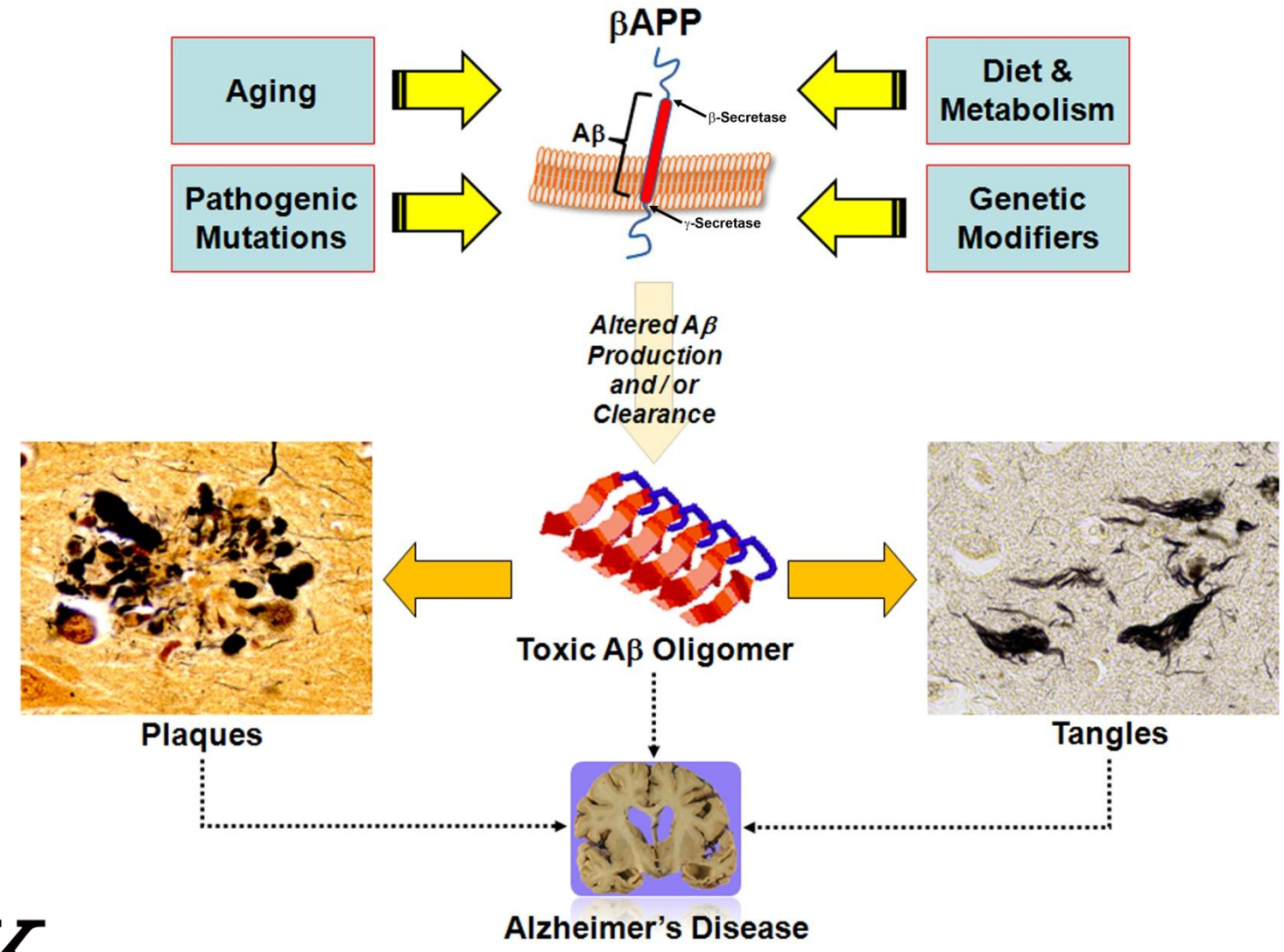
Diet, Exercise, NSAIDs, and Inclusion Body Myositis

