheimer’s Disease*
Human autoantibodies dramatically accelerate Abeta42 deposition in pyramidal neurons in mouse brain

Adult mouse brain slice cultures

100 nM Abeta42 alone

Human serum + 100 nM Abeta42

Result:
Very little Abeta42 internalization

Result:
Heavy Abeta42 internalization
Human serum autoantibodies enhance intraneuronal Abeta42 deposition in mouse brain slice cultures with different potencies.
A New Therapeutic Target – Aim for the BBB
(e.g., Darapladib (GlaxoSmithKline) blocks BBB breakdown in diabetic/hypercholesterolemic (DMHC) pigs

Atherosclerosis
Cerebrovascular amyloidosis
Vascular inflammation
Traumatic brain injury

Receptor stripping
Dendrite collapse

Blood Vessel
Leak Cloud

Dendritic Degeneration
Intraneuronal Aβ42 Accumulation

Ancharya et al., 2013  J. Alzheimer’s Disease
Pig Model of Long-Term Diabetes Mellitus (DM) and Hypercholesterolemia (HC)

DMHC induction for 4 wks (streptozotocin + cholesterol feeding)

Condition of DMHC was maintained for 24 weeks during animal treatment

Brain and retinal tissue was fixed or frozen

Biochemical and immunohistochemical analyses

Funded by GlaxoSmithKline
DMHC pigs showed the greatest extent of BBB leak. Darapladib reduces vascular leak to near control levels.

Amount of material leaking from arterioles, venules and capillaries.

### Treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>P-value</th>
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<tbody>
<tr>
<td>DMHC/C</td>
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<tr>
<td>DMHC/Rx</td>
<td>0.097645</td>
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<tr>
<td>Rx/C</td>
<td>0.298917</td>
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</tbody>
</table>
Abeta42 accumulates exclusively in pyramidal neurons in DMHC pigs

Acharya et al., 2013  J. Alzheimer's Disease
DMHC pigs show the highest density of amyloid (Aβ42)-positive neurons.

### Density of Aβ42 Neurons

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>P-value</th>
<th>n = 3</th>
<th>n = 13</th>
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<tr>
<td>DMHC / Control</td>
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<td>DMHC / Rx</td>
<td>0.005</td>
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<tr>
<td>Rx / C</td>
<td>0.986</td>
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</tr>
</tbody>
</table>
Hypothesis – Chronic BBB breakdown is a common mechanism connecting delirium and dementia.

Aging  |  Diabetes  |  High Cholesterol  |  Stroke and Traumatic Brain Injury  |  High Blood Pressure  |  Anesthesia

Infections

Disrupt BBB

Influx of Plasma Components into Brain (including soluble Abeta and autoantibodies)

Loss of brain homeostasis  
Abeta and autoantibodies bind to neurons

Acute Delirium

POCD

Dementia

Site and extent of BBB breach determines type of symptoms and rate of progression
Proposed: Brain-reactive AutoABs and Breakdown of the Blood-Brain Barrier A Common Thread That Can Drive Multiple Neuropathologies

Disease type and symptoms depend on the specific brain region affected.

Disease severity depends on extent of BBB breakdown, presence and titer of brain-reactive autoantibody and the presence and abundance of neuronal surface target antigen.
Early Alzheimer’s and Parkinson’s Disease Diagnostics
I think we all agree

Early treatment of Alzheimer’s disease (in fact any disease), has the potential benefit of slowing or stopping disease progression before too much brain devastation and loss of function has occurred.

But

Early treatment requires early diagnosis
Current AD Diagnostic Methods

Tools used to diagnose AD:

- A detailed patient history
- Information from family and friends
- Physical and neurological exams and lab tests
- Neuropsychological/cognitive tests
- Imaging tools such as CT scan, or magnetic resonance imaging (MRI).

Problem: These assess symptoms. AD pathology is already pretty far advanced by the time symptoms appear and a definitive diagnosis can be made using these methods.

