Early Changes in the Brain Proteome Associated with Alzheimer's Disease Risk

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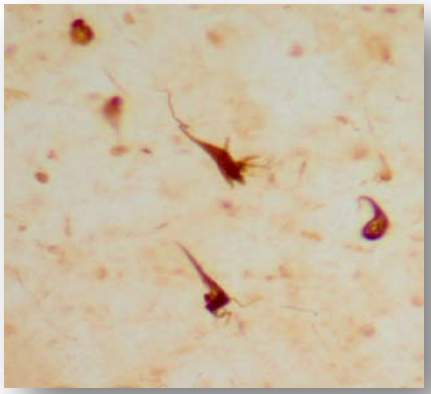
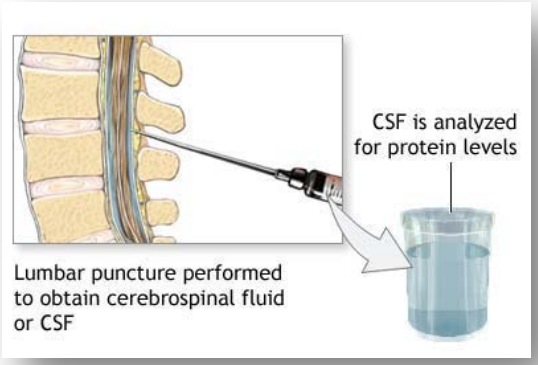
Proteomics at the Emory ADRC



Pathological Aggregates

Synapse-rich

Proteome



Cerebral Spinal Fluid (CSF)

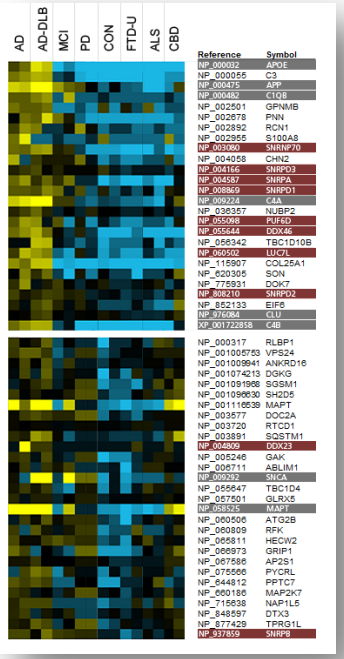
Plasma/Platelets

Proteomics in Alzheimer's Disease (AD)

Specific Goal

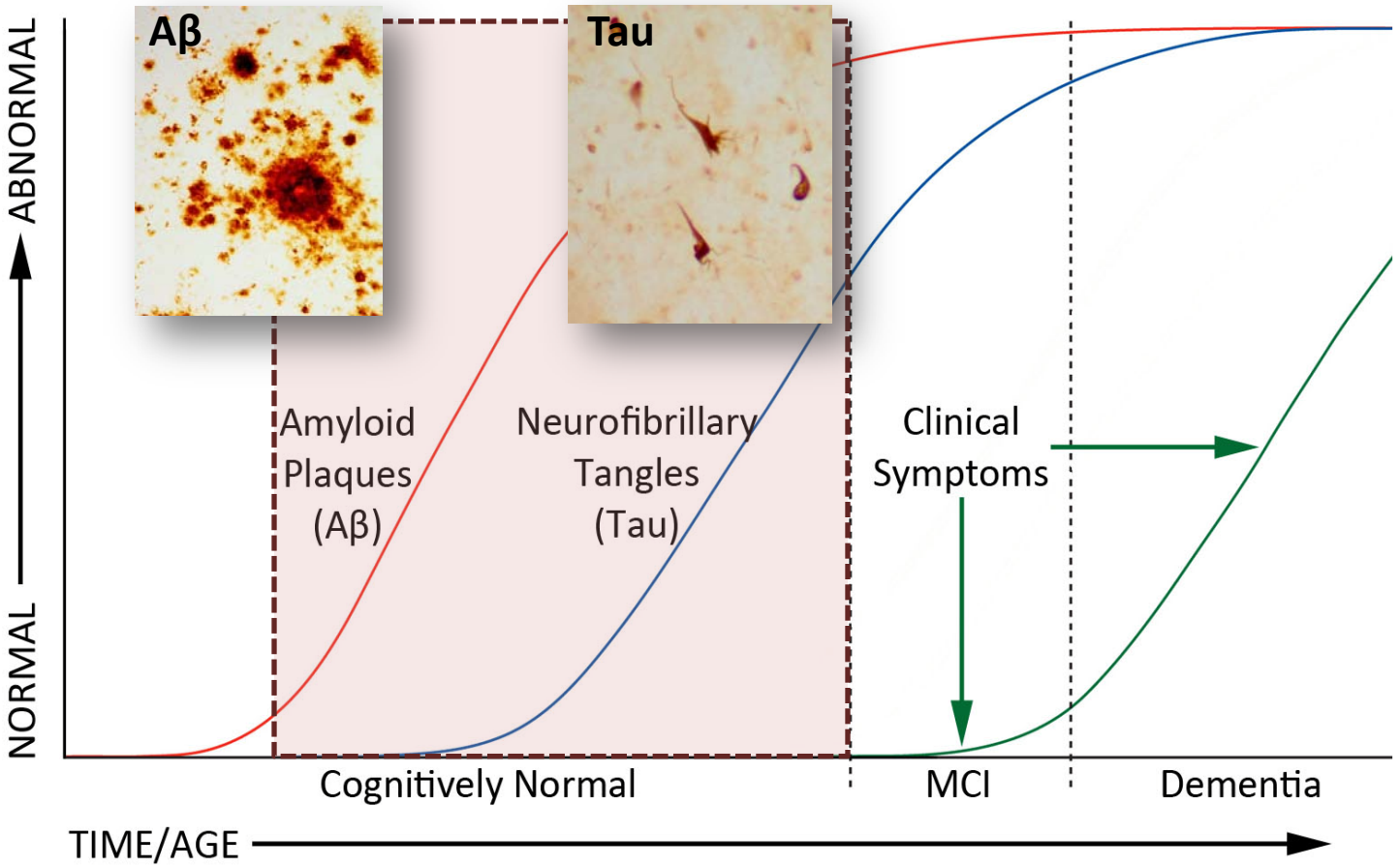
- Develop accurate and precise method to quantify proteins in brain tissue

Ultimate goal: Can we use proteomics to better define pre-clinical stages of AD and target key molecular pathways that associate with cognitive decline?



The Alzheimer's Continuum

Preclinical or Asymptomatic AD: The period between the first appearance of AD neuropathology and the onset of clinically detectable symptoms of disease.



(Jack CR et al 2010, Lancet Neurology)

Evidence for Asymptomatic AD

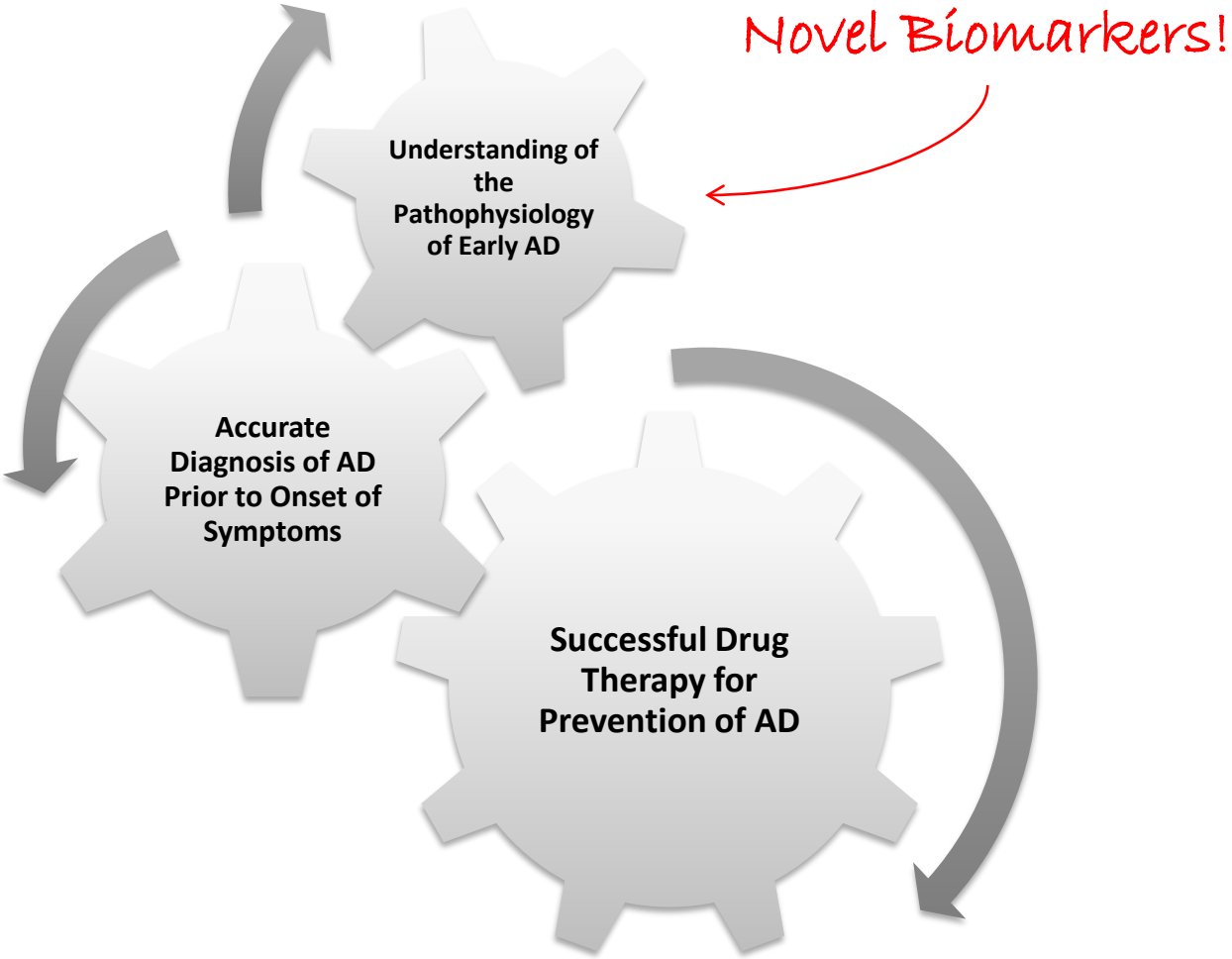
- Post-mortem neuropathological and *in vivo* PET imaging studies suggest that a substantial proportion of **cognitively normal** older individuals demonstrate evidence of **Aβ** and **tau** tangle accumulation, approximately **30%**.