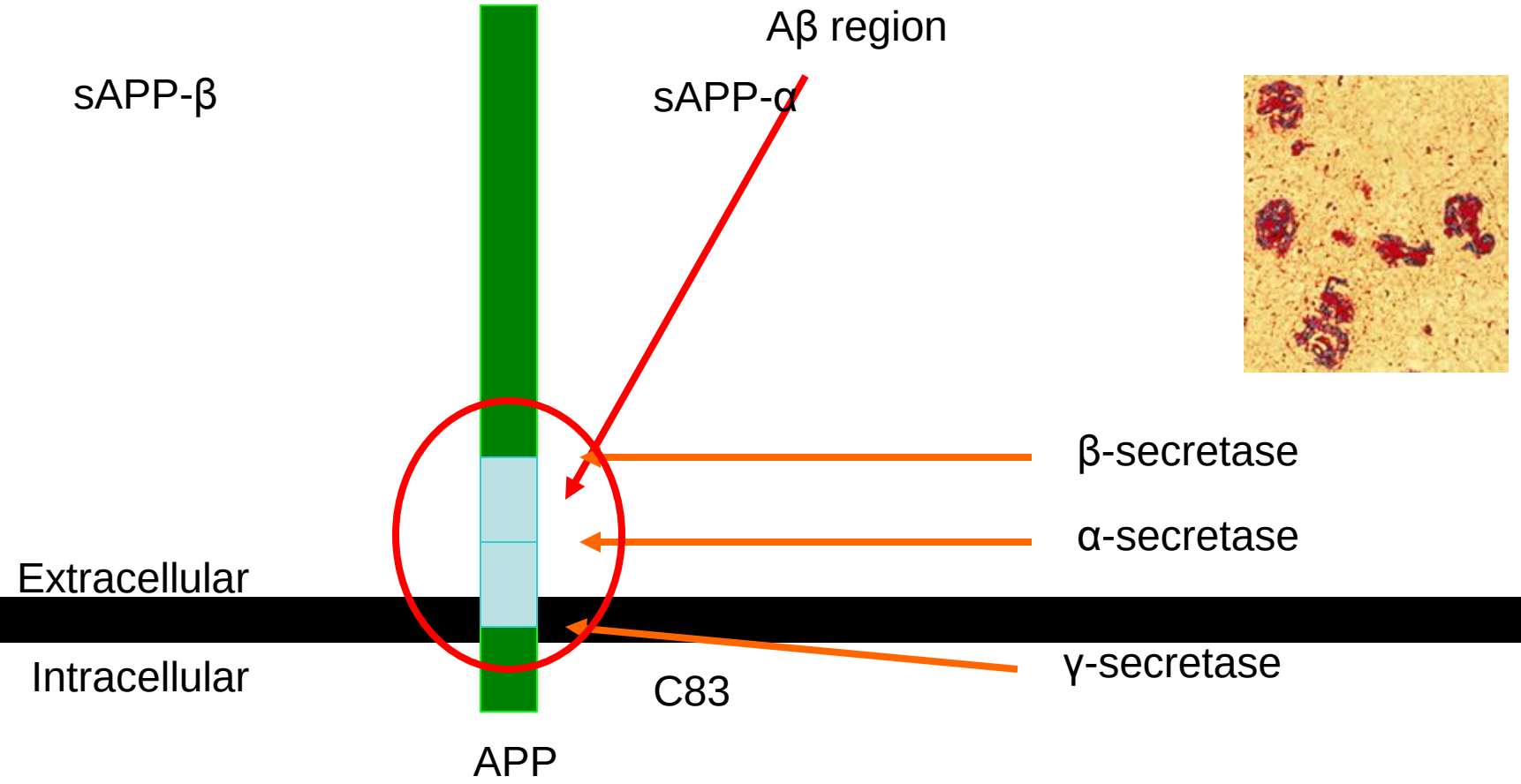
M. Paul Murphy

Associate Professor
Molecular and Cellular Biochemistry
& Sanders-Brown Center on Aging
University of Kentucky

What is AD?


Aging
Pathogenic Mutations
βAPP
Aβ
β-Secretase
γ-Secretase
Diet & Metabolism
Genetic Modifiers
Altered Aβ Production and/or Clearance
Toxic Aβ Oligomer
Plaques
Tangles
Alzheimer's Disease

Amyloid Precursor Protein and β-Amyloid

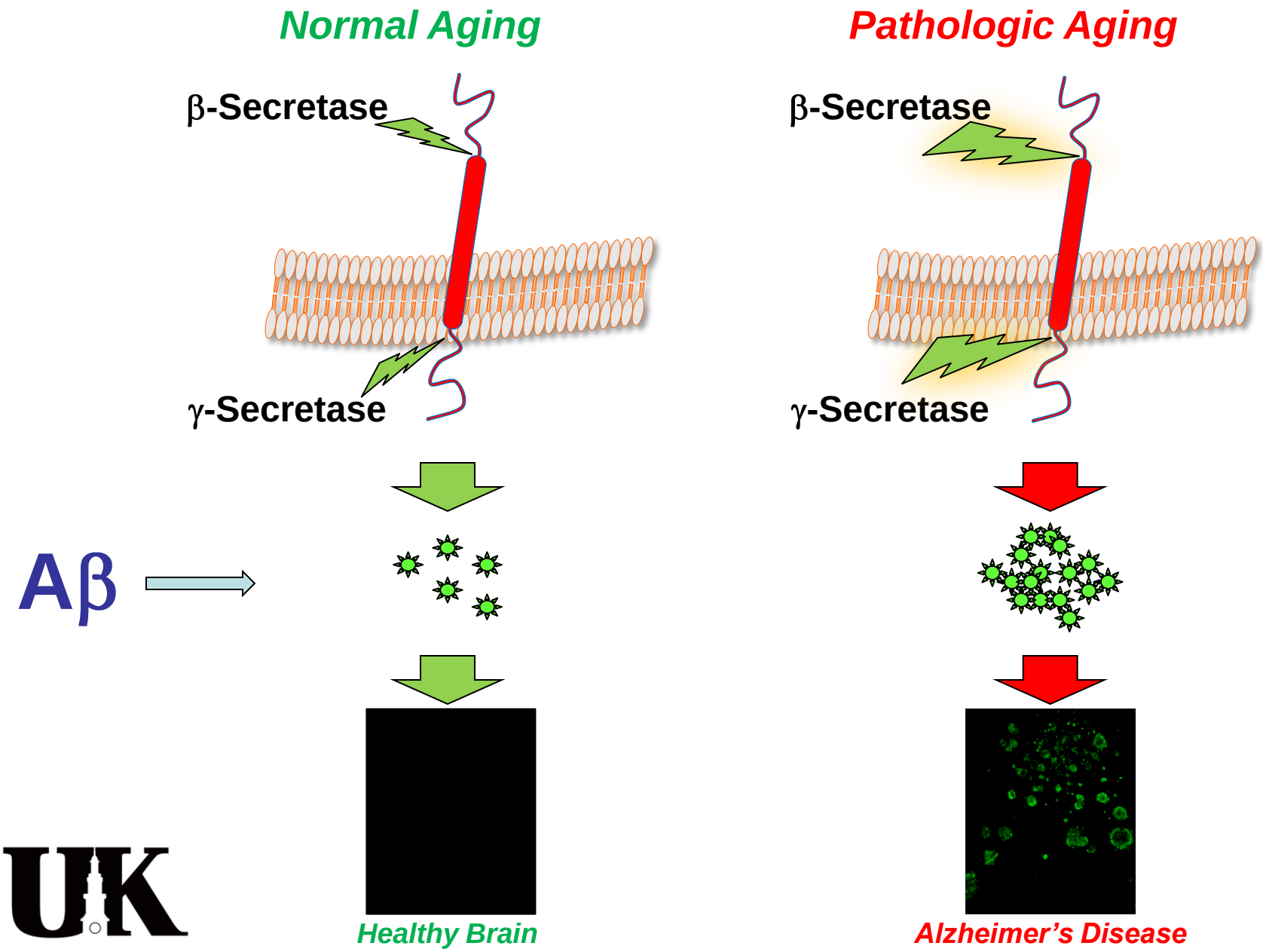


Amyloidogenic Processing (~10%)

