BIOMARKERS IN NEURODEGENERATIVE DISEASES

Tanja Krainz
Frontiers
Wipf Group Seminar
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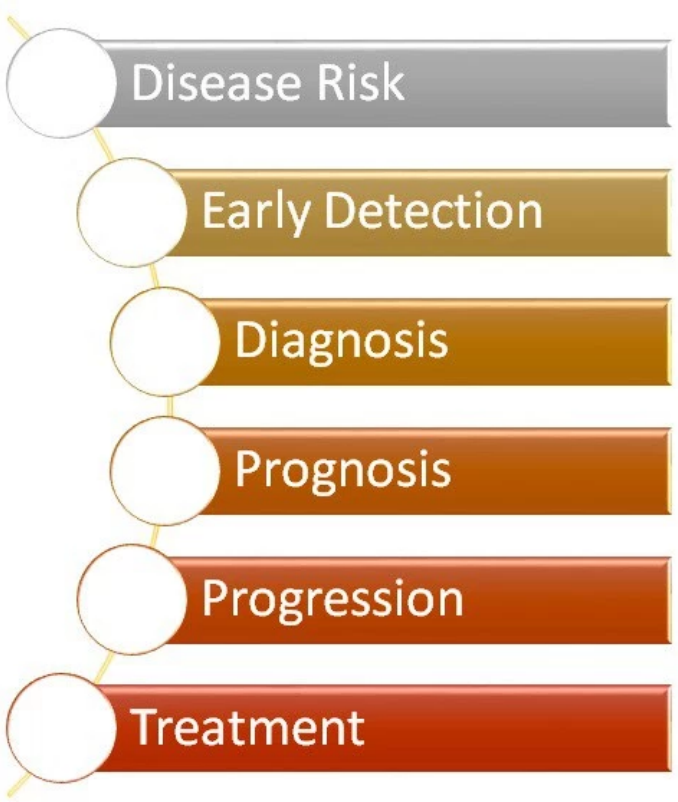
What is a Biomarker?



"..a biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test result."

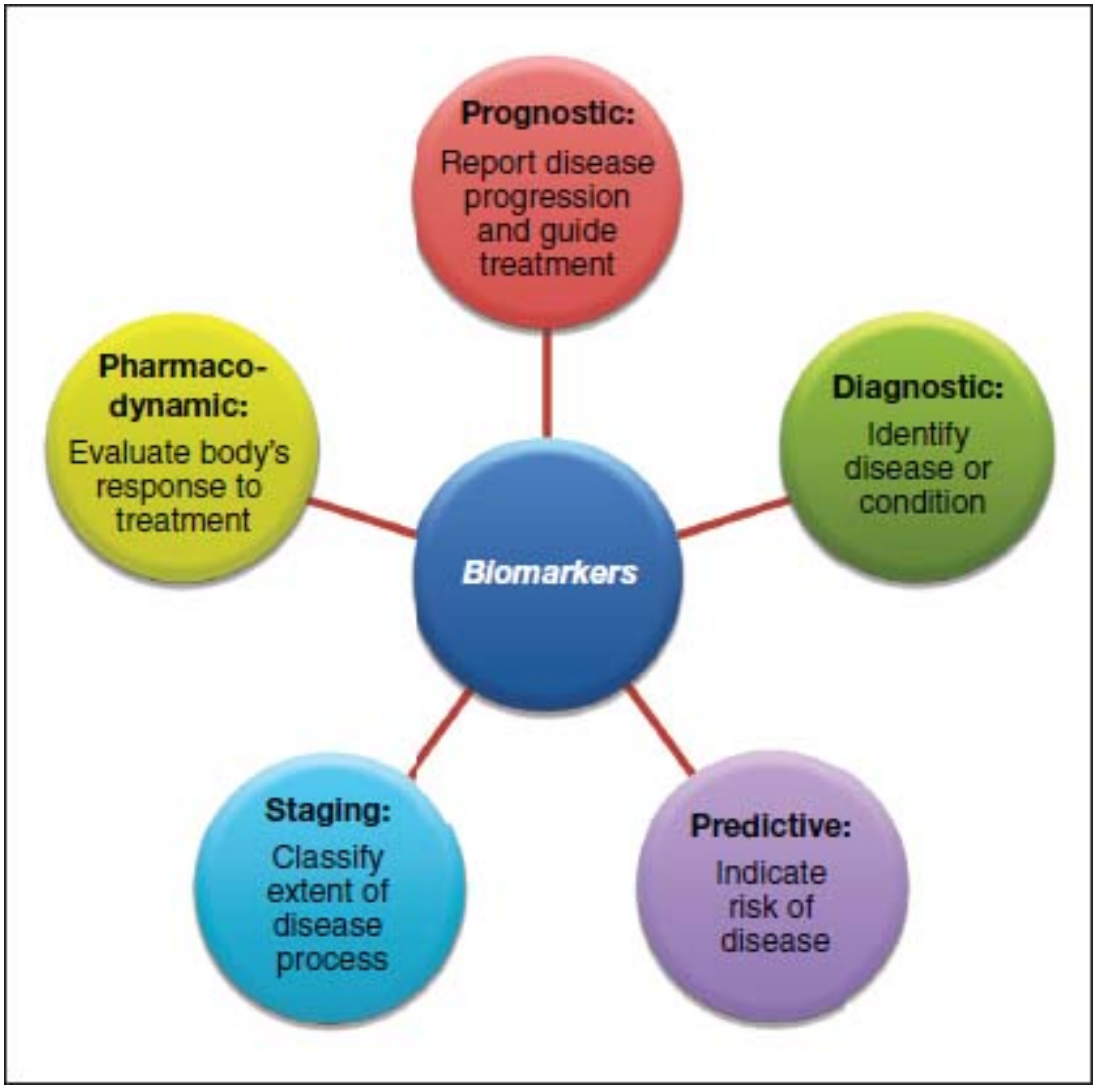
From FDA pharmacogenomics guidance

Importance of Biomarkers

- **Earlier Diagnosis** before onset of symptoms
  - Predict specific **organ involvement** and disease flares
  - Identify clinically meaningful **diseases subsets**
  - Predict and monitor **response to therapy**
  - Describe organ or **tissue damage**
- Can be useful in identifying genetic **predisposition to disease** or environmental triggers
- May provide early information about **potential efficacy** of experimental agents or **mechanism underlying drug actions**
- Can become validated **surrogate endpoints** for prevention trials

While disease symptoms are subjective, biomarkers can provide an objective, measurable way to characterize a disease.

