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Amyloid futures in the expanding pathology of brain aging and dementia

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Abstract

PET imaging studies of AD patients show progressive increases of fibrillar A β - amyloid. Because current PET ligands underestimate non-fibrillar forms, we assayed soluble A β in AD and controls. To identify mechanisms responsible for soluble A β in AD brains, we examined lipid rafts (LR), where APP is enzymatically processed. Frontal cortex was compared with cerebellum, which has minimal AD pathology. Compared with cognitively normal controls (Braak 0–1), elevations of soluble A β 40 and A β 42 were similar for intermediate and later stage AD (Braak 2–3 and 4–6). Clinical grade AD had greater increase of soluble A β 40 than A β 42 relative to CTL. LR raft yield per gram AD frontal cortex was 20% below controls, while cerebellar LR did not differ by Braak score. The extensive overlap of soluble A β levels in controls with AD contrasts with the PET findings on fibrillar A β . These findings further support fibrillar A β as a biomarker for AD treatments and show the need for more detailed postmortem analysis of diverse soluble and insoluble A β aggregates in relation to PET.

Narrative.

Amyloidogenic peptides are strongly associated with AD neurodegeneration, but little is known about the relationships of these peptides to ‘normal brain aging’, which begins decades before signs of cognitive decline and dementia. Two hypotheses are proposed: 1) Because age-matched controls are shown here to have high levels of soluble A β , we hypothesize that non-fibrillar β -amyloid peptides increase decades before AD in the cognitively normal. Analysis of lipid rafts, the site of amyloid precursor protein (APP) processing [1], shows decreased β -amyloid peptide formation in AD frontal cortex relative to age-matched controls. 2) Because the β -amyloid peptide transporter low density lipoprotein receptor-related protein 1 (LRP1) is also decreased in AD [2,3], we further hypothesize that impaired β -amyloid peptide clearance, rather than increased synthesis is a key to the elevations of toxic amyloids in both normal aging and clinical dementia.

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Conflicts of Interest. The authors report no conflicts of interest.

In 1854 Rudolph Virchow observed macroscopic structures in brain tissue resembling starch and named them amyloid derived from the Latin word *amylum* and Greek *amylon* [4]. These structures were soon resolved by Friedreich and Kekulé as protein rather than starch [5]. The six recent decades of research on brain amyloids definitively show fibrillary β -amyloid protein in senile plaques. The β -amyloid fibrils were then characterized as aggregates of so-named β -amyloid peptides containing 38–43 amino acids, primarily A β 40 and A β 42 [6]. Neurofibrillary tangles (NFT) from hyperphosphorylated tau aggregates also increase at later ages in association with cognitive decline [7].

Causal links of cognitive decline to fibrillar β -amyloid are elusive because immunotherapy to remove fibrillar β -amyloid did not consistently improve cognitive deficits in multiple clinical trials [8–10]. Moreover, only one-third of terminal cognitive decline was explained by postmortem levels of fibrillar β -amyloid and tau [11,12]. Both longitudinal studies documented a wide range of multiple pathologic lesions in older brains in addition to β -amyloid plaques and NFT, together with proteinopathies of aggregated α -synuclein (Lewy bodies) and TDP-43 protein, as well as cerebral vascular amyloid pathology and microbleeds. Clinical dementia at later ages indicative of AD rarely occurs with only amyloid plaques and NFT. Unresolved issues include how neuropathological heterogeneity changes with older ages and by ethnicity. The aging brain is a jungle.

We propose that a life course approach will help resolve relationships of β -amyloid peptides to cognitive declines and other brain aging changes. For starters, consider that levels of β -amyloid peptides A β 40 and A β 42 increase *exponentially* during normal aging in frontal cortex [13] (Fig. 1D,E). These individuals lacked AD-grade pathology or history of dementia. After age 60, A β 42 was at least ten-fold higher than levels before 30 years. This extraction method and other [14] did not distinguish solubilized and aggregated β -amyloid peptides, which we further resolve. Age increases of β -amyloid peptides is also found in lab rodents without AD transgenes (Fig. 1A) [15,16], monkey (Fig. 1C), and domestic dogs [17]. This commonality suggests that non-pathological increases of β -amyloid peptides is a broadly shared process of mammalian brain aging.

Concurrent with increasing β -amyloid peptides after age 30 years are slow declines in cognition processing and synapse density from young adulthood [18]. Longitudinal studies concur that cognitive skills entailing fluid reasoning or the ability to solve novel problems reach a peak by the mid 30s, followed by slow declines [19–21]. Underlying mechanism may be the progressively slowed processing of information [22,23] and the progressive loss of synapses in cerebral cortex [24] and basal ganglia [25]. Similar loss of monoamine receptors was shown by us for monoamine receptors 4 decades ago [26]. The loss of synapses in middle aged humans [27] and rodents are best attributed to atrophy of neuronal cell body and synaptic arbor, rather than death of neurons, as shown for cerebral cortex of cognitively healthy brains up to age 90 [28]. The blood-brain barrier also progressively weakens during middle-age, without amyloid or tau pathology, as observed by in vivo brain imaging [29,30]. We hypothesize that the accelerating risk of dementias after age 60 years arises at some critical threshold of elevated β -amyloid peptides during ‘normal aging’ when aging goes from ‘bad to worse’ with emergence of multifarious brain pathologies.

