Alzheimer's Disease Mechanisms and Early Diagnosis at Mild Cognitive Impairment

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RowanSOM

DISCLOSURES

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

Beren Technologies, Inc.
Founder , Chief Scientific Officer
Stockholder

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Michael J Fox Foundation
Alzheimer's Association
Osteopathic Heritage Foundation
Foundation Venture Capital Group
Boye Foundation
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AD Statistics

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.

Alzheimer's Disease Hits Home

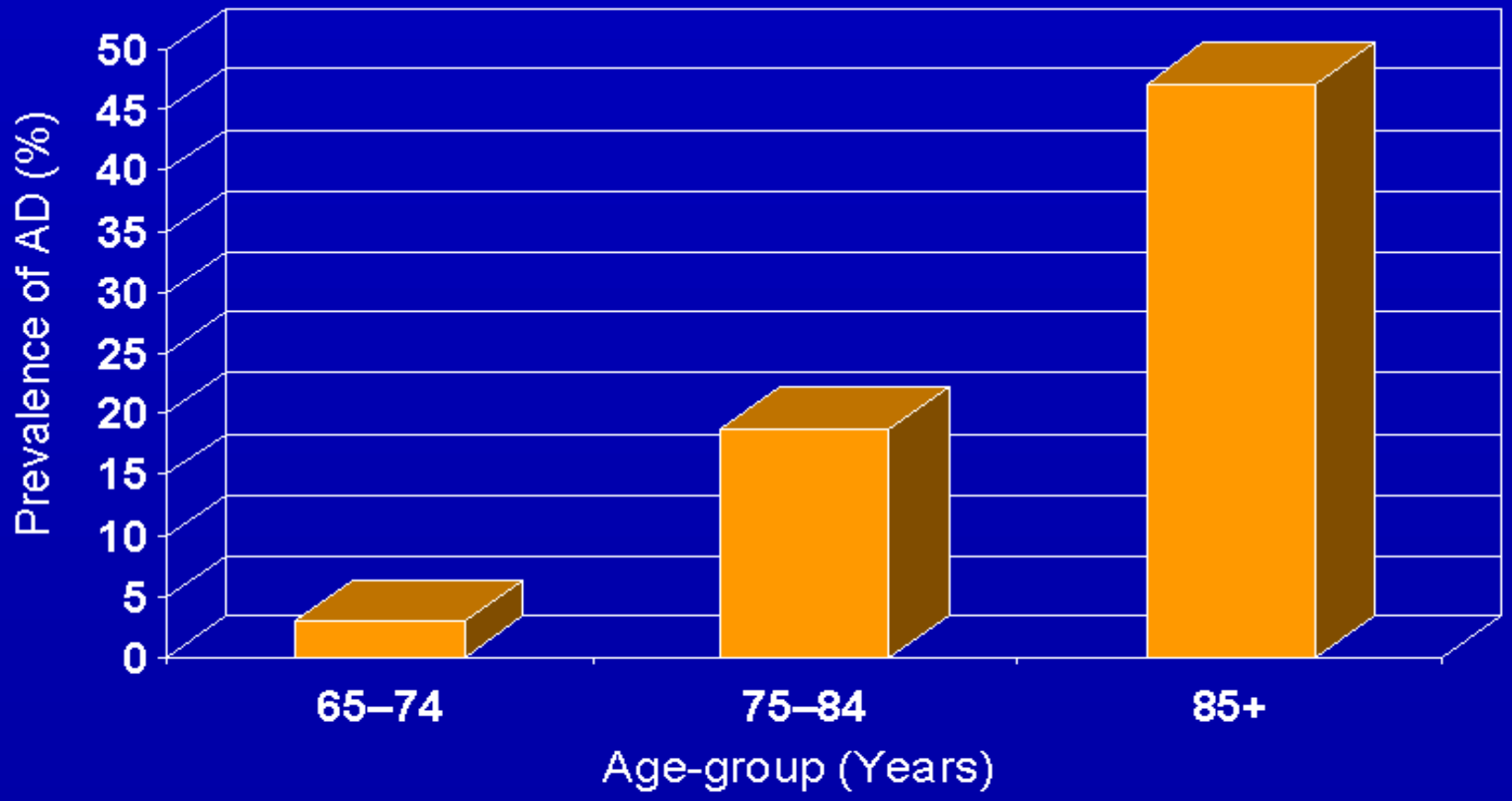
Nagele Family

My Grandparents

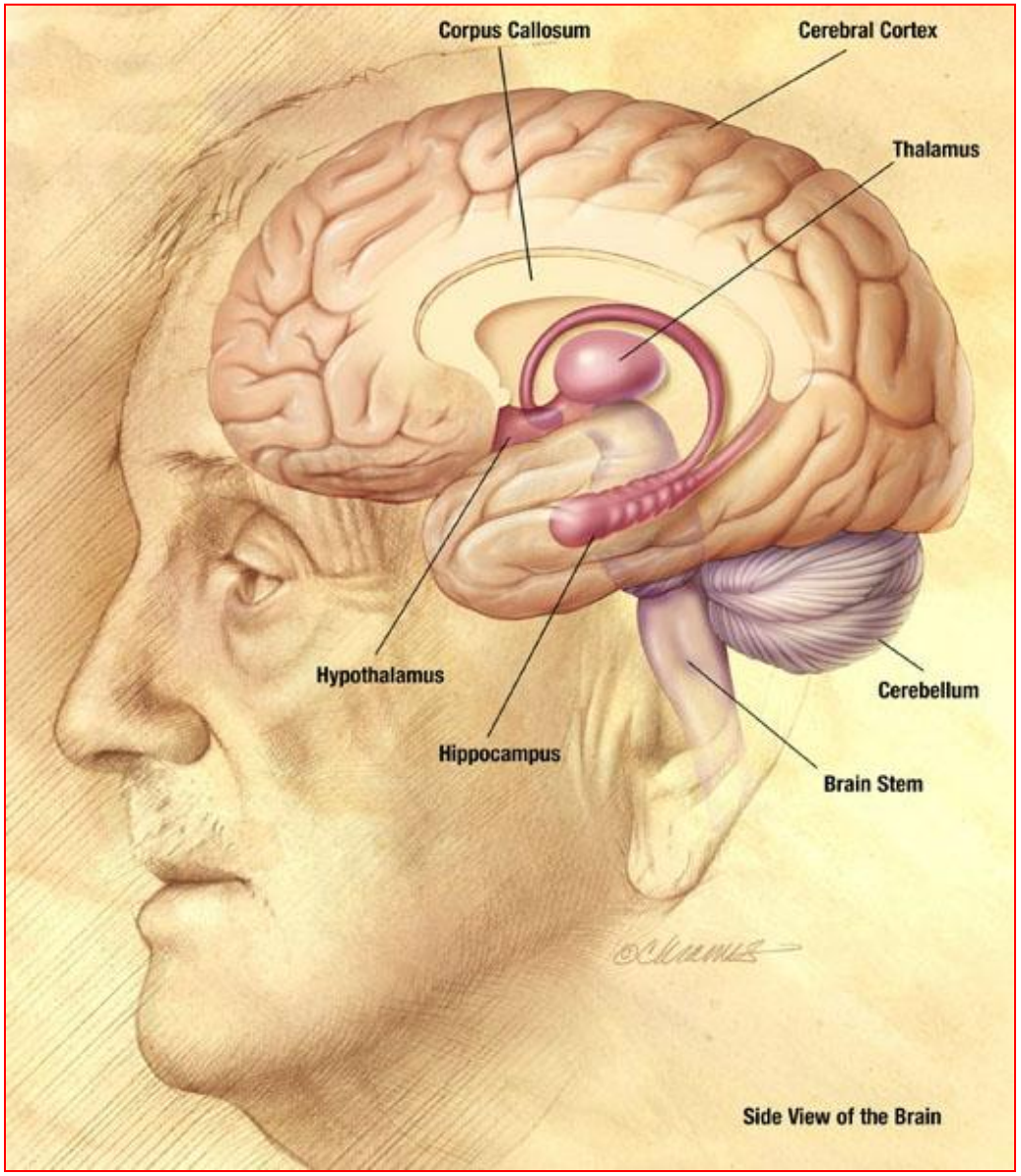
My Mother's Siblings

And now my father and mother-in-law

Prevalence of AD by Age



How Does AD Happen?



Inside the Human Brain

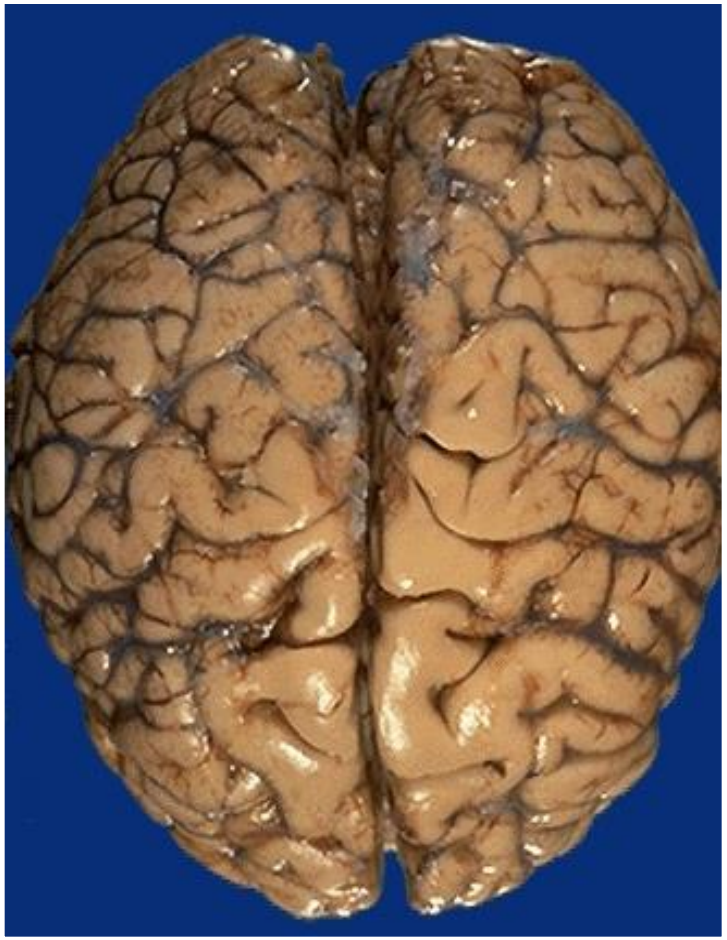
To understand AD, it's important to know a bit about the brain…

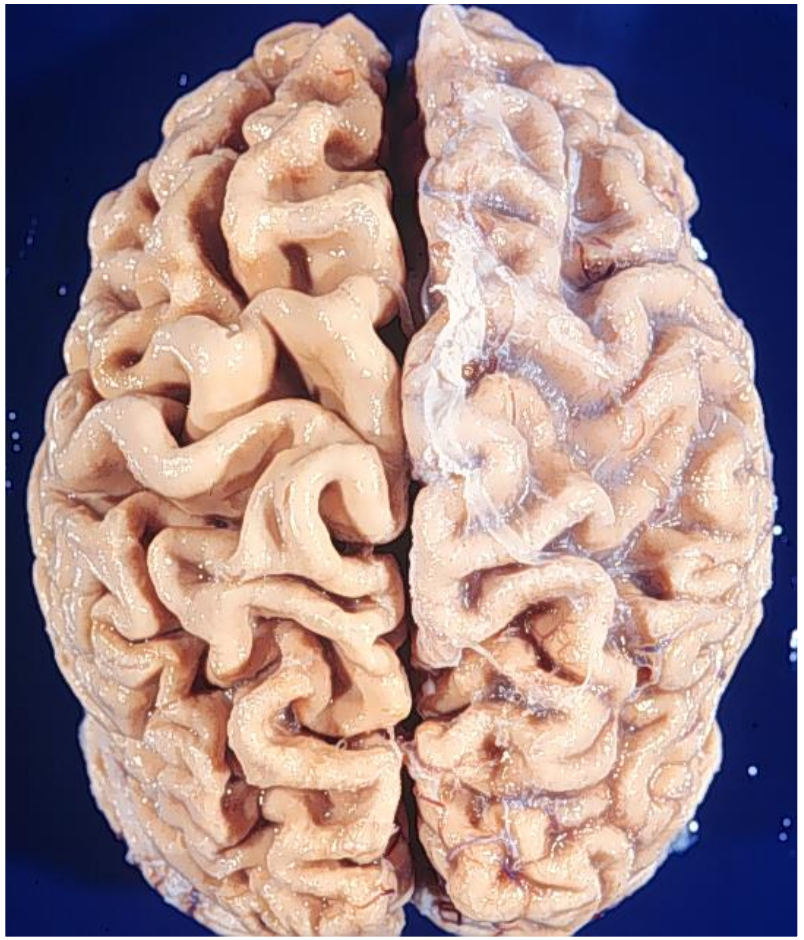
The Brain's Vital Statistics

- Adult weight: about 3 pounds
- Adult size: a medium cauliflower
- Number of neurons: 100,000,000,000 (100 billion)
- Number of synapses (the gap between neurons): 100,000,000,000,000 (100 trillion)

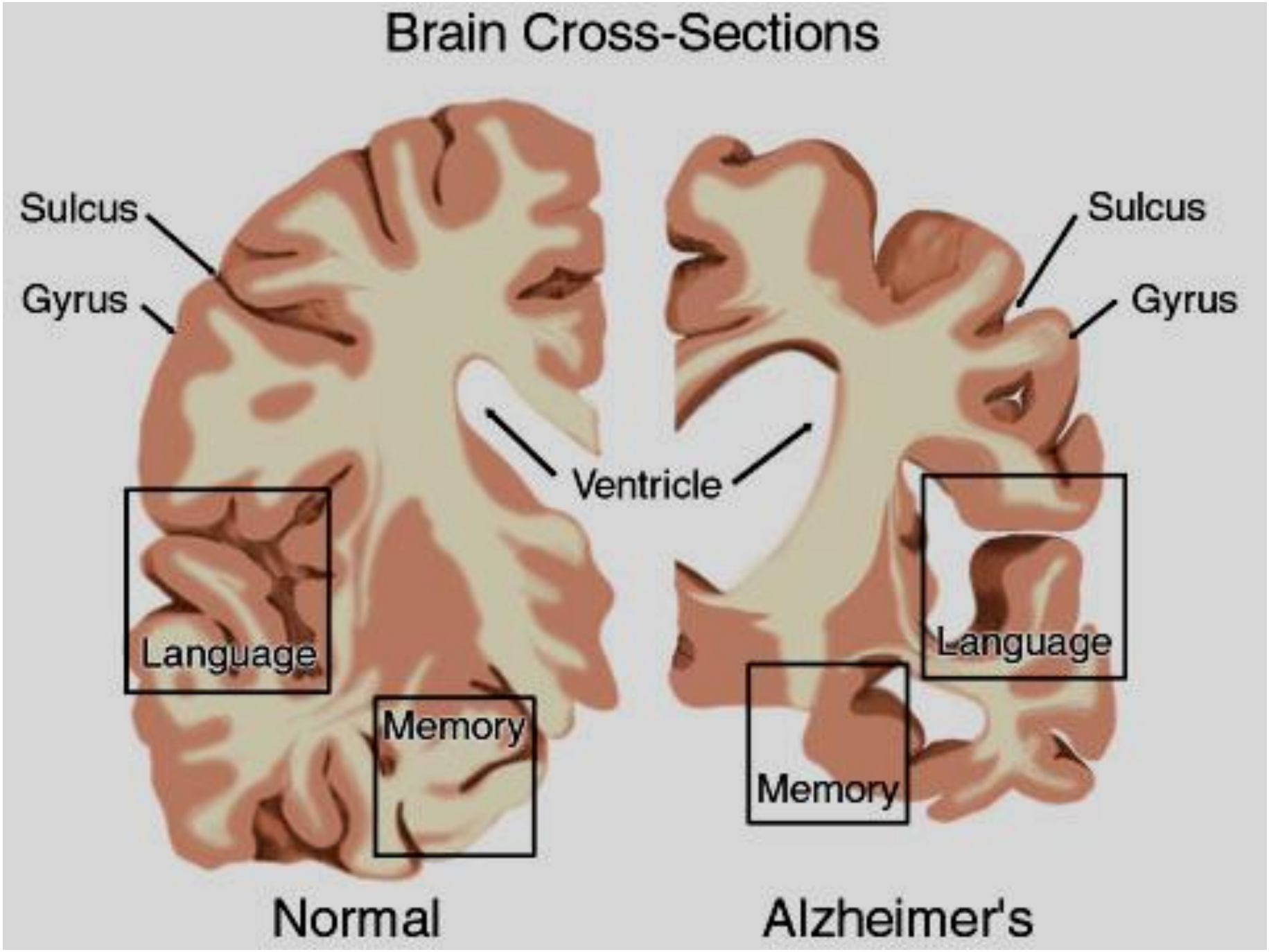
Normal vs Alzheimer Brain

Gross Pathology

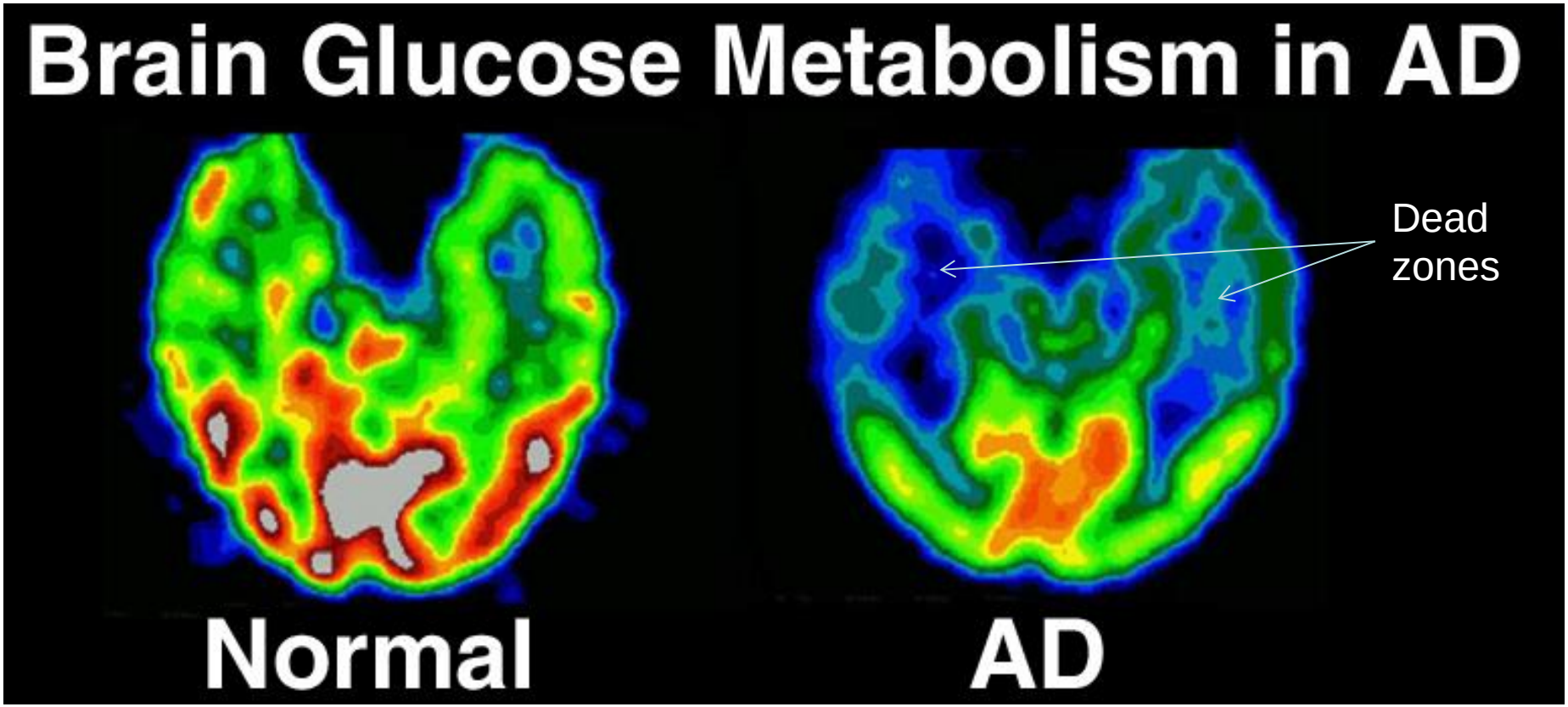




Brain shrinkage – thinning of gyral folds – broadening of fissures


Brain Cross-Sections
Sulcus
Gyrus
Ventricle
Language
Memory
Sulcus
Gyrus
Language
Memory
Normal
Alzheimer's

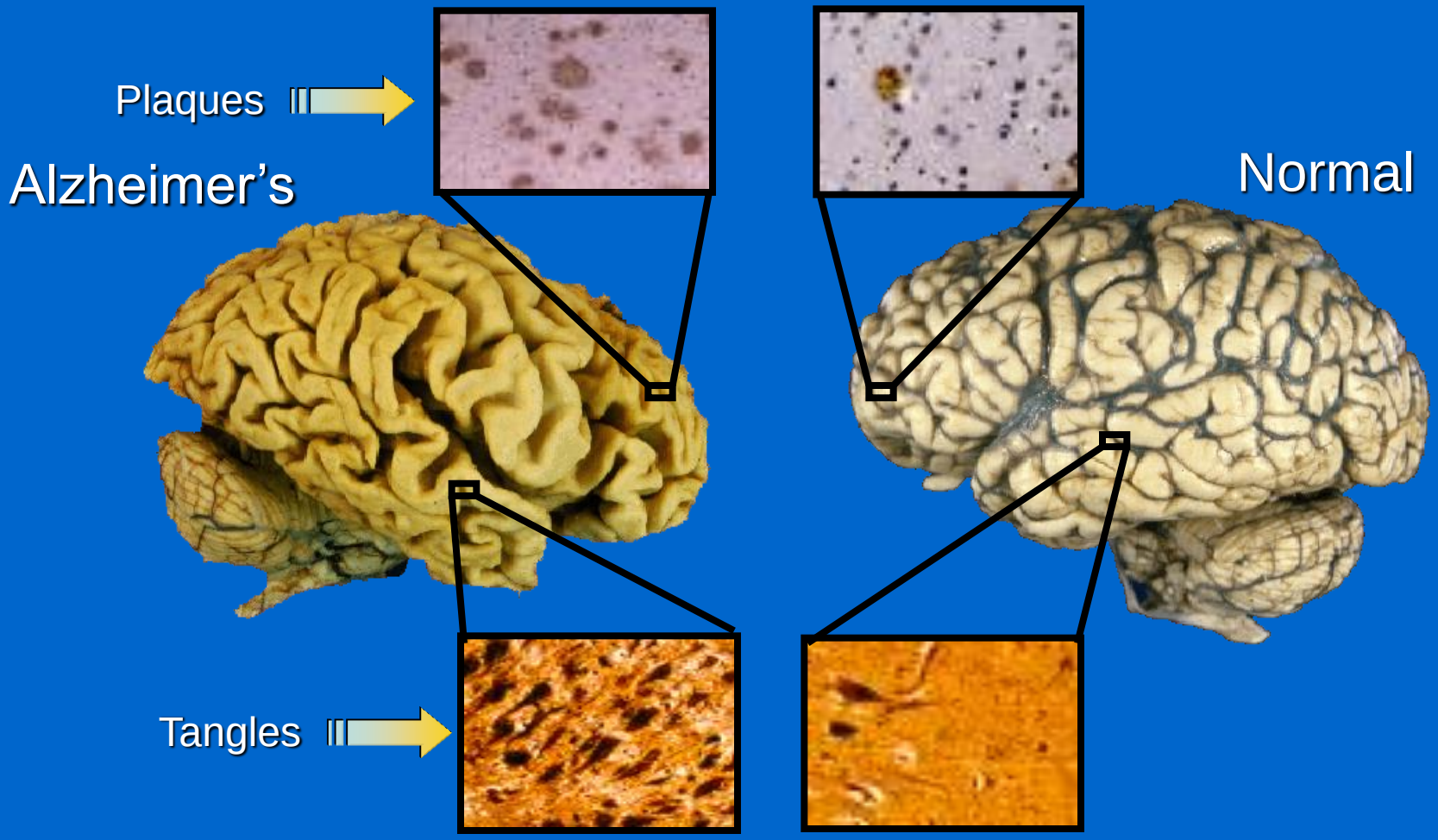
PET scans show much reduced glucose utilization in the AD brain compared to controls