Types of Biomarkers



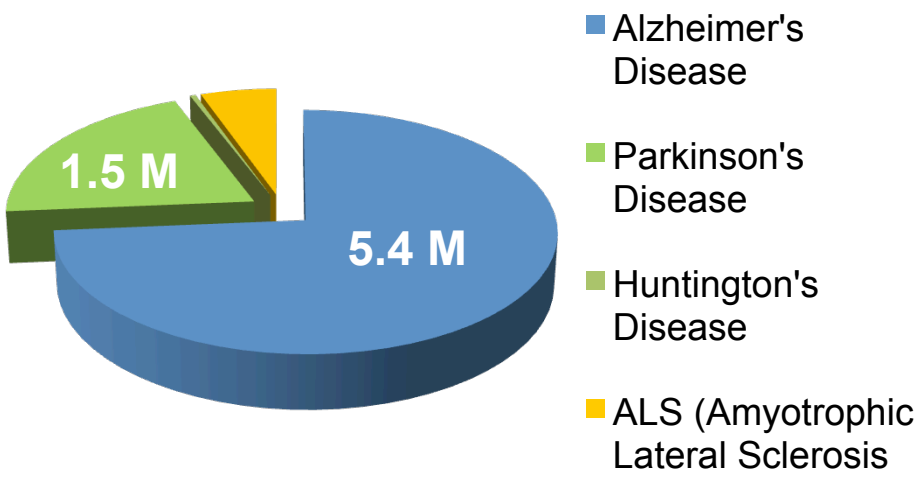
AJN, American Journal of Nursing. **2012**, 112(9):52-58

Neurodegenerative Diseases

- Central Nervous System (CNS) disorder characterized by the progressive loss of neurons/neural tissue.

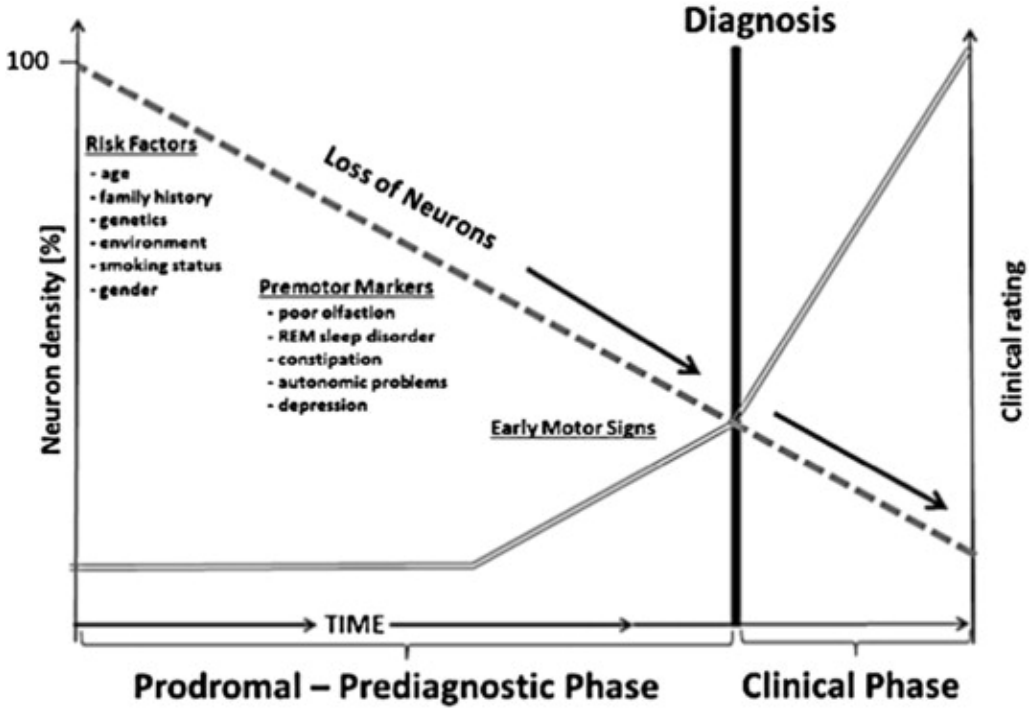
- CNS can not regenerate on their own after cell death or damage.

Prevalence in US (2015)



Lesser known ND
Prion disease
spinocerebellar ataxia or spinal muscular atrophy

Challenges in Neurodegenerative Diseases



At stage of diagnosis, 60-70% of neurons already lost

Criteria for Biomarkers:

(1) prodromal, preclinical or premotor stage biomarkers;

(2) biomarkers of risk or susceptibility; and

(3) motor stage biomarkers.

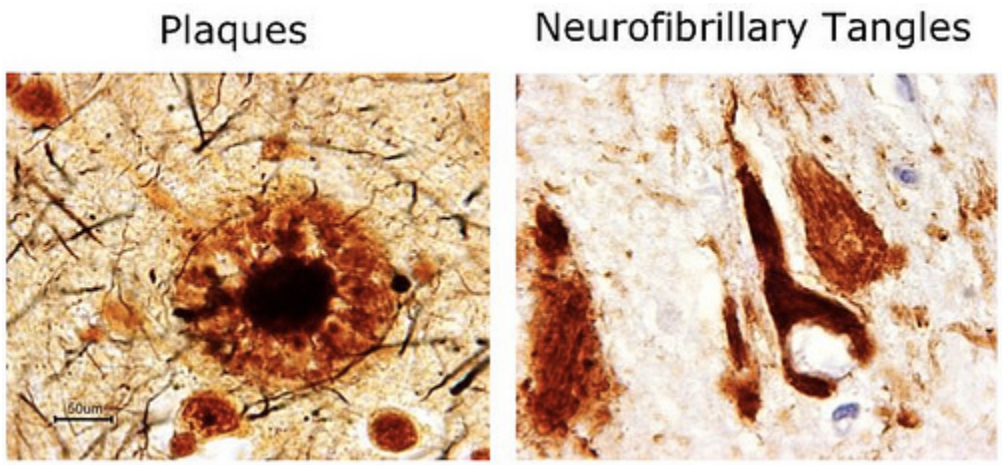
Biomarkers addressing these categories could be based on clinical, imaging, genetic, proteomic, or biochemical factors or various combinations of these

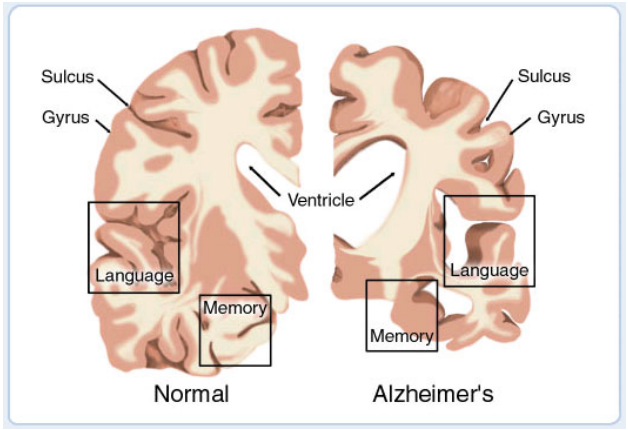
Alzheimer's Disease

Neuropathology:

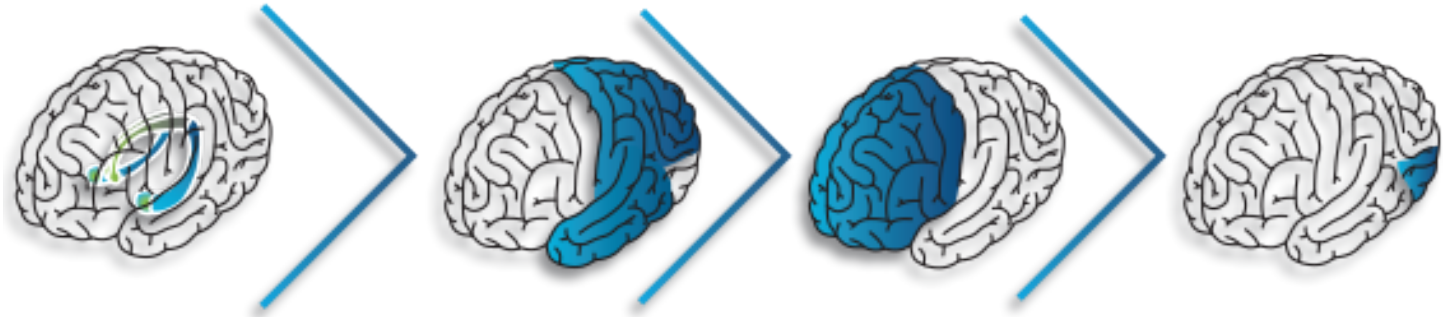
- ***Extracellular amyloid plaque***
- ***Intracellular fibrillary tangles*** *(misfolded, aggregated AB peptides, and hyperphosphorylated tau protein)*
- ***Neuroinflammation***

- Decreased brain metabolism
- Brain atrophy





Stages of Alzheimer's Disease



Mild Cognitive Impairment

Duration: 7 years

Disease begins in Medial Temporal Lobe

Symptoms:
- Short-term memory loss

Mild Alzheimer's

Duration: 2 years

Disease spreads to Lateral Temporal & Parietal Lobes

Symptoms include:
- Reading problems
- Poor object recognition
- Poor direction sense

Moderate Alzheimer's

Duration: 2 years

Disease spreads to Frontal Lobe

Symptoms include:
- Poor judgment
- Implusivity
- Short attention

Severe Alzheimer's

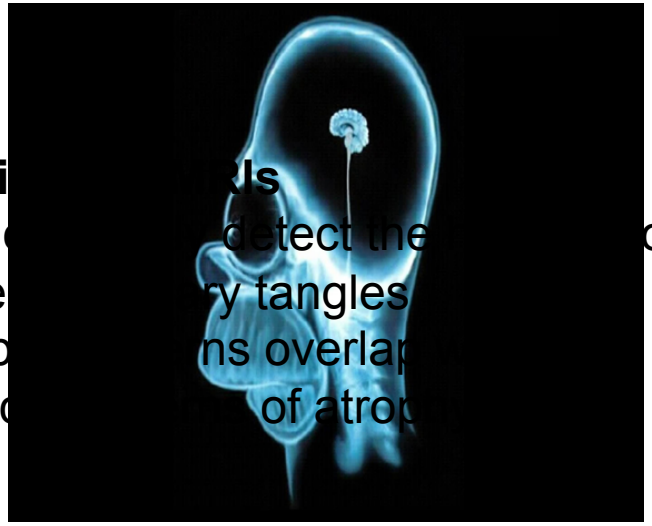
Duration: 3 years

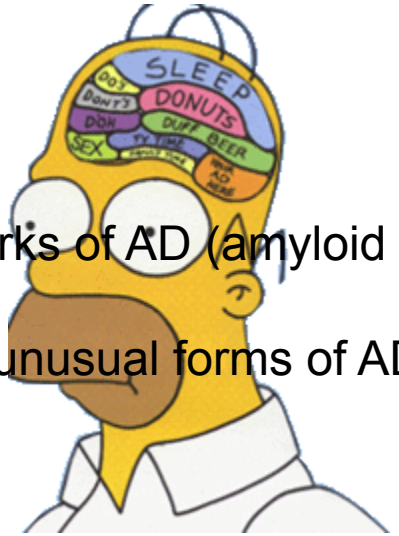
Disease spreads to Occipital Lobe

Symptoms include:
- Visual problems

Brain Imaging in Neurodegenerative Diseases

- ***Structural MRI***
  - Assessing atrophy (volumes)
  - Changes in tissue characteristics (signal alterations on certain sequences such as white matter hyperintensities
- ***Functional MRI***
  - assessing brain function

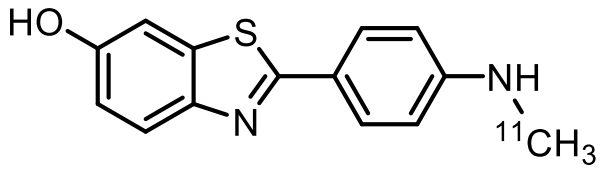




Limitati **RIs**

- cann detect the ological hallmarks of AD (amyloid plaques or ne y tangles
- Atrop ns overlap diseases and unusual forms of AD have atypi of atrop

PiB-PET Imaging

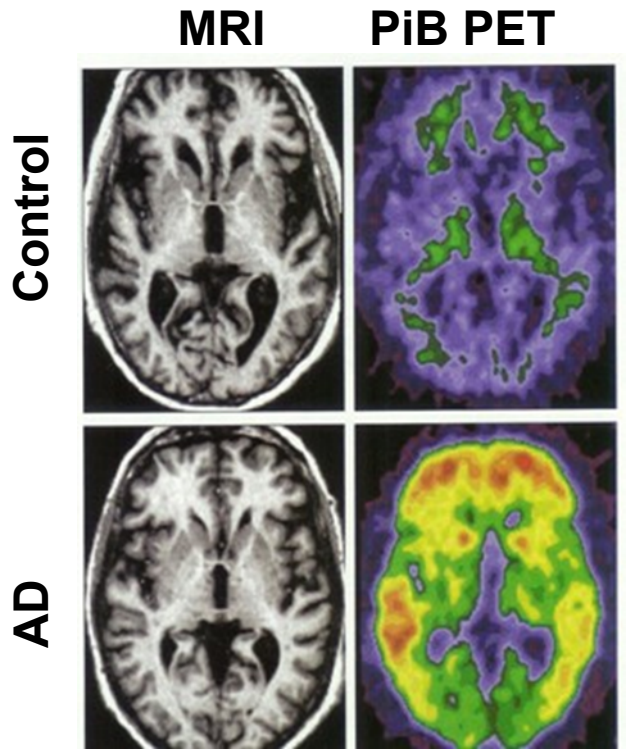


Pittsburgh Compound B

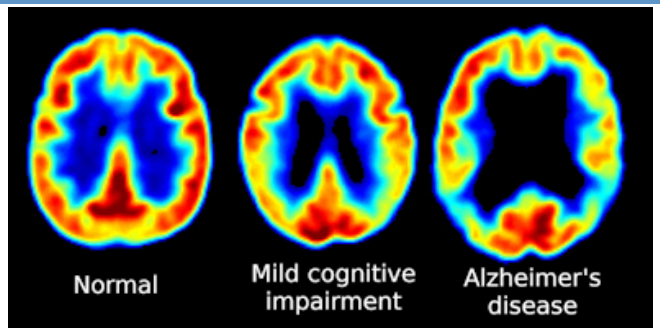
- **PiB-Pittsburgh Compound B**

Williams E. Klunk (geriatric psychiatrist)
Chester A. Mathis (radiochemist)

- Radiolabelled thioflavin T derivative
- Selectively binds to beta-amyloid plaque and cerebrovascular amyloid
- C-11 isotope, very short half-life of 20 min