Consolidated Results:

Because of these gaps in consistent assay methods for soluble β -amyloid peptides, we measured soluble and insoluble brain β -amyloid peptides by a protocol similar to that for the above data on normal aging (Fig. 2A). The initial isotonic homogenate yielded data not reported for normal aging in comparison with clinical grade dementia. The level of neurodegeneration was categorized by Braak scores 0 to 6 for NFT density [31].

Soluble A β 40 increased progressively by Braak score in frontal cortex; A β 42 increased less (Fig. 2B). The 5-fold range of soluble β -amyloid peptides in cognitively normal elderly brain overlapped with the clinically demented cases. The wide range of A β 40 and-42 peptides within all Braak scores was further validated by dot blot quantification with 'internal standards'. As expected, fibrillar β -amyloid, extracted by formic acid (Fig. 2A), increased progressively with Braak score by 3-fold (Fig. 2C).

Possible causes of the elevated soluble peptides could include increased production and or decreased clearance mechanisms. β -Amyloid peptide production from the amyloid precursor protein APP was examined in lipid rafts (LR), a subcellular fraction containing the secretase enzymes BACE1 and PSEN 1 that cleave APP. Yields of LR wet weight from frontal cortex decreased at later stages of AD with higher Braak scores (Fig. 3A). The decreased yield was in proportion to neuronal loss on frontal cortex, assessed by the neuron marker NeuN (Fig. 3D). APP levels were 25% lower in clinical grade AD with Braak stages 4–6 (Fig. 3C). The cerebellum showed nonsignificant differences between AD and nondemented controls for soluble and fibrillar β -amyloid peptides, see below, consistent with this region's minimal neurodegeneration [32] and was also relatively unaffected with aging in healthy brains (Fig. 1F).

The clearance of A β peptides was evaluated with proteins that bind to A β and target it for degradation [2]. In AD frontal cortex, LRP1 decreased by 40% in correlation with ApoE levels (Fig. 4). Soluble A β 40 varies inversely with LR yield, LRP1, and NeuN (Fig. 5).

Discussion:

These findings document the wide range of soluble brain β -amyloid peptides A β 40 and-42 in frontal cortex of nondemented control brain and their extensive overlap with levels observed in AD. A similarly wide range of concentrations were shown for formic acid soluble β -amyloid peptides in brains that had minimal histochemical amyloid by standard postmortem criteria (Fig. 1D,E). This is the first quantification of postmortem soluble A β peptides by Braak scores using an assay based on standard curves of A β peptides added to brain extracts, which showed linearity over a 100-fold range. This is also the first comparison of β -amyloid peptides with parallel extracts of bulk brain tissue in relation to lipid rafts.

We do not know the level of aggregation in β -amyloid peptides extracted by formic acid in these human brains, or in wild-type mice, across a wide age range (Fig 1A). Most soluble A β peptides are aggregated as oligomers in postmortem extracts [33] and in cerebrospinal fluid from living patients [34]. There is minimal neuron loss in non-demented

cerebral cortex of ‘normal aging’ mice and humans, despite elevated A β . This fact is not incompatible with the decades of research showing the neurotoxicity of A β oligomers [35] because in vivo oligomers are complexed with ApoE, ApoJ (CLU), and other proteins [33,36,37]. The term ‘amyloid’ thus needs to be carefully defined.

Is soluble A β elevated in AD above normal aging because of increased synthesis, or clearance from the brain? Lipid rafts, the subcellular site of APP cleavage, are decreased in proportion to neuron loss in frontal cortex. While the enzymes of APP cleavage are unchanged in AD, APP levels are further decreased. Secretase enzymes of APP processing and APP levels did not statistically correlate with soluble A β 40 and –42 in cerebral cortex and cerebellum. Neurons import A β through LRP1 for subsequent lysosomal degradation [38]. The large decrease of LRP1 suggests that impaired clearance rather than increased synthesis is a cause of increased soluble A β in AD. Multiple clearance mechanisms may differ by cell type and Braak stage [3].