In other words, it may be too late for treatments to be effective.
Diagnosis of AD is expensive and based on detection of telltale symptoms, results from neurological and neuropsychiatric tests and brain imaging. The pathology has already been underway for years.

Accurate early diagnosis of AD at the mild cognitive impairment (MCI) stage is not yet possible.

No blood or laboratory tests for AD exist – the annual world market for a diagnostic test that can detect AD at the early MCI stage is $3.5 billion.

An intensive worldwide search is underway for useful AD biomarkers and a diagnostic blood test for AD.

Ideal criteria for any diagnostic test include that it must be:
  - specific
  - reliable and reproducible
  - non-invasive or minimally invasive
  - simple to perform
  - affordable

Early detection of AD at the MCI stage is the goal – because it allows early treatment
Thousands of autoantibodies are present in all human serum

Nearly 2,000 autoantibodies are detected using 9,486 human protein targets - this reflects only 1/3 of the total human proteome

### Effects of Age and Gender on Number of Autoantibodies in the Blood

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>% Female</th>
<th>Antibody Count</th>
<th>P value</th>
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<tbody>
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<td>&lt; 45</td>
<td>10</td>
<td>33.3</td>
<td>1498.2 ± 545.7</td>
<td>&lt;45 vs. 45-65: 0.0021</td>
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<tr>
<td>45 - 65</td>
<td>32</td>
<td>18.2</td>
<td>2335.6 ± 1009.5</td>
<td>45-65 vs. &gt;65: 0.37</td>
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<tr>
<td>&gt; 65</td>
<td>15</td>
<td>60</td>
<td>2647.8 ± 1139.2</td>
<td>&lt;45 vs. &gt;65: 0.0028</td>
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</table>

<table>
<thead>
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<th>Sex</th>
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<th>Age</th>
<th>Antibody Count</th>
<th>P value</th>
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<td>Female</td>
<td>18</td>
<td>57.6 ± 18.7</td>
<td>2772.5 ± 714.8</td>
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<tr>
<td>Male</td>
<td>39</td>
<td>53.1 ± 15.1</td>
<td>2039.3 ± 1092.7</td>
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<tr>
<td>Total</td>
<td>166</td>
<td>62.4 ± 16.3</td>
<td>1996.9 ± 1051.9</td>
<td></td>
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</tbody>
</table>

Nagele et al., 2013 *PLoS ONE*
This Study Showed That.....

1. All human serum typically contains thousands of autoantibodies targeting a wide variety of proteins.

2. Individual autoantibody profiles are stable over long time periods (spanning many years in healthy individuals).

3. Similarly complex autoantibody profiles are also found in the rat, mouse and swine, suggesting evolutionary conservation among all mammals.

4. The total number of detectable autoantibodies is strongly influenced by age, gender and the presence of disease.
This discovery has pointed the way to new disease diagnostics

Natural IgG Autoantibodies Are Abundant and Ubiquitous in Human Sera, and Their Number Is Influenced By Age, Gender, and Disease

Eric P. Nagele¹,³,⁹, Min Han¹,²,⁹, Nimish K. Acharya¹,², Cassandra DeMarshall¹,², Mary C. Kosciuk¹, Robert G. Nagele¹*

¹ Biomarker Discovery Center, New Jersey Institute for Successful Aging, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America, ² University of Medicine and Dentistry of New Jersey-Graduate School of Biomedical Sciences at the School of Osteopathic Medicine, Stratford, New Jersey, United States of America, ³ Durin Technologies, Inc., New Brunswick, New Jersey, United States of America