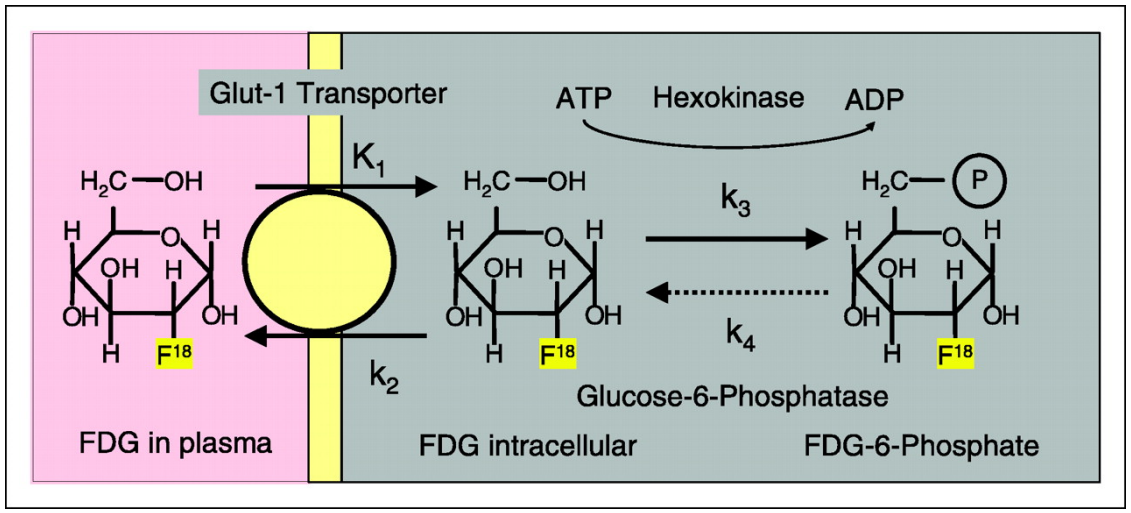


FDG PET Scans



[^{18}F]Fluorodeoxyglucose PET Scans

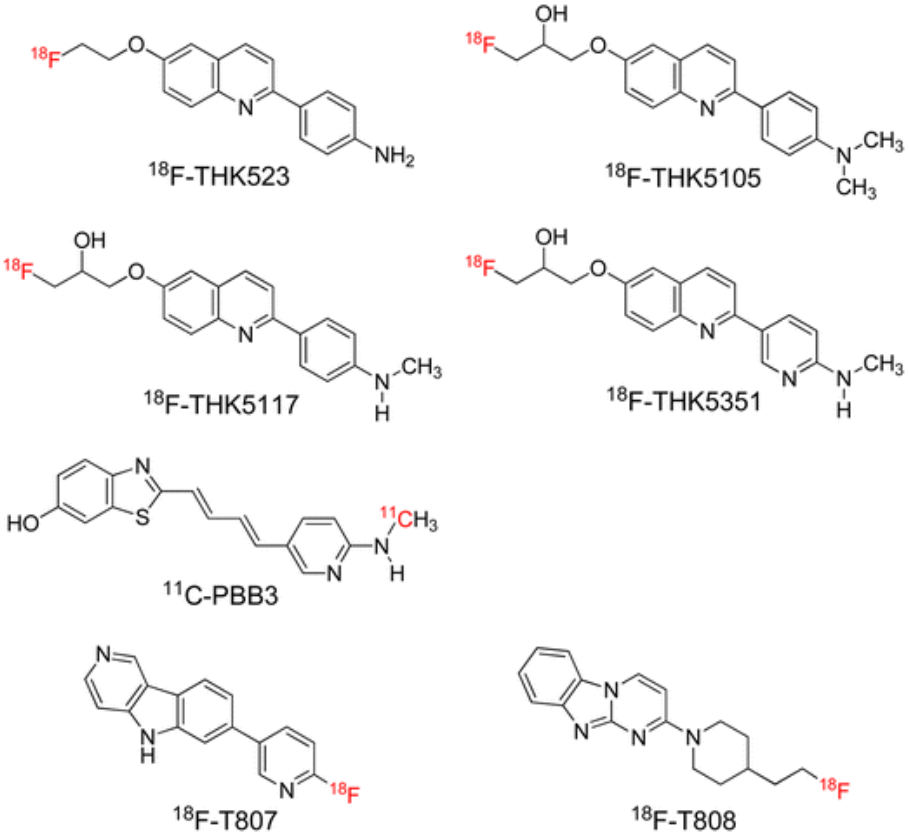
[^{18}F]FDG PET imaging is used to measure cerebral metabolic rates of glucose an index of brain synaptic activity and density



Limitations of FDG PET Imaging:

- Expensive
- Requires intravenous access and involves exposure to radioactivity (levels well below significant known risk)
- Brain FDG retention is a nonspecific indicator of metabolism that can be deranged for a variety of reasons (e.g., ischemia or inflammation)

Currently available tau imaging radiotracers

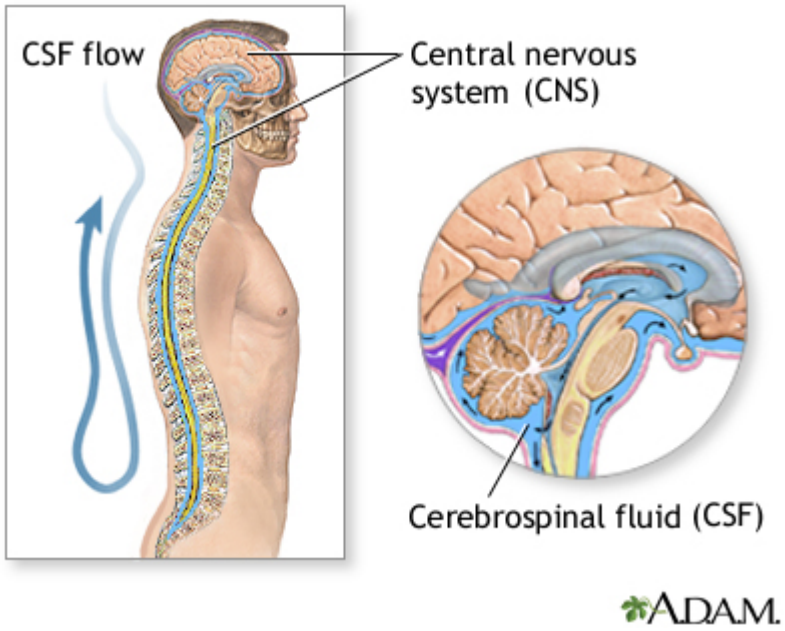


Key general properties for radiotracers:

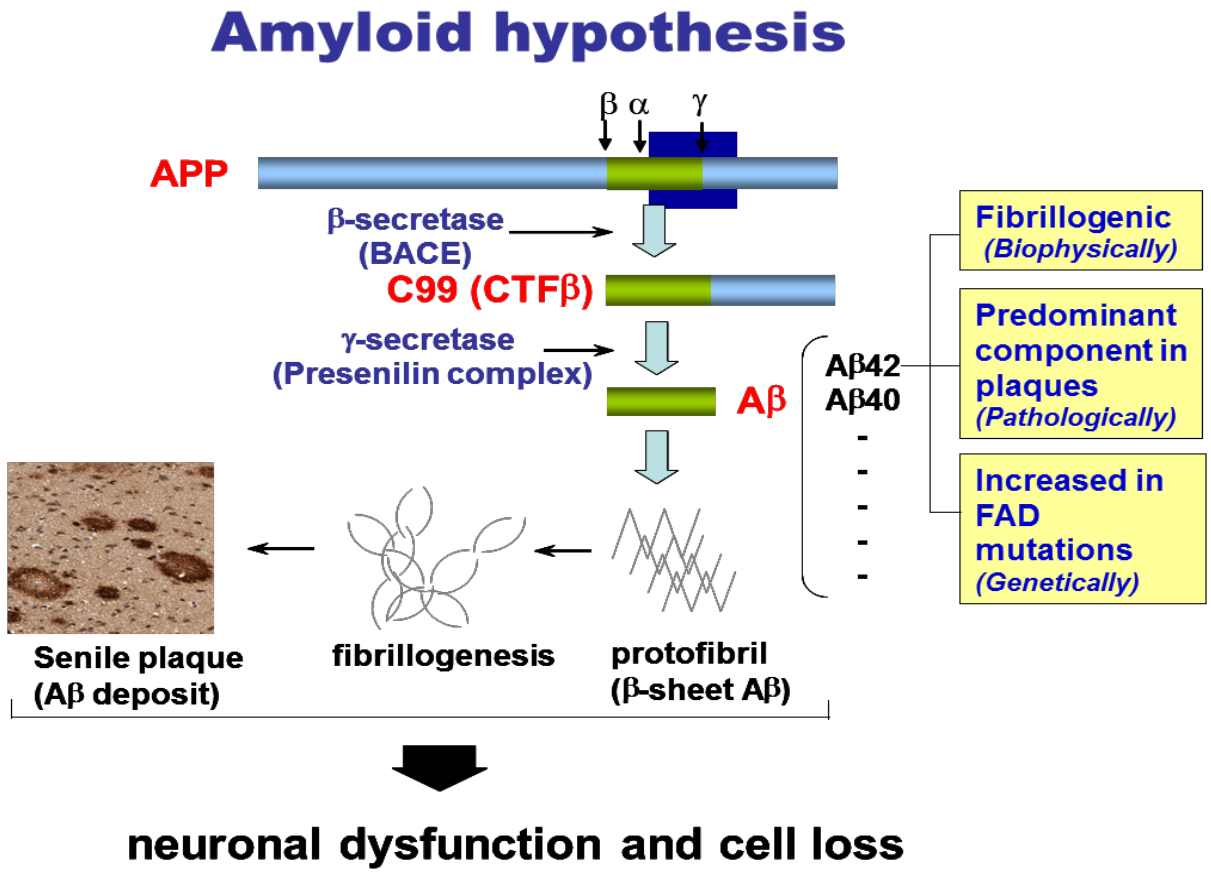
- nontoxic lipophilic molecules of low molecular weight (<450) that cross the BBB
- rapid clearance from blood and preferably not metabolized, whilst reversibly binding to its target in a specific and selective fashion.

Cerebrospinal Fluid (CSF) Biomarkers for AD

- CSF is a translucent body fluid
- “liquid cushion” providing basic mechanical and immunological protection to the brain
- Most informative fluid biomarker for ND prognosis due to
  - Physical contact between CSF and the brain
  - CSF not separated from the brain by blood-brain-barrier (BBB)

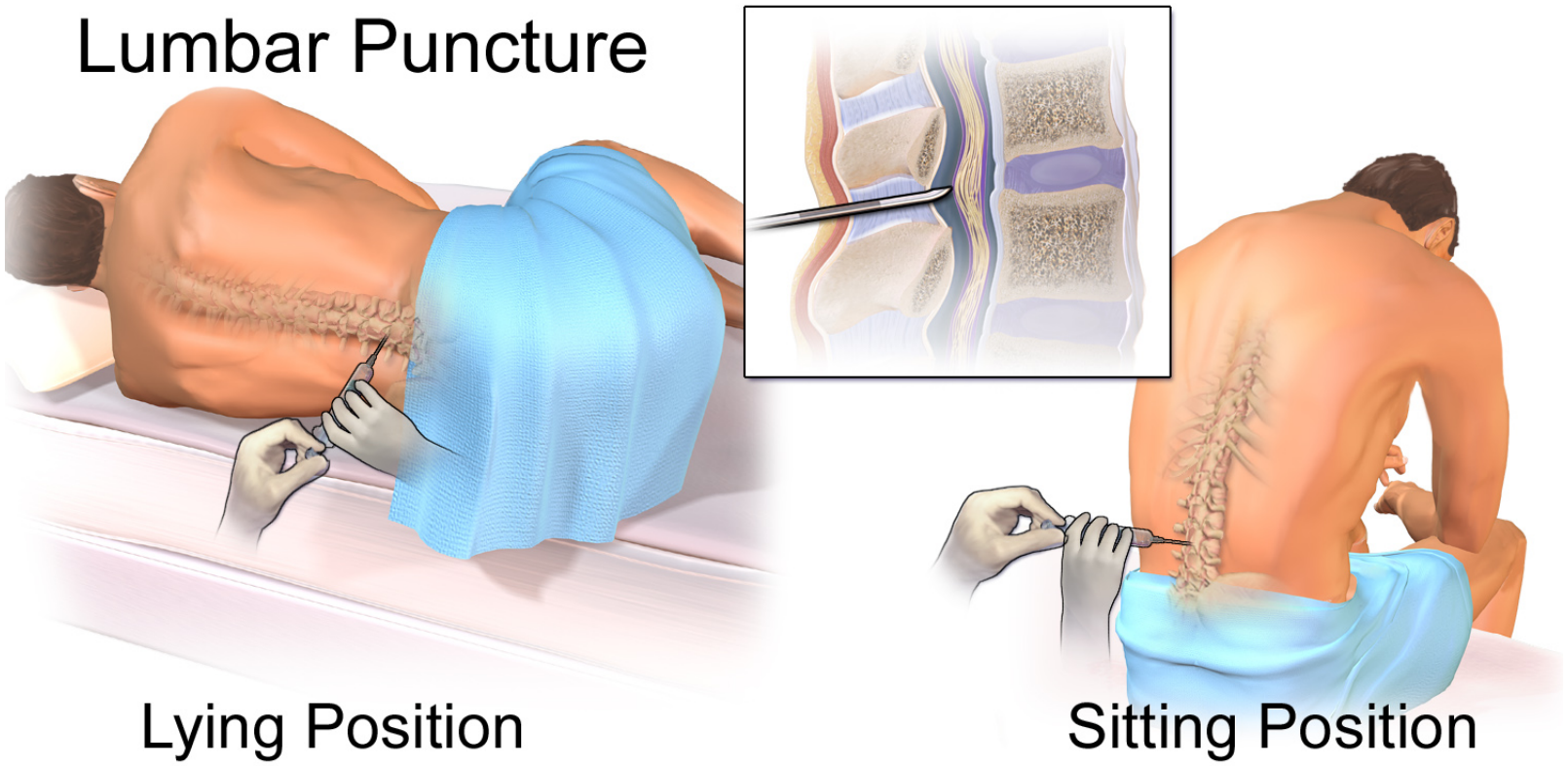


CSF Biomarkers for AD



- APP sticks through the neuron membrane.
- Enzymes cut the APP into fragments of protein, including beta-amyloid.
- Isoforms of β-amyloid can go through a slow nucleation stage, followed by fast elongation resulting in the formation of mature aggregated fibrils.
- Overproduction and ineffective clearance of Aβ causes plaque deposition.