- The distribution of amyloid deposition in these **cognitively normal** older individuals tends to occur in a pattern similar to that found in AD.

- **Summary:** This suggests that **Aβ** and **neurofibrillary** pathologies are necessary, but **not sufficient alone** to explain the onset of cognitive decline.

(Rowe et al. 2010, Mintum et al. 2006, Jack et al. 2008, Gomperts et al. 2008)

Relevance of Asymptomatic AD

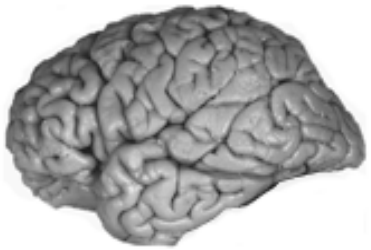


Biomarker Criteria in AD

1. Detect a fundamental feature of AD neuropathology.
2. Be validated in autopsy-confirmed cases of the disease.
3. Have a diagnostic specificity for distinguishing and sensitivity for detecting AD from other dementias.
4. Diagnostic laboratory tests should be reliable, reproducible, non-invasive and simple to perform.

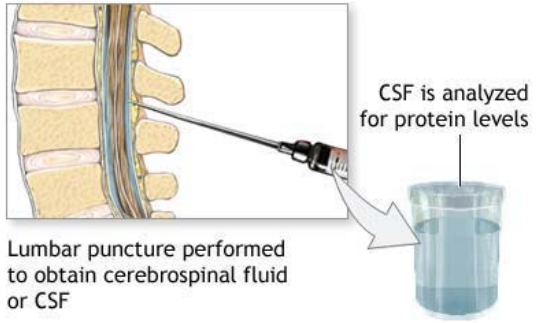
Trojanowski, J.Q. (2004) *Practical Neurology*, 3:30-34
Frank RA, (2003) Neurobiol. Aging, 24:521-536

Brain



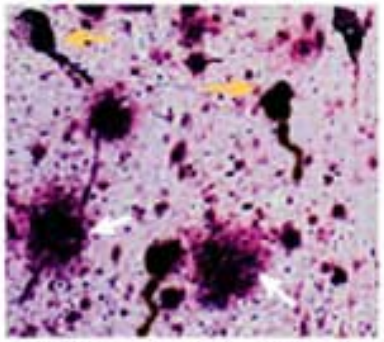
Orbitrap MS

CSF

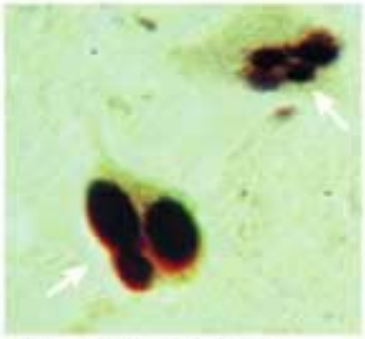


Where do we first look for biomarkers in AD?

β-amyloid
Tau



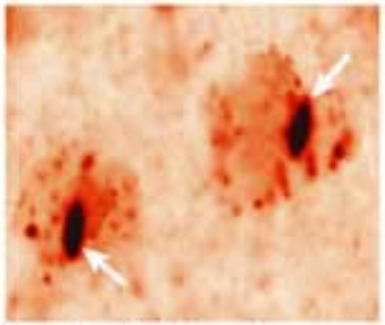
Alzheimer's plaques and tangles



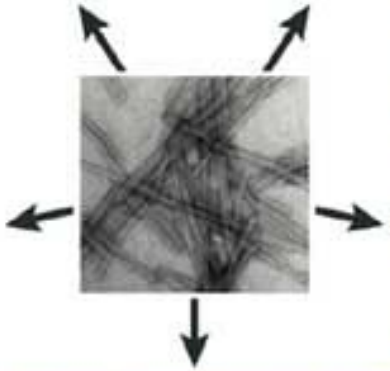
Parkinson's Lewy bodies

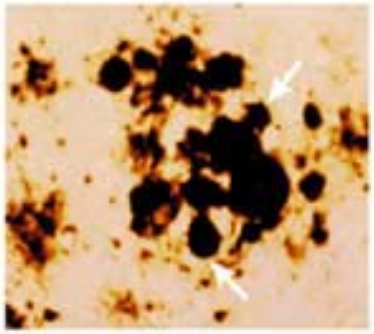
α-synuclein

Huntingtin



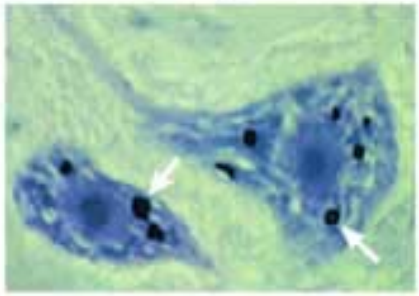
Huntington's intranuclear inclusions





Prion amyloid plaques

PrP



Amyotrophic lateral sclerosis aggregates

TDP-43
SOD1
FUS

1) Human brain tissue
2) Detergent insoluble-fraction

Soto C. (2003) *Nature Reviews Neuroscience* **4**, 49-60

Biomarker Discovery in AD

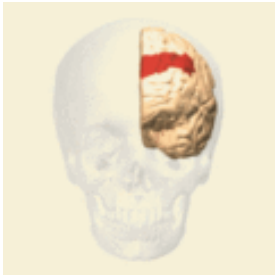
Hypothesis:

Conserved sets of, yet unidentified, proteins are pathologically altered in AD and contribute to disease pathogenesis.

Approach:

Analysis of the detergent-resistant (i.e., insoluble) brain proteome from control and seven neurodegenerative disease sub-groups by quantitative MS-based proteomics

Enriching for the detergent-resistant pathological proteins



(Pre-frontal cortex)

↓

Homogenization buffer (low salt)

↓

1% Sarkosyl buffer and high salt (Homogenate (H)) (sonicate)

↓

Spin 200,000 g for 30 mins (Supernatant =(S))

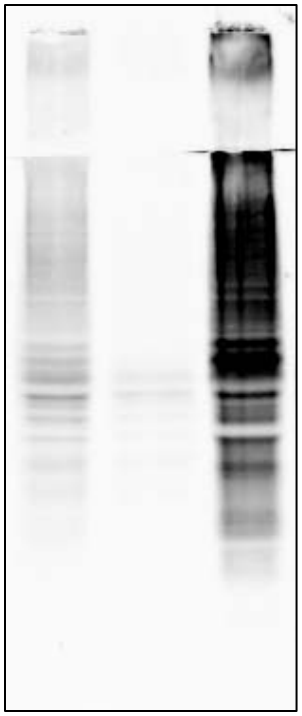
↓

Wash Pellet

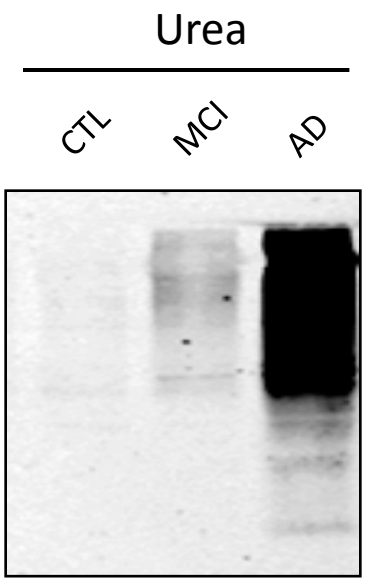
↓

8 M Urea, 1% SDS, 50 mM Tris-HCl, pH 7.8

Urea soluble Pellet (U)