Abstract

The presence of self-reactive IgG autoantibodies in human sera is largely thought to represent a breakdown in central tolerance and is typically regarded as a harbinger of autoimmune pathology. In the present study, immune-response profiling of human serum from 166 individuals via human protein microarrays demonstrates that IgG autoantibodies are abundant in all human serum, usually numbering in the thousands. These IgG autoantibodies bind to human antigens from organs and tissues all over the body and their serum diversity is strongly influenced by age, gender, and the presence of specific diseases. We also found that serum IgG autoantibody profiles are unique to an individual and remarkably stable over time. Similar profiles exist in rat and swine, suggesting conservation of this immunological feature among mammals. The number, diversity, and apparent evolutionary conservation of autoantibody profiles suggest that IgG autoantibodies have some important, as yet unrecognized, physiological function. We propose that IgG autoantibodies have evolved as an adaptive mechanism for debris-clearance, a function consistent with their apparent utility as diagnostic indicators of disease as already established for Alzheimer’s and Parkinson’s diseases.

What is the function of all of these autoantibodies?

Hypothesis

Autoantibodies are involved in the clearance of debris generated by the body on a day-to-day basis.

If so, then……..

The presence of disease leads to production of excessive debris from the organ affected

….and this leads to an increased abundance of autoantibodies responsible for the clearance of disease-associated debris
Detecting Disease-Specific Autoantibody Profiles Using Human Protein Microarrays

Identification of disease specific autoantibody profiles in serum or plasma employing patterns of protein targets.

Microarrays contain 9,486 full-sized human proteins

Preparation and Processing

1. **Human protein targets**
2. **Slide with fluorescent targets**
3. **Incubation with human serum**
4. **Incubation with labeled secondary antibodies**
5. **Autoantibody biomarkers bind to targets**
6. **Labeled secondary antibodies bind to autoantibodies**
7. **Analysis of fluorescent signal patterns and detection of disease-specific autoantibody biomarkers**

**Preparation and Processing**

**Mild-moderate Alzheimer’s**
- Sensitivity: 96.0%
- Specificity: 92.5%

**Mild-moderate Parkinson’s**
- Sensitivity: 93.1%
- Specificity: 100%

Autoantibody production

1. **Neurons die** – which type of neurons depends on brain region affected
2. **Dead neurons release debris** into the surrounding cerebrospinal fluid (CSF)
3. **Debris enters bloodstream and activates the immune system**
4. **Immune system generates many autoantibodies to clear debris**
5. **Detect disease-related autoantibodies as biomarkers**

Neurons die – which type of neurons depends on brain region affected

Dead neurons release debris into the surrounding cerebrospinal fluid (CSF)

Debris enters bloodstream and activates the immune system

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Detect disease-related autoantibodies as biomarkers

1 drop of blood

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Immune system generates many autoantibodies to clear debris

Detect disease-related autoantibodies as biomarkers

1 drop of blood
Mild-Moderate Alzheimer’s Disease Results

1. Detected **451 autoantibodies** showing significantly higher prevalence in AD compared to controls (p < 0.01) – these are potentially useful as AD biomarkers.

2. Selected **the top 10 autoantibodies** showing the largest difference in group prevalence as our diagnostic indicators.

3. **Using only the top 10 indicators**, AD sera were distinguished from control sera with a **sensitivity of 96.0% and specificity of 92.5%**
Diagnosis of Alzheimer’s Disease Based on Disease-Specific Autoantibody Profiles in Human Sera

Eric Nagele, Min Han, Cassandra DeMarshall, Benjamin Belinka, Robert Nagele

1 Durin Technologies, Inc., New Brunswick, New Jersey, United States of America, 2 Graduate School of Biomedical Sciences, School of Osteopathic Medicine, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America, 3 New Jersey Institute for Successful Aging, School of Osteopathic Medicine, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America

Abstract

After decades of Alzheimer’s disease (AD) research, the development of a definitive diagnostic test for this disease has remained elusive. The discovery of blood-borne biomarkers yielding an accurate and relatively non-invasive test has been a primary goal. Using human protein microarrays to characterize the differential expression of serum autoantibodies in AD and non-demented control (NDC) groups, we identified potential diagnostic biomarkers for AD. The differential significance of each biomarker was evaluated, resulting in the selection of only 10 autoantibody biomarkers that can effectively differentiate AD sera from NDC sera with a sensitivity of 96.0% and specificity of 92.5%. AD sera were also distinguishable from sera obtained from patients with Parkinson’s disease and breast cancer with accuracies of 86% and 92%, respectively. Results demonstrate that serum autoantibodies can be used effectively as highly-specific and accurate biomarkers to diagnose AD throughout the course of the disease.