Reason: Rampant cell death means less cells capable of metabolizing glucose

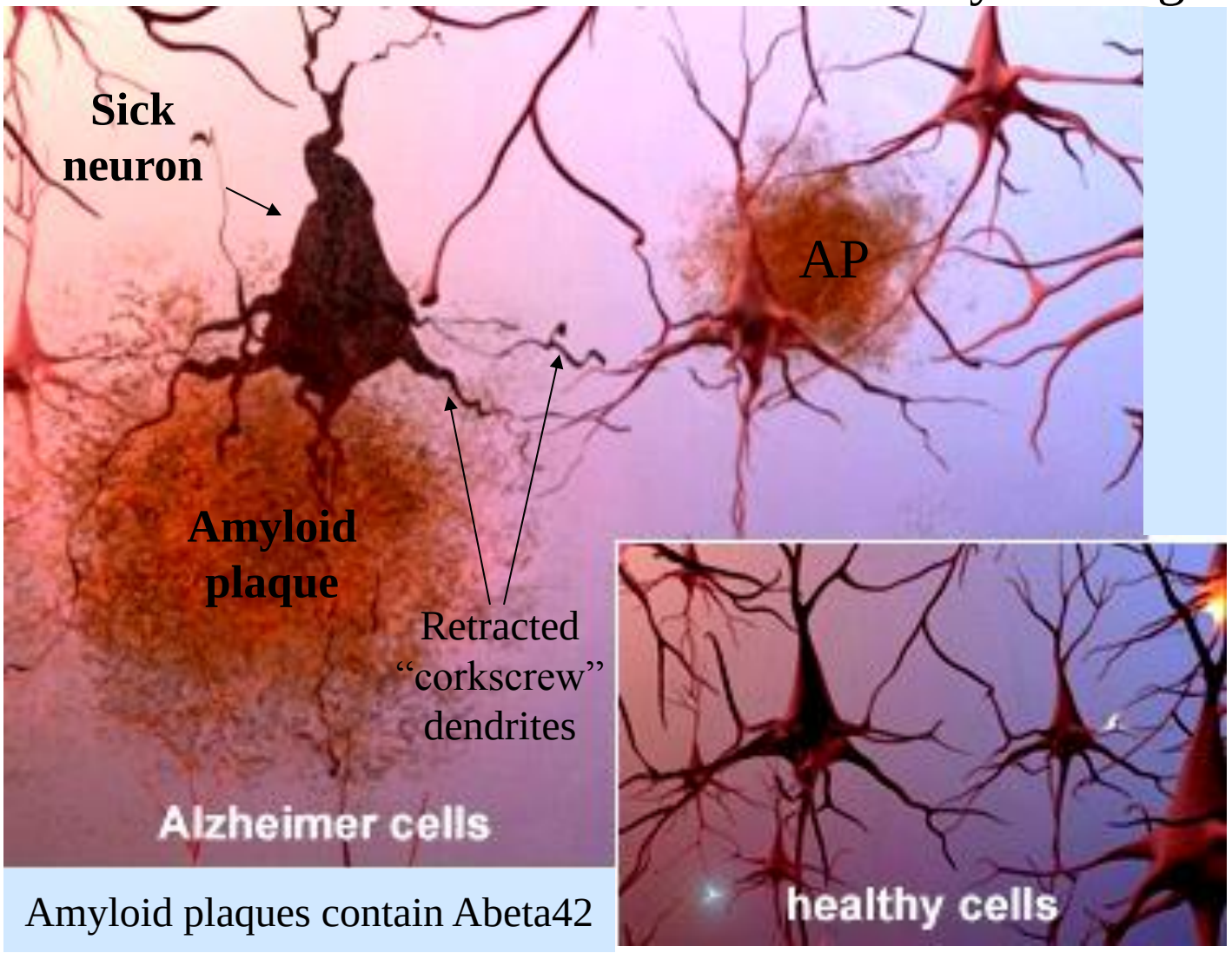
Amyloid Plaques and Neurofibrillary Tangles

Pathological Hallmarks of Alzheimer's Disease



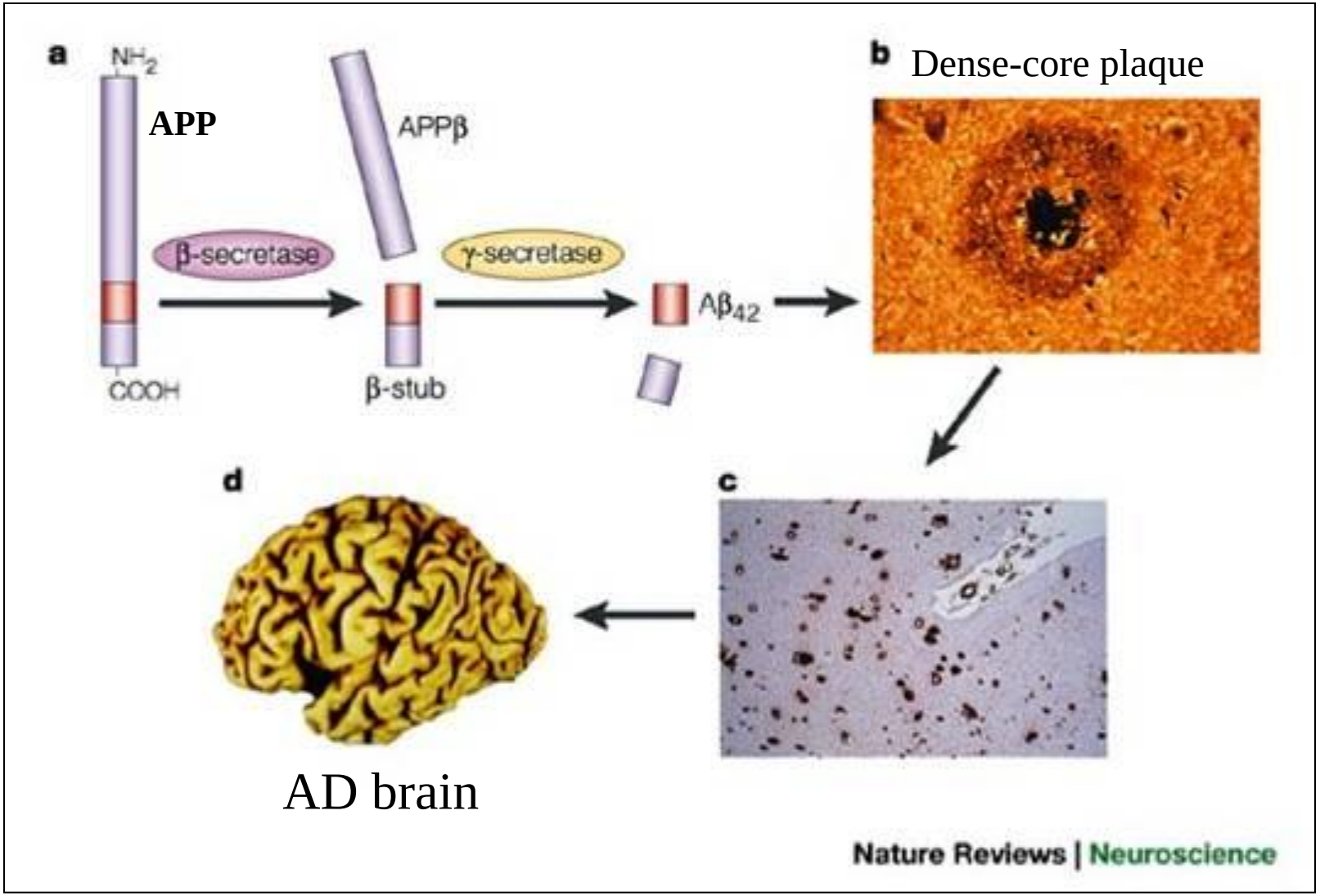
Courtesy of Harry Vinters, MD.

Amyloid (Abeta42) deposition makes neurons sick.
Sick neurons retract their axons and dendrites and lose synaptic connections with each other → Loss of memory and cognition



Amyloid plaques contain Abeta42

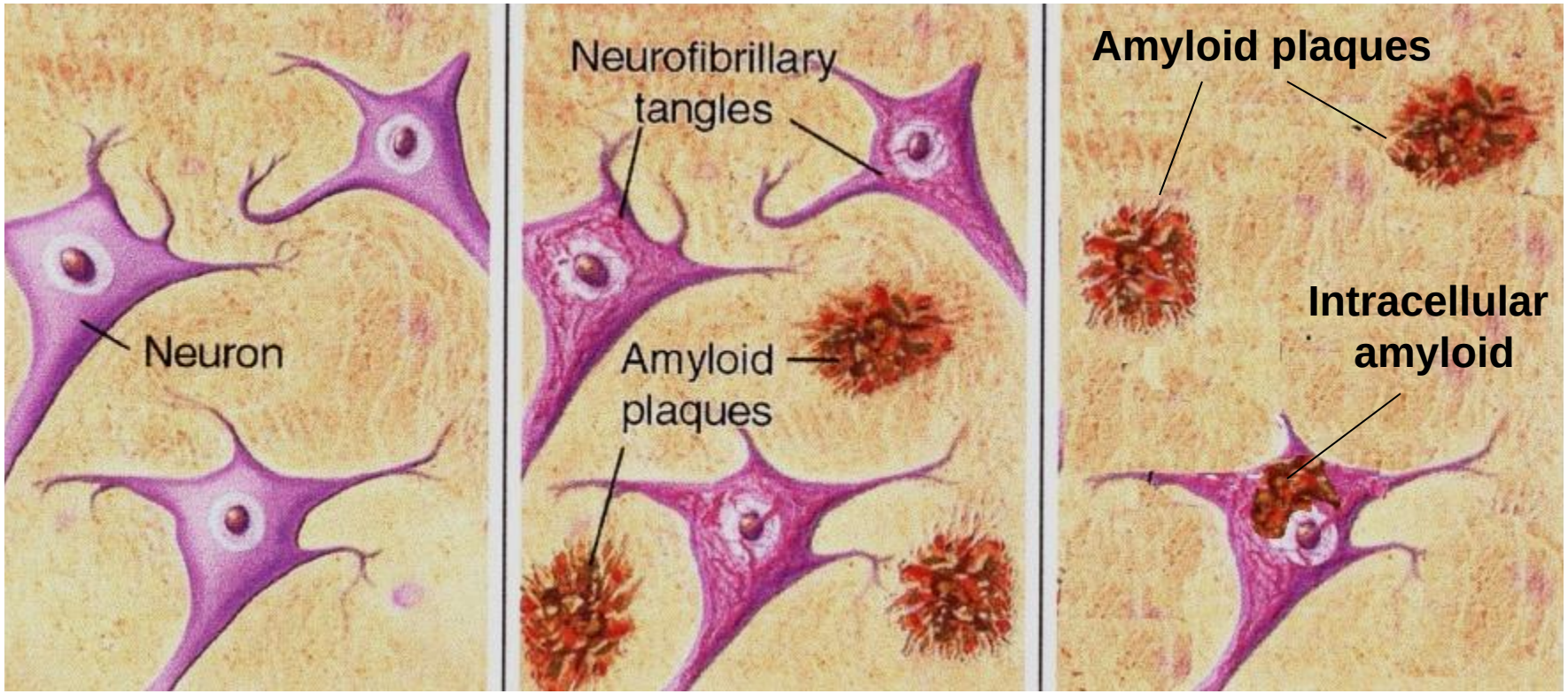
Amyloid beta (Abeta42) deposits in the brain and arises from sequential cleavage of the amyloid precursor protein



Alzheimer's Disease

How Amyloid (Abeta42) Deposits in the Brain

Old Model | **Our New Model**



Normal healthy brain

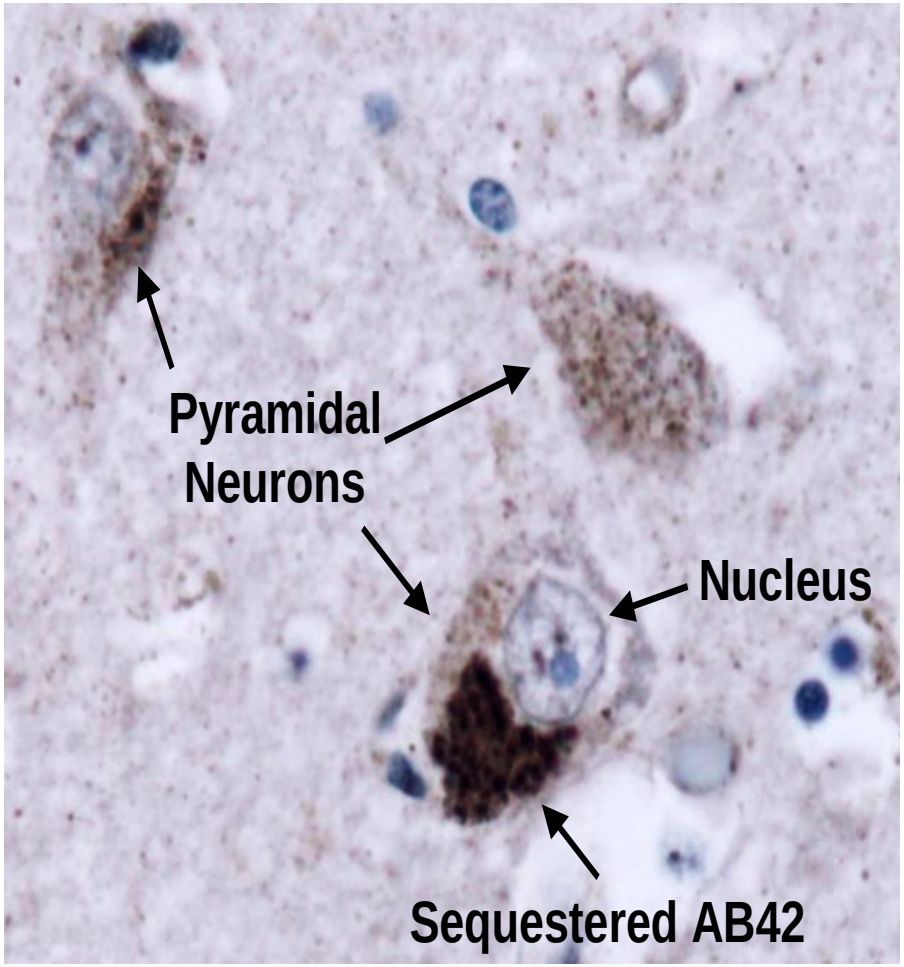
AD brain
Extracellular amyloid

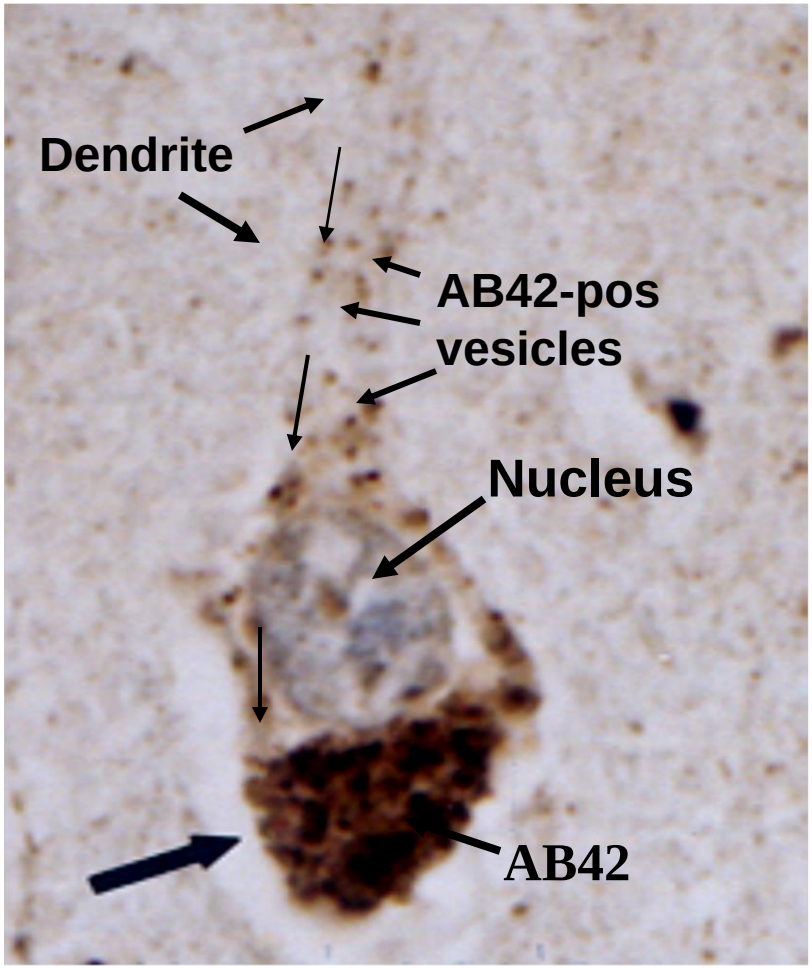
AD brain
Intracellular amyloid

New Model Means New Potential Drug Targets

In AD, neurons accumulate excessive Abeta42 (amyloid) prior to cell lysis and amyloid plaque formation

AD brain sections immunostained for Abeta42 (AB42)

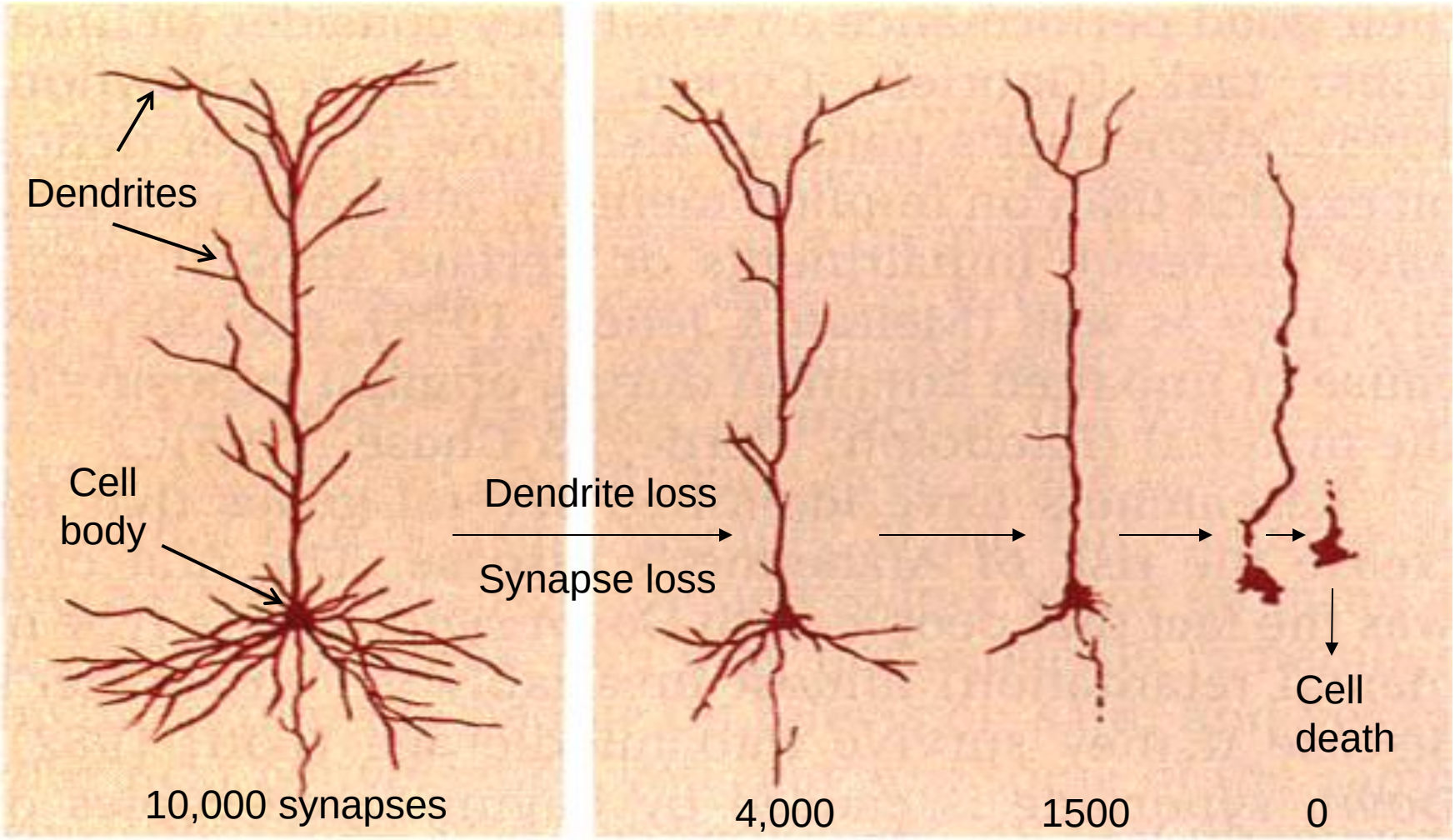




Note abundant Abeta42-positive (presumably) endocytic vesicles

Alzheimer's is a synaptic loss disease

Normal versus progressively degenerating pyramidal neuron



Months to years – Synaptic loss is tied to onset of symptoms

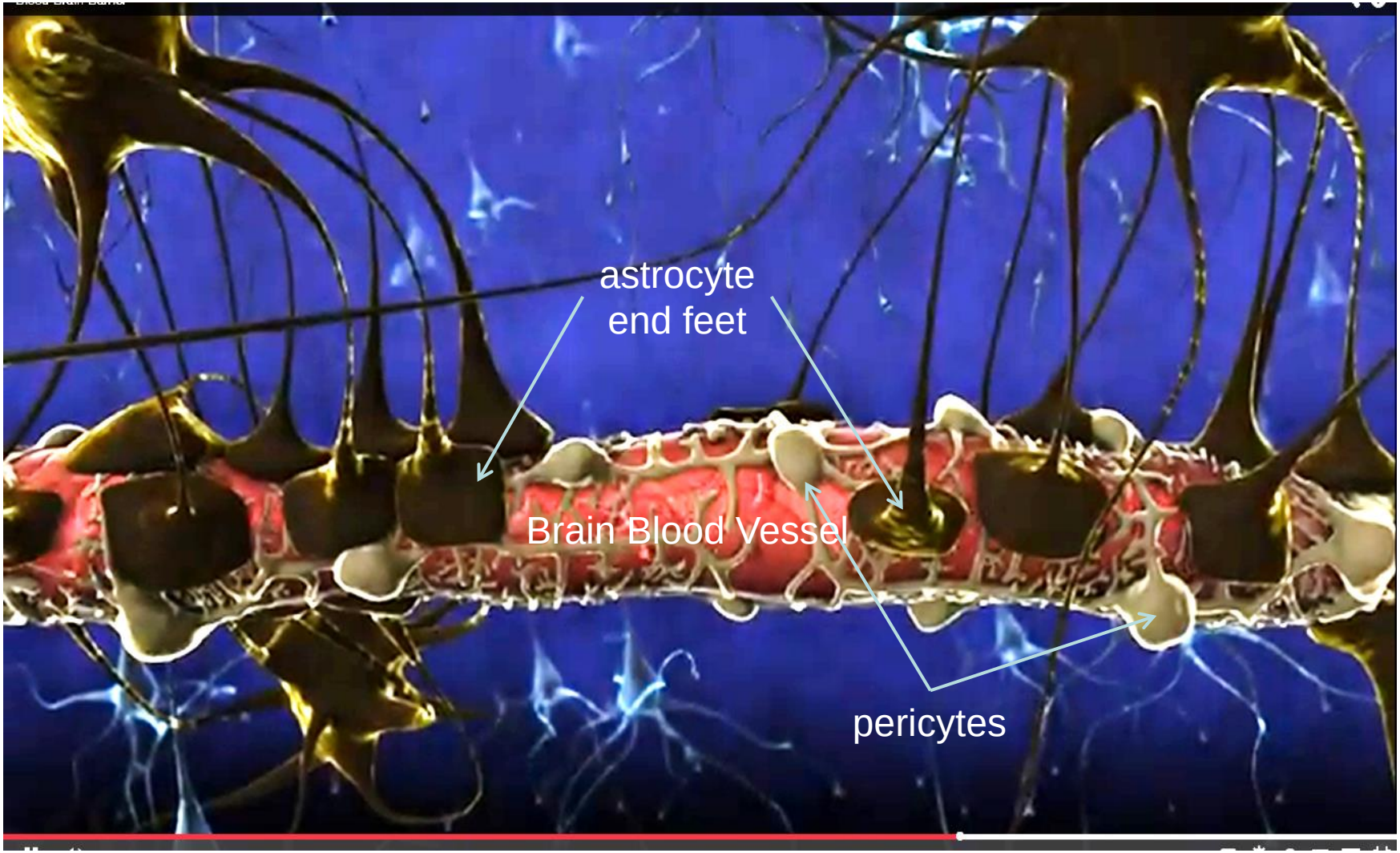
How can we stop amyloid deposition in the brain?