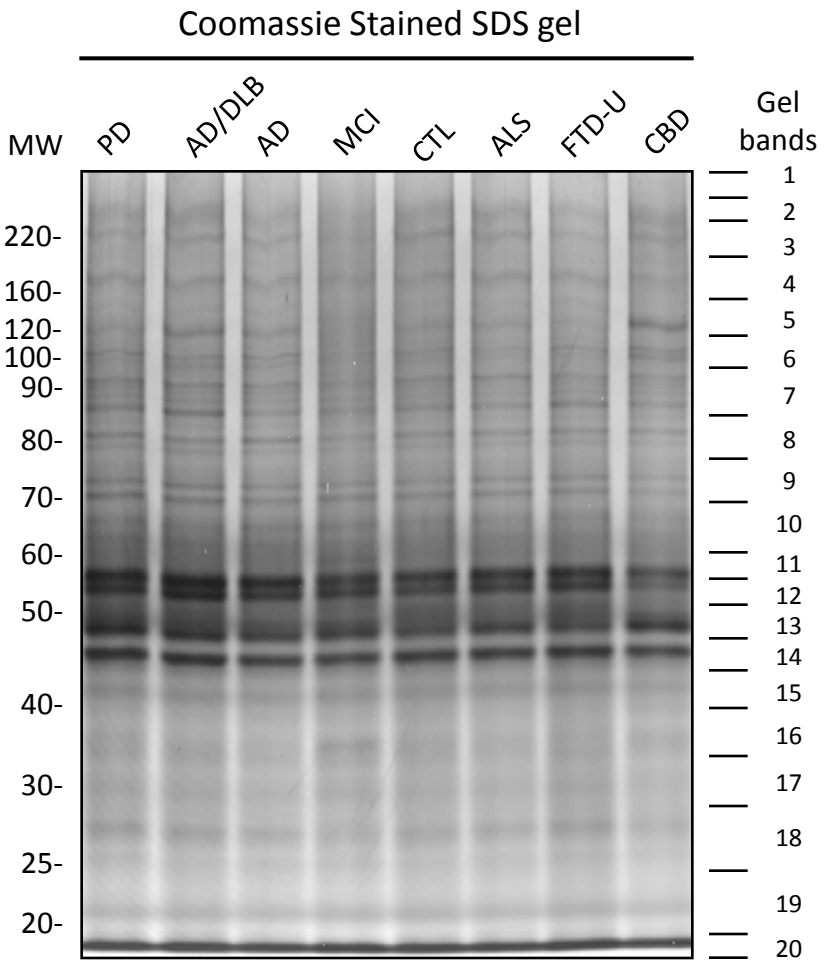
WB: Tau



WB: Phospho-tau

Identifying AD specific markers in the insoluble proteome

A



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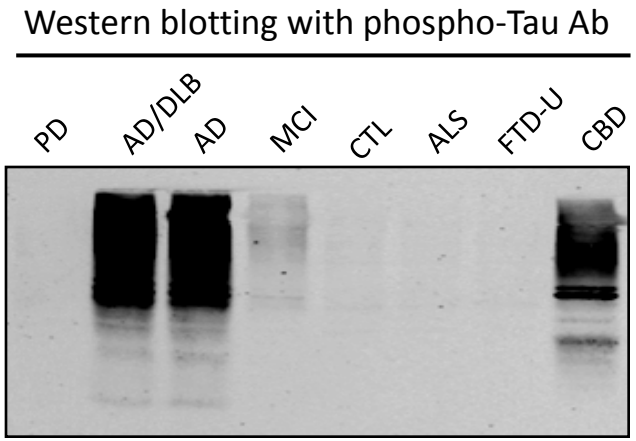
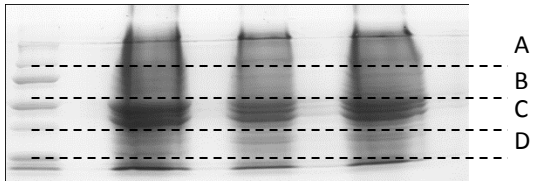


Brain Tissue (Pre-frontal cortex) was provided by the Emory ADRC Neuropath Core

Proteomic Workflow

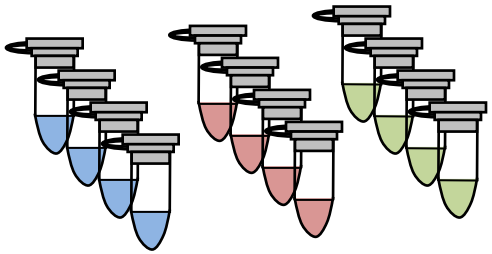
Control MCI AD

Detergent Insoluble Fractions



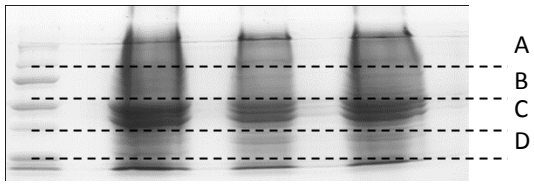
SDS-PAGE

In-gel trypsin digestion



Orbitrap MS

LC-MS/MS based proteomics — identify and quantify



SDS-PAGE

Trypsin digest

peptides

nanoLC

peptide identification

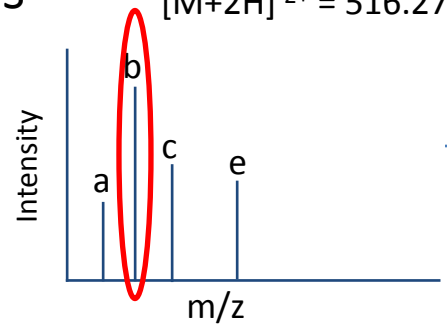
SEQUEST

protein identification

protein quantitation

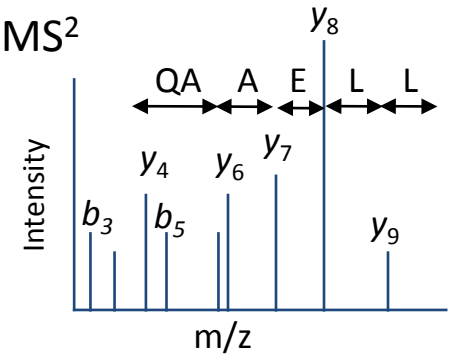
MS

[M+2H] $^{2+}$ = 516.27



CID

MS^2

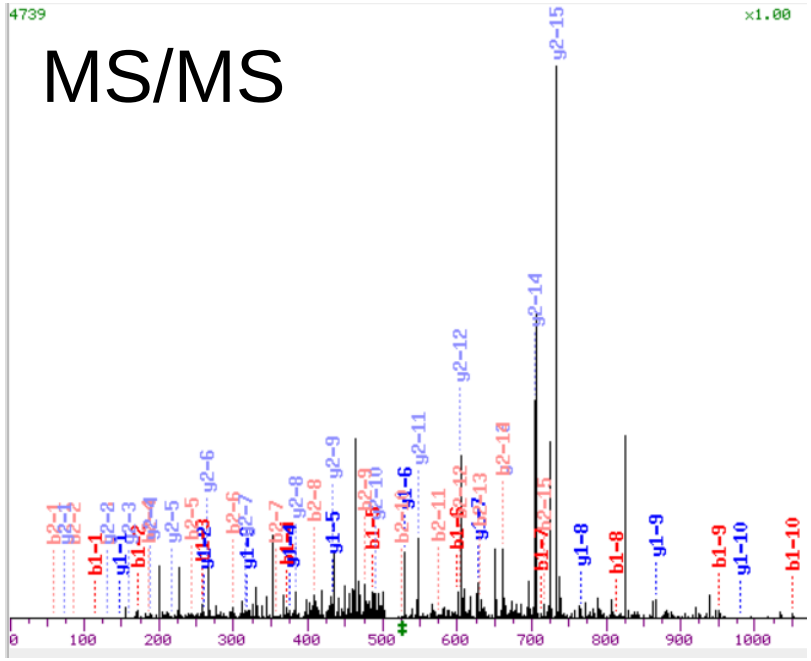


[M+2H] $^{2+}$ = 516.27

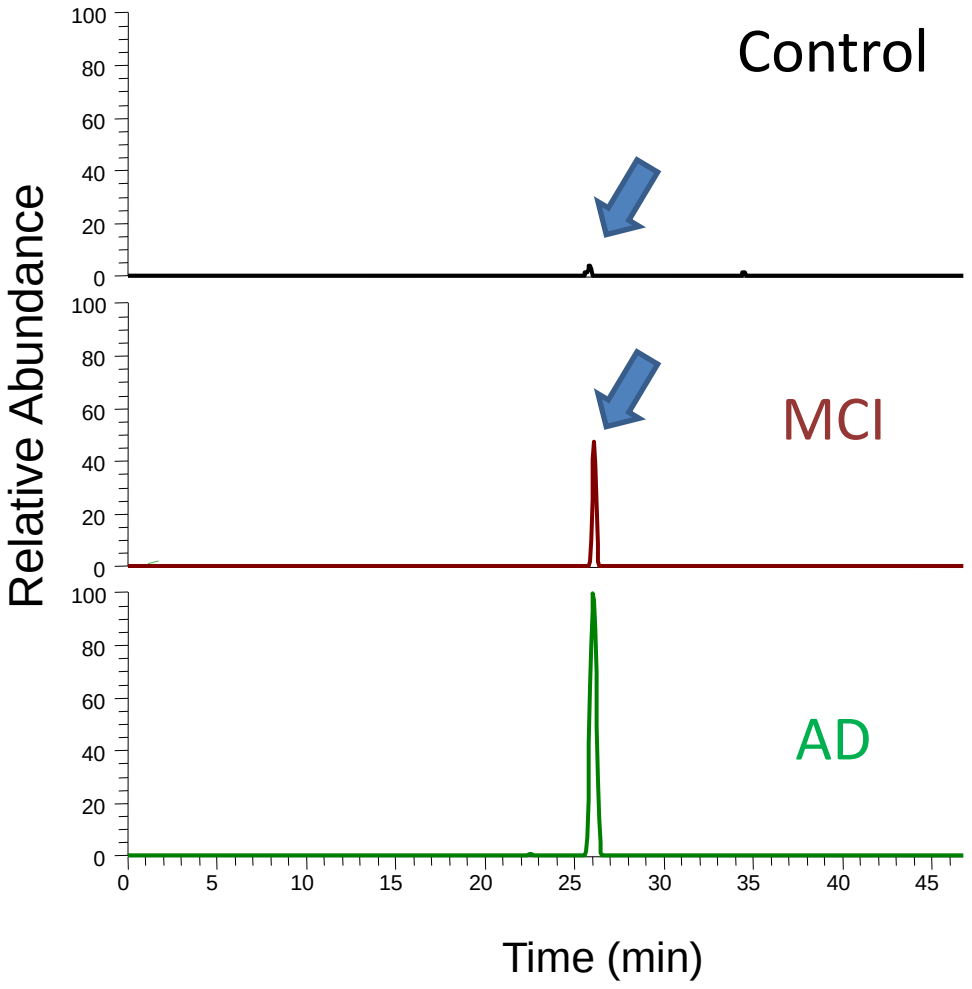
LLEAAQSTK

Microtubule Associated Protein Tau

K.IGSLDNITHVPGGGNK.K
(amino acids 354-369)