Diagnosis of Parkinson’s Disease Based on Disease-Specific Autoantibody Profiles in Human Sera

Min Han¹,², Eric Nagele³, Cassandra DeMarshall¹,², Nimish Acharya¹,², Robert Nagele²,³*

¹ University of Medicine and Dentistry of New Jersey-Graduate School of Biomedical Sciences at the School of Osteopathic Medicine, Stratford, New Jersey, United States of America, ²New Jersey Institute for Successful Aging, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America, ³Durin Technologies, Inc. New Brunswick, New Jersey, United States of America

Abstract
Parkinson’s disease (PD), hallmarked by a variety of motor disorders and neurological decline, is the second most common neurodegenerative disease worldwide. Currently, no diagnostic test exists to identify sufferers, and physicians must rely on a combination of subjective physical and neurological assessments to make a diagnosis. The discovery of definitive bloodborne biomarkers would be a major step towards early and reliable diagnosis. Despite attention devoted to this search, such biomarkers have remained elusive. In the present study, we used human protein microarrays to reveal serum autoantibodies that are differentially expressed among PD and control subjects. The diagnostic significance of each of these autoantibodies was evaluated, resulting in the selection of 10 autoantibody biomarkers that can effectively differentiate PD sera from control sera with a sensitivity of 93.1% and specificity of 100%. PD sera were also distinguishable from sera obtained from Alzheimer’s disease, breast cancer, and multiple sclerosis patients with accuracies of 86.0%, 96.6%, and 100%, respectively. Results demonstrate that serum autoantibodies can be used as highly specific and accurate biomarkers for PD diagnosis throughout the course of the disease.

Results:
Mild/moderate PD sera were differentiated from control sera with a 97.1% overall accuracy; sensitivity of 93.1% and specificity of 100%
Early Detection of Parkinson’s Disease

In our first study, we achieved early detection of PD with 90.5% accuracy!
Diagnosis of Early- and Mild-Moderate-Stage PD
Proposed origin of autoantibodies useful for PD diagnostics
Early-Stage Parkinson’s Disease Diagnostic Results
Using a Panel of the Top 50 PD Biomarkers

<table>
<thead>
<tr>
<th>Early-Stage PD (n = 103) vs.</th>
<th>Age Matched Controls</th>
<th>Age Matched and Young Controls</th>
<th>Mild-Moderate PD</th>
<th>Mild-Moderate AD</th>
<th>Multiple Sclerosis</th>
<th>Breast Cancer</th>
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<tbody>
<tr>
<td>n</td>
<td>111</td>
<td>56</td>
<td>29</td>
<td>50</td>
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<tr>
<td>Sensitivity %</td>
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<td>94.2</td>
<td>98.1</td>
<td>98.1</td>
<td>98.1</td>
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<tr>
<td>Specificity %</td>
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<td>PPV %</td>
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<td>99.0</td>
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<td>Overall Accuracy %</td>
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<td>97.7</td>
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<td>Overall Error %</td>
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<td>1.5</td>
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<td>3.0</td>
<td>2.3</td>
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</table>

The performance of the top 50 early-stage PD autoantibody biomarkers was assessed. PPV, positive predictive value; NPV, negative predictive value.

Note: Early-stage PD was diagnosed with 90% confidence and confirmed with subsequent patient follow-up.