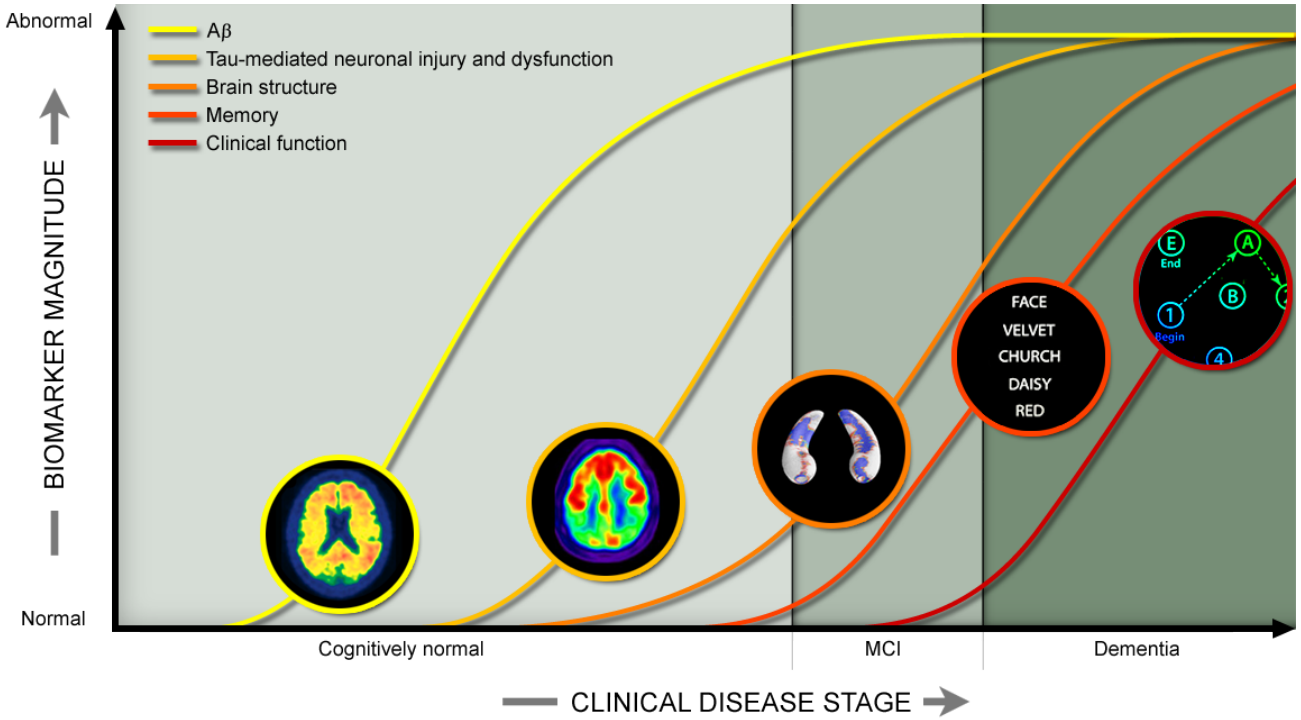
Disadvantage: CSF Collection



Other Biomarkers for AD and PD

- **Blood, Plasma, and Serum**
  - Currently investigation of microRNAs
- **Urine**
  - Contains proteins, RNAs, small molecules and metabolites
  - 8-hydroxydeoxyguanosine (8-OHdG) levels in urine correlate well with stage of PD
  - AD7c (neuronal thread protein) correlates well with severity of dementia in AD
- **Saliva**
  - Submandibular gland (where saliva is produced) expresses both tau and APP

Summary AD

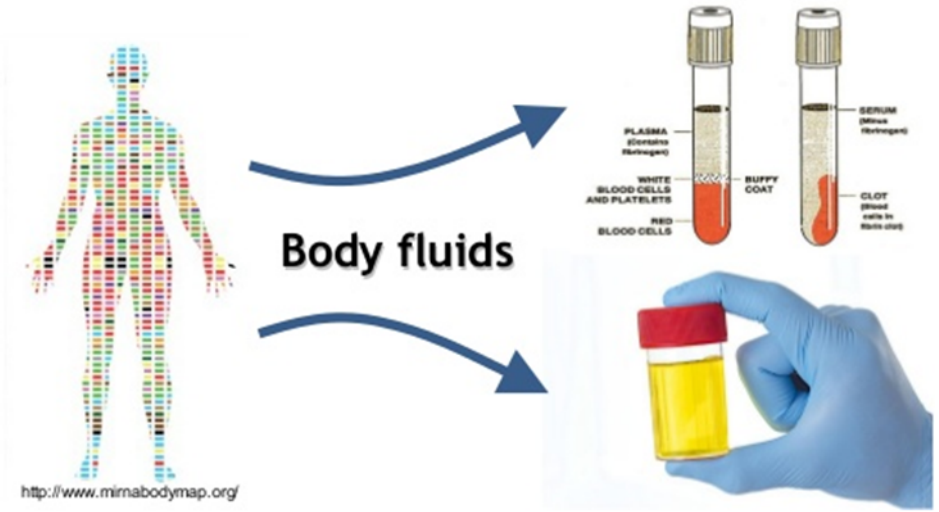


1. **Amyloid beta** imaging detected in CSF and PET amyloid imaging
2. Neurodegeneration detected by rise of **CSF tau species** and synaptic dysfunction, measured via FDG-PET
3. **Brain atrophy** and neuron loss measured with MRI (most notably in hippocampus, caudate nucleus, and medial temporal lobe)
4. **Memory loss** measured by cognitive assessment
5. **General cognitive decline** measured by cognitive assessment

MicroRNA as a new Biomarker

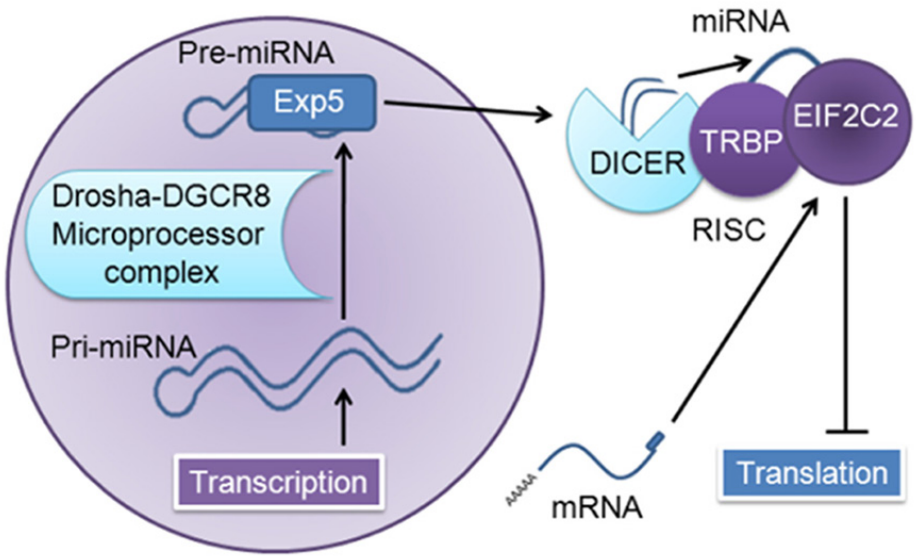
- microRNA can be detected in serum, plasma, urine and other biological fluids
- microRNA are non-coding RNAs found only in eukaryotic cells
- Small in size with an average lengths of 22 nucleotides
- Transcribed by RNA polymerase II from independent genes
- Play important gene regulatory roles in plants and animals