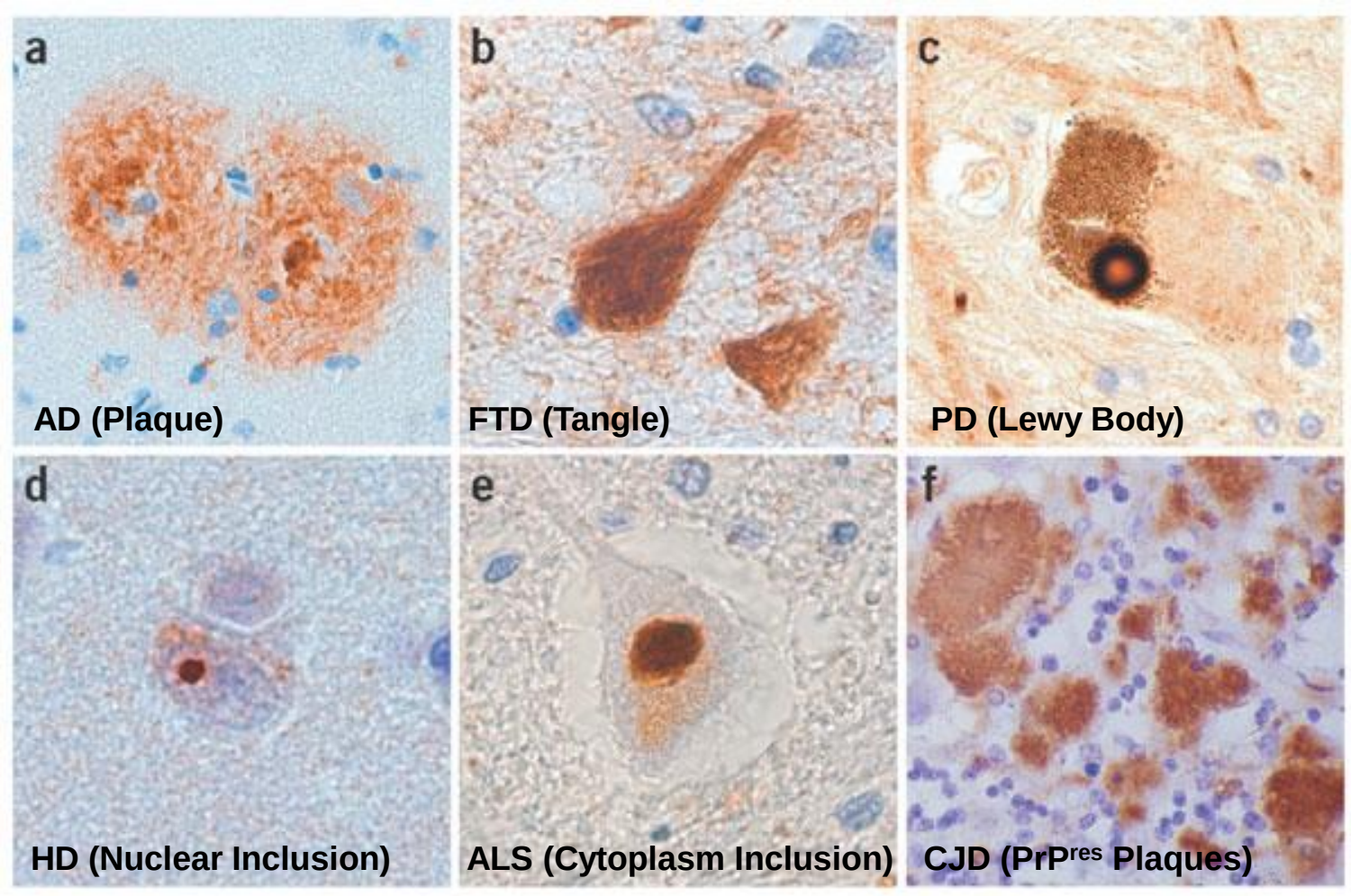
Constitutive Processing (~90%)

Adam Weidner

Is AD a loss of Repression on the Aβ Producing Enzymes?



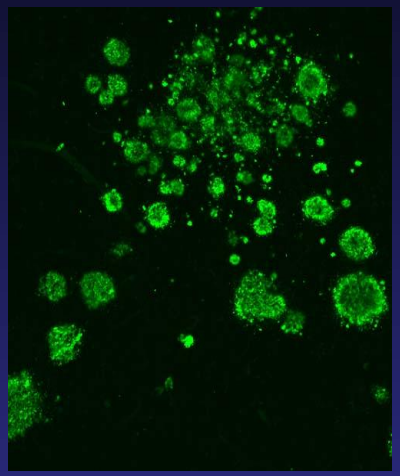
Amyloid Pathology is Generic



Adapted from: Forman *et. al.*, *Nature Medicine*, 10: 1055-63, 2004

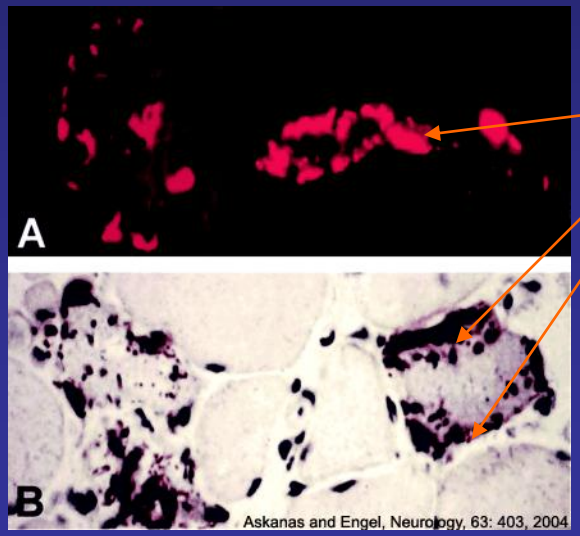
IBM and AD

- In the brain of AD patients the Aβ forms plaques that are *outside* of the cells



Aβ
(in green)

- In the muscle of IBM patients the Aβ forms inclusions that are *inside* of the cells



Connection…?