Extracted Ion Intensity



Identifying AD specific markers in the insoluble proteome

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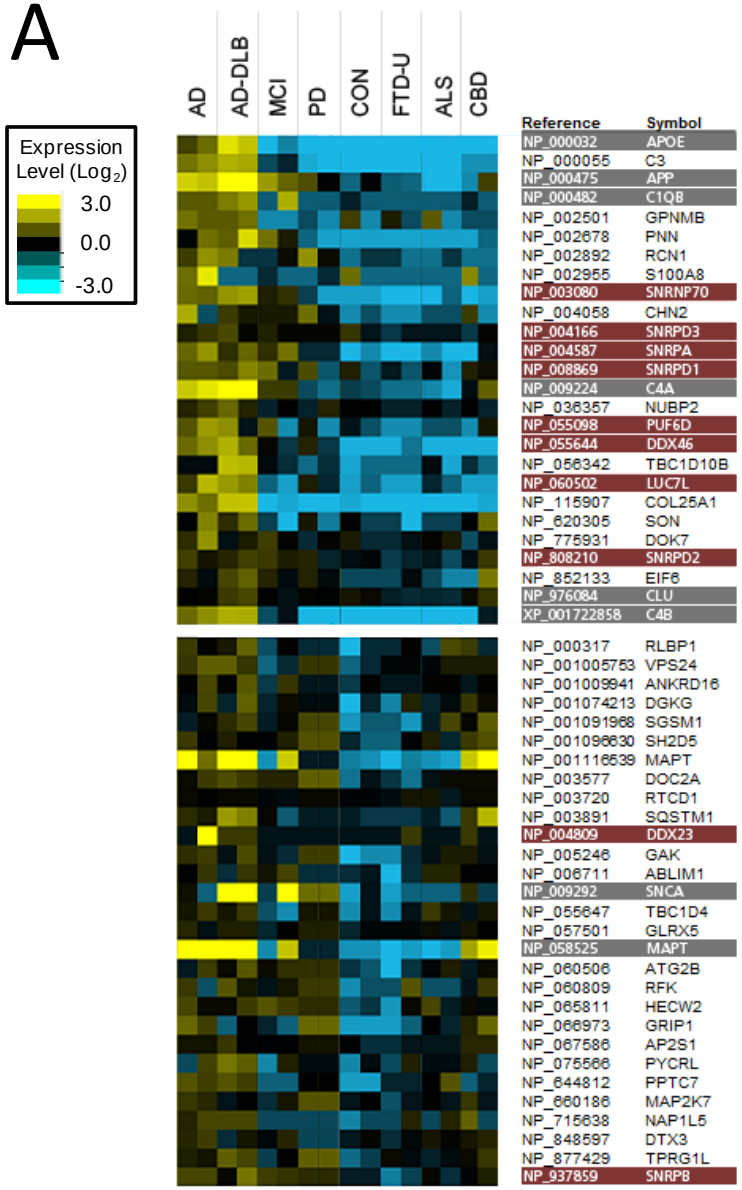
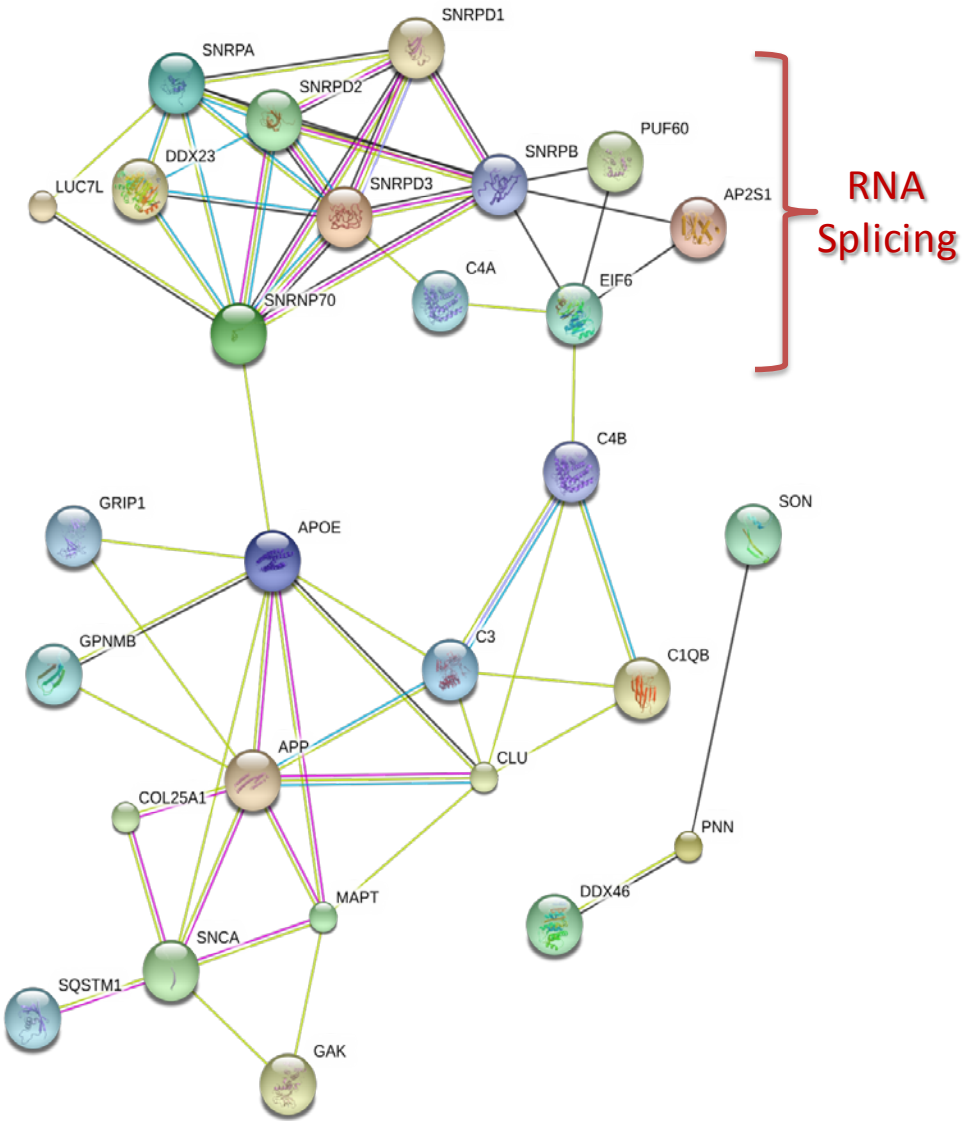


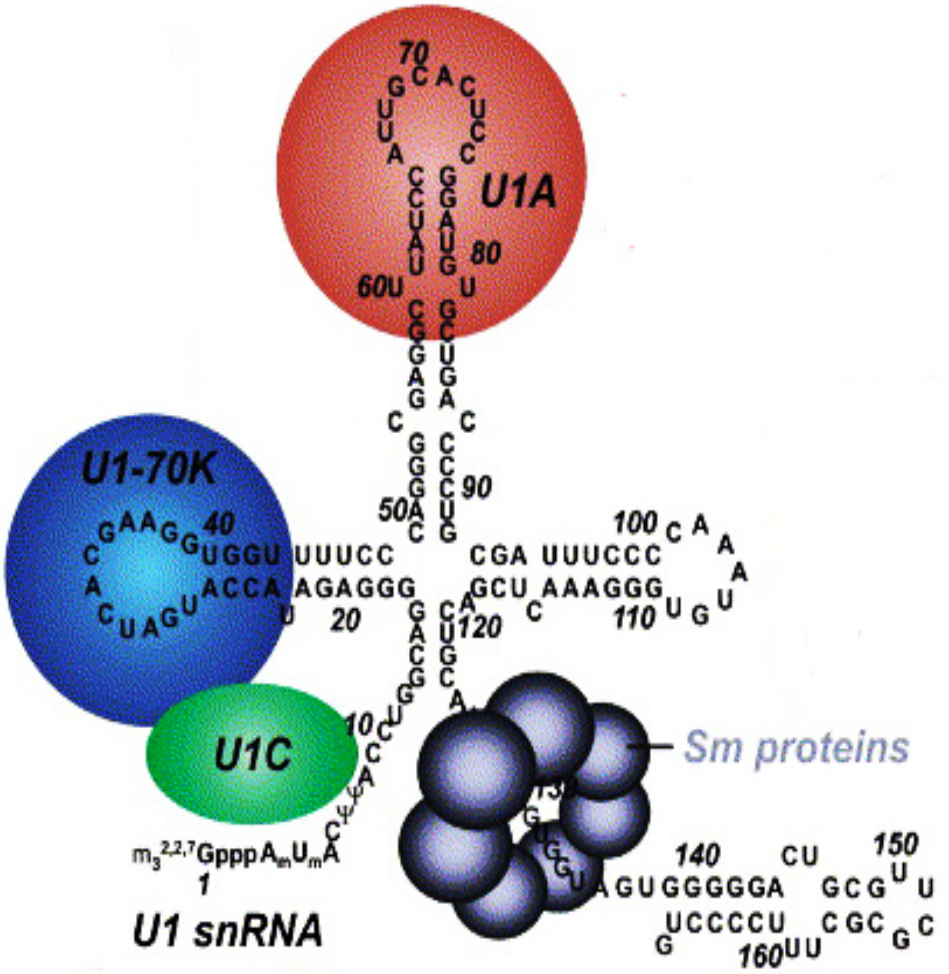
Reference	Symbol
NP_000032	APOE
NP_000055	C3
NP_000475	APP
NP_000482	C1QB
NP_002501	GPNMB
NP_002678	PNN
NP_002892	RCN1
NP_002955	S100A8
NP_003080	SNRNP70
NP_004058	CHN2
NP_004166	SNRPD3
NP_004587	SNRPA
NP_008869	SNRPD1
NP_009224	C4A
NP_036357	NUBP2
NP_055098	PUF6D
NP_055644	DDX46
NP_056342	TBC1D10B
NP_060502	LUC7L
NP_115907	COL25A1
NP_620305	SON
NP_775931	DOK7
NP_808210	SNRPD2
NP_852133	EIF6
NP_976084	CLU
XP_001722858	C4B
NP_000317	RLBP1
NP_001005753	VPS24
NP_001009941	ANKRD16
NP_001074213	DGKG
NP_001091968	SGSM1
NP_001096630	SH2D5
NP_001116539	MAPT
NP_003577	DOC2A
NP_003720	RTCD1
NP_003891	SQSTM1
NP_004809	DDX23
NP_005246	GAK
NP_006711	ABLIM1
NP_009292	SNCA
NP_055647	TBC1D4
NP_057501	GLRX5
NP_058525	MAPT
NP_060506	ATG2B
NP_060809	RFK
NP_065811	HECW2
NP_066973	GRIP1
NP_067586	AP2S1
NP_075566	PYCRL
NP_644812	PPTC7
NP_660186	MAP2K7
NP_715638	NAP1L5
NP_848597	DTX3
NP_877429	TPRG1L
NP_937859	SNRPB