Answer: Find the source

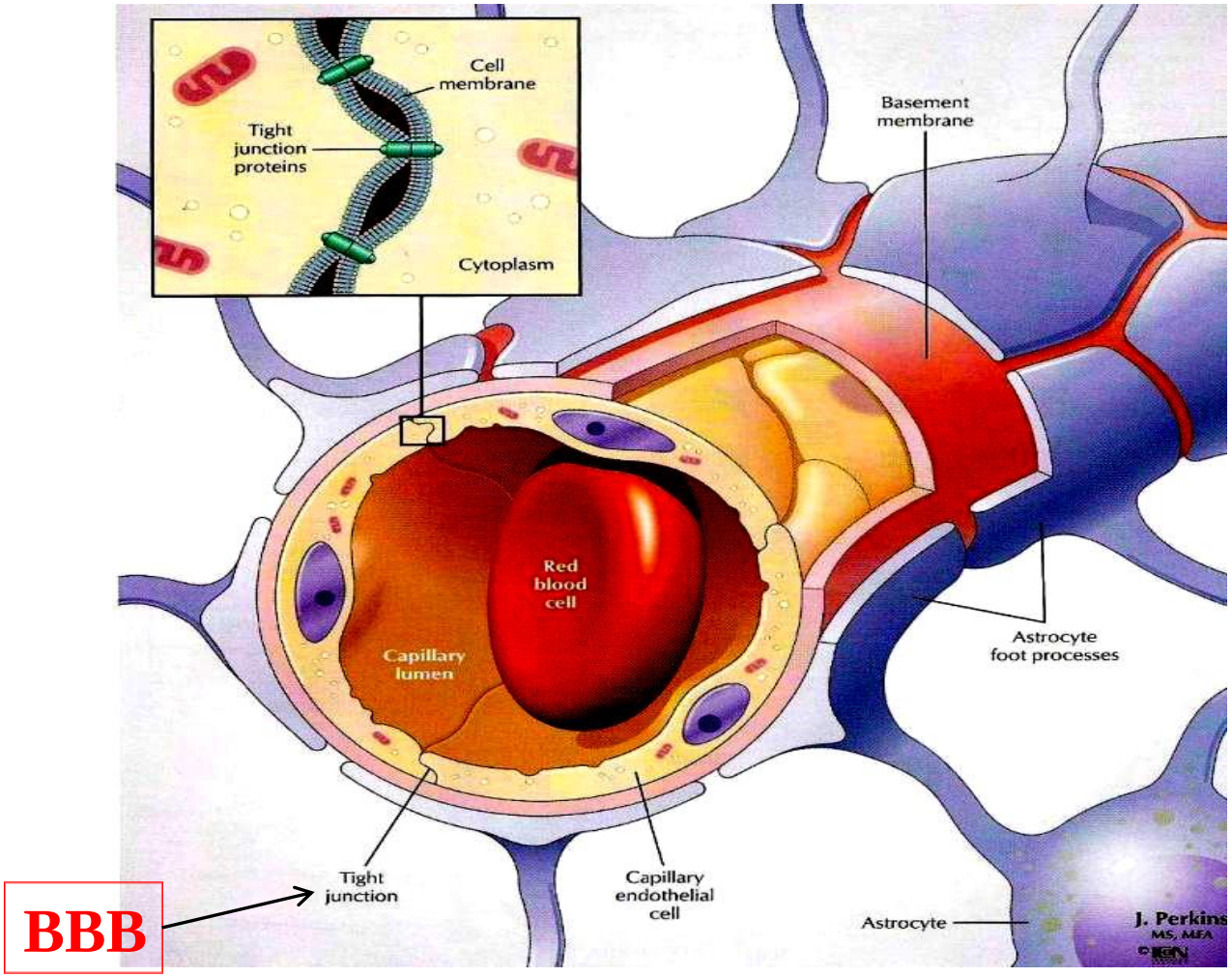
The blood is a major source.

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

Blood-brain barrier



Tight junctions:
the primary structural correlate of the BBB



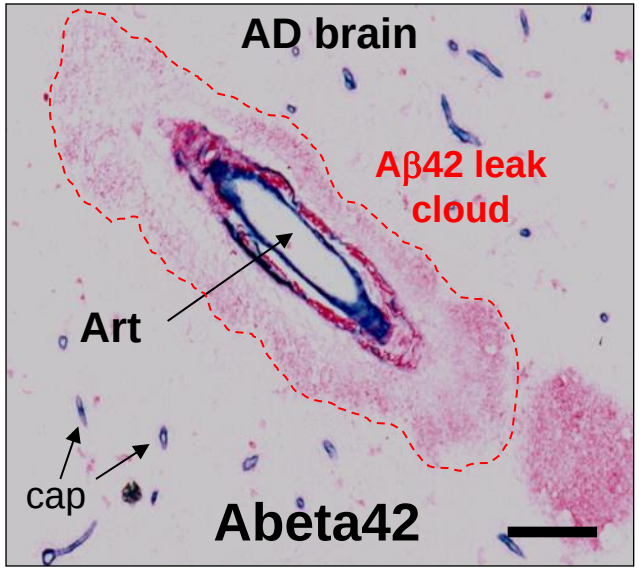
BBB

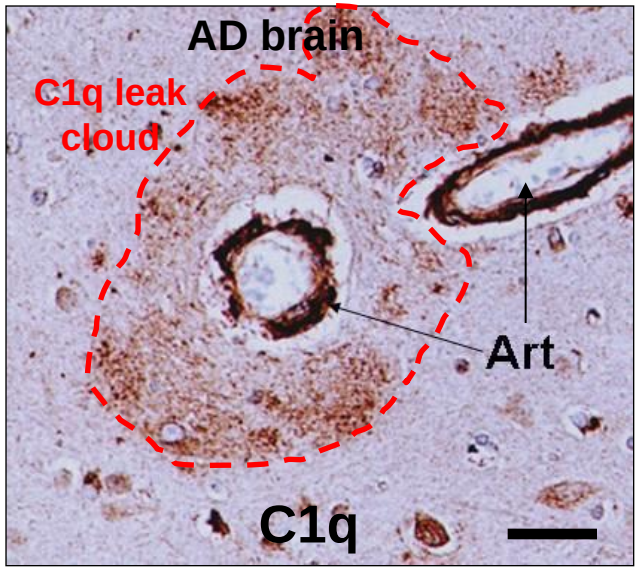
The BBB prevents the entry of most plasma components into the brain

In all AD brains, the BBB is defective

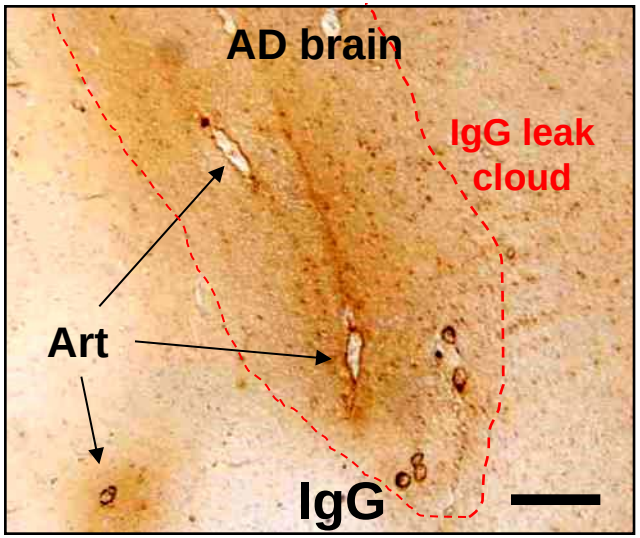
Plasma components (including Abeta42) can now leak into the brain and form vascular leak clouds

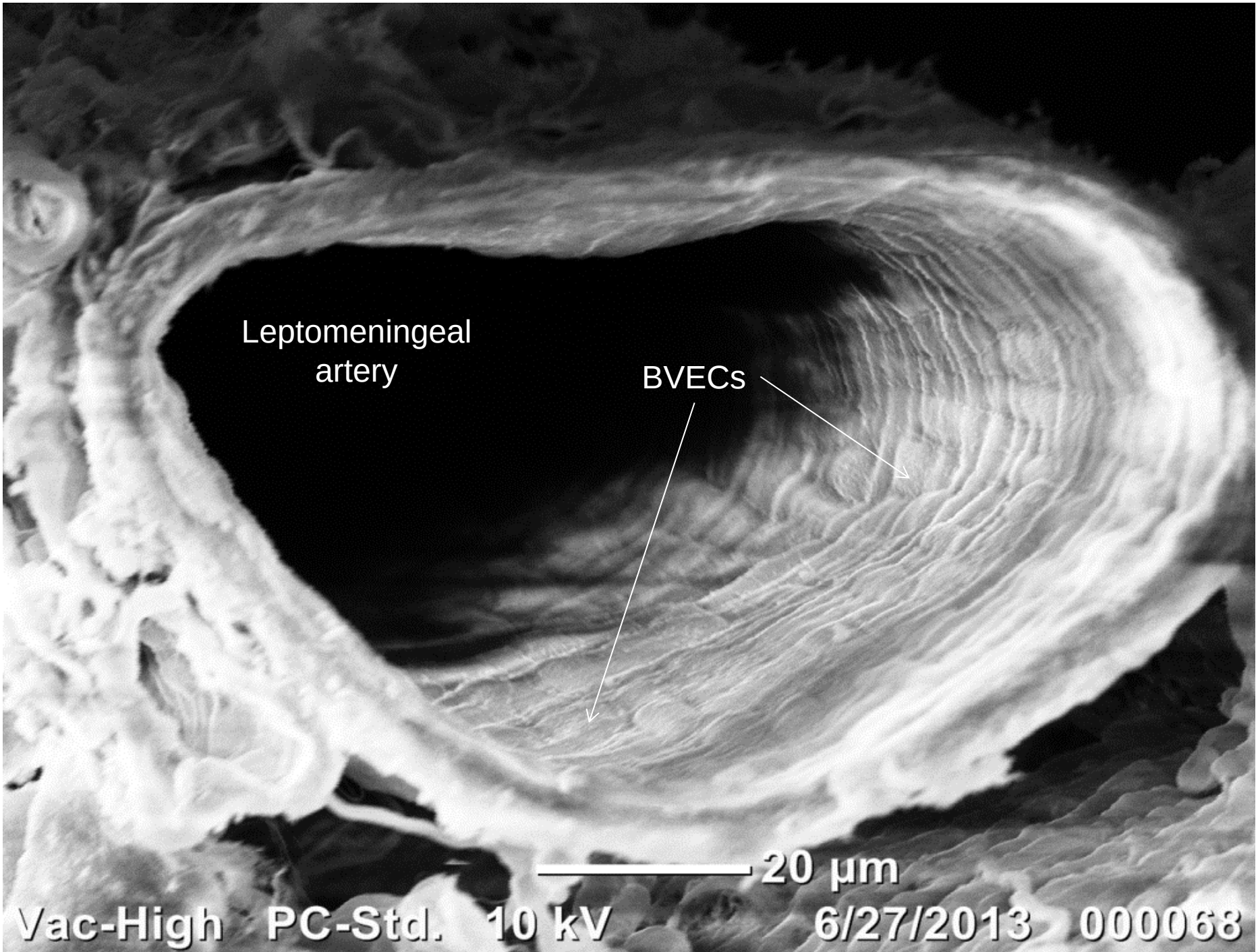
Abeta42



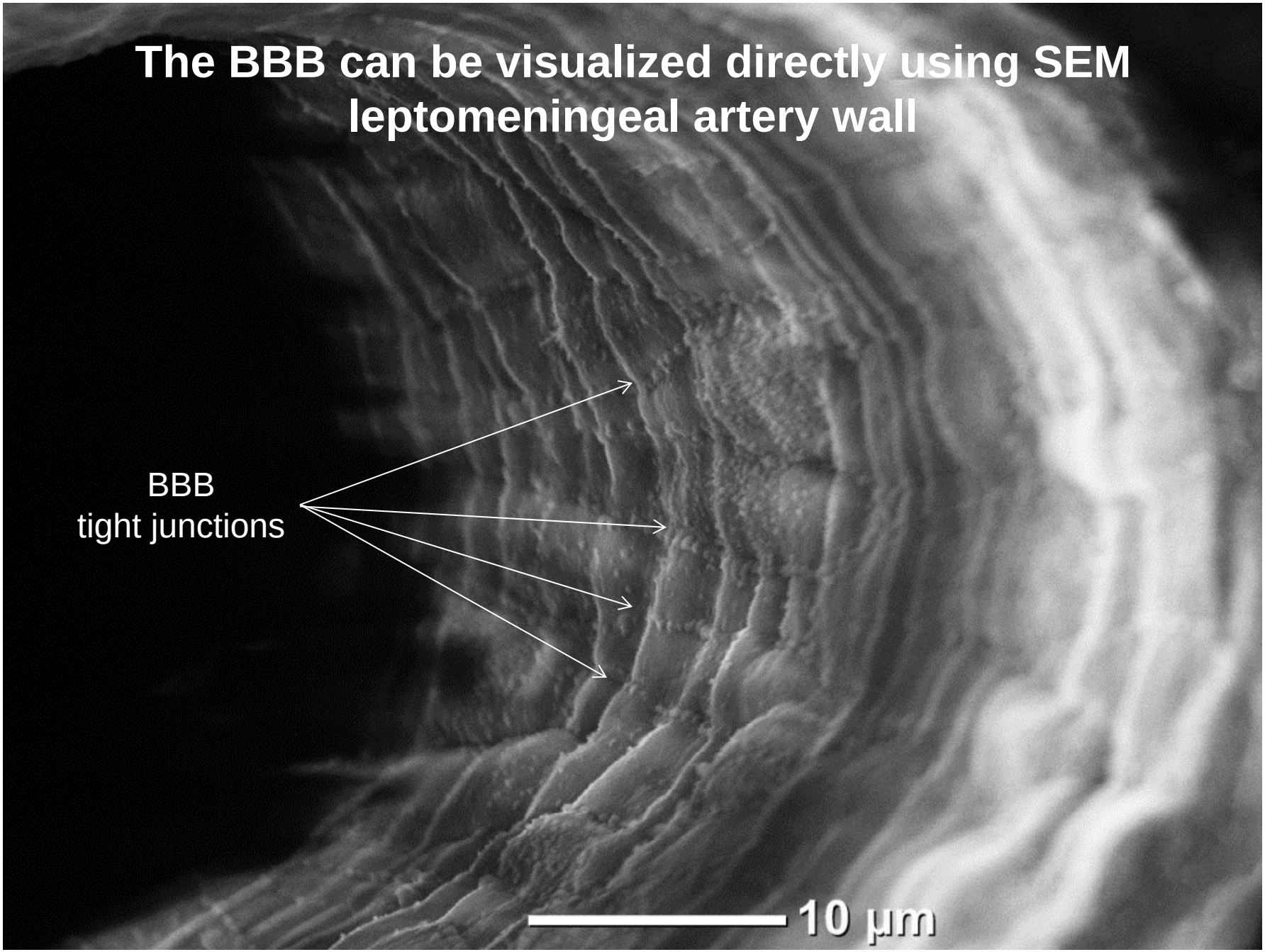


Abeta42 leak suggests that the main source of soluble amyloid beta (Abeta) in the brain is the blood

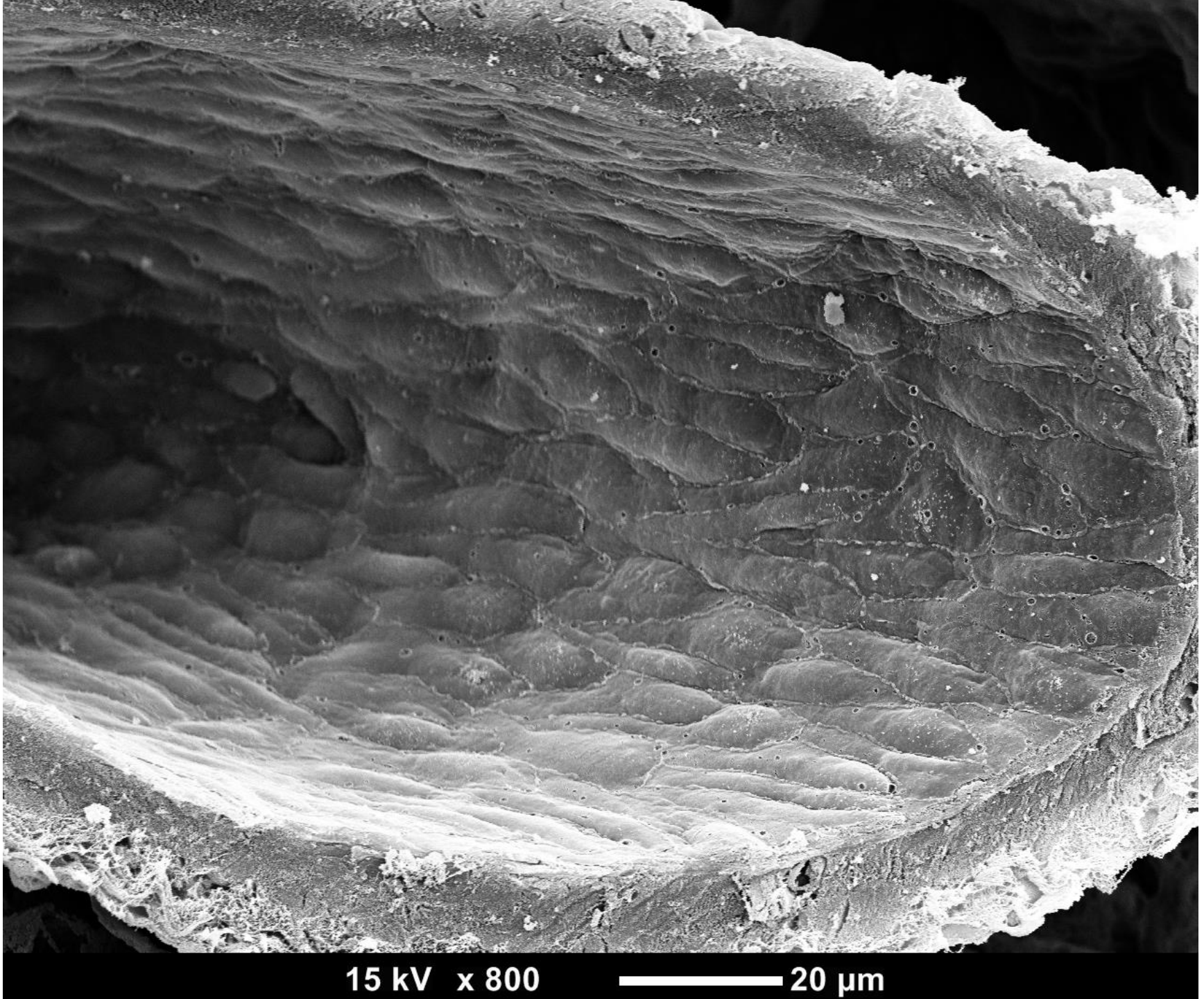



Leptomeningeal
artery
BVECs
20 µm
Vac-High PC-Std. 10 kV 6/27/2013 000068

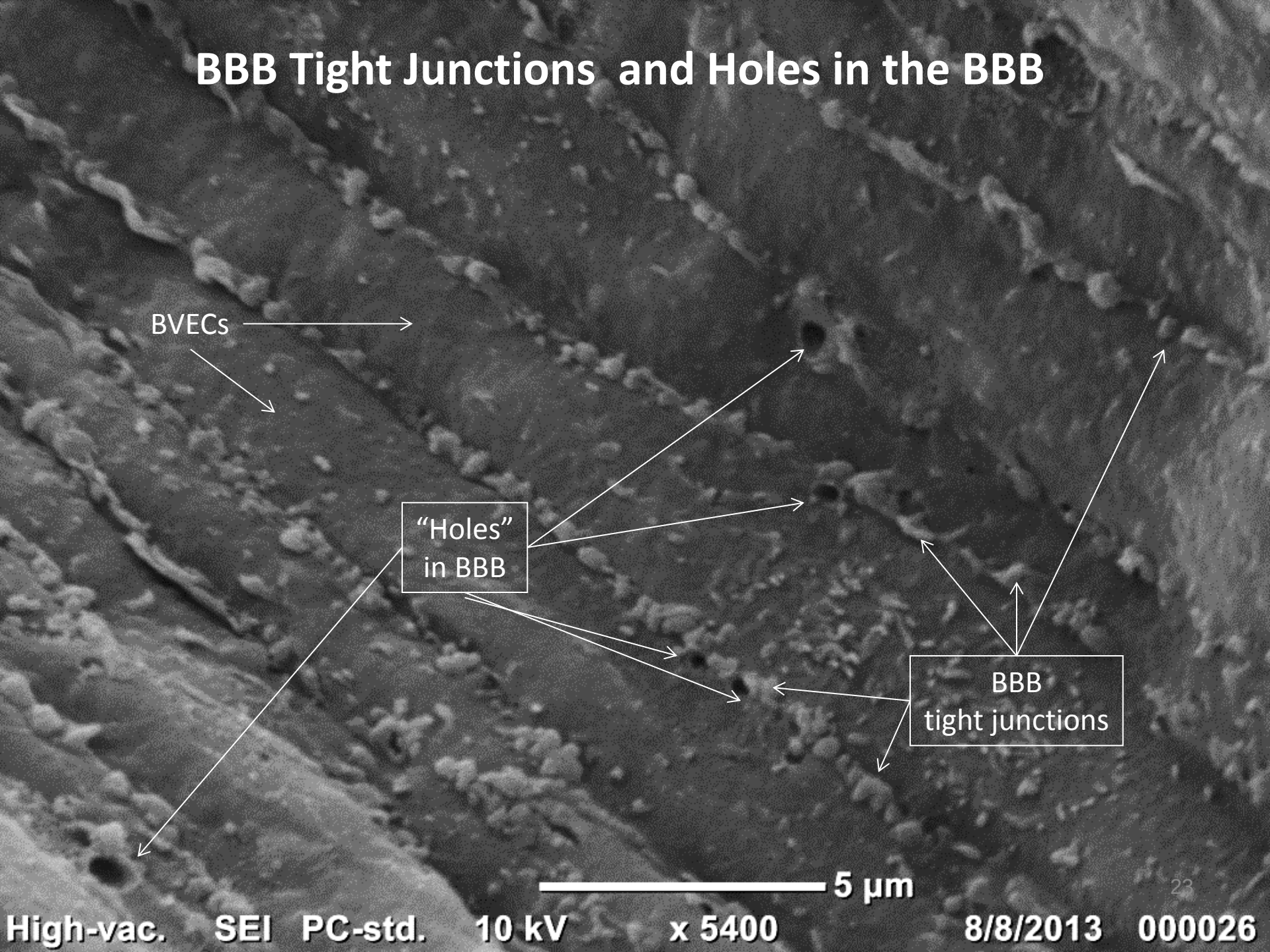
The BBB can be visualized directly using SEM
leptomeningeal artery wall