1) Aβ-positive, non-congophilic deposits appear prior to frank vacuolization in IBM affected muscle fibers
2) mRNA for APP, the larger protein from which Aβ is derived, is increased in sIBM, as is the APP protein itself
3) β-secretase, a major amyloidogenic enzyme involved in AD, is up regulated in IBM (as well as in AD and aging in general)
4) Overexpression of APP and/or APP fragments containing Aβ in the muscle of transgenic mice leads to degenerative changes similar to the disease state in humans

UK

IBM Mouse Models

- One of the only options to resolve the actual role of Aβ in the disease process, is to perform experiments in animal models.
- IBM-T7A6 mouse: reported in 2002 in collaboration with Frank LaFerla, UCI
- Overexpresses full length APP in muscle under the direction of a creatine kinase promoter

Inclusion body myositis-like phenotype induced by transgenic overexpression of βAPP in skeletal muscle

Michael C. Sugarman*, Tritia R. Yamasaki*, Salvatore Oddo*, Julio C. Echegoyen*, M. Paul Murphy†, Todd E. Golde†, Mehrdad Jannatipour*, Malcolm A. Leissring*, and Frank M. LaFerla*‡

*Department of Neurobiology and Behavior, University of California, Irvine, CA 92697; and †Department of Neuroscience and Pharmacology, Mayo Clinic Jacksonville, Jacksonville, FL 32224

Edited by Laszlo Lorand, Northwestern University Medical School, Chicago, IL, and approved February 14, 2002 (received for review October 12, 2001)

Inclusion body myositis (IBM), the most common age-related muscle disease in the elderly population, is an incurable disorder leading to severe disability. Sporadic IBM has an unknown etiology, although affected muscle fibers are characterized by many of the pathobiochemical alterations traditionally associated with neurodegenerative brain disorders such as Alzheimer's disease. Accumulation of the amyloid-β peptide, which is derived from proteolysis of the larger amyloid-β precursor protein (βAPP), seems to be an early pathological event in Alzheimer's disease and also in IBM, where in the latter, it predominantly occurs intracellularly within affected myofibers. To elucidate the possible role of βAPP mismetabolism in the pathogenesis of IBM, transgenic mice were derived in which we selectively targeted βAPP overexpression to skeletal muscle by using the muscle creatine kinase promoter. Here we report that older (>10 months) transgenic mice exhibit intracellular immunoreactivity to βAPP and its proteolytic derivatives in skeletal muscle. In this transgenic model, selective overexpression of βAPP leads to the development of a subset of other histopathological and clinical features characteristic of IBM, including centric nuclei, inflammation, and deficiencies in motor performance. These results are consistent with a pathogenic role for βAPP mismetabolism in human IBM.

Brain and skeletal muscle are the only known tissues in humans marked by the pathological accumulation of the amyloid-β (Aβ) peptide. In brain, Aβ deposition is associated with several genetically related neurodegenerative disorders including Alzheimer's disease (AD), Down syndrome, and hereditary cerebral hemorrhage with amyloidosis–Dutch type (1). On the basis of genetic evidence, Aβ accumulation seems to be an early pathogenic event, although it remains to be determined whether Aβ directly leads to cell degeneration or whether cell degeneration is mediated by other downstream factors induced by it. In muscle, Aβ accumulation is associated with inclusion body myositis (IBM), the most common muscle disorder to afflict the elderly. IBM is the first human disorder marked by the pathological accumulation of this amyloidogenic peptide outside the central nervous system. Notably, Aβ and/or other Aβ-containing fragments produced by proteolysis of the amyloid-β precursor protein (βAPP) are not implicated in other myopathies, suggesting that βAPP mismetabolism is an integral component of the molecular pathogenesis of IBM.

Like AD, IBM is an age-related degenerative disorder with a slowly progressive clinical course for which no effective treatment is available. Clinically characterized by muscle weakness and atrophy involving both proximal and distal muscle groups (2–4), IBM was first recognized as its own disorder in the early 1970s (5); before that, it was often diagnosed as polymyositis (6). That Aβ-containing fragments are involved in the pathogenesis of IBM is somewhat surprising because no obvious genetic link exists to either the βAPP gene or to other AD-related genes such as apolipoprotein E (7, 8). Nevertheless, IBM and AD share many pathobiochemical features, including twisted intracellular tubulofilaments consisting of hyperphosphorylated tau (9) and the aberrant accumulation of other "dementia"-related proteins, including apoE, presenilin, prion protein, and α-synuclein (10–13). These data suggest that after an initial insult, a coordinated molecular cascade occurs, triggering the accumulation of these "dementia"-related proteins both in muscle and in brain. Along these lines, AD patients also contain slightly elevated levels of amyloidogenic $A\beta_{1-42}$ peptides in their muscle, but these elevated levels seem to be without pathological consequence, perhaps because of low steady-state levels (14). Curiously, it has been reported that myoglobin can also form amyloid fibrils, but whether this ability plays a role in muscle disease is not yet established (15).

One interesting distinction between IBM and AD involves the location of the Aβ deposits. Whereas the AD brain is characterized by accumulation of amyloid deposits in extracellular plaques, Aβ accumulates *intracellularly* in IBM (16). Although Aβ accumulates intraneuronally according to reports (17, 18), it has not been established whether this intracellular form of Aβ is relevant in the neurodegenerative cascade. Despite this cytogeographical difference, Aβ-containing fragments seem to play a critical pathogenic role in IBM. Whereas increased expression of βAPP, aberrant proteolysis, and/or diminished clearance of Aβ-containing fragments in muscle could contribute to amyloid accumulation in IBM muscle fibers, clear evidence exists of excessive βAPP transcripts in IBM (19). Moreover, the subcellular distribution of βAPP seems to be altered in IBM myofibers, away from the postsynaptic domain of the neuromuscular junction to a subsarcolemmal location in IBM fibers (19). Further underscoring the potential pathological consequences of βAPP overexpression in muscle, it has been shown that transfection of normal cultured muscle cells with *βAPP* mRNA leads to IBM-like changes including Congo red-positive amyloid and cytoplasmic tubulofilaments (20, 21).