Future studies could compare immunoassays with LC-MS for other β -amyloid peptides, and for correspondence with PET-imaging of amyloids. The aggregation state of A β and its complex with other proteins [33] may provide insights into the mechanisms underlying slow loss of synapses and cognitive processing during normal aging. Studies of APP processing in aging wild-type mice may be informative together modulators of APP processing [39]. Further analysis should include middle-aged brains and CSF for the roles of synthesis and clearance in A β elevations that precede AD pathology by decades. Amyloid futures may be sought in earlier development, when gene expression is established for β -amyloid peptide production and clearance.

Detailed Methods and Results

Methods:

Tissue.—Case-matched (Ctx;52, Cbl;58) frozen samples ages 85 ± 1 ; 66–99 years from University of Southern California (N=38), University of California Irvine (N=13), and University of Washington (N=8); equal sex distribution; cognition by MMSE, 93 ± 55 months pre-mortem (range 1–1,220 months; median 36 months); PMI, 6.6 ± 0.5 hours (range 0–19 hours; median 5.8 hours). The brain tissue used in this project was provided by the University of Southern California Alzheimer’s Disease Research Center, the University of California Irvine Alzheimer’s Disease Research Center (UCI-ADRC) and the Institute for Memory Impairments and Neurological Disorders, and the University of Washington’s Alzheimer’s Disease Research Center. Further information is presented in Table 1. All human subjects provided informed consent. IRB protocol #UP-20-00014-EXEMPT.

Amyloid Extraction.—Full thickness sections were homogenized by motorized pestle in RIPA buffer (30 mg tissue:150 μ L; [40]) containing 1% Nonidet P-40, and inhibitors of proteases and phosphatases (Fig. 6A). RIPA did not contain SDS which alters A β aggregation [41]. Homogenates were centrifuged at 10,000g for 1 hour at 4°C, yielding supernate (S1) with soluble amyloids. The pellet (P1) was resuspended in 70% formic acid [42] and sonicated (80% power/10s). After nutating for 2 hours at room temperature, the resulting S2 was fully solubilized, without visible pellets after 10,000g for 1 hour. S2

was neutralized with 20 volumes of 1M Tris and concentrated for 4 hours with a vacuum concentrator (Labconco, Kansas City, MO).

Lipid Raft Isolation.—Total LR was isolated from 40–60 mg of human frontal cortex and cerebellum by column chromatography (LR-039; Invent Biotechnologies, Plymouth, MN). LR yield was determined by weighing isolated rafts before resuspension in PBS+1% Triton and sonicated. Lipid rafts were assayed for cholesterol (Cell Biolabs, San Diego, CA) and used for western blotting. Lipid raft isolations were compared to Optiprep (Sigma Aldrich, St. Louis, MO) which were previously validated (Fig. 8C–F)[43].

Western Blots.—20 μ g of S1 or 5 μ g of lipid raft lysates were resolved on 4–15% gradient gels by Criterion Cell (Bio-Rad, Hercules, CA). Gels were transferred for 1 hour at 100V using a Criterion Blotter in an ice bath onto 0.45 μ m PVDF membranes. Total protein was stained (Revert 700, LI-COR Biosciences, Lincoln, NE), and imaged (LI-COR Odyssey 9120). Membranes were destained and incubated 1 hour with Intercept blocking buffer. Membranes were incubated with primary antibodies for 16 hours: GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA), H3, BACE1, PSEN1, LRP1 (Cell Signaling Technology, Danvers, MA), APP, NeuN (Abcam, Waltham, MA), Flotillin1, Flotillin2, Caveolin1, RFTN1, ADAM10, PSD95, and β III tubulin (Proteintech, Rosemead, IL). Membranes were visualized using fluorescent-conjugated secondary antibodies for image analysis by ImageJ and corrected by total protein load.

Dot Blots.—25 μ g of S1 or S2 lysates were loaded onto a dot blot apparatus (Bio-Rad, Hercules, CA) and were filtered through 0.45 μ m PVDF for 2 hours by gravity filtration. Membranes were stained with Revert 700, imaged, and blocked for 1 hour with Intercept blocking buffer before incubation for 16 hours with primary antibodies. Antibodies against A β 40, A β 42 (Biolegend, San Diego, CA), and amyloid fibrils M78 (conformation specific for A β fibrils [44], gift of Dr. Charles Glabe, UCI) were used. Membranes were visualized using fluorescent-conjugated secondary antibodies for image analysis by ImageJ and corrected by total protein load. A β 40 and A β 42 were quantified with A β peptides (Biolegend, San Diego, CA). Standard curves were created by loading S1 lysates with known concentrations of A β peptides (Fig. 6B,C). Oligomeric A β was not assayed because of concerns for its stability during freezing and thawing of brain tissues, and lability to detergents used during extraction [45].

Statistics.—Groups were compared by one way ANOVA using Tukey's HSD post hoc test for multiple comparisons. Significant differences for non-parametric data were calculated by Kruskal-Wallis with Dunn's post hoc test. Correlation plots and matrices were constructed by Spearman correlation.