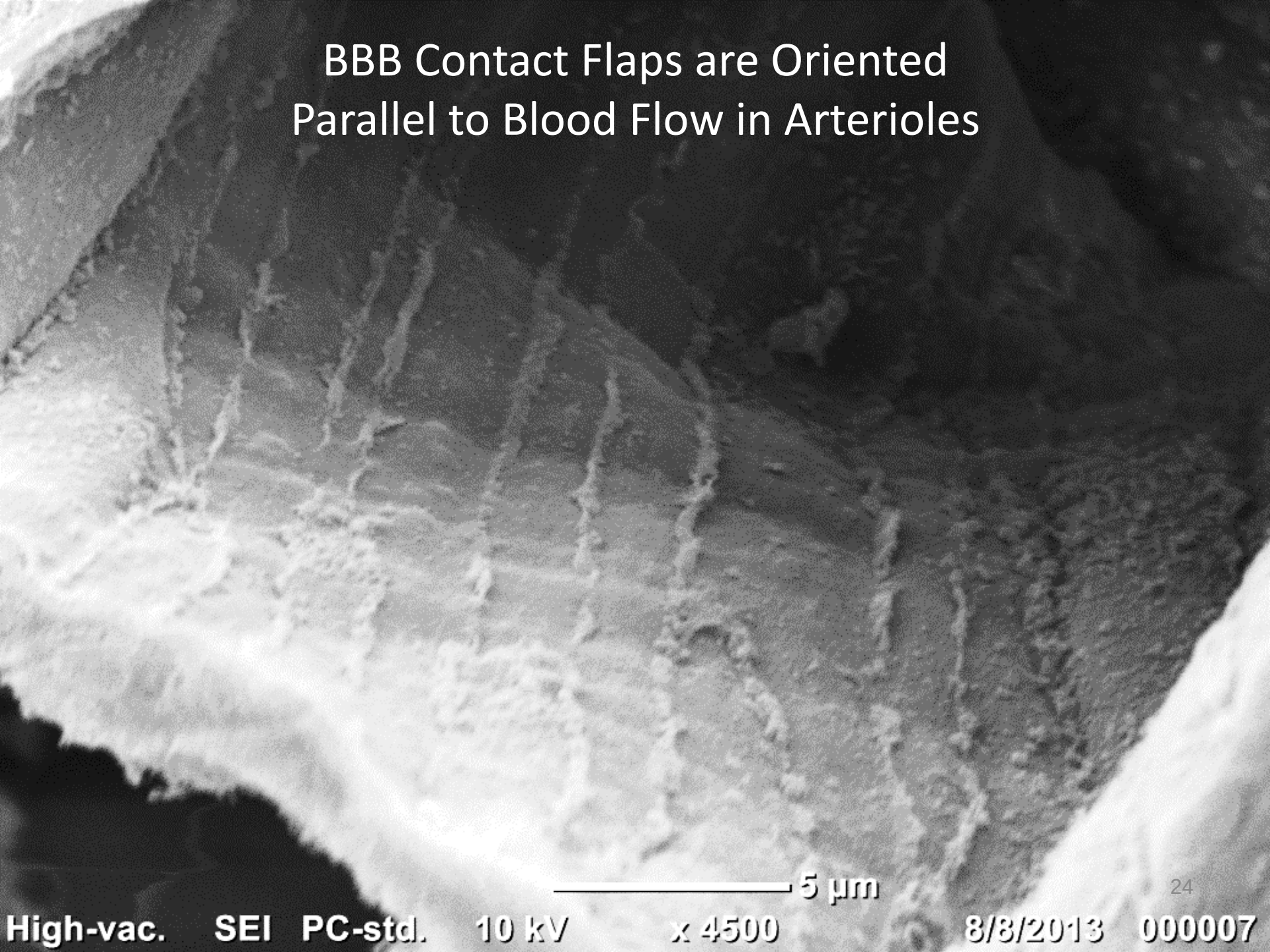
Arrows point to BBB – looks like rows of dots


15 kV x 800
20 µm

BBB Tight Junctions and Holes in the BBB

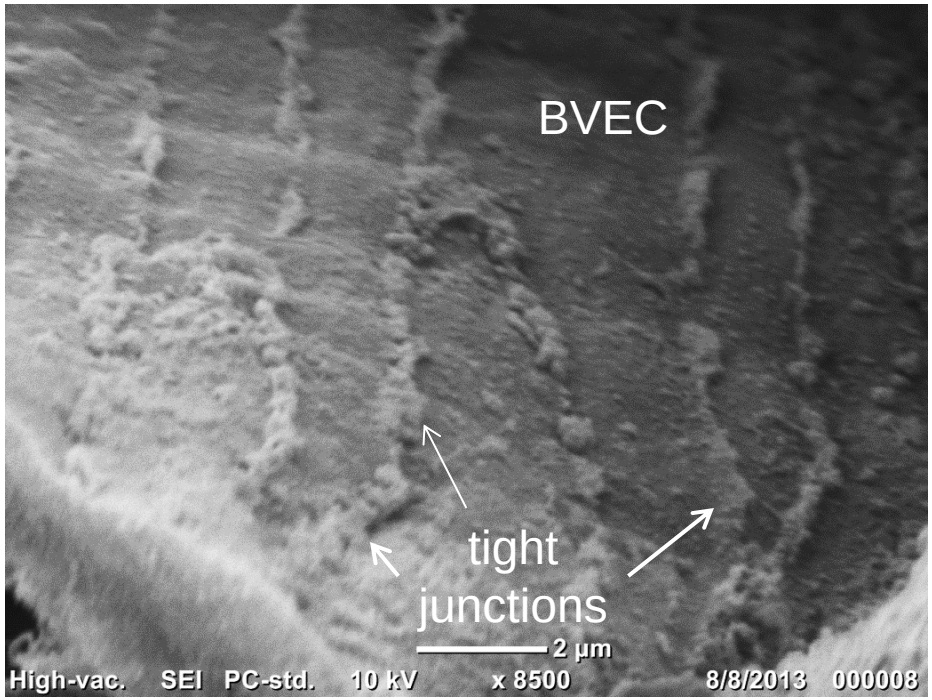


BBB Contact Flaps are Oriented Parallel to Blood Flow in Arterioles

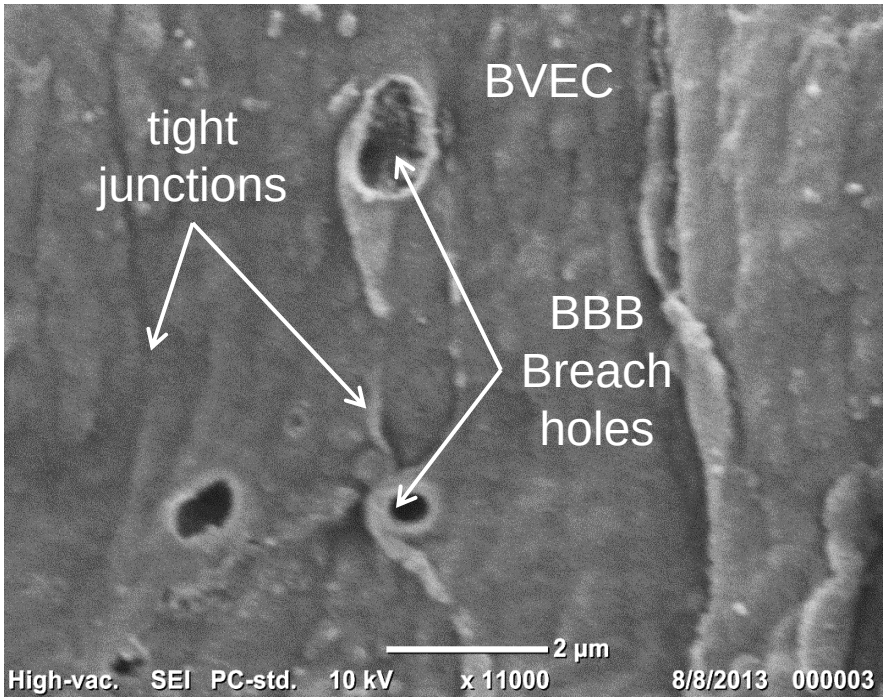


Anesthesia causes short-term BBB breakdown

- Probable mechanism of post-surgical delirium and trigger of dementia-



Control



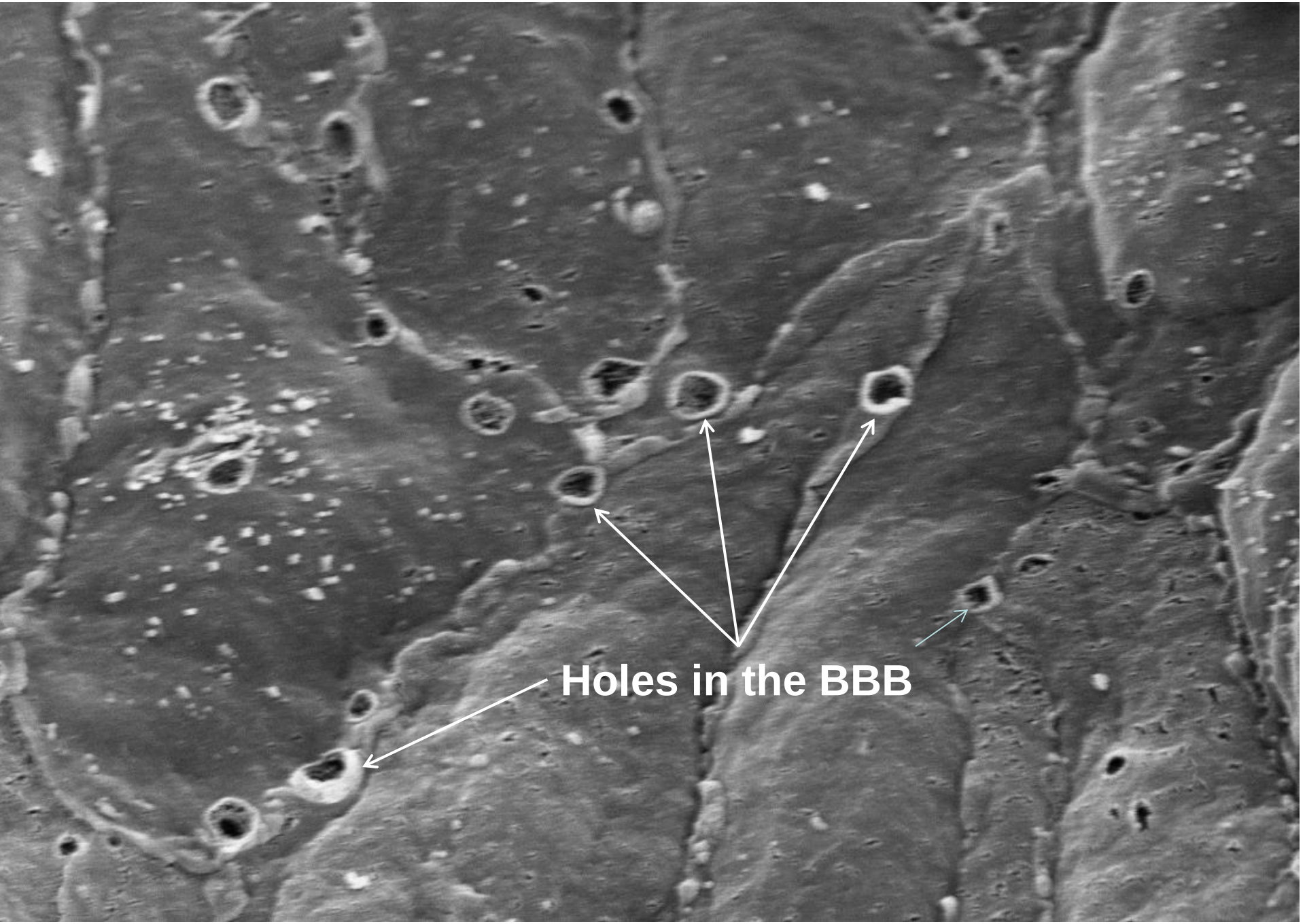
3 hr surgical plane anesthesia
sevoflurane

Results

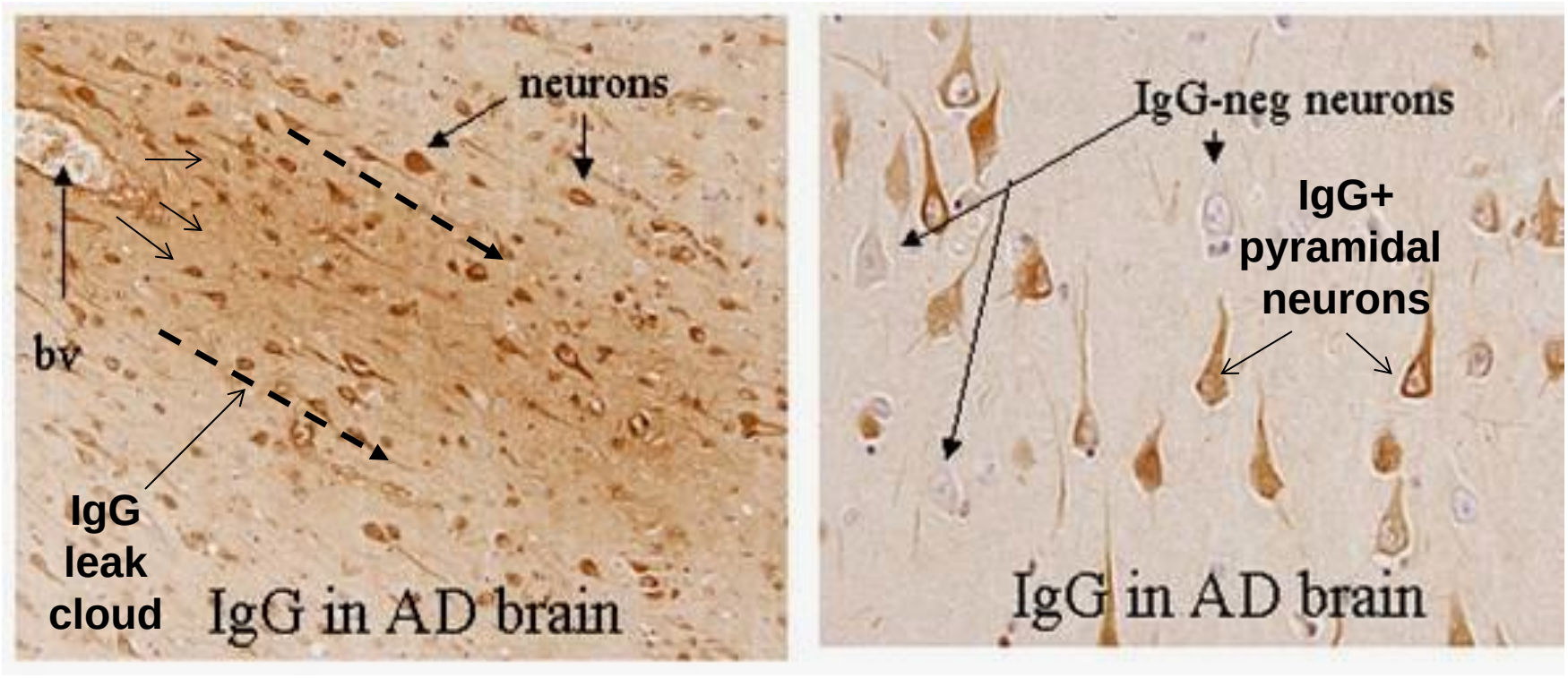
1. Anesthesia (**sevoflurane** and **isoflurane**) induces immediate changes in the surfaces of brain vascular endothelial cells (BVECs), including a profound **smoothing of surface membranes** and visible **holes in the BBB.**

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

*Collaboration with Dept. of Anesthesiology at Johns Hopkins via **Eli Levin DO-PhD***
Manuscript in preparation


Holes in the BBB

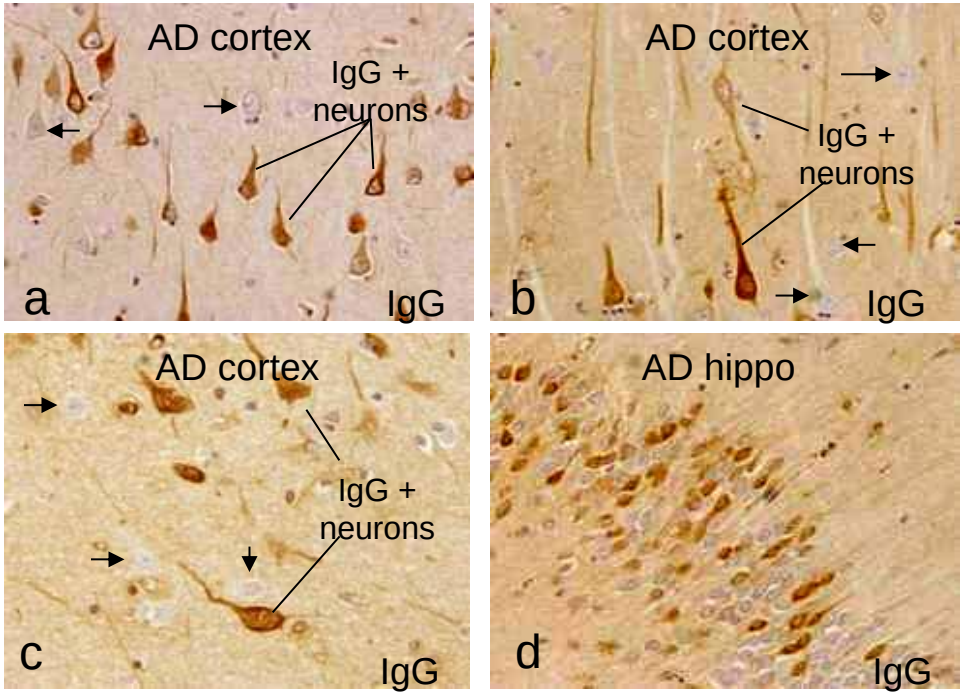
Brain-reactive antibodies leak from blood vessels and bind to pyramidal neurons in AD brains



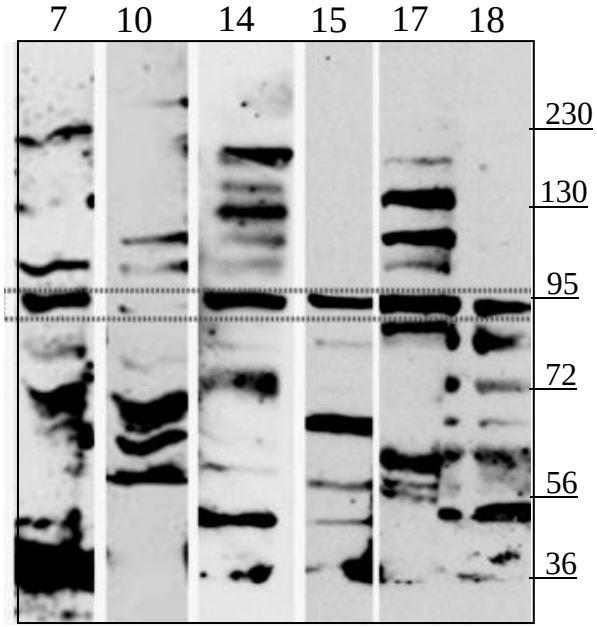
AD brain cerebral cortex

BBB breakdown, autoantibody influx and Alzheimer's disease

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



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



Human brain protein probed with human sera

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[a,b], Nimish K. Acharya[b], Min Han[b], Semah B. Zavareh[b], Jonathan C. Sedeyn[b], Venkateswar Venkataraman[c], Robert G. Nagele[a,]*

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[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.

How do neurons respond to autoantibody binding?

Answer

They clean their surfaces by internalizing surface-bound autoantibodies via endocytosis and degrade them in lysosomes

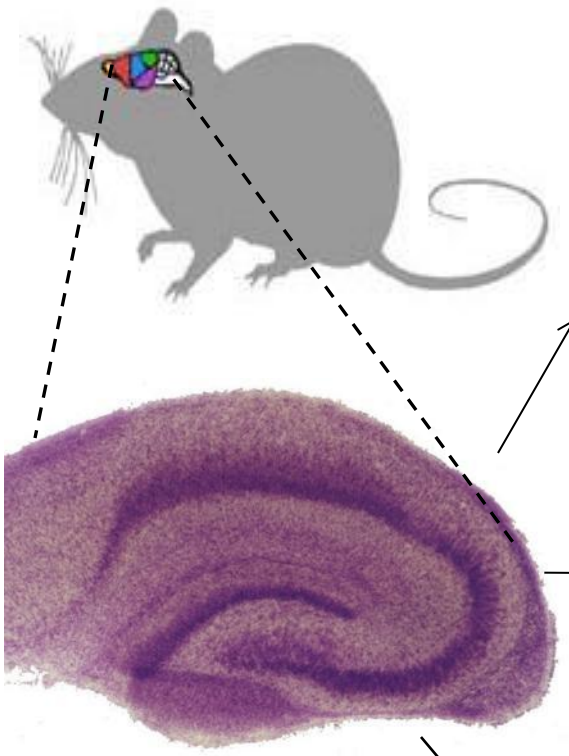
The pathological significance

Abeta42 (amyloid) 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 mouse neurons *in vitro*



Mouse Brain Slice Cultures

No Treatment – 24 h

a IHC – human IgG

HS1 only – 24 h

ML

PCL

b IHC - human IgG

HS1 only – 24 h

IgG+ neurons

c IHC - human IgG

AB42 only - 24 h

d IHC – human IgG

AB42 only - 24 h

AB42+ neurons

e IHC – AB42

HS1 + AB42 - 24 h

AB42+ neurons

f IHC – AB42

HS1 + AB42 – 24 h

IgG+ neurons

g IHC – human IgG

HS1 + AB42 – 24 h

AB42+ neurons

h IHC – AB42

HS8 + AB42 – 24 h

AB42+ neurons

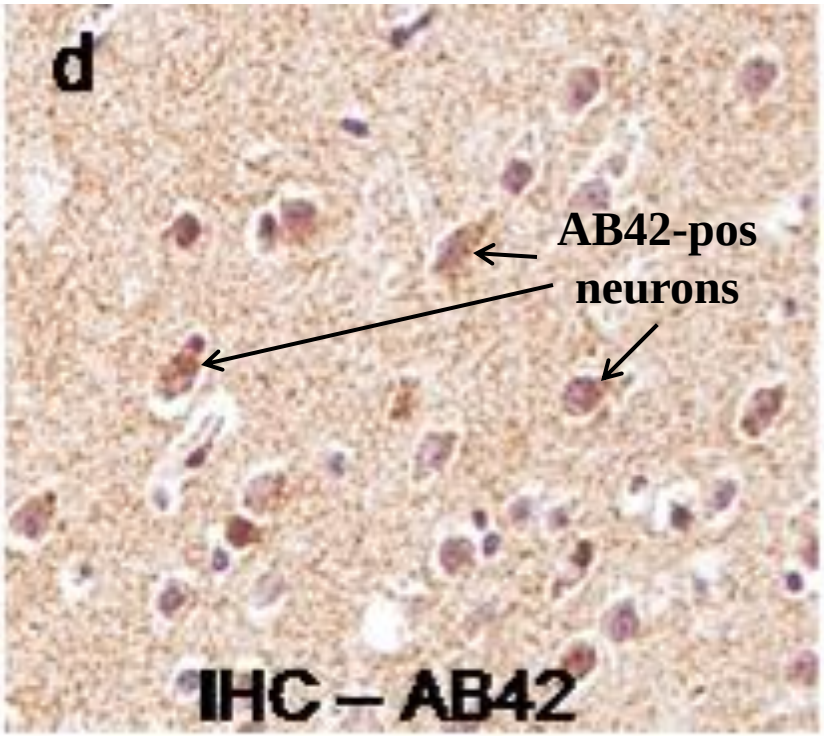
i IHC – AB42

Nagele et al. 2011
J. Alzheimer's Disease

Result: Human autoantibodies dramatically accelerate amyloid deposition in pyramidal neurons in mouse brain

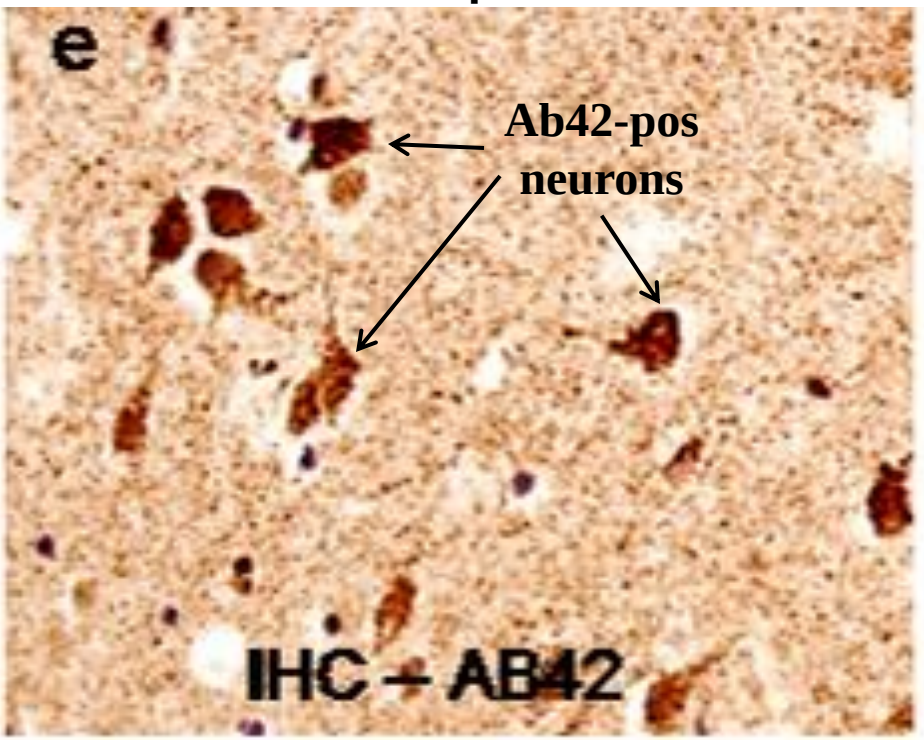
Adult mouse brain slice cultures treated with human serum Ig and Abeta42