B



U1 snRNP – structure and function

U1 Small Nuclear Ribonucleoprotein Complex



Structure

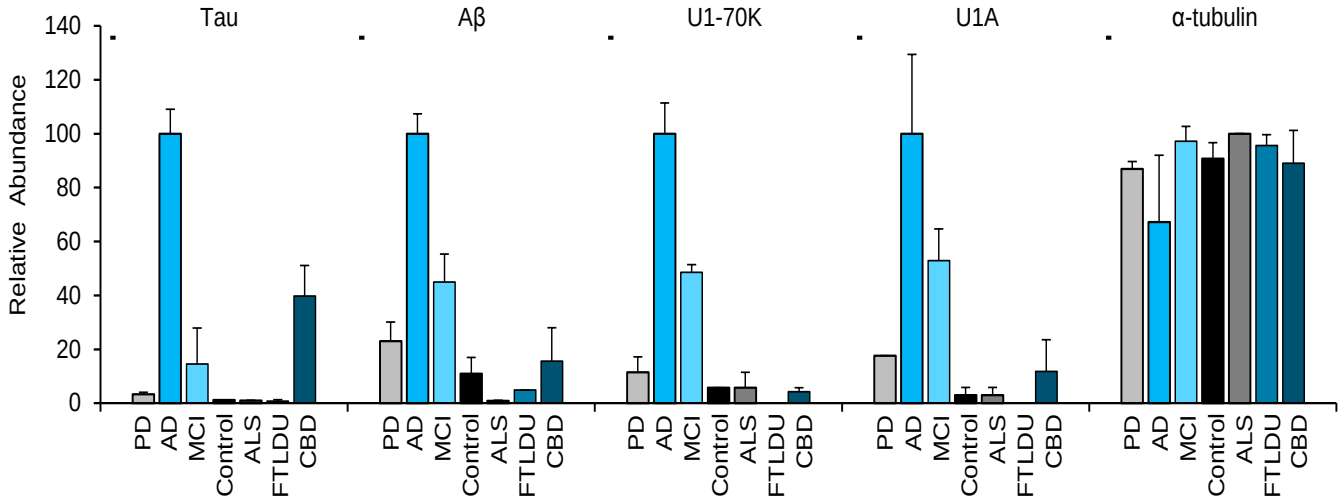
i. U1 snRNA
ii. U1 proteins
- U1-A
- U1-70K
- U1-C

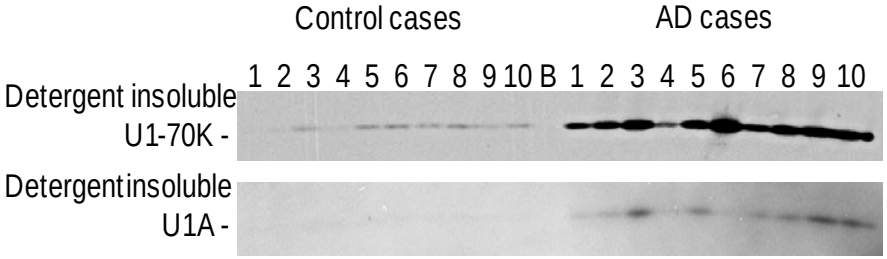
iii. 7 Sm proteins (SMN complex)

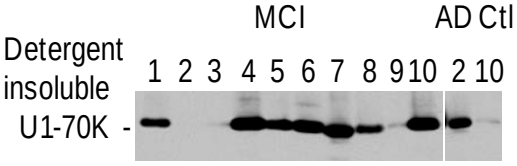
Function

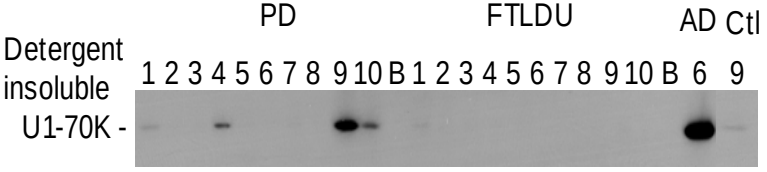
i. Binds 5' splice site of pre-mRNA to initiate intron removal and alternative splicing of 95% of transcripts.
ii. Functional form localized to nucleus

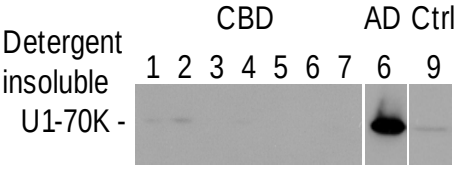
U1 snRNP insolubility is highly specific to AD