To test the hypothesis that βAPP mismetabolism is an early component of the molecular pathogenesis of IBM, we derived transgenic mice that selectively overexpress full-length human βAPP in skeletal muscle. These mice develop age-related myopathological and behavioral changes resembling those observed in IBM patients, including intracellular immunoreactivity to Aβ and Aβ-containing fragments, cellular inflammation, and motor deficits. These mice may prove useful for evaluating novel therapeutic interventions and for studying the pathophysiological consequences of intracellular Aβ accumulation outside the central nervous system, which may provide insights into the pathogenesis of AD as well.

Material and Methods

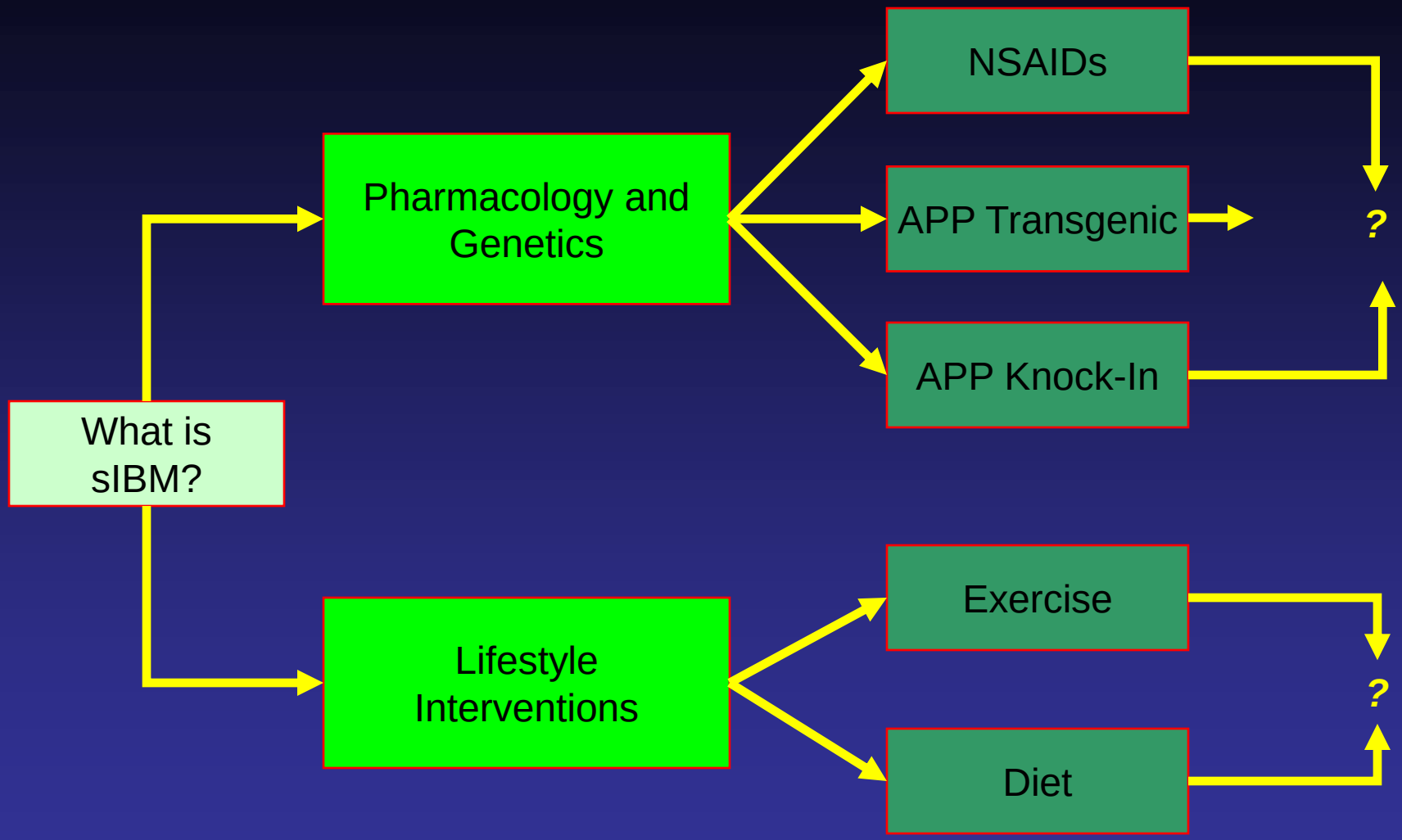
Generation of Transgenic Mice. Human *βAPP* cDNA harboring the Swedish double mutation was subcloned downstream of the 1.3-kb

This paper was submitted directly (Track II) to the PNAS office.

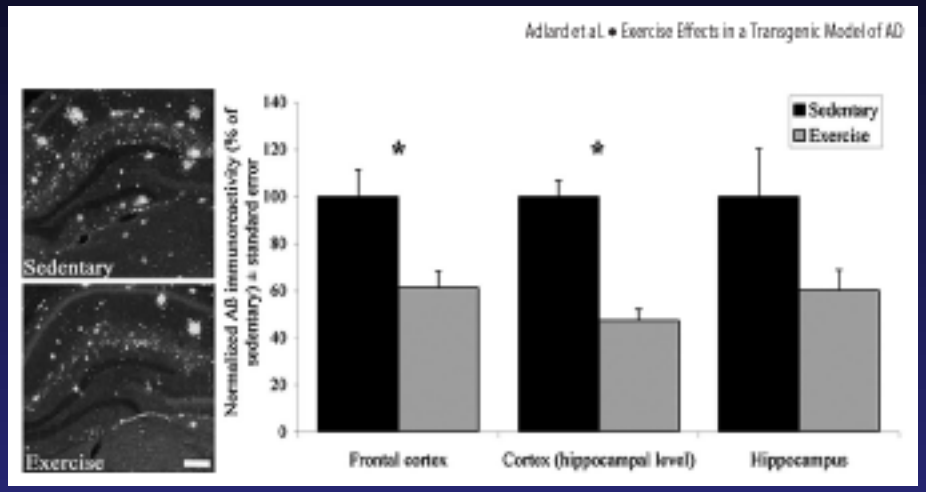
Abbreviations: IBM, inclusion body myositis; AD, Alzheimer's disease; βAPP, amyloid-β precursor protein; Aβ, amyloid-β.