Highly expressed in the CNS, including brain and spinal cord



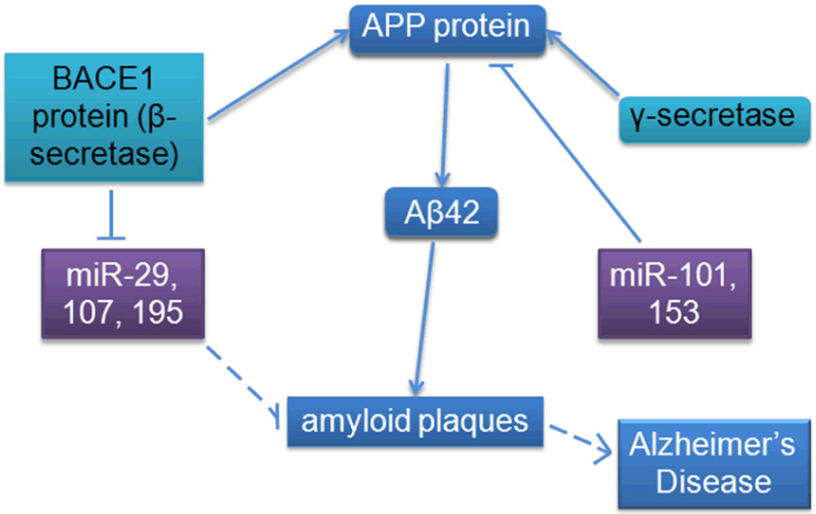
MicroRNA as a new Biomarker

- MicroRNAs (miRNAs) are **small non-coding RNA** which have been shown to **regulate gene expression**.
- It has been demonstrated that specific miRNAs are expressed in the central nervous system (CNS), where they regulate neuronal differentiation, synaptic plasticity and neurite outgrowth



- miRNA is generated as a long precursor sequence in the nucleus; cleaved to form a shorter stem-loop precursor,
- transported to the cytoplasm, and further processed in the RNA-induced silencing complex (RISC) by the ribonuclease DICER1 into short (~22 nucleotide) double-strand sequence;
- this is then unwound and one-strand is loaded onto EIF2C2, which consequently inhibits translation or results in cleavage of target messenger RNAs

MicroRNA as a new Biomarker



- miRNAs involved in regulatory functions of the amyloid pathway, including regulation of amyloid protein precursor (APP) and beta-site APP cleaving enzyme 1 (BACE1 or β-secretase), a protease that cleaves APP to generate Aβ
- 8 major microRNAs identified that correlate with disease pathology

Advantages/Limitations of microRNA biomarkers

- Mature miRNAs are small, stable molecules
- Resistant to RNA degradation
- Highly conserved amongst species
- Differentiate pathological phenotypes
- Non-invasive biomarkers
- Could lead to early diagnosis
- Might enable to get patients on treatment earlier

- It's difficult to measure each miRNA contribution to the disease
- Cost of miRNA sequencing

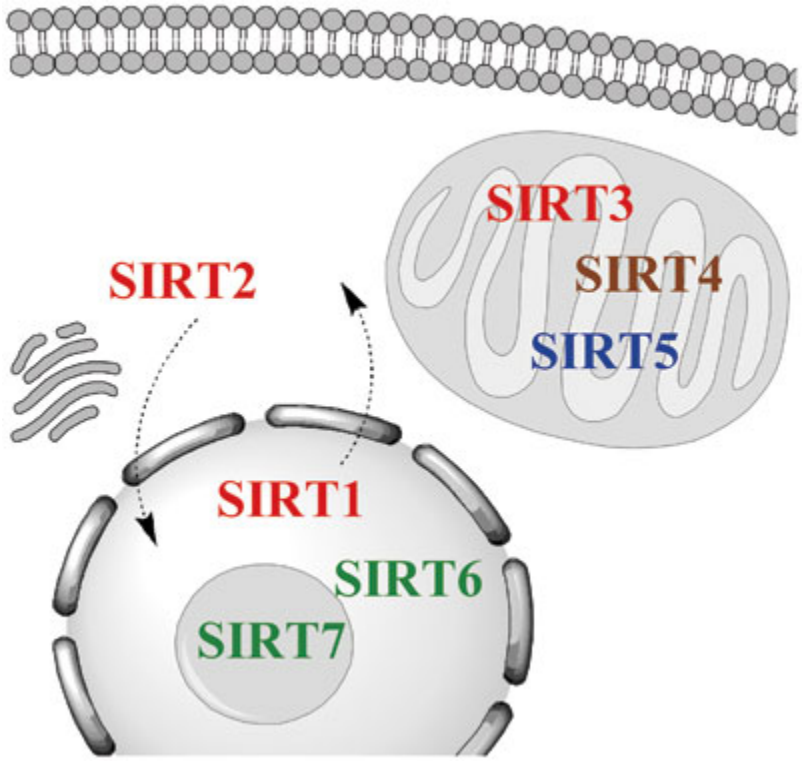
Sirtuins as a biomarker for Neurodegenerative Diseases?

Silent **I**nformation **R**egulator (SIRTuins)
NAD-dependent deacteylase

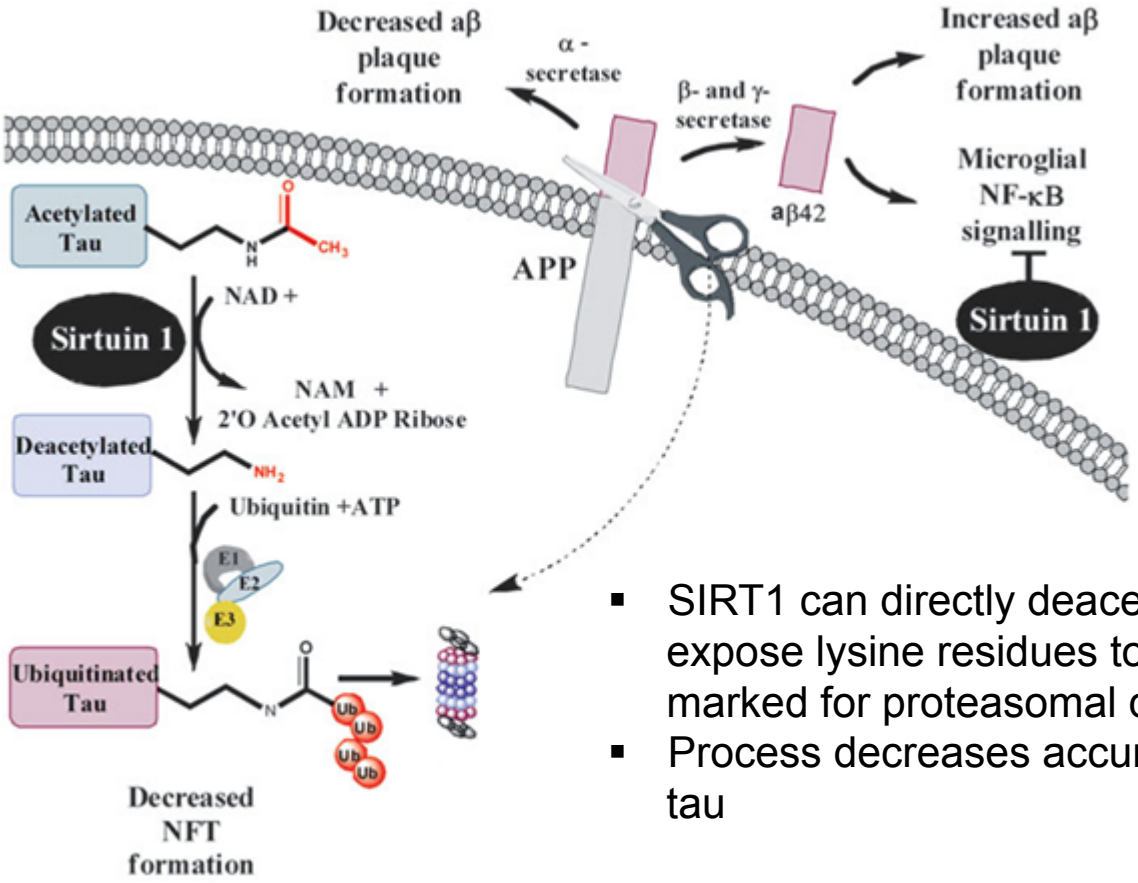
Modulate major biological pathways, such as