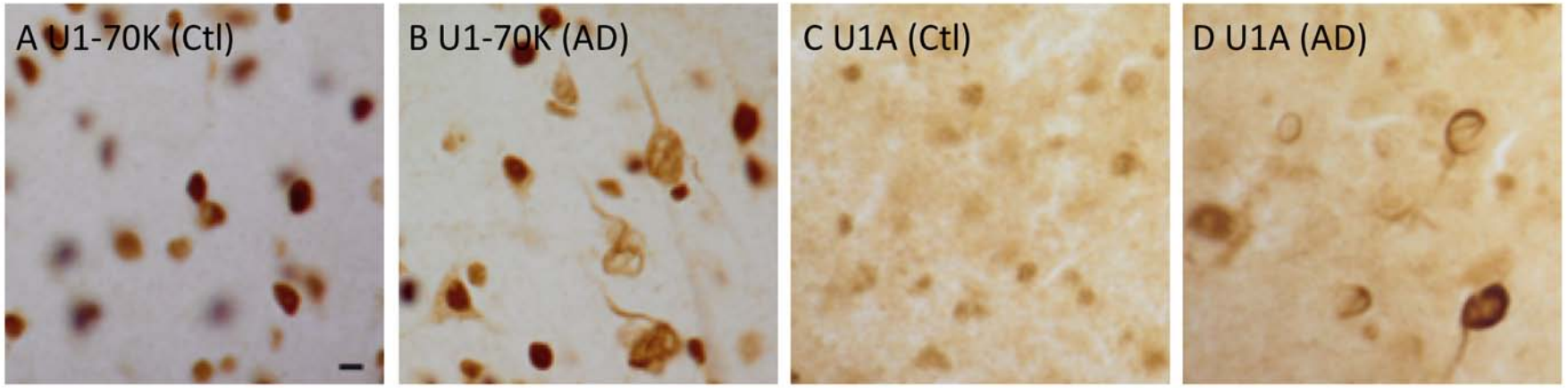






Bai *et. al., Proc Natl Acad Sci U S A.* (2013) **110**:16562-7

U1 snRNP is associated specifically with AD pathology



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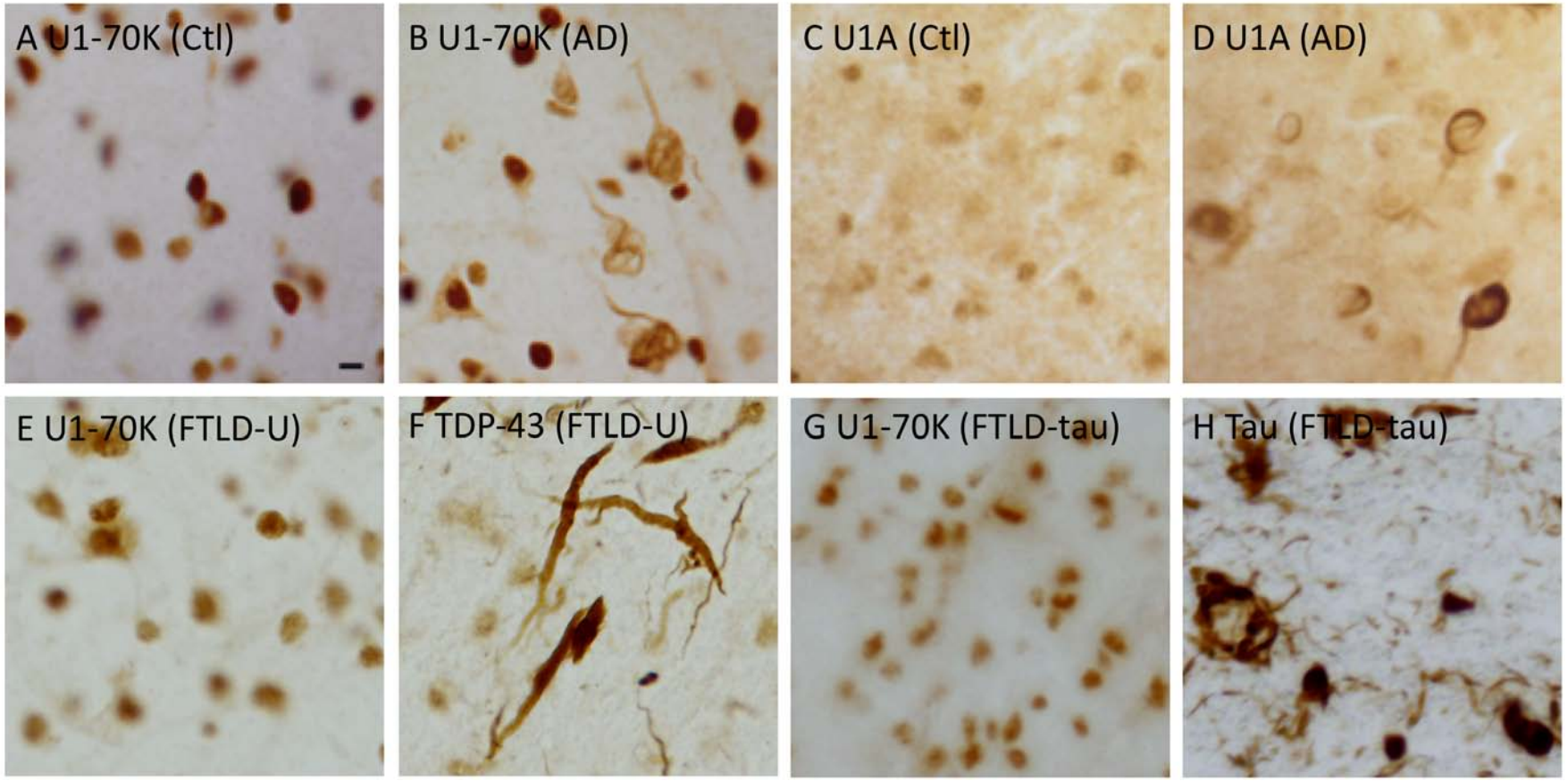

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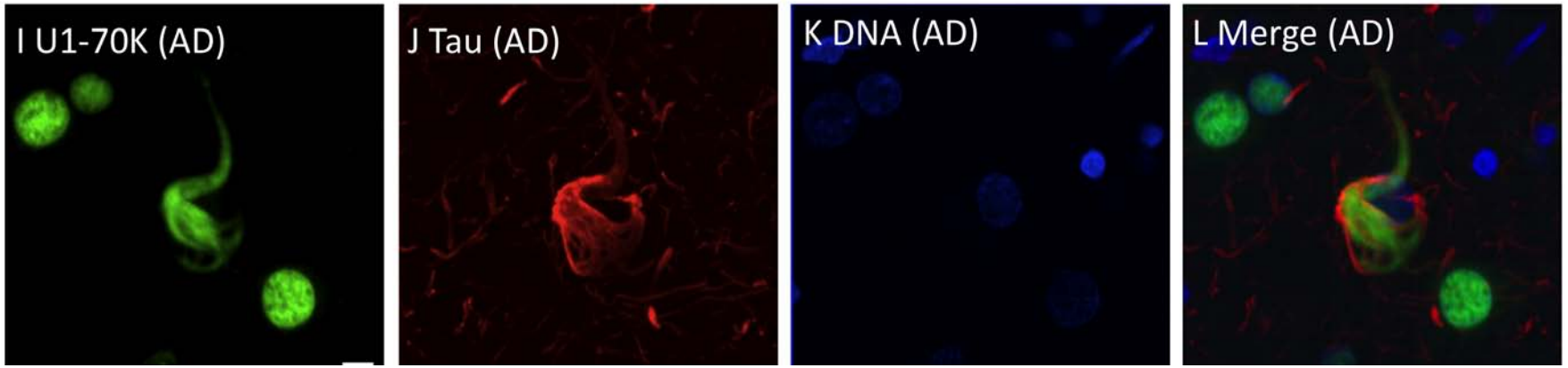
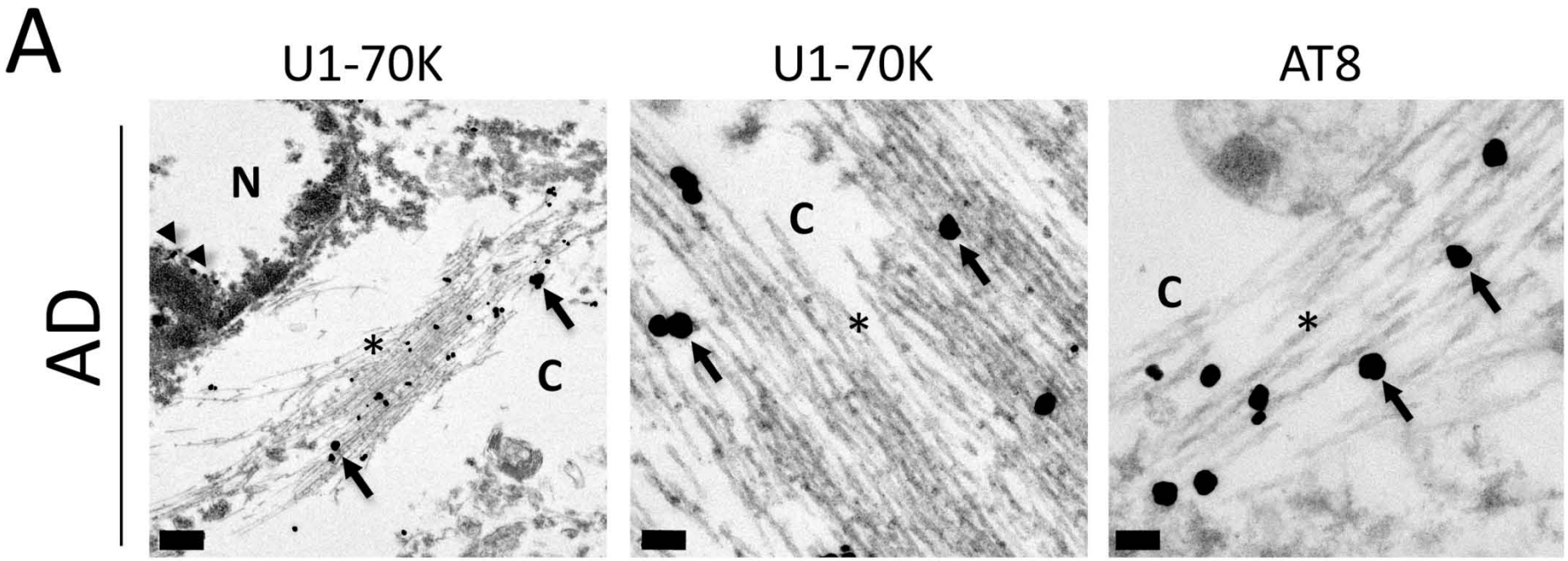


Bai *et. al., Proc Natl Acad Sci U S A.* (2013) **110**:16562-7

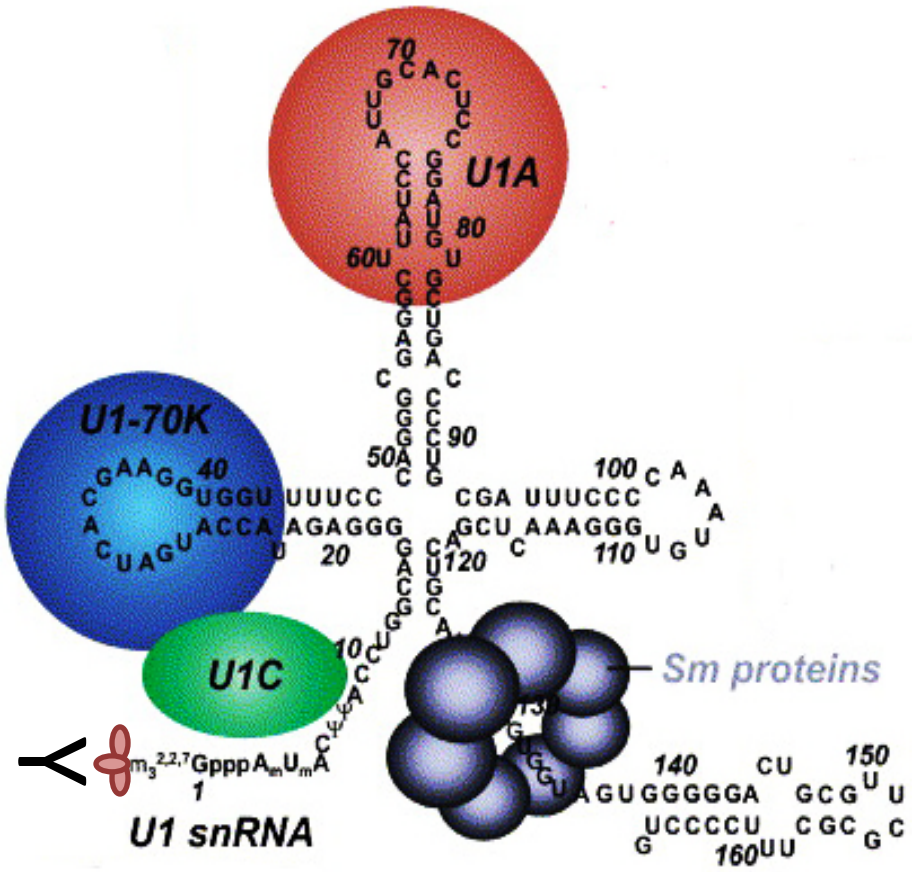
Does U1 snRNP directly associated with NFTs in AD?