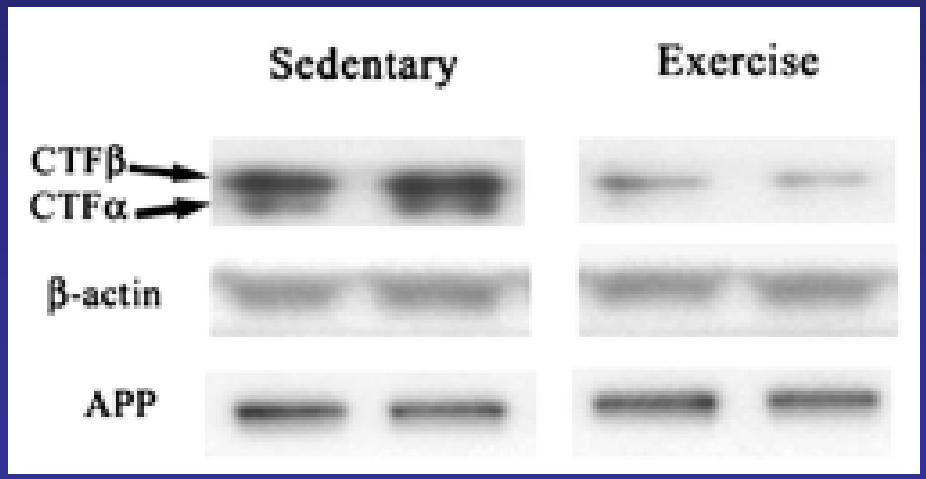
‡To whom reprint requests should be addressed. E-mail: laferla@uci.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.


What is sIBM?
Pharmacology and Genetics
Lifestyle Interventions
NSAIDs
APP Transgenic
APP Knock-In
Exercise
Diet
?
?

Exercise

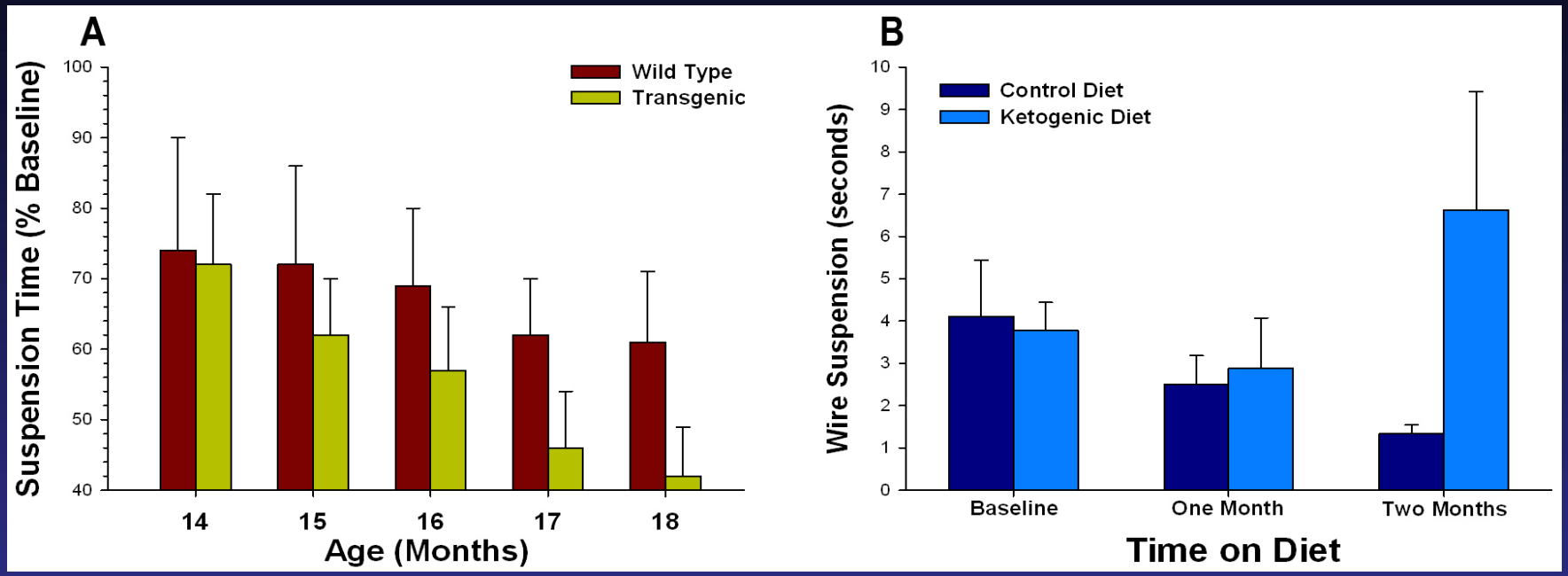




- Nice resonance for a muscle disease
- Several anecdotal reports indicate that moderate exercise may help IBM patients
- Simple running wheel activity reduces pathology in several mouse models of AD
- However, minimal effect in sIBM mice