DeMarshall et al. 2015 Immunology Letters
Funded by The Osteopathic Heritage Foundation and the Michael J Fox Foundation
Early-Stage Parkinson’s Disease – Only 4 Biomarkers Required!

ROC Curve Assessment of the Utility of PD Biomarkers for Detection of Early-Stage PD

Total Number of Subjects = 214; PD (n=103) vs. Age-matched Controls (n=111)

Comparison of early-stage PD vs. age-matched controls using a panel of 50 (red line) or 4 (blue line) biomarkers shows that these biomarker panels can be used to detect early-stage PD with a relatively high overall accuracy. Dashed line represents line of no discrimination. ROC AUC represents “area under the curve” at 95% confidence.

ROC AUC = 0.93 (95% CI)

ROC AUC = 0.92 (95% CI)
### Early Detection and Staging of Parkinson’s Disease

**Early Detection** – 89.2% overall accuracy  
**Staging** – 98.5% overall accuracy

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<td><strong>NPV %</strong></td>
<td>94.0</td>
<td>95.9</td>
<td>93.6</td>
<td>96.1</td>
<td>93.3</td>
<td>93.6</td>
</tr>
<tr>
<td><strong>Overall Accuracy %</strong></td>
<td>89.2</td>
<td>91.9</td>
<td>98.5</td>
<td>98.0</td>
<td>97.0</td>
<td>97.7</td>
</tr>
<tr>
<td><strong>Overall Error %</strong></td>
<td>10.8</td>
<td>8.1</td>
<td>1.5</td>
<td>2.0</td>
<td>3.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Performance of the top 50 early-stage PD autoantibody biomarkers in patients known to have early-stage PD with 90% confidence.
Comparison of early-stage PD (n=103) vs. mild-moderate PD (n=29) using a panel of 50 (red line) or 4 (blue line) biomarkers showing that autoantibody biomarkers can be used to accurately distinguish different stages of PD progression.

ROC AUC = 0.98 (95% CI)
ROC AUC = 0.99 (95% CI)
Lots of Bad News Recently Regarding Failed Clinical Trials of Potential Alzheimer’s Drugs

The Wall Street Journal
Lilly Alzheimer's Drug Disappoints in Trials
Aug. 24, 2012
The drug, solanezumab, failed to meet its primary goals in each study of slowing the erosion of memory and basic abilities

NY Times
Trials for Alzheimer’s Drug Halted After Bapineuzimab Poor Results
Johnson & Johnson and Pfizer
August 6, 2012

ABC News
Pfizer, Medivation Pull Plug on Alzheimer's Drug Dimebon
Jan 17. 2012
Another failure in phase III clinical testing. Dimebon finally sent to the trash heap

Why?

Answer: The disease is too far advanced. We need early diagnosis, so that early treatments become possible – early treatments are much more likely to be effective.
Detection of Early Stage AD MCI or even pre-symptomatic stages

Alzheimer’s Disease (AD):
AD pathology is underway 8-10 years before symptoms emerge

Estimate MMSE as a function of time

Minimental Exam

Early Detection Allows Early Treatment (prior to appearance of symptoms)

Funded by the Osteopathic Heritage Foundation

It takes 8 years to reach as score of 24/30 points, the cutoff for AD
Confirmation of 100% diagnostic accuracy for AD-driven MCI using only Testing Set subjects. Here, we note that our panel of 50 MCI biomarkers was derived from the analysis of 25 MCI subjects (called the Training Set). Here, as an indisputable test of diagnostic accuracy, we used the Training Set biomarkers to diagnose a Testing Set of subjects (those not used in biomarker discovery) and achieved the same 100% overall accuracy. In addition, we tested the specificity of the biomarker panel (the ability to distinguish AD-driven MCI from other diseases) and achieved specificity values exceeding 95%. These are fantastic results and we are very excited about it. This suggests that we have arrived at our final biomarker panel that will be used to design the test kit for Specific Aim 3.