Abeta42 alone



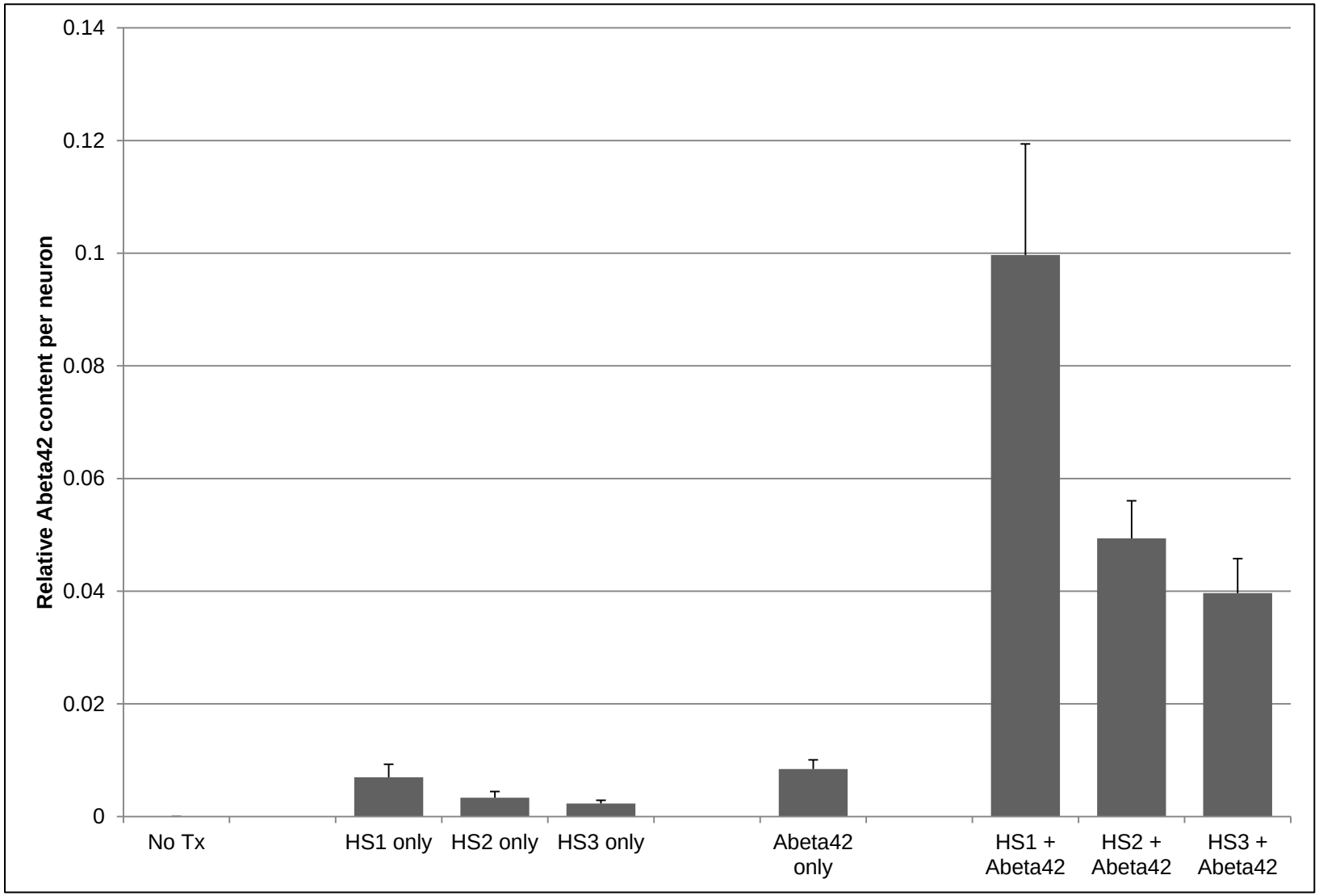
Very little AB42 internalization

Human serum plus Abeta42



Heavy AB42 internalization

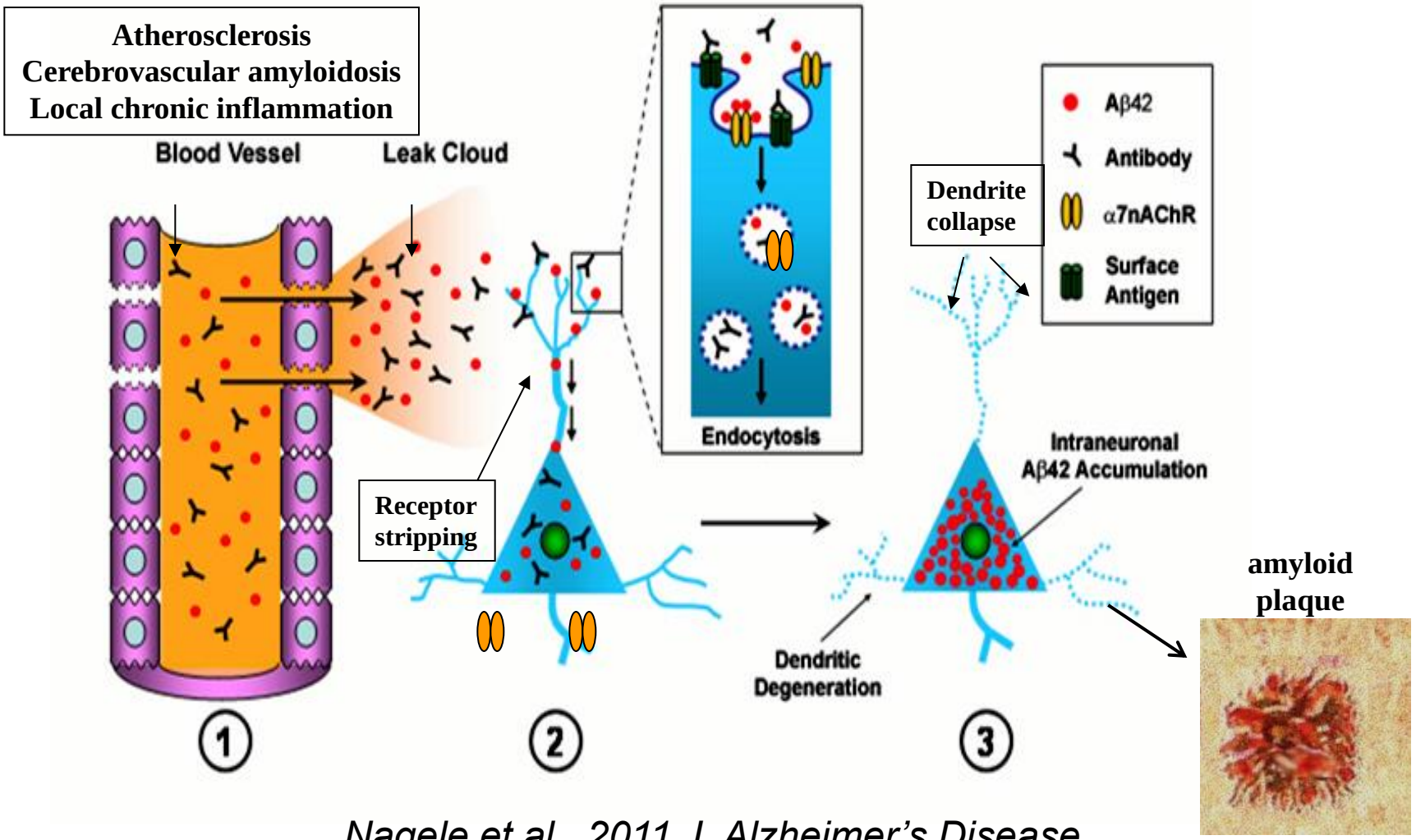
Human autoantibodies enhance intraneuronal Aβ42 deposition in mouse brain slice cultures with different potencies



BBB breakdown triggers Alzheimer's disease

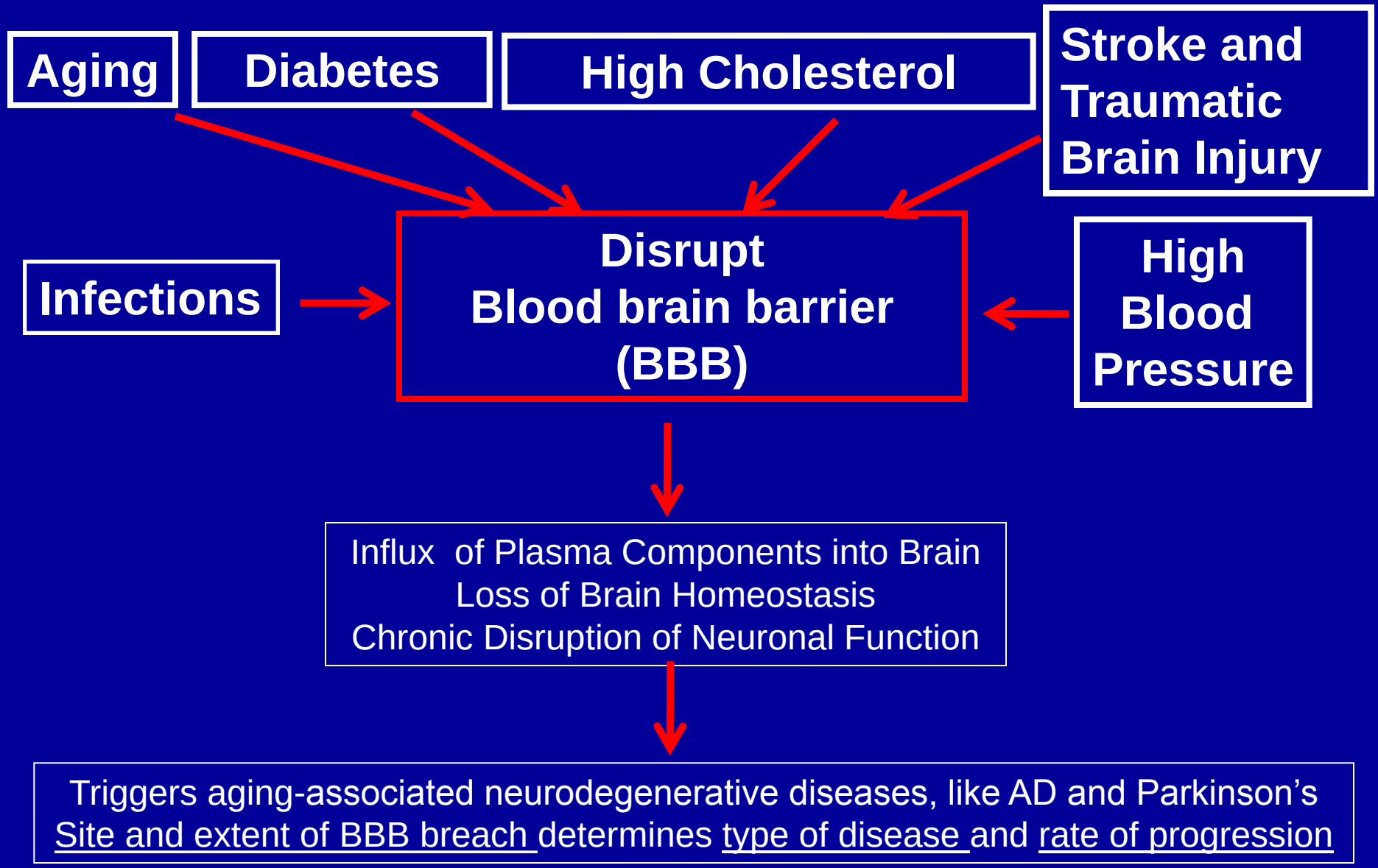
Causes intraneuronal amyloid (Abeta) deposition

Binding of autoantibodies to targets on neuronal surfaces induces endocytosis
Chronic endocytosis drives surface-bound Abeta42 into neurons



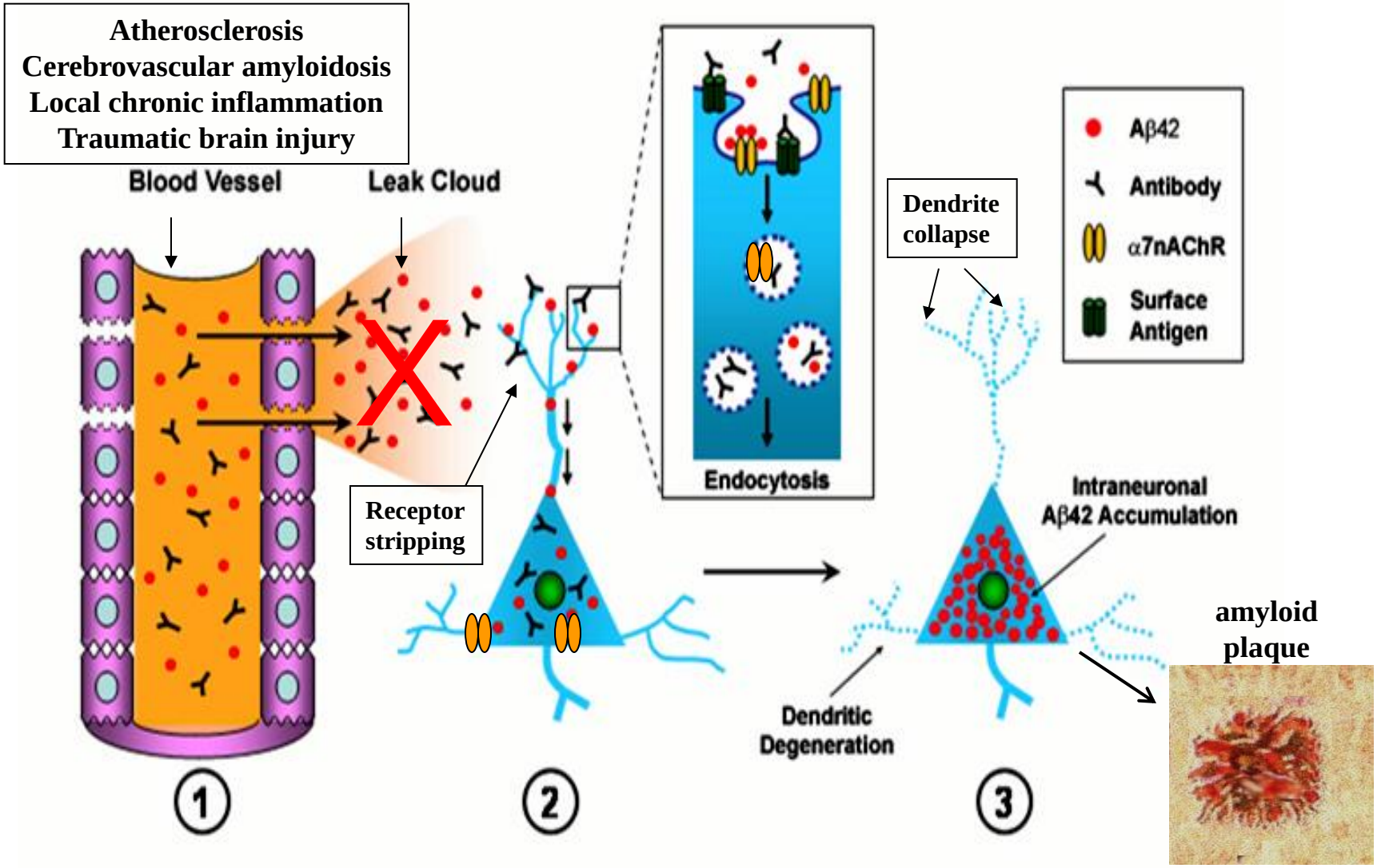
Nagele et al., 2011 J. Alzheimer's Disease

Hypothesis – BBB breakdown can trigger neurodegenerative diseases



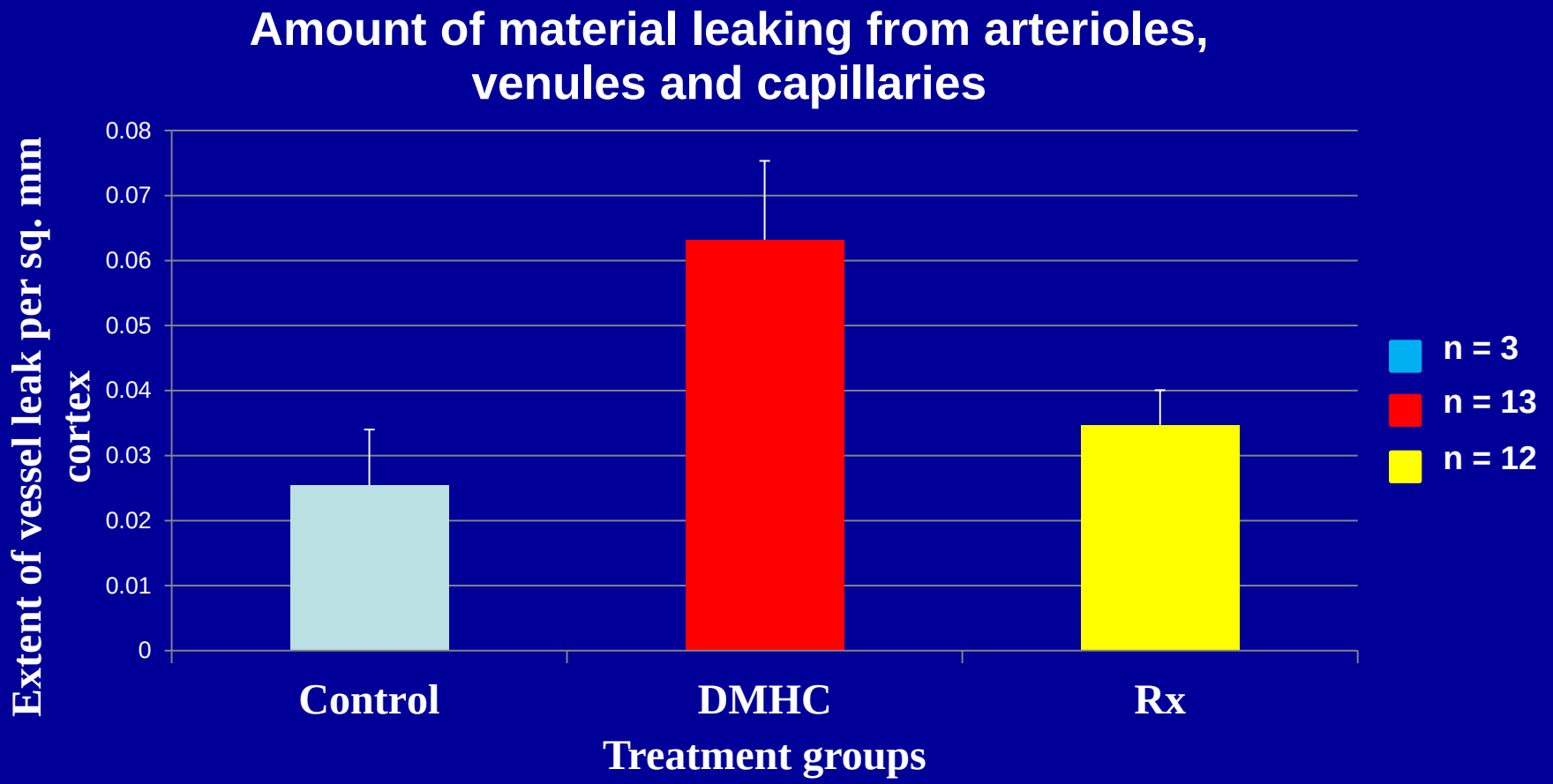
Develop drugs/therapies that maintains/repairs BBB integrity

(e.g., Darapladib blocks BBB breakdown in diabetic/hypercholesterolemic (DMHC) pigs



Acharya et al., 2013 J. Alzheimer's Disease

Diabetic/hypercholesterolemic (DMHC) pigs showed the greatest extent of BBB leak and Darapladib reduces vascular leak to control levels



Treatment groups	P-value
DMHC/C	0.033088
DMHC/Rx	0.097645
Rx/C	0.298917

Darapladib Blocks Blood-Brain Barrier Breakdown
A Global Treatment for All Neurodegenerative Diseases?

Journal of Alzheimer's Disease 35 (2013) 179–198
DOI 10.3233/JAD-122254
IOS Press

Diabetes and Hypercholesterolemia Increase Blood-Brain Barrier Permeability and Brain Amyloid Deposition: Beneficial Effects of the LpPLA2 Inhibitor Darapladib

Nimish K. Acharya[a,e], Eli C. Levin[a,e], Peter M. Clifford[a,e], Min Han[a,e], Ryan Tourtellotte[f], Dean Chamberlain[f], Michael Pollaro[f], Nicholas J. Coretti[a,e], Mary C. Kosciuk[a], Eric P. Nagele[a], Cassandra DeMarshall[a], Theresa Freeman[b], Yi Shi[b], Chenbing Guan[c], Colin H. Macphee[d], Robert L. Wilensky[g] and Robert G. Nagele[a,*]

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Handling Associate Editor: Thomas Shea

Funded by GlaxoSmithKline

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.

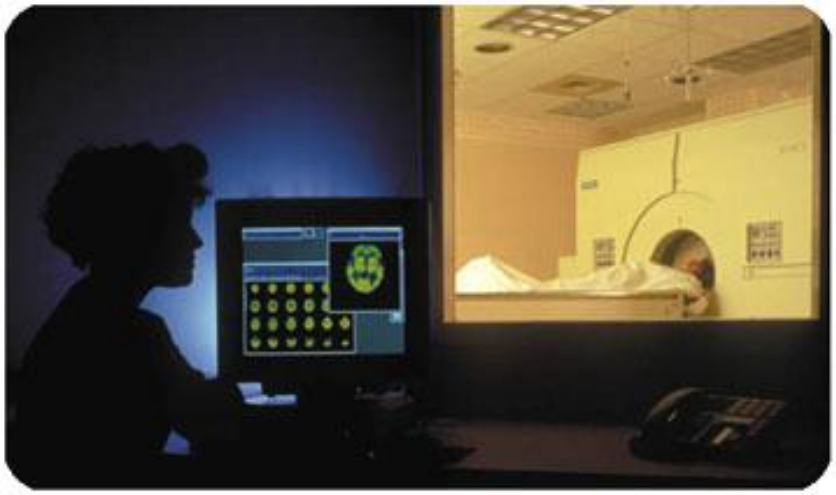
But

Early treatment requires early diagnosis

Current AD Diagnostic Methods

Tools used to diagnose AD:

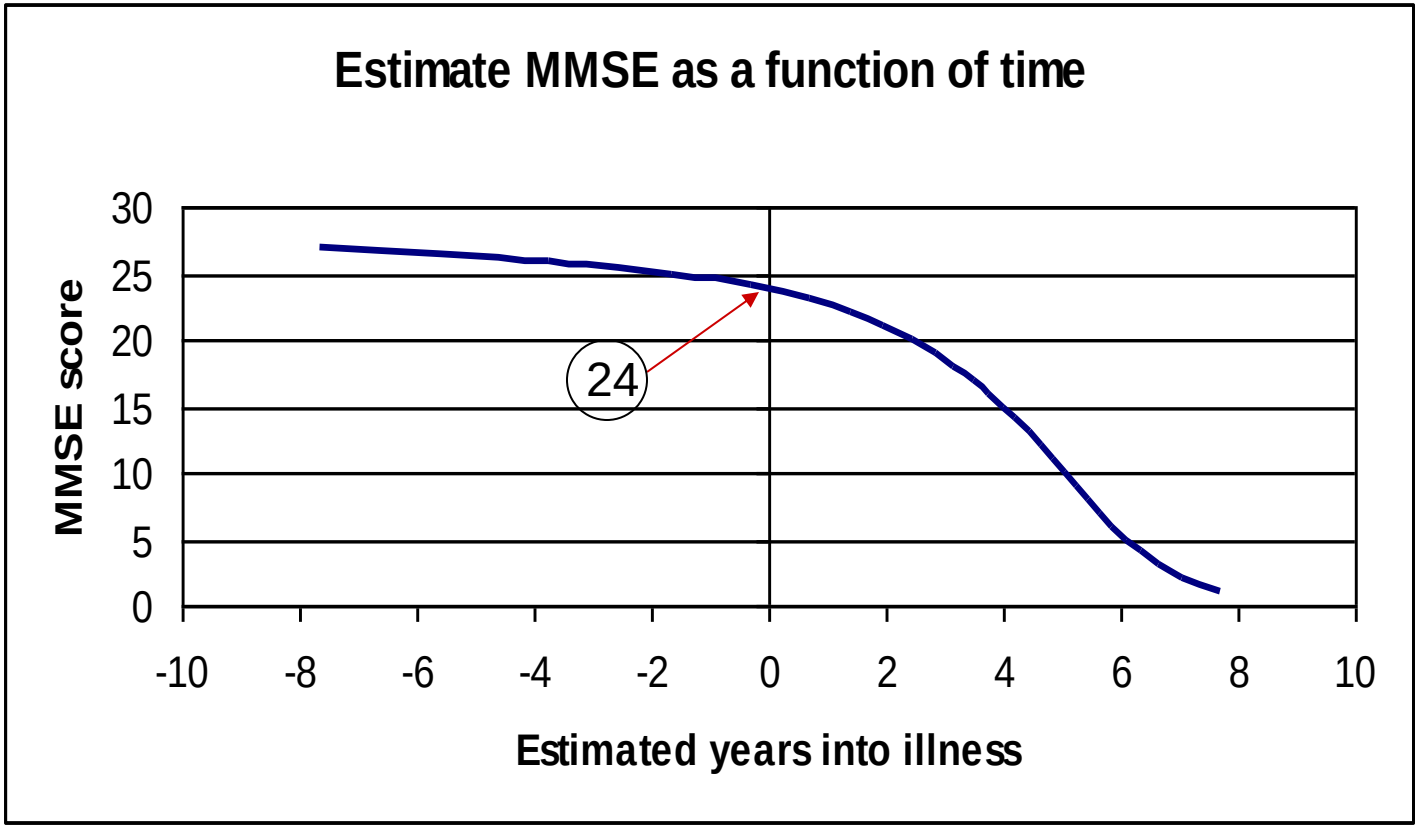
- A detailed patient history
- Information from family and friends
- Physical and neurological exams and lab tests
- Neuropsychological/cognitive tests
- Imaging tools such as CT scan, or magnetic resonance imaging (MRI).