UK

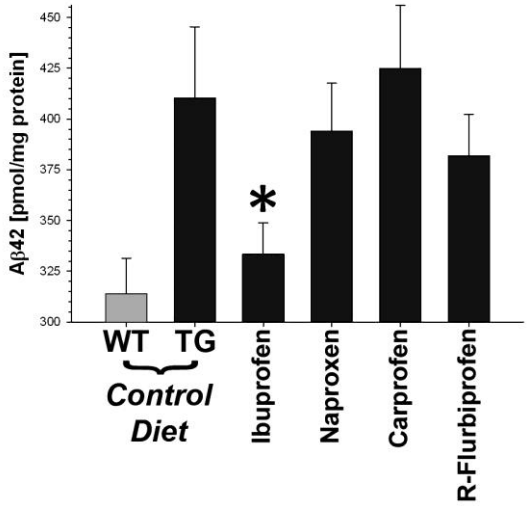
Diet

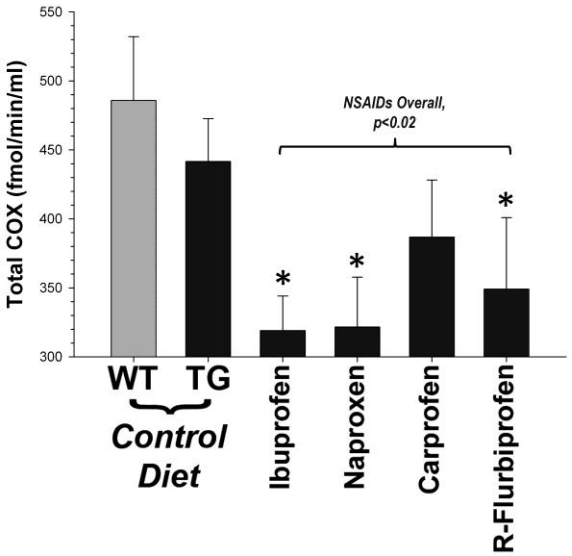


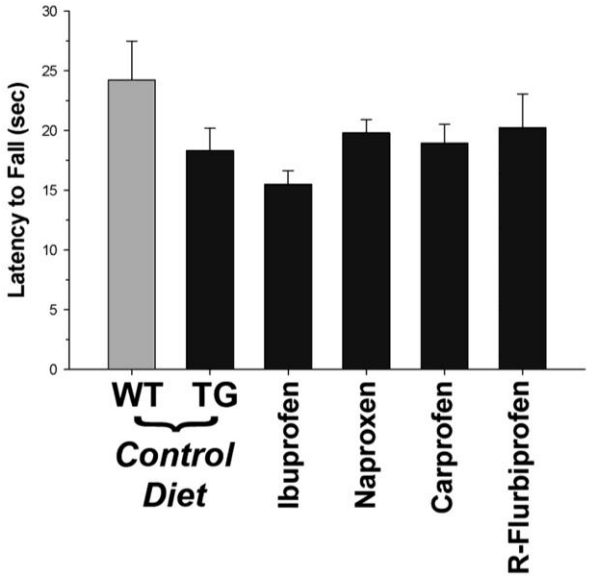
		Genotype							
		Wild-type		APP		PS1		APP/PS1	
		control	KD	control	KD	control	KD	control	KD
Weight (g)	Baseline	25±3	24±3	23±1	23±1	27±2	25±2	25±1	25±1
	1 month[a]	28±2	25±2	28±1	25±1	31±2	25±2	29±1	27±1
Ketones (mM)	Baseline	0.37±0.07	0.3±0.06	0.36±0.02	0.48±0.09	0.46±0.07	0.44±0.10	0.37±0.03	0.42±0.04
	1 month[a]	0.37±0.22	1.0±0.22	0.36±0.10	1.09±0.10	0.34±0.17	0.98±0.17	0.36±0.12	1.18±0.12
Glucose (mg/dL)	Baseline	143±28	133±4	140±7	151±9	126±15	142±22	140±10	125±9
	1 month	89±13	106±13	104±6	111±6	98±10	91±10	109±7	115±7
Insulin (ng/mL)	[a]	1.74±0.37	0.42±0.37	1.82±0.17	0.52±0.17	1.88±0.29	0.64±0.29	2.13±0.20	0.71±0.20

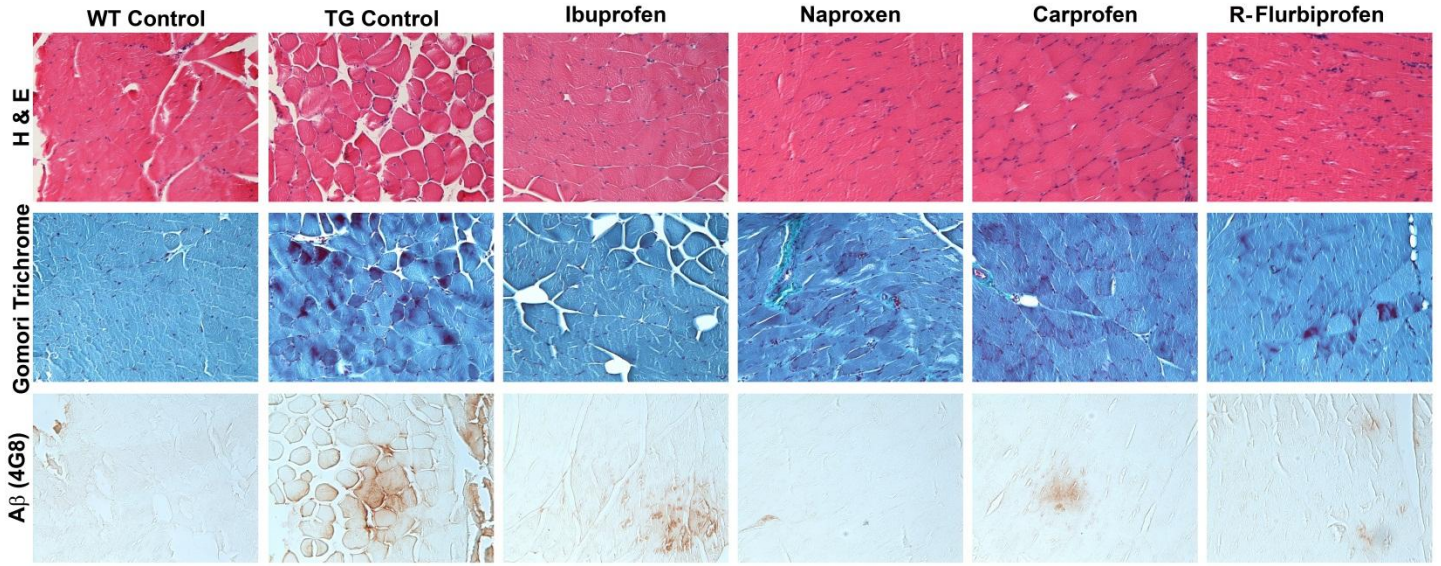
Mice do get a little better in muscle function, but NO effect on the underlying pathology

NSAIDs


Aβ42 [pmol/mg protein]
450
425
400
375
350
325
300
*
WT
TG
Control Diet
Ibuprofen
Naproxen
Carprofen
R-Flurbiprofen