### Diagnosis of Alzheimer’s Disease at the MCI Stage

**ADNI MCI subjects with Low CSF Abeta42 Levels**

100% overall diagnostic accuracy

<table>
<thead>
<tr>
<th>MCI (n = 25) vs.</th>
<th>Age Matched Controls</th>
<th>Early-Stage PD</th>
<th>Mild-Moderate AD</th>
<th>Multiple Sclerosis</th>
<th>Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td>*50</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Specificity %</td>
<td>100.0</td>
<td>96.0</td>
<td>98.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>PPV %</td>
<td>100.0</td>
<td>96.2</td>
<td>96.2</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>NPV %</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Accuracy %</td>
<td>100.0</td>
<td>98.0</td>
<td>98.7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Error %</td>
<td>0</td>
<td>2.0</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

This data is derived from Testing Set ADNI subjects only; i.e., subjects not involved in biomarker discovery.

*DeMarchall et al. 2015 manuscript in preparation*
Confirmation of 100% diagnostic accuracy for AD-driven MCI using only Testing Set subjects. Here, we note that our panel of 50 MCI biomarkers was derived from the analysis of 25 MCI subjects (called the Training Set). Here, as an indisputable test of diagnostic accuracy, we used the Training Set biomarkers to diagnose a Testing Set of subjects (those not used in biomarker discovery) and achieved the same 100% overall accuracy. In addition, we tested the specificity of the biomarker panel (the ability to distinguish AD-driven MCI from other diseases) and achieved specificity values exceeding 95%. These are fantastic results and we are very excited about it. This suggests that we have arrived at our final biomarker panel that will be used to design the test kit for Specific Aim 3.

### Staging of Alzheimer’s Disease

Distinguishing MCI from mild-moderate AD with 98.7% overall accuracy

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<td>100.0</td>
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<tr>
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<td>96.2</td>
<td>96.2</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>NPV %</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Accuracy %</td>
<td>100.0</td>
<td>98.0</td>
<td>98.7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Error %</td>
<td>0</td>
<td>2.0</td>
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<td>0</td>
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### Disease Specificity of MCI Biomarkers
Distinguishing MCI from other diseases
Nearly 100% overall accuracy

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<tr>
<th>MCI (n = 25) vs.</th>
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<td>96.2</td>
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</tr>
<tr>
<td>NPV %</td>
<td>100.0</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Accuracy %</td>
<td>100.0</td>
<td>98.0</td>
<td>98.7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Error %</td>
<td>0</td>
<td>2.0</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AD MCI biomarkers can distinguish MCI from early-stage PD, multiple sclerosis and breast cancer with nearly 100% overall accuracy.
# Capabilities of Our New AD and PD Diagnostic Tests

## Early Diagnosis

## Patient Management

## Test of Drug Efficacy

1. **Early detection and diagnosis** of AD and PD.

2. **Staging the disease** - allows physicians to **follow disease progress** in individual patients.

3. **Evaluate patient response to drug therapy** by monitoring patient progression through clinical stages.

4. **Confirmation of disease** in subjects enrolling in **clinical trials** of new drugs.

5. Allows **early enrollment** into clinical trials.

6. Evaluate **patient response to therapy (drug efficacy)** in clinical trials of new drugs.
Detection of Psychosis
Development of a panel of 50 Biomarkers

Total Subjects: 40
20 psychosis samples
20 age/gender matched healthy control samples

Training set (n = 20; used to select 50 biomarkers):
Testing set (n = 20; not involved in biomarker selection):

Clinical Diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>90.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Specificity %</td>
<td>90.0</td>
<td>100.0</td>
</tr>
<tr>
<td>PPV %</td>
<td>90.0</td>
<td>100.0</td>
</tr>
<tr>
<td>NPV %</td>
<td>90.0</td>
<td>90.9</td>
</tr>
<tr>
<td>Overall Accuracy %</td>
<td>90.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Overall Error %</td>
<td>10.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Collaboration underway with Tom Pollak – King’s College London
Biomarkers for Risk for Delirium
Collaboration with Johns Hopkins