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

ALZHEIMER'S DISEASE ASSESSMENT
Mini Mental State Exam (MMSE)



Limitations:
False-positives (usually depression with pseudodementia)
False negatives (early dementia in high functioning patients)
Lack of comprehensiveness

Important Note: *AD begins 8-10 years before symptoms are detectable!!*

Criteria and Market for an Early Alzheimer's Diagnostic

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

Autoantibodies as Blood-Based Disease-Specific Biomarkers: We have discovered that thousands of autoantibodies are present in all human serum

Protein Microarray

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

Age	N	% Female	Antibody Count	P value
< 45	10	33.3	1498.2 ± 545.7	<45 vs. 45-65: 0.0021
45 - 65	32	18.2	2335.6 ± 1009.5	45-65 vs. >65: 0.37
> 65	15	60	2647.8 ± 1139.2	<45 vs. >65: 0.0028

Sex	N	Age	Antibody Count	P value
Female	18	57.6 ± 18.7	2772.5 ± 714.8	0.004
Male	39	53.1 ± 15.1	2039.3 ± 1092.7	
Total	166	62.4 ± 16.3	1996.9 ± 1051.9	

Nagele et al., 2013 PLoS ONE

This discovery has pointed the way to our new disease diagnostics

OPEN ACCESS Freely available online

PLOS ONE

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

Eric P. Nagele[1,3], Min Han[1,2], Nimish K. Acharya[1,2], Cassandra DeMarshall[1,2], Mary C. Kosciuk[1], Robert G. Nagele[1]*

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.

Citation: Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, et al. (2013) Natural IgG Autoantibodies Are Abundant and Ubiquitous in Human Sera, and Their Number Is Influenced By Age, Gender, and Disease. PLoS ONE 8(4): e60726. doi:10.1371/journal.pone.0060726

This Study Shows 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. Similar autoantibody profiles are also found in the rat, mouse and swine, suggesting evolutionary conservation among all mammals.

4. The total number of different autoantibodies is strongly influenced by age, gender and the presence of disease.

What is the function of all of these autoantibodies?

Hypothesis

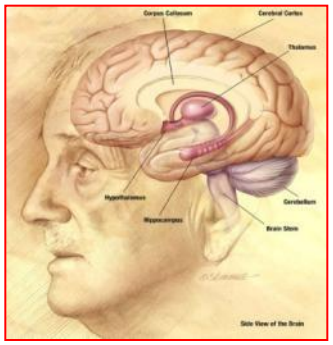
Autoantibodies are involved in the clearance of debris generated by the body on a day-to-day basis.

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



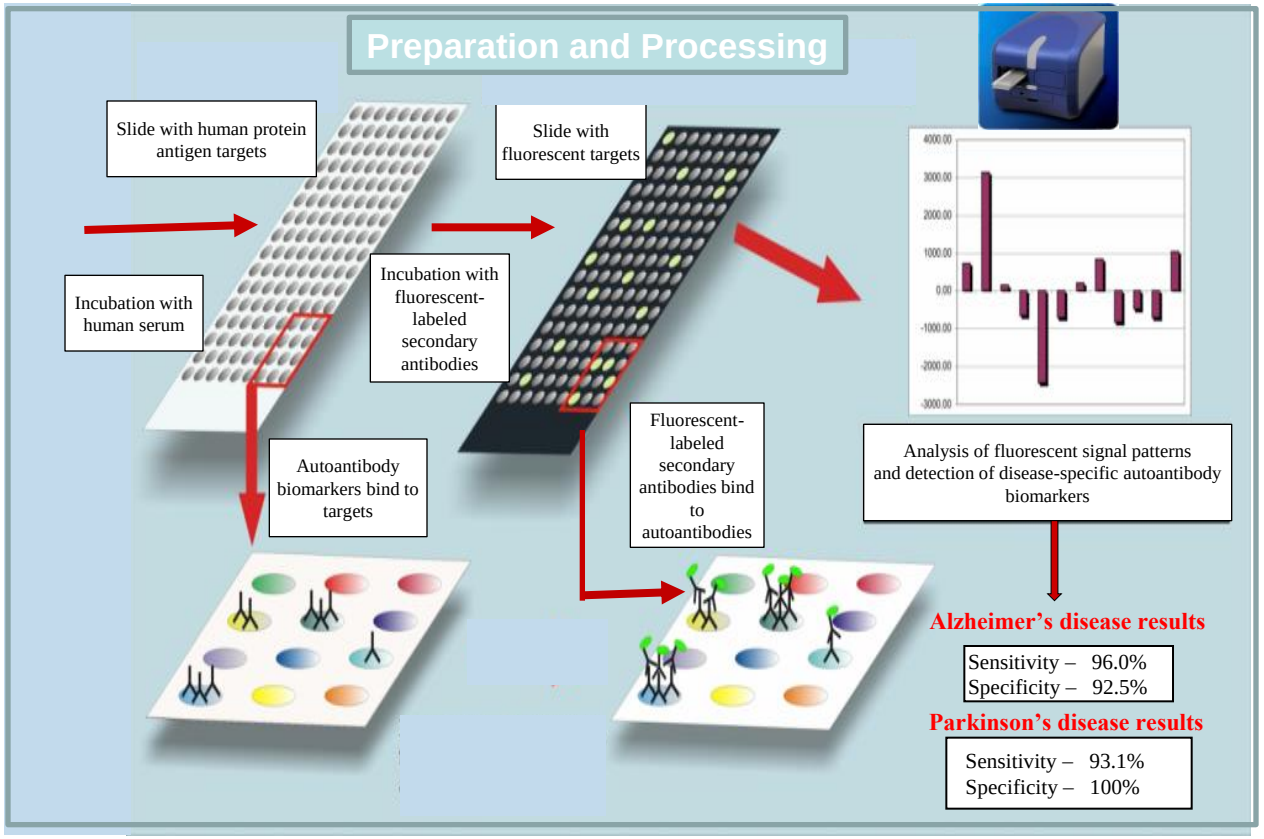
Alzheimer's disease

Autoantibody production

1. Neurons die – which type of neurons depends on brain region affected
2. Dead neurons release their 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. Durin detects disease-specific autoantibodies as biomarkers

1 drop of blood

Technology based upon the identification of disease specific autoantibody profiles in blood samples employing proprietary patterns of protein targets.



Alzheimer's Disease – completed!
Parkinson's Disease – completed!
Multiple Sclerosis – nearly complete
Autism – in progress

Funded by The Osteopathic Heritage Foundation

Autoantibody Biomarker Selection Strategy
Training and Testing Sets

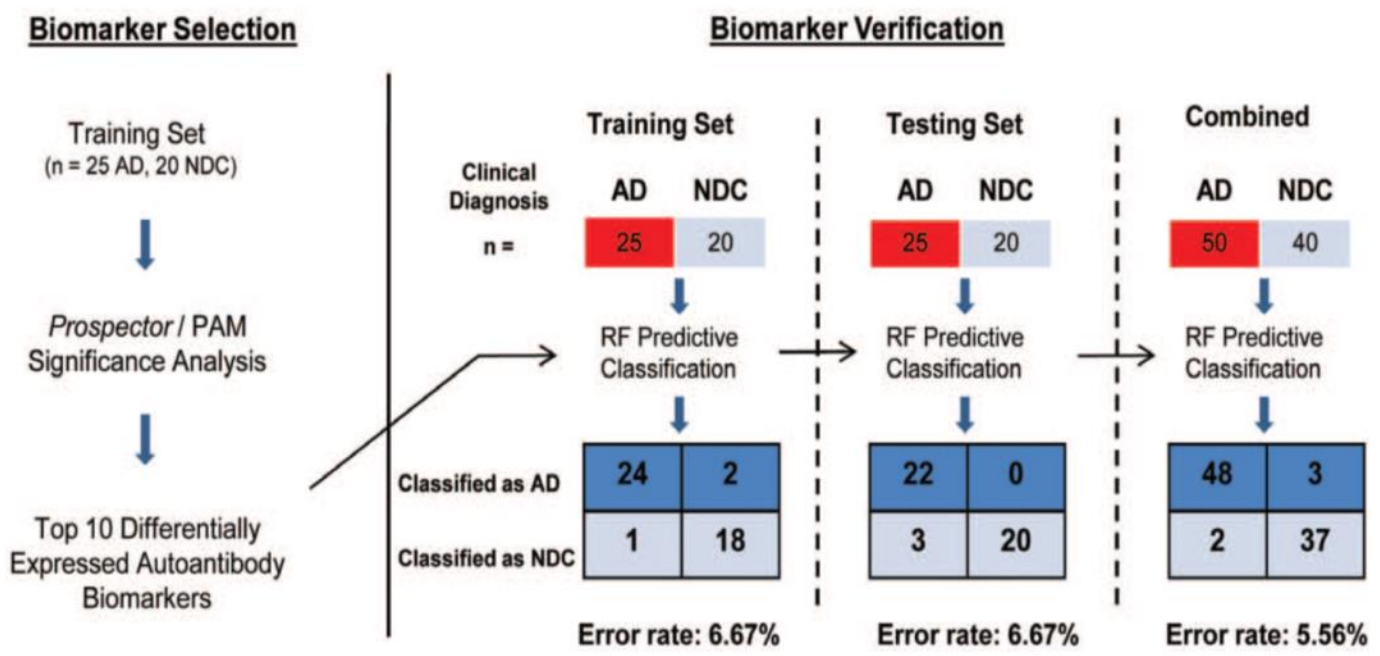


Figure 1. Biomarker selection and Training / Testing Analysis. Before biomarker selection, our total sample pool was split into two randomized groups: the Training Set and Testing Set. *Prospector* and *PAM* statistical analyses were performed on the Training Set to identify the top 10 most significant autoantibody classifiers of AD and NDC. We then verified the diagnostic accuracy of these selected biomarkers by using *Random Forest* to predict sample classification in the Training Set, Testing Set, and then both sets combined.
doi:10.1371/journal.pone.0023112.g001

Alzheimer's Disease Results

Mild-moderate disease

1. *D*etected 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%**

Diagnostic accuracies of the 10 selected AD biomarkers in different sample demographics

Table 2. Diagnostic accuracies of selected biomarkers.

	AD (n = 50) vs.					Earlier-stage AD (n = 35) vs.		Later-stage AD (n = 15) vs.	
	All NDC	Older Control	Younger Control	PD*	Breast Cancer	All NDC	Older Control	All NDC	Older Control
	n = 40	n = 20	n = 20	n = 29	n = 30	n = 40	n = 20	n = 40	n = 20
Sensitivity %	96.0	98.0	98.0	90.0	98.0	97.1	97.1	86.7	93.3
Specificity %	92.5	85.0	90.0	79.3	83.0	92.5	90.0	97.5	90
PPV%	94.1	94.2	96.1	88.2	90.7	91.9	94.4	92.9	87.5
NPV %	94.9	94.4	94.7	82.1	96.2	97.4	94.7	95.1	94.7

**The biomarkers used for this classification are those of Table 5; all others are the biomarkers identified in Table 3.*

doi:10.1371/journal.pone.0023112.t004

Biomarker Performance Details

1. The 10 autoantibody biomarkers diagnosed AD blood samples with 96.0% sensitivity and 92.5% specificity (n=90; 50 AD, 40 Non-Demented Controls).

2. The top 10 selected biomarkers diagnosed AD with over 90% accuracy in the following subgroups tested
   1. earlier-stage AD – makes early AD diagnosis possible
   2. later-stage AD,
   3. all stages of AD (early plus late)

3. AD patients could be distinguished from Parkinson's disease and breast cancer patients, the latter confirming that our test shows no general bias for disease.

Alzheimer's Diagnostic Proof of Concept Study
Published August 3, 2011

OPEN ACCESS Freely available online

PLoS one

Diagnosis of Alzheimer's Disease Based on Disease-Specific Autoantibody Profiles in Human Sera

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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.

Citation: Nagele E, Han M, DeMarshall C, Belinka B, Nagele R (2011) Diagnosis of Alzheimer's Disease Based on Disease-Specific Autoantibody Profiles in Human Sera. PLoS ONE 6(8): e23112. doi:10.1371/journal.pone.0023112

Parkinson's Disease
A Multi-Disease Diagnostic Strategy!!

OPEN ACCESS Freely available online

PLoS one

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

Min Han[1,2☯], Eric Nagele[3☯], Cassandra DeMarshall[1,2], Nimish Acharya[1,2], Robert Nagele[2,3*]

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, 2 New Jersey Institute for Successful Aging, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America, 3 Durin Technologies, Inc. New Brunswick, New Jersey, United States of America

Abstract

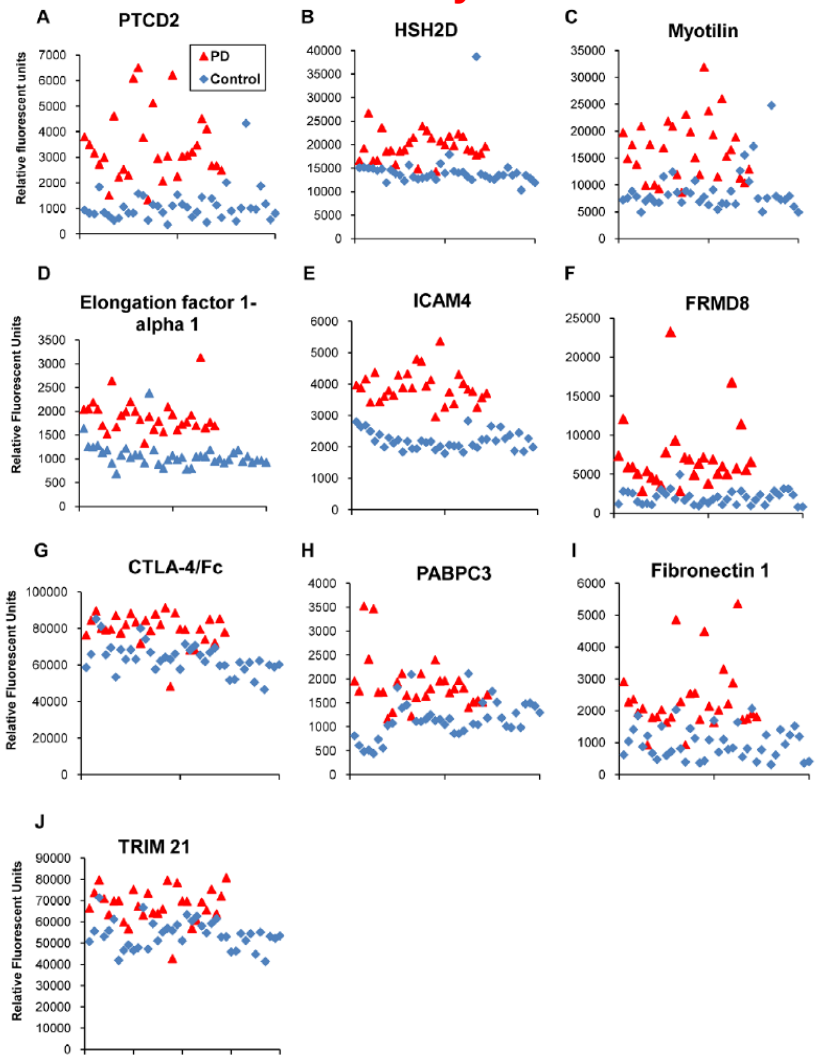
Parkinson's disease (PD), hallmarked by a variety of motor disorders and neurological decline, is the second most common neurodegenerative disease worldwide. Currently, no diagnostic test exists to identify sufferers, and physicians must rely on a combination of subjective physical and neurological assessments to make a diagnosis. The discovery of definitive blood-borne 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%

Differential Expression of Top 10 Biomarkers for Mild-Moderate PD

Microarray Data



Dot-blot confirmation

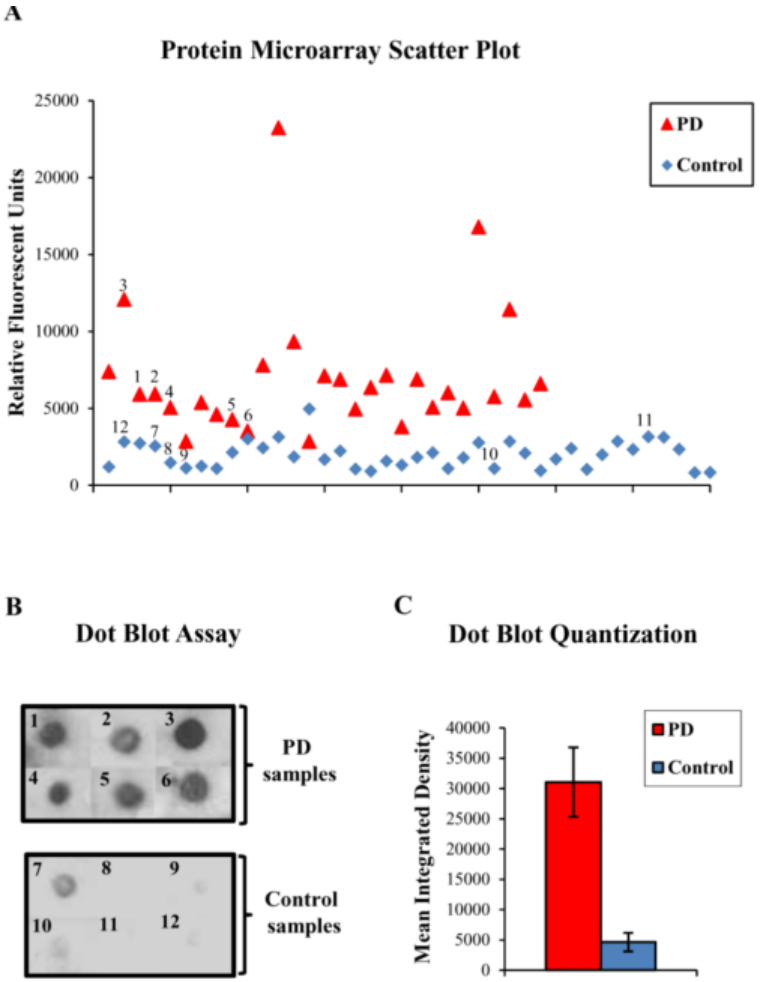


Figure 2. Differential expression of identified PD-specific autoantibody biomarkers in PD and control sera. Microarray fluorescence values reflecting individual serum autoantibody titers demonstrate the differences in the serum expression of the selected ten PD-specific autoantibody biomarkers in PD (n = 29) and control (n = 40) sera (A–I).
doi:10.1371/journal.pone.0032383.g002