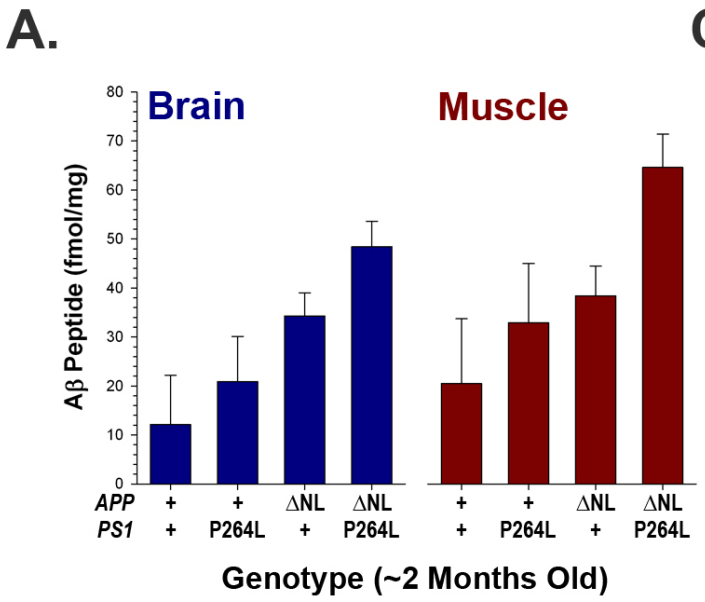

Total COX (fmol/min/ml)
550
500
450
400
350
300
NSAIDs Overall, p<0.02
*
*
*
WT
TG
Control Diet
Ibuprofen
Naproxen
Carprofen
R-Flurbiprofen

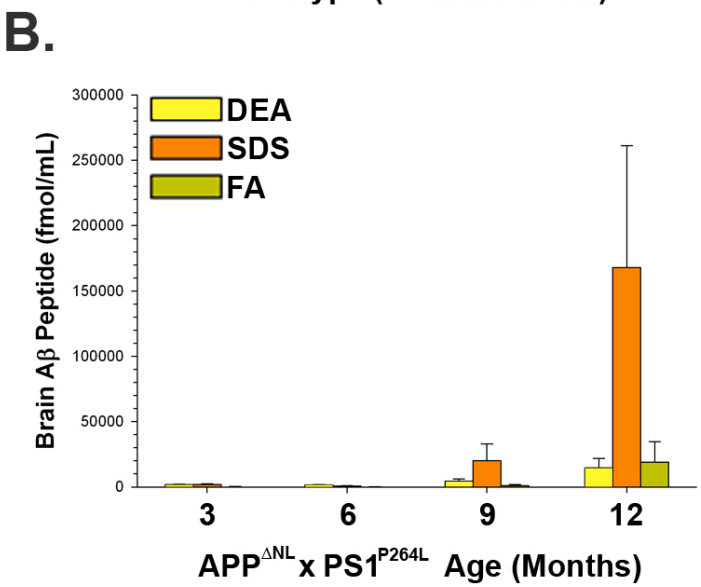

Latency to Fall (sec)
30
25
20
15
10
5
0
WT
TG
Control Diet
Ibuprofen
Naproxen
Carprofen
R-Flurbiprofen

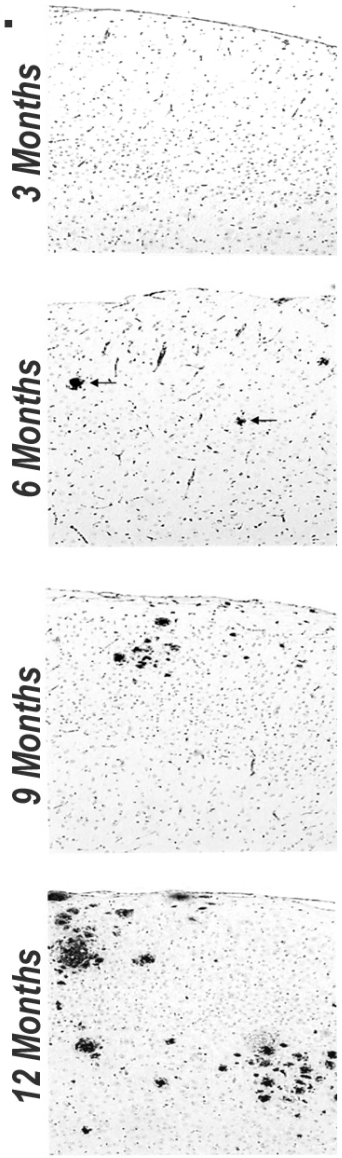

WT Control
TG Control
Ibuprofen
Naproxen
Carprofen
R-Flurbiprofen
H & E
Gomori Trichrome
Aβ (4G8)

UK

Other Mice?


A.
Brain
Muscle
Aβ Peptide (fmol/mg)
0
10
20
30
40
50
60
70
80
APP + + ΔNL ΔNL + + ΔNL ΔNL
PS1 + P264L + P264L + P264L + P264L
Genotype (~2 Months Old)


B.
DEA
SDS
FA
Brain Aβ Peptide (fmol/mL)
0
50000
100000
150000
200000
250000
300000
3
6
9
12
APP^ΔNL x PS1^P264L Age (Months)


C.
3 Months
6 Months
9 Months
12 Months

	APP	Aβ	Inflam.	Weak?
C57 Mice	✓✓	✓✓	✓	Yes
B6C3 Mice	✓✓	✓	-	No
Knock-In Mice	✓	✓	-	No
NSAIDs (+)	-	✓	✓	No Effect
NSAIDs (-)	-	-	✓	No Effect
Diet	-	-	-	Improve

NIH (R01 NS058382, P20 RR020171, P01 AG005119, P30 AG005144, T32 HL086341), KSEF, NSF, AFAR, CART Foundation, The Myositis Association, The Alzheimer's Association, and The American Heart Association

Rachel Ahmed, Grad Student
Kristen Kingry, Research Assistant
Robin Webb, Grad Student
Angela Spinelli, Research Assistant
Chris Holler, Grad Student
Feng Li, Research Technician
Katharina Kohler, Research Technician
Moshe Khurgel, Visiting Scientist
Tina Beckett, Research Analyst
Dana Niedowicz, Post-Doc
Thomas Platt, Grad Student
Adam Weidner, Post-Doc
Christa Studzinski, Post-Doc