Compare serum/CSF from patients with history of delirium

Can we Identification of brain-reactive autoantibodies?
If yes, this may reveal the identities of many pathological autoantibodies.
Multiple Sclerosis: Another autoimmune disease

In small patient group (n=20) – Relapsing – Remitting – 95% accuracy
Multiple Sclerosis (MS) Pilot study
Results: >95% overall accuracy

Diagnosis made using 15 protein targets specific for binding to MS autoantibody biomarkers

Patients samples = 10 with MS

Control samples = 20

Diagnostic Sensitivity – 96.8%

Diagnostic Specificity – 96.7%

Although only a small pilot study, results suggest that this approach will be successful. We have recently received funding from the Boye Foundation for a larger study, which is currently underway.
Concussion and Traumatic Brain Injury

- Work Underway
  - Collaboration with Dr. Randel Swanson, DO-PhD
    - University of Pennsylvania
Schizophrenia?
Do autoantibodies cause it? Can autoantibodies diagnose it?

Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood–brain barrier integrity

In 2007, a multifaceted syndrome, associated with anti-NMDA receptor autoantibodies (NMDAR-AB) of immunoglobulin-G isotype, has been described, which variably consists of psychosis, epilepsy, cognitive decline and extrapyramidal symptoms. Prevalence and significance of NMDAR-AB in complex neuropsychiatric disease versus health, however, have remained unclear. We tested sera of 2817 subjects (1325 healthy, 1081 schizophrenic, 263 Parkinson and 148 affective-disorder subjects) for presence of NMDAR-AB, conducted a genome-wide genetic association study, comparing AB carriers versus non-carriers, and assessed their influenza AB status. For mechanistic insight and documentation of AB functionality, in vivo experiments involving mice with deficient blood–brain barrier (ApoE−/−) and in vitro endocytosis assays in primary cortical neurons were performed. In 10.5% of subjects, NMDAR-AB (NR1 subunit) of any immunoglobulin isotype were detected, with no difference in seroprevalence, titer or in vitro functionality between patients and healthy controls. Administration of extracted human serum to mice influenced basal and MK-801-induced activity in the open field only in ApoE−/− mice injected with NMDAR-AB-positive serum but not in respective controls. Seropositive schizophrenic patients with a history of neurotrauma or birth complications, indicating an at least temporarily compromised blood–brain barrier, had more neurological abnormalities than seronegative patients with comparable history. A common genetic variant (rs524991, P = 6.15E−08) as well as past influenza A (P = 0.024) or B (P = 0.006) infection were identified as predisposing factors for NMDAR-AB seropositivity. The > 10% overall seroprevalence of NMDAR-AB of both healthy individuals and patients is unexpectedly high. Clinical significance, however, apparently depends on association with past or present perturbations of blood–brain barrier function.

Molecular Psychiatry advance online publication, 3 September 2013; doi:10.1038/mp.2013.110

Collaboration with Prof. H. Ehrenreich at Max Planck, Gottingen, Germany
Detection of the Presence and Extent of BBB Breakdown In Post-Surgical Delirium Patients

Detect Autoantibodies in the Cerebrospinal Fluid (CSF)

BBB Intact
- No Abs in CSF

READOUT
BBB Leak – Abs in CSF

Collaboration with F Sieber at John’s Hopkins.

Protein Microarray
Stages of Breast Cancer

0. Abnormal cells in lining of the ducts or sections of the breast. Results in increased risk of developing cancer in both breasts.

1. Cancer in the breast tissue tumor less than 1 inch across.

2. Cancer in the breast tissue tumor less than 2 inches across. Cancer may also spread to auxiliary lymph nodes.

3. Tumor is larger than 2 inches across with extensive spread to auxiliary or nearby lymph nodes. Possible dimpling, inflammation or change of skin color.