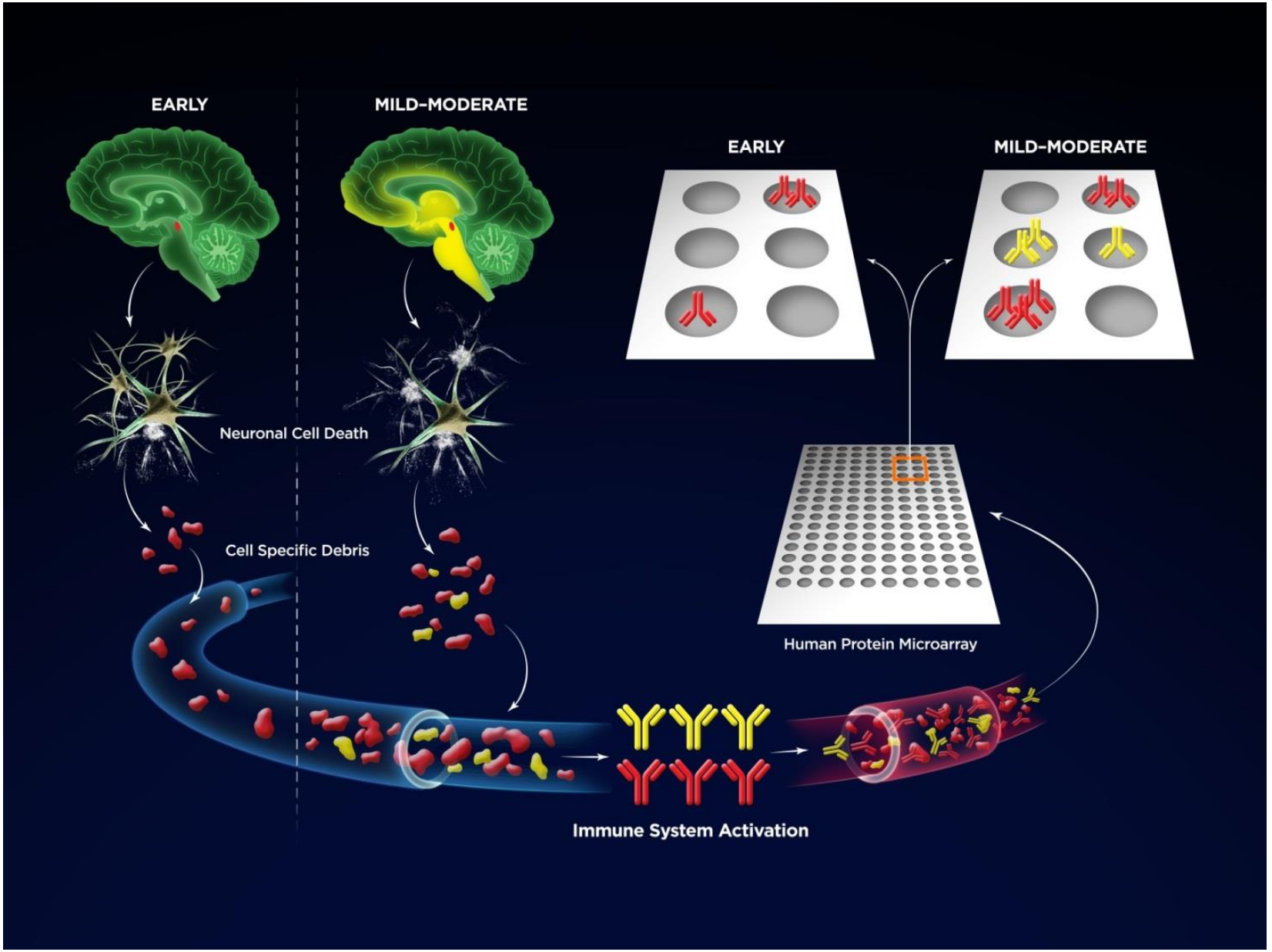
Early Detection of Parkinson's Disease

THE MICHAEL J. FOX FOUNDATION
FOR PARKINSON'S RESEARCH

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



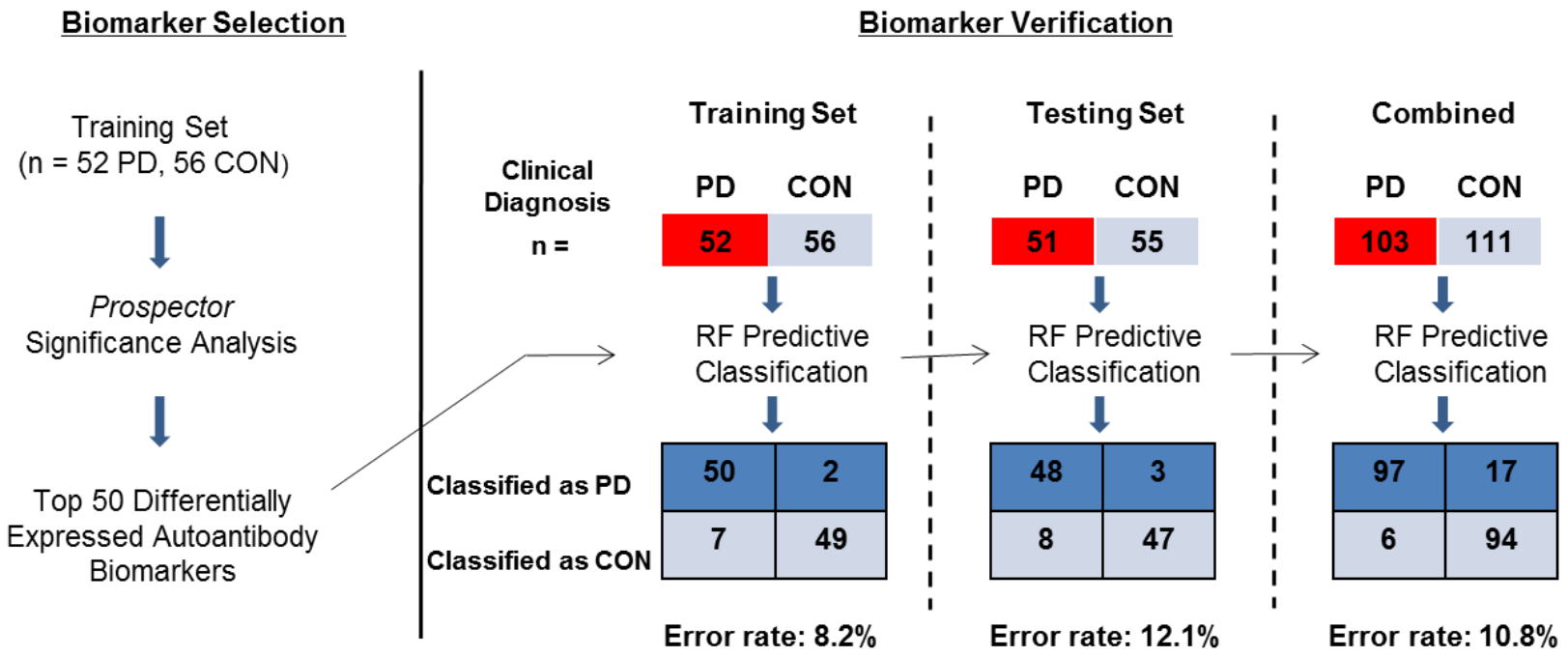
Subject Demographics for Early Parkinson's Study

Early-Stage PD Subjects Obtained From MJ Fox Foundation and Parkinson's Study Group

Group	n	Age		Sex	Ethnicity	UPDRS	Hoehn & Yahr
		(Years)	(Range)	(% Male)	(% Caucasian)		
Parkinson's disease	132	65.1 ± 10.3	37-88	57	89	-	-
-Early-Stage	103	62.7 ± 9.3	37-79	58	98	38.1 ± 16.8	2.1 ± 0.6
-Mild-Moderate	29	74.3 ± 9.0	53-88	55	55	-	-
Controls	156	55.0 ± 15.6	19-87	56	76	-	-
-Age-Matched	111	63.1 ± 8.4	51-87	56	78	-	-
-Non Age-Matched	45	34.9 ± 10.2	19-50	49	71	-	-
Alzheimer's disease	50	78.5 ± 8.8	61-97	42	88	-	-
Multiple Sclerosis	20	51.0 ± 9.2	36-67	33	97	-	-
Breast Cancer	30	46.9 ± 5.8	32-54	0	97	-	-

For each disease group the number of individuals (n), age, range of age, gender, and ethnicity are listed. For the early-stage PD subjects, the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr scores are included as indices of PD severity.

Early-Stage Parkinson's Disease
Biomarker Selection and Training/Testing Set Strategy



The total sample pool (n = 214) was randomly split into two groups: the Training Set and Testing Set. *Prospector* statistical analysis was performed on the Training Set to identify the top 50 most significant autoantibody classifiers of early stage PD. The diagnostic accuracy of these selected biomarkers was tested by using *Random Forest* to predict sample classification in the Training Set, Testing Set, and both sets combined.

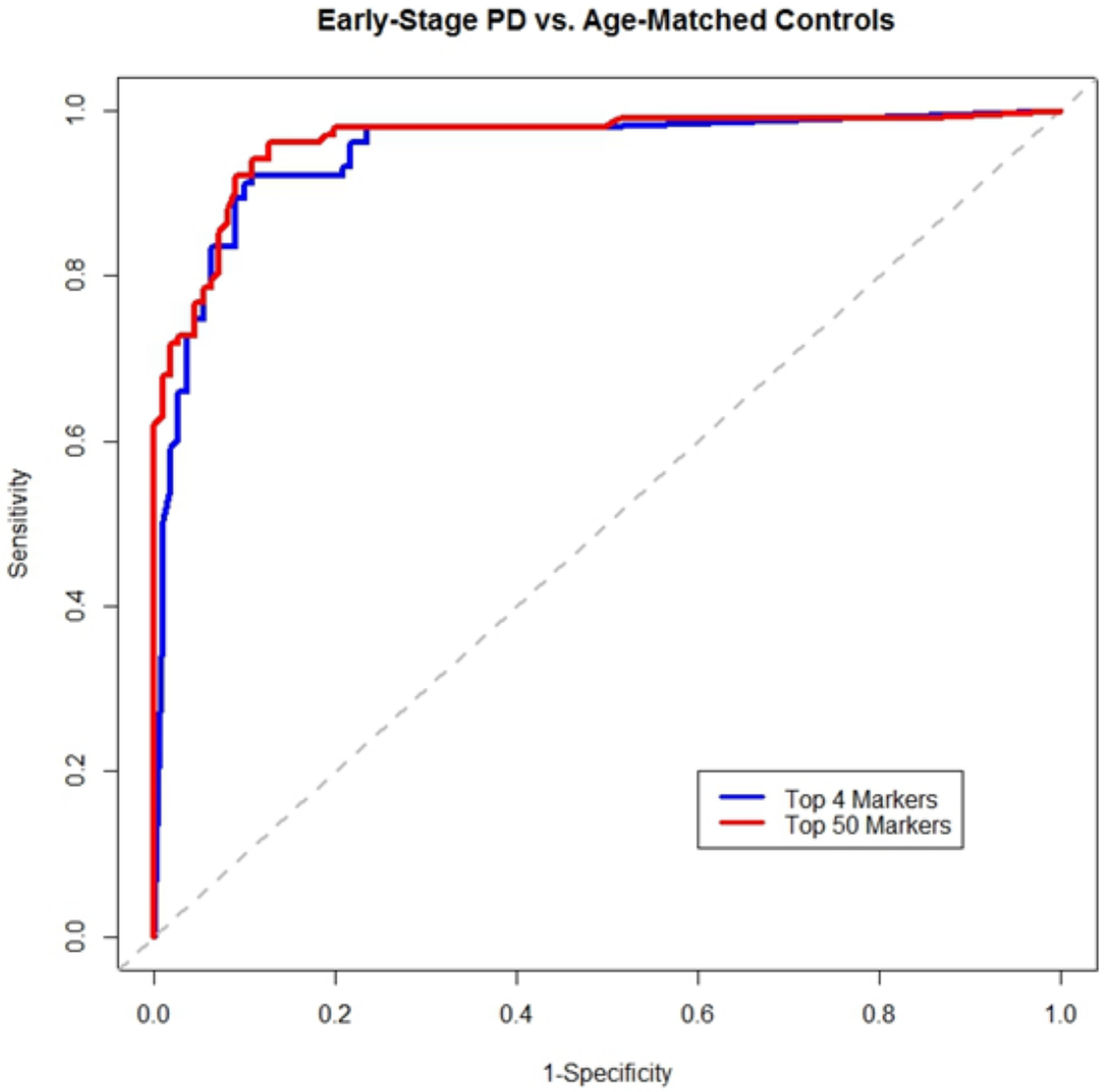
Diagnostic Results Using A Panel of 50 Early-Stage PD Biomarkers and *Random Forest (RF)*.

Early-Stage PD (n = 103) vs.	Age Matched Controls	Age Matched and Young Controls	Mild-Moderate PD	Mild-Moderate AD	Multiple Sclerosis	Breast Cancer
n	111	56	29	50	30	30
Sensitivity %	94.2	94.2	98.1	98.1	98.1	98.1
Specificity %	84.7	90.4	100.0	98.0	93.3	96.7
PPV %	85.1	86.6	100.0	99.0	98.1	99.0
NPV %	94.0	95.9	93.6	96.1	93.3	93.6
Overall Accuracy %	89.2	91.9	98.5	98.0	97.0	97.7
Overall Error %	10.8	8.1	1.5	2.0	3.0	2.3

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

Detection of Early-Stage Parkinson's Disease

ROC Curve Assessment of the Utility of PD Biomarkers for Detection of Early-Stage 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 = 0.93 (95% CI)
ROC AUC = 0.92 (95% CI)

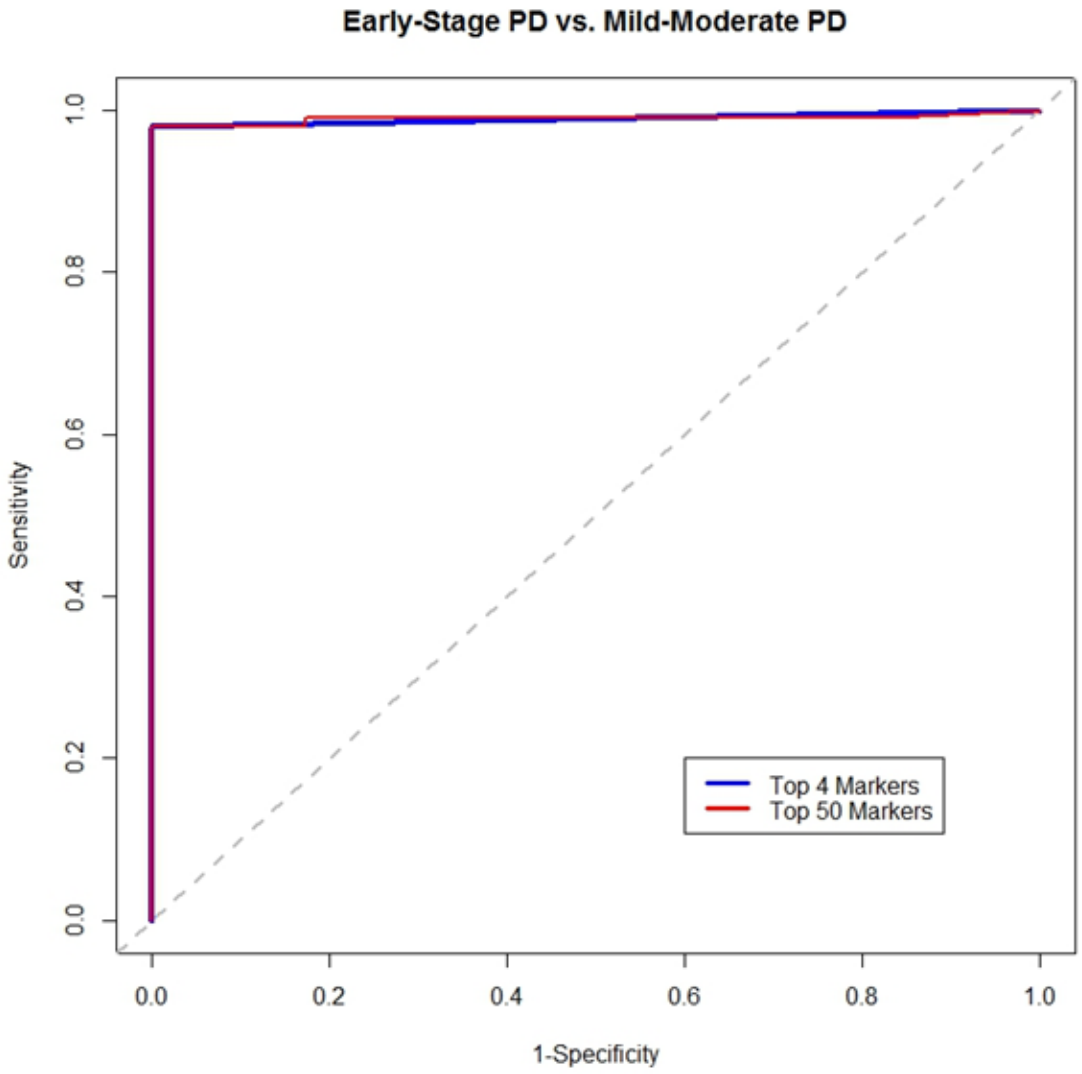
ROC Curve Analysis of Diagnostic Utility Using 50 or Only the Top 4 Early-Stage PD Biomarkers

	50 Markers			4 Markers		
Early-Stage PD (n=103) vs.	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Age Matched Controls (n=111)	0.93 (.88-.99)	0.92 (.84-.98)	0.87 (.78-.94)	0.92 (.86-.97)	0.84 (.74-.94)	0.87 (.78-.94)
Age Matched and Younger Controls (n=156)	0.96 (.93-.98)	0.91 (.85-.96)	0.94 (.90-.97)	0.96 (.93-.98)	0.91 (.85-.96)	0.94 (.90-.97)
Mild-Moderate PD (n=29)	0.98 (.97-1)	0.98 (.95-1)	1.00	0.99 (.97-1)	0.98 (.95-1)	1.00
Mild-Moderate AD (n=50)	0.99 (.97-1)	0.98 (.95-1)	1.00	0.99 (.97-1)	0.98 (.95-1)	0.98 (.94-1)
Multiple Sclerosis (n=30)	0.98 (.97-1)	0.98 (.95-1)	1.00	0.99 (.97-1)	0.98 (.95-1)	1.00
Breast Cancer (n=30)	0.99 (.98-1)	0.98 (.93-1)	1.00	0.99 (.97-1)	0.98 (.95-1)	0.97 (.90-1)

ROC curve analyses was used to assess the diagnostic utility of the full panel of 50 or only the top 4 selected biomarkers for distinguishing early-stage PD subjects from age-matched controls from the subject groups listed. Areas under the curve (AUC) at 95% confidence are listed along with values for sensitivity and specificity derived from the ROC curve output data.

Staging of Parkinson's Disease

ROC Curve Assessment of the Utility of PD Biomarkers for Distinguishing Early-Stage vs. Mild-Moderate PD



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)

Conclusions for the Early PD Detection Study

Using this diagnostic strategy, early-stage PD patients were correctly diagnosed with an overall accuracy of 89.2%, and sensitivity of 94.2% and a specificity of 84.7%.

These biomarkers were also capable of staging the disease, differentiating patients with early-stage PD from those with mild-moderate PD with an overall accuracy of 98.5%.

Early-stage PD autoantibody biomarkers are different from those that diagnose mild-moderate PD.

This appears to be a multi-disease diagnostic strategy, since it now seems to be useful for early-stage PD, mild-moderate PD, mild-moderate Alzheimer's disease, and mild cognitive impairment (MCI) driven by Alzheimer's disease.

This diagnostic strategy can be used to detect early stages of disease with minimal ongoing pathology.

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

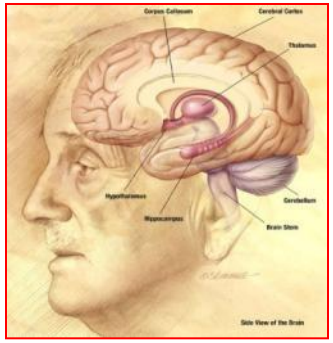
ABC News
Pfizer, Medivation Pull Plug on Alzheimer's Drug Dimebon
Jan 17. 2012
Another failure in phase III clinical testing. Dimebon finally sent to the trash heap

WHY?

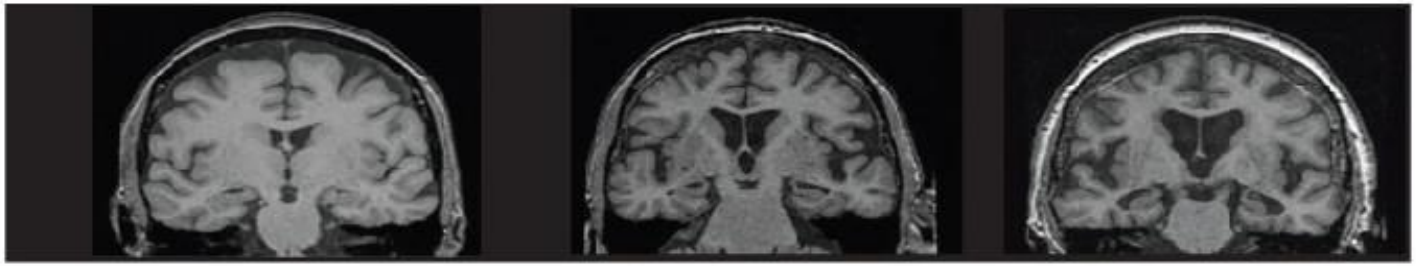
Answer: The disease is too far advanced. We need early diagnosis, so that early treatments become possible – early treatments are much more likely to be effective.

Detection of AD at the Mild Cognitive Impairment Stage

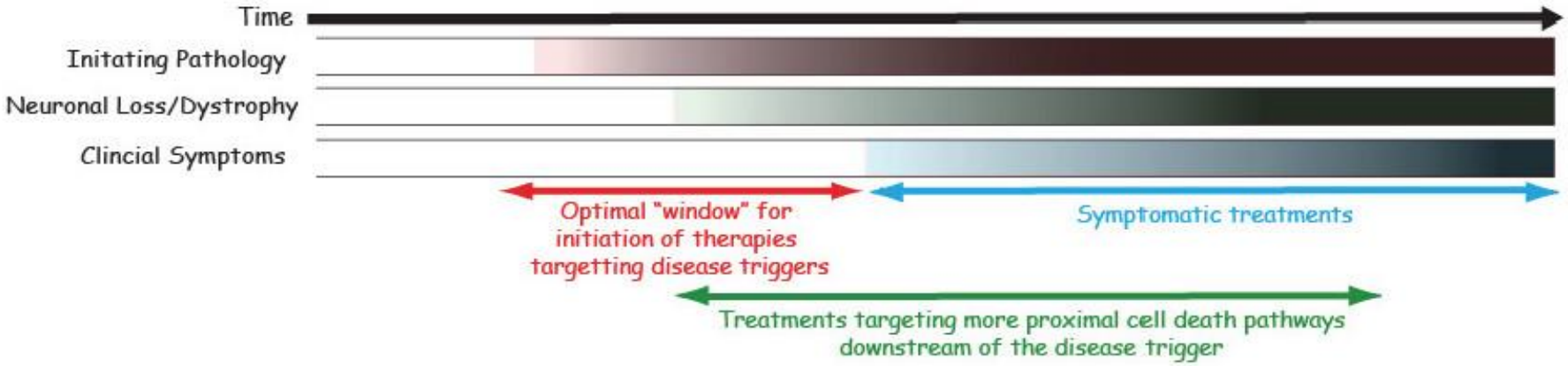
Our goal is to detect Alzheimer's disease in earlier, MCI and pre-symptomatic phases



Alzheimer's pathology is underway 8-10 years before symptoms appear



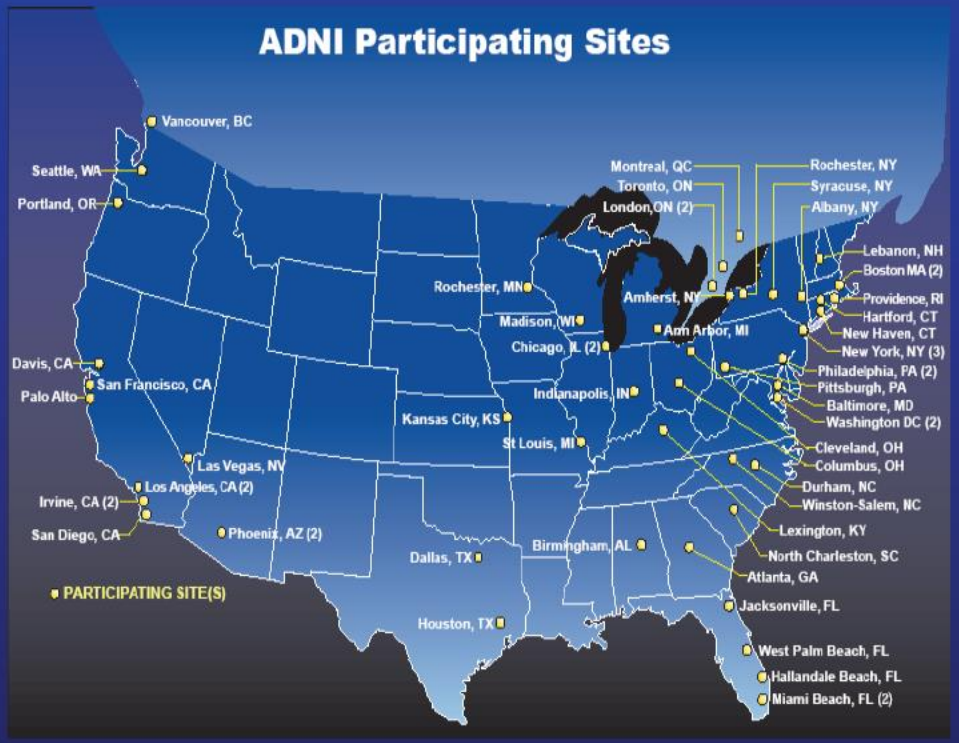
Normal | Initial Presentation with Functional Impairement | Rapidly Progressing Disease



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

Funded by the Osteopathic Heritage Foundation

ADNI-1: Naturalistic study of AD progression



- 200 NORMAL 3 yrs
- 400 MCI 3 yrs
- 200 AD 2 yrs
- Visits every 6 mo

- 57 sites
- Clinical, blood, LP
- Cognitive Tests
- 1.5T MRI

Some also have

- 3.0T MRI (25%)
- FDG-PET (50%)
- PiB-PET (approx 100)

All data in public database:
UCLA/LONI/ADNI
No embargo of data

Diagnosis of Mild Cognitive Impairment (MCI) in ADNI Patients with Low CSF Abeta42 Levels

Top 10 Biomarkers

All ADNI MCI patients had low CSF Abeta42 levels as surrogate biomarker for AD-related pathology

ADNI MCI (n=50)
vs.
Age-Matched Controls (n=35)

ADNI MCI ~74 yr old; controls ~73 yr old

Confusion matrix:

	CON	MCI	classification error
CON	34	1	0.02857143
MCI	0	50	0.00000000

Error rate: 1.18%
Sensitivity = 100.0%
Specificity = 97.1%
PPV = 98.0%
NPV = 100.0%

ADNI MCI (n=50)
vs.
All Controls (n=111)

ADNI MCI ~75 yr old; controls ~63 yr old

Confusion matrix:

	MCI	CON	classification error
MCI	49	1	0.02000000
CON	2	109	0.01801802

Error rate: 1.86%
Sensitivity = 98.0%
Specificity = 98.2%
PPV = 96.1%
NPV = 99.1%