EM of immunogold-labeled U1-70K aggregates in cytoplasm

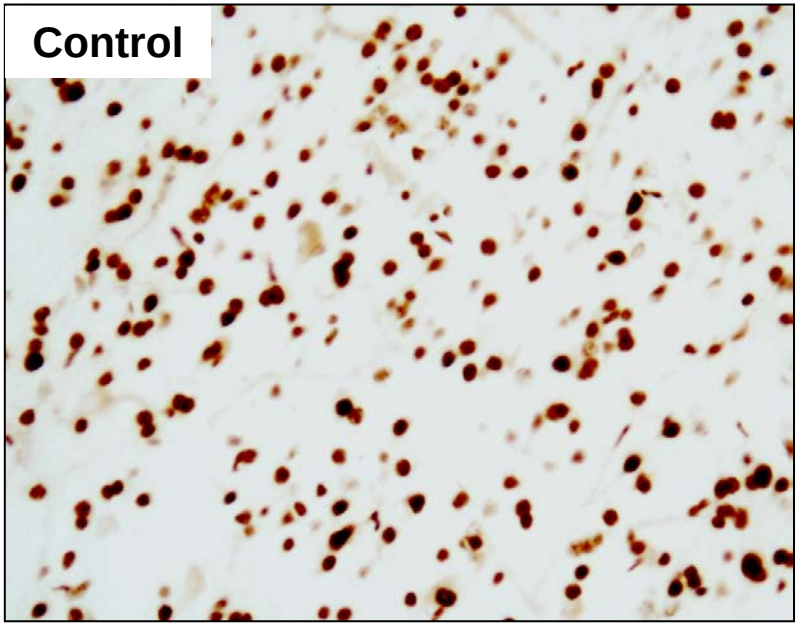


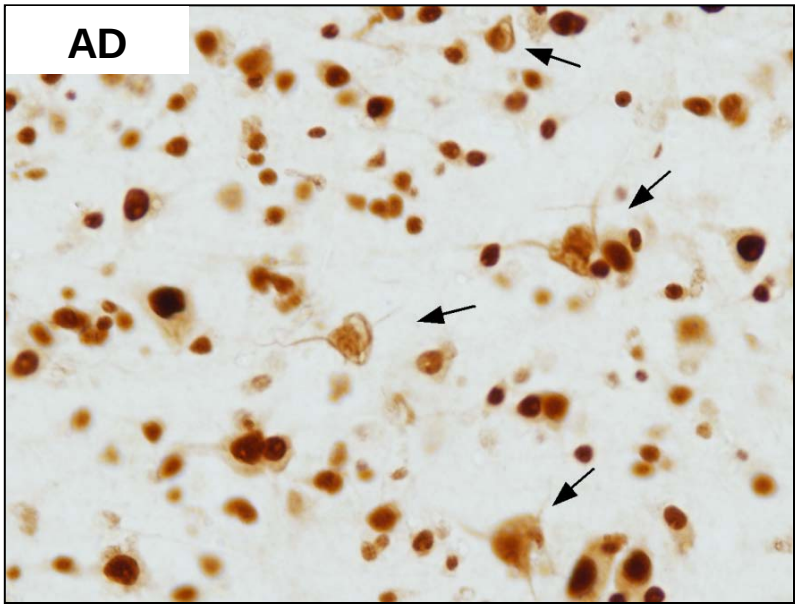
Hales *et. al.,* (2014) *Mol. Neurodegeneration* (In press)

Is U1-70K aggregation dependent on RNA?



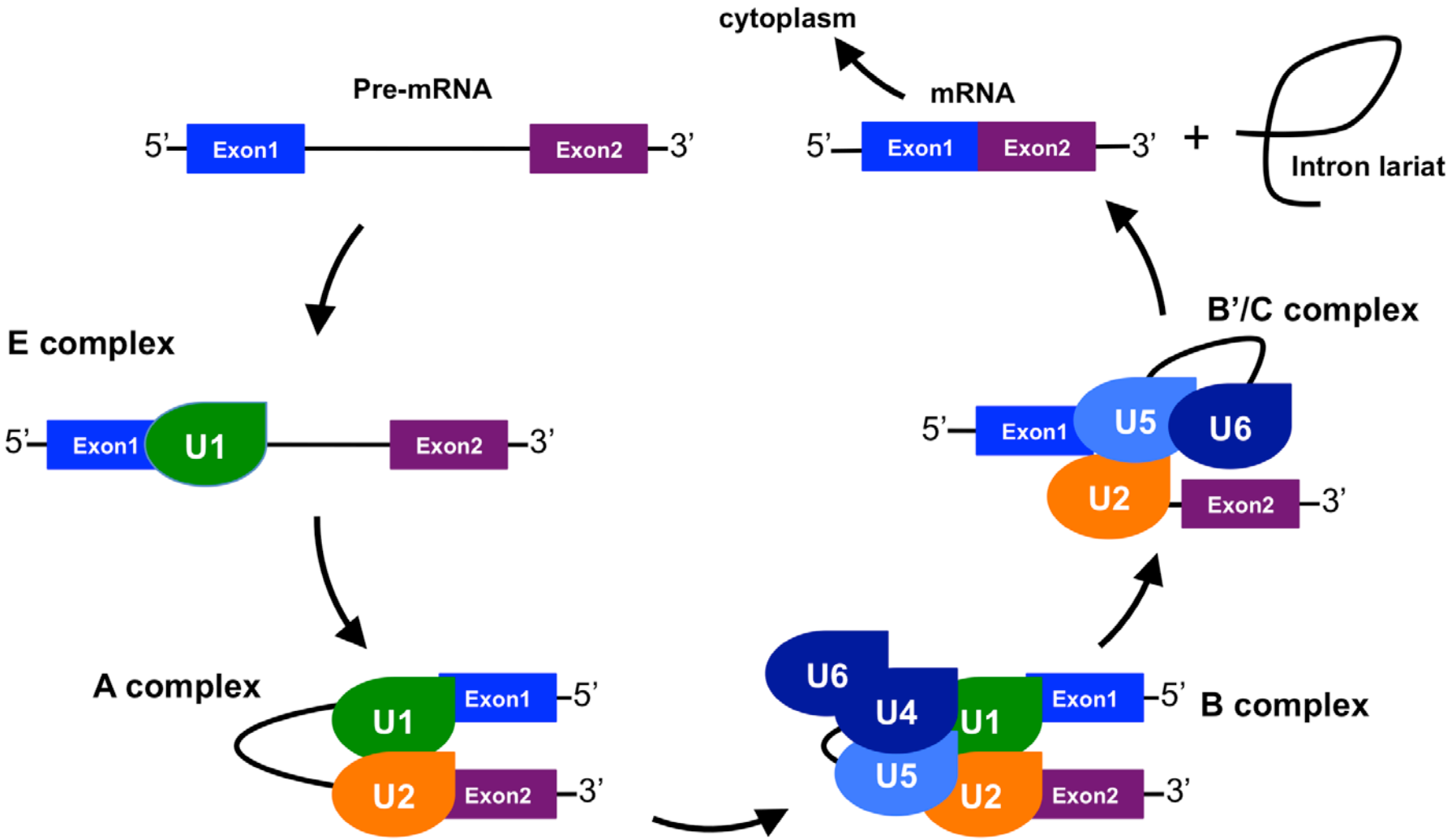
2,2,7-trimethylguanosine cap of U1 snRNA evident in tangles





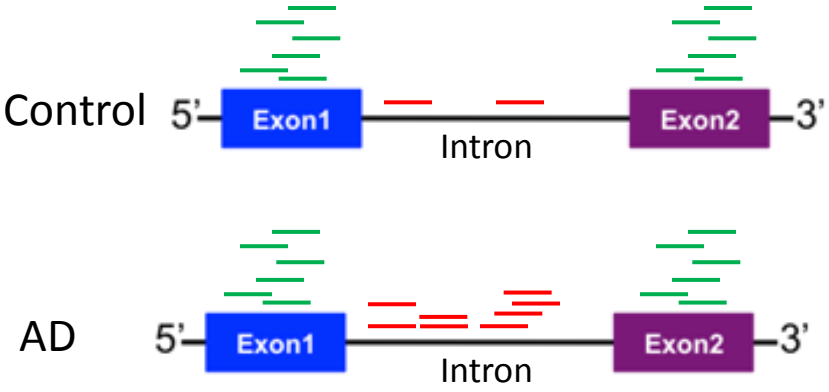
Hales *et. al.,* (2014) *Brain Pathol*. In press

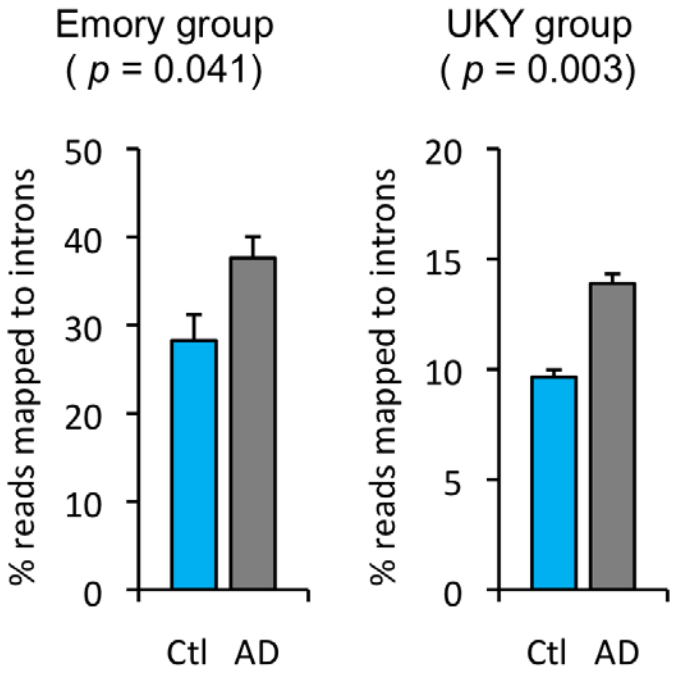
Are there defects in RNA maturation in AD brain?