- stress response
- protein aggregation
- inflammatory processes

That are involved in the aging and age-related diseases



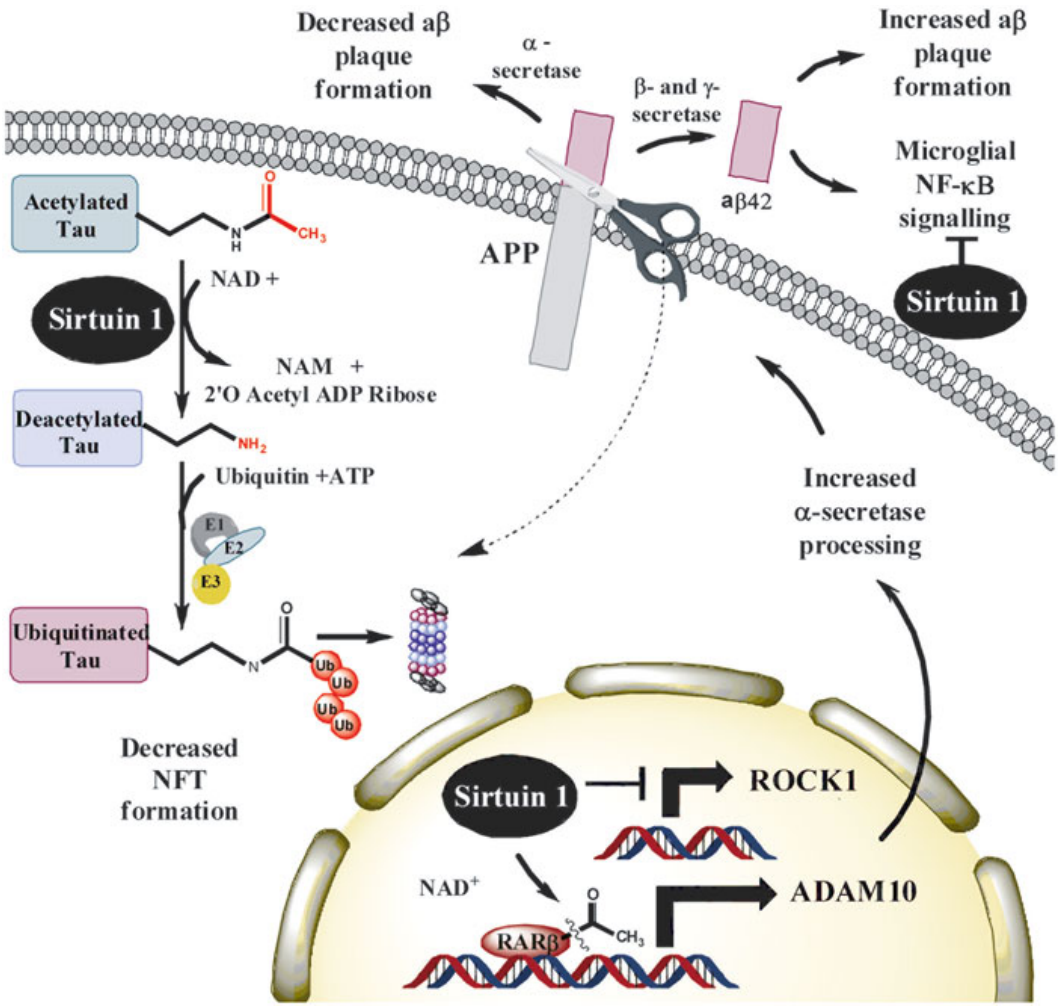
Why Sirtuins?



- SIRT1 can directly deacetylase tau protein which may expose lysine residues to ubiquitin ligases so tau protein is marked for proteasomal degradation
- Process decreases accumulation of hyper phosphorylated tau

Cell Research (2013) **23**:746–758

Why Sirtuins?



- Overexpression of SIRT1 <u>decreases the plaque burden</u> and improve behavioral phenotypes by deacetylating retinoic acid and receptor β, a transcriptional activator of ADAM10.

- ADAM10 is a component of the α-secretase
- SIRT1 expressen may decrease ROCK1, a serine/threonine Rho kinase previously shown to regulate Aβ metabolism.

Biomarker Need in Pharmaceutical Drug Development

Biomarkers as an essential part of clinical development

- 20 drugs withdrawn by the FDA in the last 10 years!
  - Mainly due to hepatotoxic and cardiotoxic effects

- Biomarkers are becoming an essential part of clinical development
- Improving clinical research
- Improve decision making
- Accelerate drug development
- Reduce development costs

The percentage of tested products entering phase I trials that eventually gain regulatory approval is about 8%

Biomarker Need in Pharmaceutical Drug Development

- Define populations with highest benefits from a drug (diagnostics, personalized medicine)
- Monitor the effects of therapy (pharmacodynamics)
- Predict clinical outcomes early (surrogate endpoints)
- Monitor adverse events (safety biomarkers)
- Ongoing feedback from clinic to generate hypotheses to drive preclinical research
- Ongoing feedback from preclinical or ex vivo studies to help design clinical trials
- Biomarkers are essential for the approach of Translational Science

Summary and Future Outlook

- Several biomarkers for neurodegenerative diseases have been established and need further evaluation in clinical trials
- MicroRNAs promising new biomarkers for early diagnosis of AD
- BUT, more comprehensive analyses of miRNA functions needed; contribution of other research areas such as epigenetics and systems biology will be necessary to gain a better understanding of miRNAs and the regulatory networks in which they are involved.
- SIRTUINS as novel biomarkers for early detection of ND

Acknowledgments

- Dr Peter Wipf
- Wipf Group Members Past & Present