Funded by The Osteopathic Heritage Foundation

Specificity of MCI Biomarkers and Staging of AD

MCI vs. Mild-Mod AD

(top 50 markers from training set)

OOB estimate of error rate: 0%

Confusion matrix:

	MCI	AD	classification error
MCI	50	0	0
AD	0	50	0

MCI vs. Early-Stage PD

(top 50 markers from training set)

OOB estimate of error rate: 2%

Confusion matrix:

	MCI	esPD	class.error
MCI	24	1	0.04
esPD	0	25	0.00

MCI vs. Multiple Sclerosis

(top 50 markers from training set)

OOB estimate of error rate: 0%

Confusion matrix:

	MCI	MS	classification error
MCI	50	0	0
MS	0	50	0

MCI vs. Breast Cancer

(top 50 markers from training set)

OOB estimate of error rate: 0%

Confusion matrix:

	MCI	BC	classification error
MCI	50	0	0
BC	0	50	0

Capabilities of Our New AD and PD Diagnostic Tests

Early Diagnosis
Patient Management
Test of Drug Efficacy

1. Early detection and diagnosis of diseases and traumatic injury (e.g., AD and PD).

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

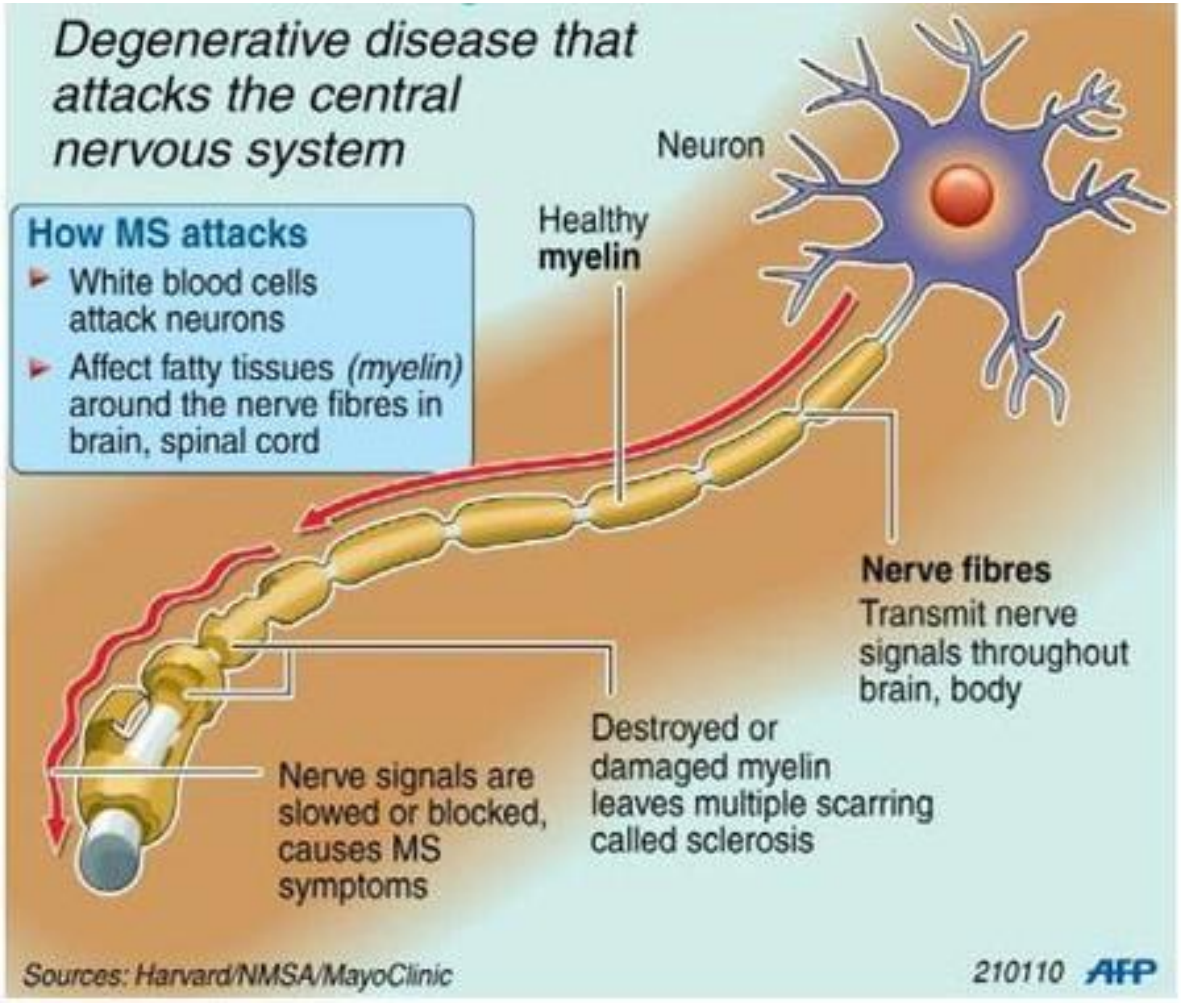
3. Evaluate patient response to drug therapy by monitoring patient progression through clinical stages.

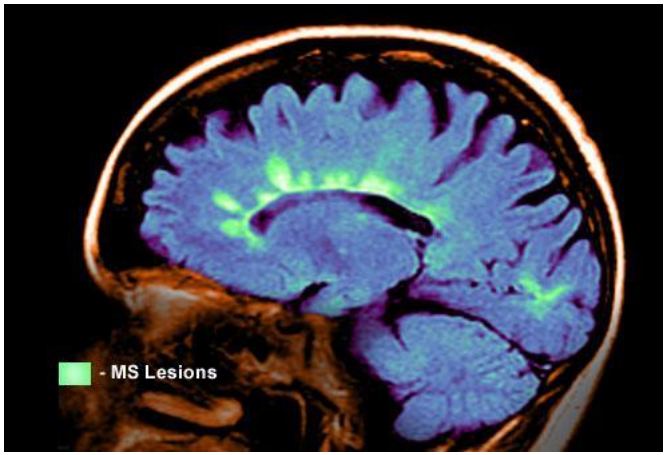
4. Confirmation of disease in subjects enrolling in clinical trials of new drugs.

5. Allows early enrollment of patients into clinical trials.

6. Evaluate patient response to therapy (drug efficacy) in clinical trials of new drugs.

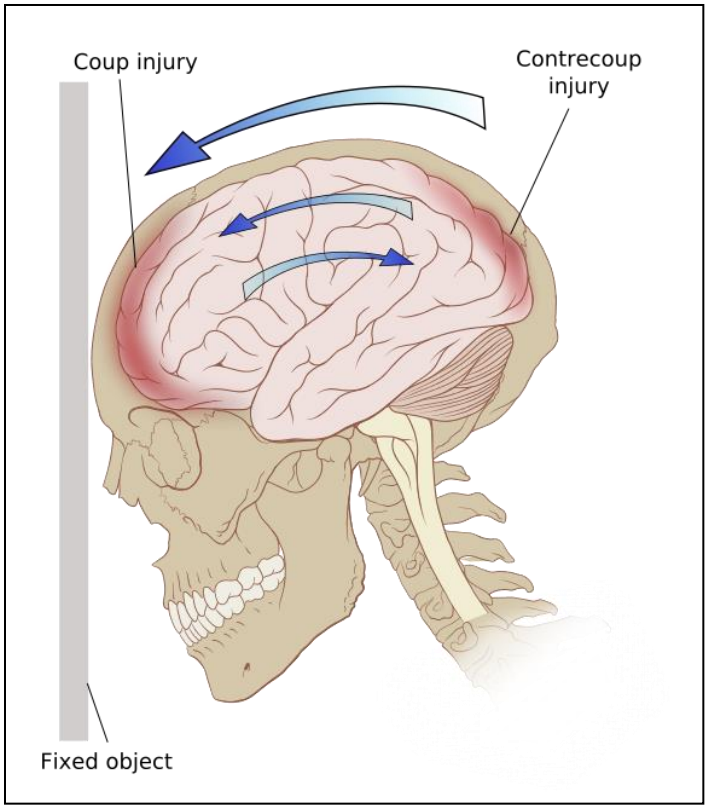
Multiple Sclerosis: Another autoimmune disease





In small patient group – 95% accuracy

Concussion and Traumatic Brain Injury

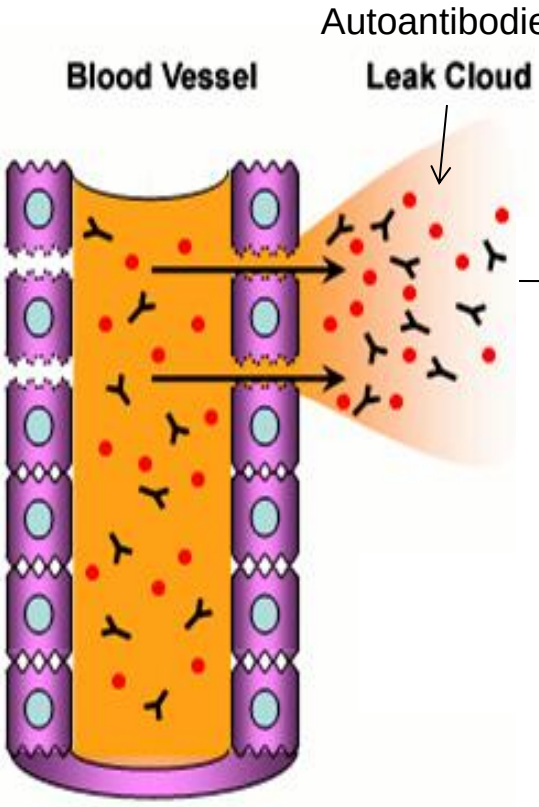


General Neurodegenerative Disease Biomarkers

- **Work Underway** -

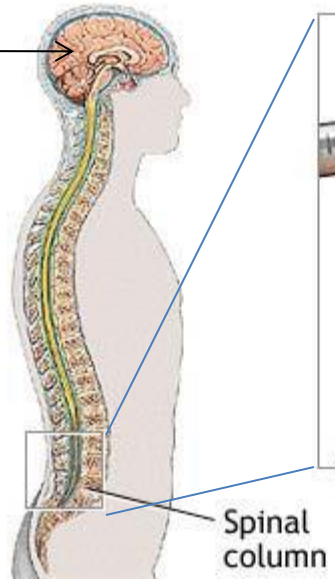
Detection of the Presence and Extent of BBB Breakdown In Post-Surgical Delirium Patients

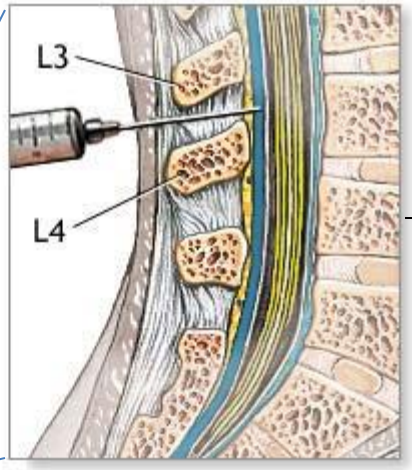
Detect Autoantibodies
in the Cerebrospinal Fluid (CSF)



Blood

→ Protein Microarray





CSF

→ Protein Microarray

READOUT

BBB Intact – No Abs in CSF
BBB Leak – Abs in CSF

Collaboration with F Sieber
at John's Hopkins.

Schizophrenia?

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

C Hammer[1], B Stepniak[1], A Schneider[2,3,4], S Papiol[1,3], M Tantra[1,3], M Begemann[1], A-L Sirén[5], LA Pardo[6], S Sperling[1], S Mohd Jofrry[1], A Gurvich[1], N Jensen[1], K Ostmeier[1], F Lühder[7], C Probst[8], H Martens[9], M Gillis[10], G Saher[11], F Assogna[12], G Spalletta[12], W Stöcker[8], TF Schulz[10], K-A Nave[3,11] and H Ehrenreich[1,3]

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

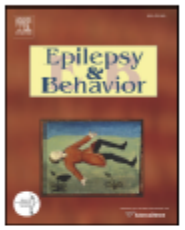
Psychosis?

Epilepsy & Behavior 36 (2014) 33–38

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh



Hypothesis

Epilepsy-related psychosis: A role for autoimmunity?

T.A. Pollak [a,b,*], T.R. Nicholson [a,b], J.D.C. Mellers [c], A. Vincent [d], A.S. David [b]

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ABSTRACT

Postictal psychosis (PIP) is a serious psychiatric complication of epilepsy that occurs in approximately 6% of patients following multiple complex partial or generalized seizures. The psychosis is classically described as having a pleomorphic phenomenology, including paranoid, grandiose, and religious delusions as well as multimodal hallucinations with prominent affective changes and agitation. Little is understood about the pathophysiology of the condition.

There has been a recent increase in interest in the relevance of autoimmunity to the pathogenesis of both epilepsy and psychosis. Studies have demonstrated the presence of antibodies directed against synaptic autoantigens (such as the N-methyl-D-aspartate receptor or the voltage-gated potassium channel complex) in approximately 10% of cases of sporadic epilepsy. These same autoantibodies are known to cause encephalopathy syndromes which feature psychiatric symptoms, usually psychosis, as a prominent part of the phenotype as well as other neurological features such as seizures, movement disorders, and autonomic dysfunction. It is beginning to be asked if these antibodies can be associated with a purely psychiatric phenotype.

Here, we hypothesize that PIP may be an autoimmune phenomenon mediated by autoantibodies against synaptic antigens. More specifically, we outline a potential mechanism whereby long or repeated seizures cause short-

Collaboration with TA Pollak at King's College London

Diagnosis of Stage 0 - 1 Beast Cancer Using Serum Autoantibodies

1. Experimental Design

Subjects	Gender	Sample size	Age
Breast Cancer	Female	30	47 ± 5.8
Control	Female	23	52 ± 16

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

2. Diagnosis of breast cancer using the top 50 biomarkers

Training Error: 4.3% on average

Testing Error: 2.5% on average

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10	Average
Training Error	2.70%	2.70%	8.10%	5.41%	5.41%	2.70%	2.70%	5.41%	5.41%	2.70%	4.3%
Testing Error	1/16	0/16	0/16	0/16	0/16	1/16	0/16	0/16	1/16	1/16	2.5%

Overall Results for Early Stage Breast Cancer - >95% accuracy!

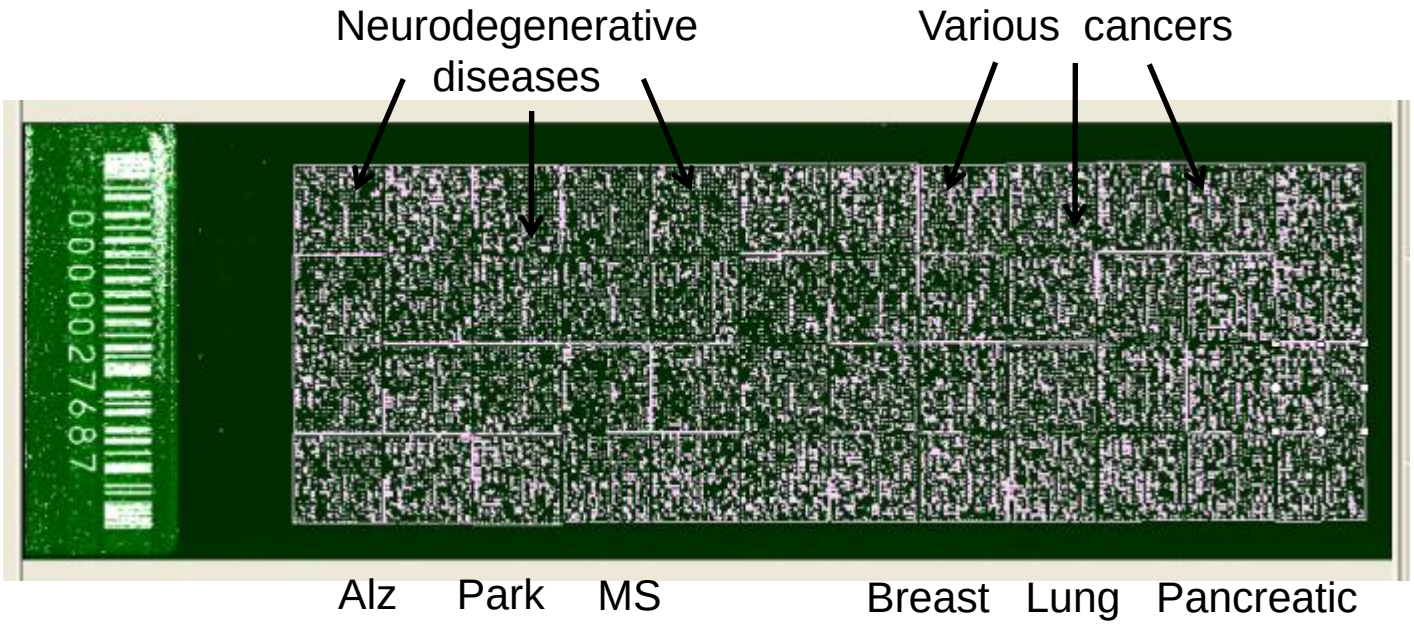
Future Plans and Directions

Short-Term Plans for MCI Diagnostic Test

1. Identify biomarkers for AD-driven MCI and patent the MCI biomarkers.
2. Complete a large-scale validation study of the MCI diagnostic using serum samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI).
3. Seek FDA approval.

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

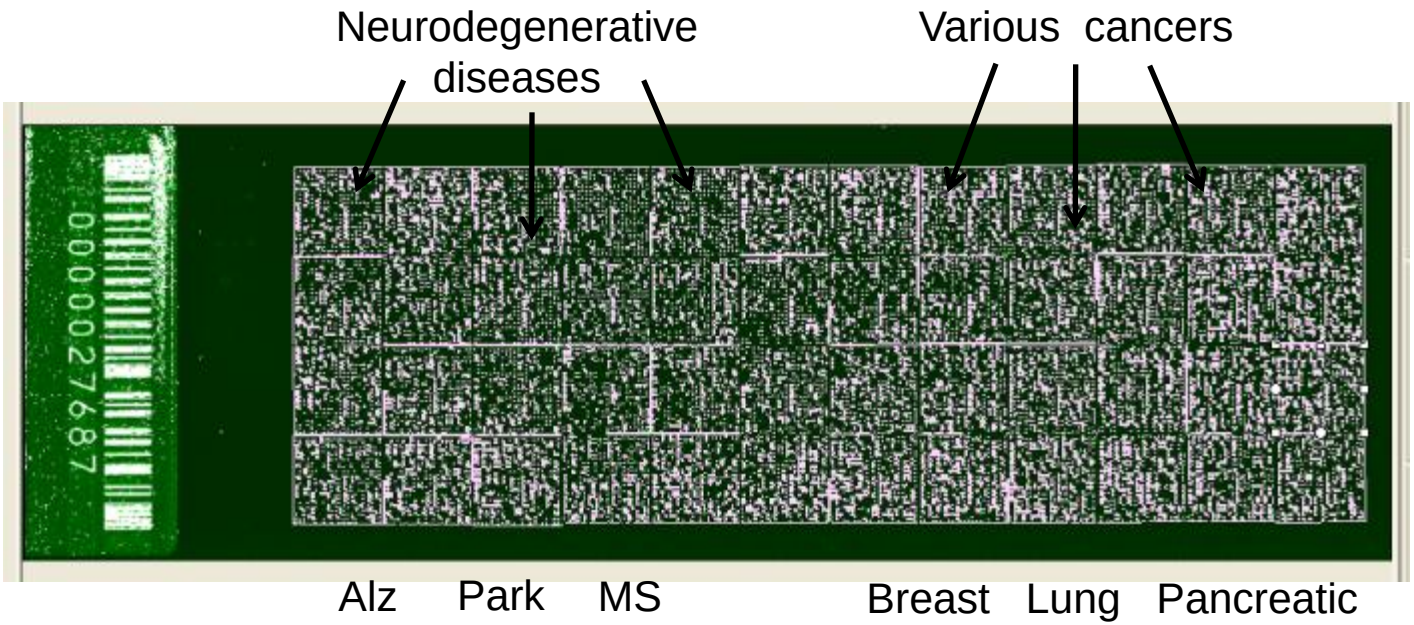
Only 20-50 autoantibodies are needed for each diagnostic.
Each array can currently hold over 20,000 protein targets.
There is plenty of room for hundreds of diagnostic tests on a single microarray.



Biomarkers of Therapeutic Efficacy

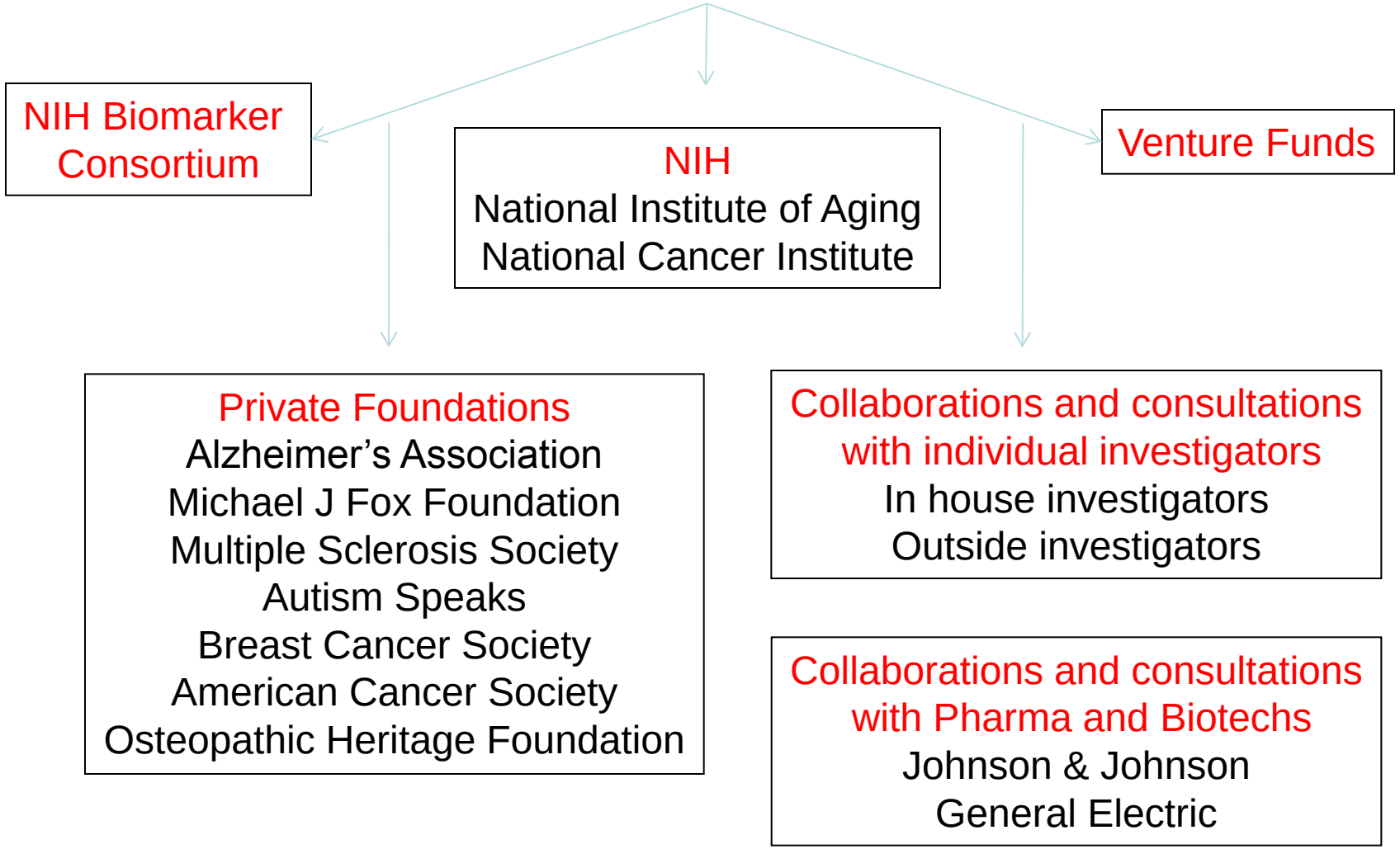
Concept

If treatments are effective, there should be less disease-associated debris production and a corresponding decrease in autoantibody biomarkers in the blood



Comparison of blood samples before and after drug treatment

RowanSOM NJISA's Biomarker Discovery Center



Some Members of My Laboratory Family

And The Osteopathic Heritage Foundation

Thank You