4. Spread of cancer beyond the immediate region of the breast.

Survival Rate:
- 100% Survival Rate
- 98% Survival Rate
- 88% Survival Rate
- 52% Survival Rate
- 16% Survival Rate
Diagnosis of Stage 0 - 1 Breast Cancer Using Serum Autoantibodies

1. Experimental Design

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Gender</th>
<th>Sample size</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>Female</td>
<td>30</td>
<td>47 ± 5.8</td>
</tr>
<tr>
<td>Control</td>
<td>Female</td>
<td>23</td>
<td>52 ± 16</td>
</tr>
</tbody>
</table>

Detected 301 autoantibodies with significant prevalence difference (P<0.01)

2. Diagnosis of breast cancer using the top 50 biomarkers

Random Shuffling → Training Group 70%, 37 samples → Testing Group 30%, 16 samples

Training Error: 4.3% on average
Testing Error: 2.5% on average

<table>
<thead>
<tr>
<th>Training Error</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Trial 6</th>
<th>Trial 7</th>
<th>Trial 8</th>
<th>Trial 9</th>
<th>Trial 10</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.70%</td>
<td>2.70%</td>
<td>8.10%</td>
<td>5.41%</td>
<td>5.41%</td>
<td>2.70%</td>
<td>2.70%</td>
<td>5.41%</td>
<td>5.41%</td>
<td>2.70%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Testing Error</td>
<td>1/16</td>
<td>0/16</td>
<td>0/16</td>
<td>0/16</td>
<td>0/16</td>
<td>1/16</td>
<td>0/16</td>
<td>0/16</td>
<td>0/16</td>
<td>1/16</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

Overall Results for Early Stage Breast Cancer - >95% accuracy!
**Future Plans and Directions**

Schizophrenia? Psychosis – collaboration with Tom Pollak – Kings College London
Multiple Sclerosis – pilot study complete – 95% accuracy for RR Type
Traumatic Brain Injury – concussion injuries – UPenn collaboration
Risk for Post-Surgical Delirium – Collaboration with Johns Hopkins

**Long-term goal:** Construct a Multi-Disease Diagnostic Blood Test

Only 20-50 autoantibodies are needed for each diagnostic.
Each array can currently hold over 20,000 protein targets.
There is plenty of room for hundreds of diagnostic tests on a single microarray.

Neurodegenerative diseases

Various cancers

Alz  Park  MS  Breast  Lung  Pancreatic
If treatments are effective, there should be less disease-associated debris production and a corresponding decrease in autoantibody biomarkers in the blood.

Concept

Comparison of blood samples before and after drug treatment
With Profound Thanks

Current Nagele Lab
Nimish Acharya, PhD
Mary Kosciuk, PhD
Cassandra DeMarshall, PhD Student
Eric Goldwaser, DO-PhD student
Abhirup Sarkar, PhD Student
George Dodsey, PhD Student
Eric Nagele, DO, Neurology Resident

Recent Past Nagele Lab Members
Peter Clifford, DO-PhD
Gilbert Siu, DO-PhD
Eli Levin, DO-PhD
Min Han, MD-PhD

Honorary Lab Members
Janice Ciesielski
Benjamin Belinka, PhD
Gerald Carey

Collaborators
RowanSOM
Anita Chopra and the NJISA
Martin Forsberg, MD
Venkat Venkataraman Lab

GlaxoSmithKline
Colin Macphee, PhD

Johns Hopkins
Frederick Sieber, MD

Univ. of Penn
Douglas Smith, MD-PhD
Randel Swanson, DO-PhD
Kings College London
Thomas Pollak, PhD

City Univ. of NY
Hoau-Yan Wang, PhD

Funding
The Osteopathic Heritage Foundation
Richard Vincent – President & CEO
Michael J Fox Foundation
Foundation Venture Capital Group
Boye Foundation
Alzheimer’s Association
Thank You