Are there defects in RNA maturation in AD brain?

RNA-seq

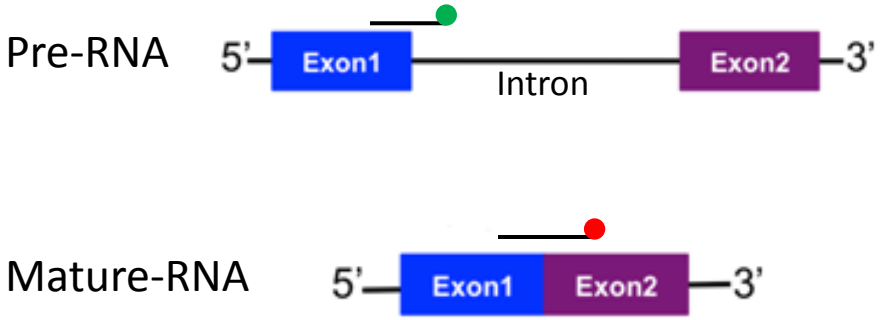


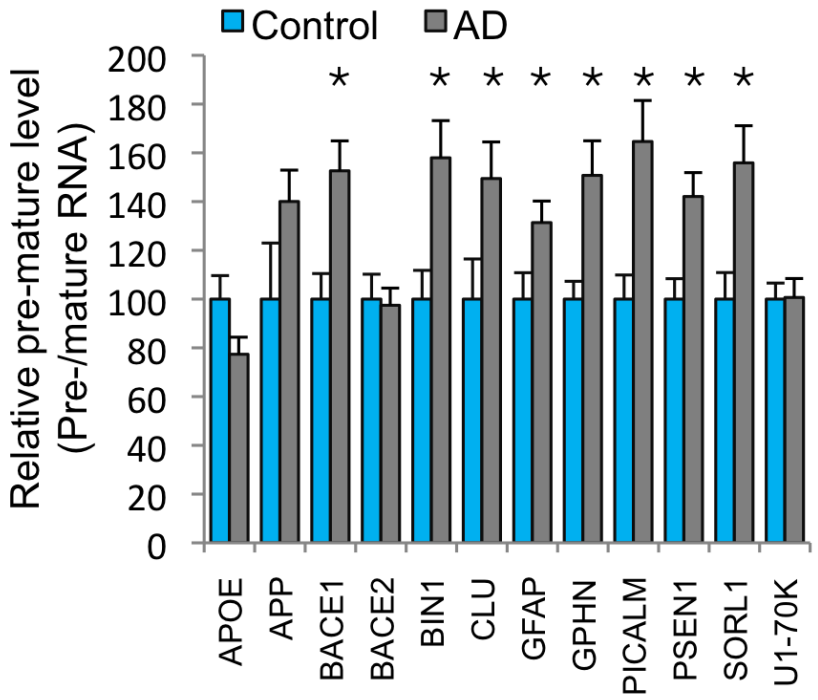


Bai *et. al., Proc Natl Acad Sci.* (2013) **110**:16562-7

Are there defects in RNA maturation in AD brain?

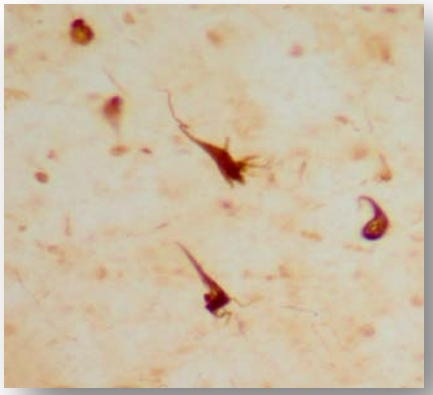
nanoString TECHNOLOGIES®





Bai *et. al., Proc Natl Acad Sci.* (2013) **110**:16562-7

Proteomics at the Emory ADRC

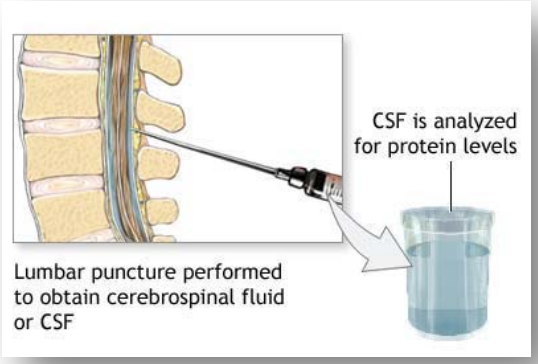


Pathological Aggregates

Synapse-rich

Individual

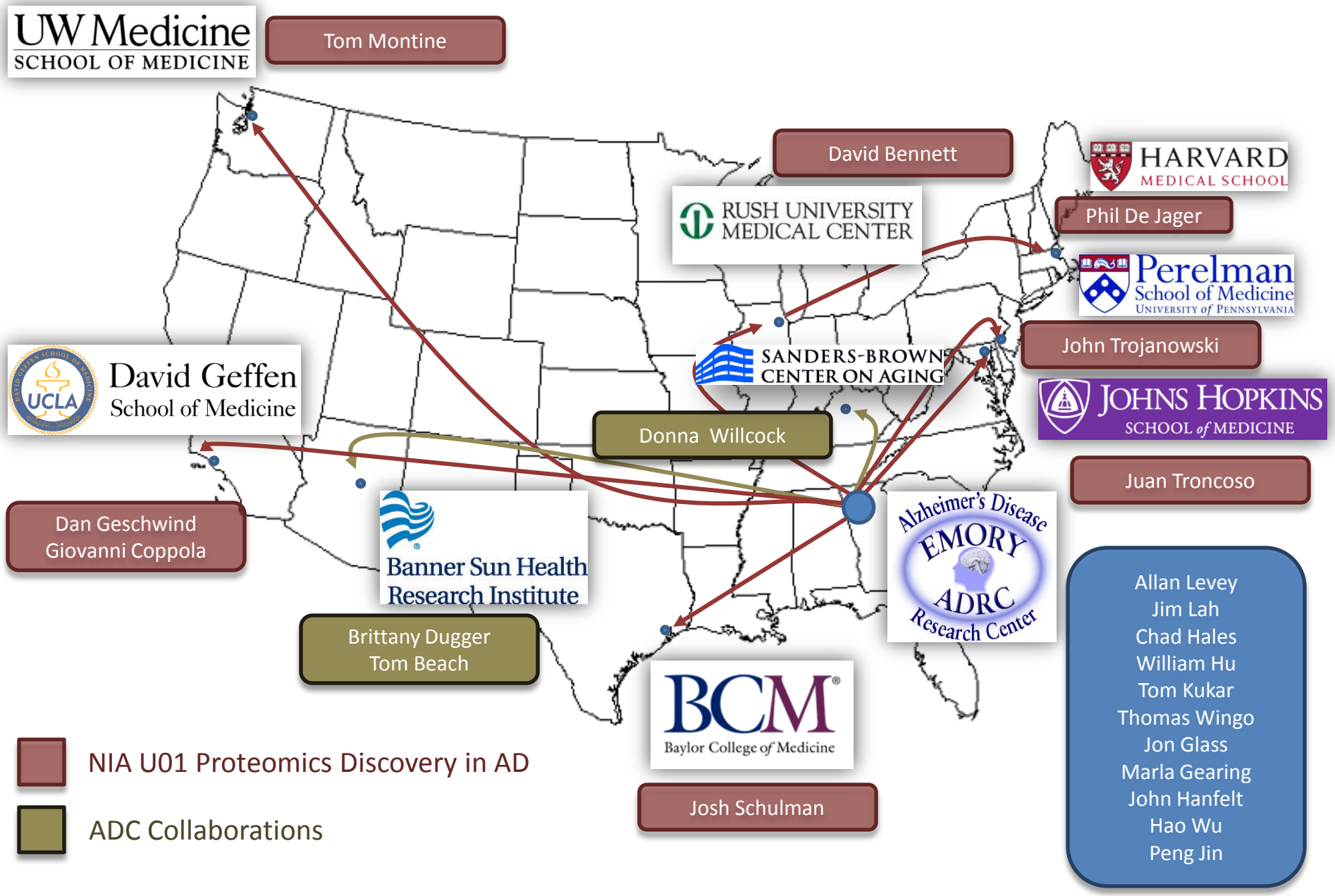
Proteomics



Cerebral Spinal Fluid (CSF)

Plasma/Platelets

Proteomics Collaborators at Emory ADRC



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