Results:

A β 40 and A β 42 in frontal cortex and cerebellum of elderly (> 65 y, N=48, equal sex) were analyzed by Braak scores: low neurodegeneration (0–1), medium (2–3), and high (4–6) (Fig. 6). Scores 0–3 mainly represent 'normal' patients without clinical grade cognitive deficits (open circles), with 8% exceptions (closed circles). Two fractions of tissue homogenates

were analyzed for soluble A β by relative fluorescence and standard curve (Fig. 6B): S1, supernates of RIPA buffer homogenate with mild non-ionic detergent (S1/NP-40); S2, pellets of S1 solubilized by formic acid (S2/FA). Because of the wide range of A β 42 levels within all Braak scores, we further validated dot blot quantification with 'internal standards' (Fig. 6B). The addition of A β peptides to S1 gave linear increments above sample fluorescence across a 100-fold range of values. A β fibrils are presented as S2 relative fluorescence units because no reagent standards are available for heterogeneous A β fibrils.

A β was assayed to determine if observations made by Fukumoto et al. 2004 [13] extended to soluble A β peptides which were not reported for their equivalent of fraction S1. Frontal cortex S1 A β 40 increased progressively up to 50% across the Braak stages (P=0.02), while A β 42 had a weak trend (P=0.4; Fig. 7): For the S2/FA, the A β 40–42 peptides and fibrillary A β with Braak scores 4–6 were at least 100% above Braak stages 0–1. Cerebellum S1 A β 40 and A β 42 did not differ by Braak stage. The total extracted cortex A β 40 and A β 42 were 2-fold higher in AD brains (Braak 4–6) than controls (Braak 0–1) (Fig. 7A).

Possible causes of the elevated soluble peptides could include increased production and or decreased clearance. These same brains were characterized for lipid rafts (LR; Fig. 8A), the subcellular site of A β production from the secretase enzymes that cleave APP. LR isolations were initially validated by flotillin1, an LR protein in cytosolic and nuclear fractions obtained from the same extraction (Fig. 8B). LRs were void of cytosolic and nuclear contaminants (Fig. 8C). Isolations were then compared to LRs obtained by ultracentrifugation (UC) previously validated [43]. Flotillin1 was more abundant in the LR by chromatography kit compared to UC (Fig. 8D). Additional lipid raft markers flotillin2, caveolin1, and RFTN1 were used to validate kit LR's (Fig. E-F). Yields of LR wet weight from frontal cortex of Braak 0–1 stages were 25% higher than Braak 4–6 stages (Fig. 9A). Cortex LR protein (Fig. 9B), LR cholesterol (Fig. 9C), and APP processing did not vary by Braak stage for frontal cortex (BACE1, PSEN1, ADAM10; Fig. 9E–G). APP levels were 25% lower for the higher Braak 4–6 stages (Fig. 9D). Cerebellum LR yield was 75% less than cerebral cortex by wet weight and protein, yet LR of both regions had similar levels of APP, BACE1, and PSEN1 by Braak stage. However, cerebellar ADAM10 increased 6-fold in Braak stages 4–6 brains compared to 0–1 (Fig. 9G). Amyloid clearance receptor LRP1 decreased in Braak stages 4–6 in frontal cortex from 0–1 and 2–3 groups; lipid raft ApoE was unchanged (Fig. 9H,I).

Neuronal markers were used to assess neuronal loss in relation to Braak score and brain region. The levels of NeuN, β III tubulin, and PSD95 decreased in showed parallel with for Braak 4–6 stages in frontal cortex compared to 0–1 but was unchanged in cerebellum (Fig. 10). Because NeuN is not found in cerebellar Purkinje neurons, β III tubulin was included [46].

Correlation matrices examined relationships between A β peptides, LRs, and LRP1 for amyloid clearance. Levels of A β peptides in bulk tissue (Fig. 7) had limited correlation of LR composition across Braak scores (Fig. 11). Cortex A β 40 was inversely correlated with LR yield, NeuN, and LRP1. In both regions, LR APP protein varied inversely with Braak scores and AD clinical status. Cerebellar correlations with AD were generally weaker than

frontal cortex (Fig. 11). A β 42 and Braak score were strongly correlated in cortex but not cerebellum.

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Research In Context

Systematic Review:

Cited literature was found using PubMed. Soluble β -amyloid peptides ($A\beta$) are not well defined by the level of Alzheimer's disease (AD) neuropathology.

Interpretation.

Elevations in soluble $A\beta$ peptides in AD frontal cortex are attributable to impaired $A\beta$ clearance rather than production.

Future Directions:

Differences by sex and Apolipoprotein E (ApoE) allele need analysis in expanded samples in relation to Braak stage and positron emission tomography (PET) amyloid. Younger ages merit analysis for the cause of progressive $A\beta$ peptide elevations during middle-age, synthesis or clearance.

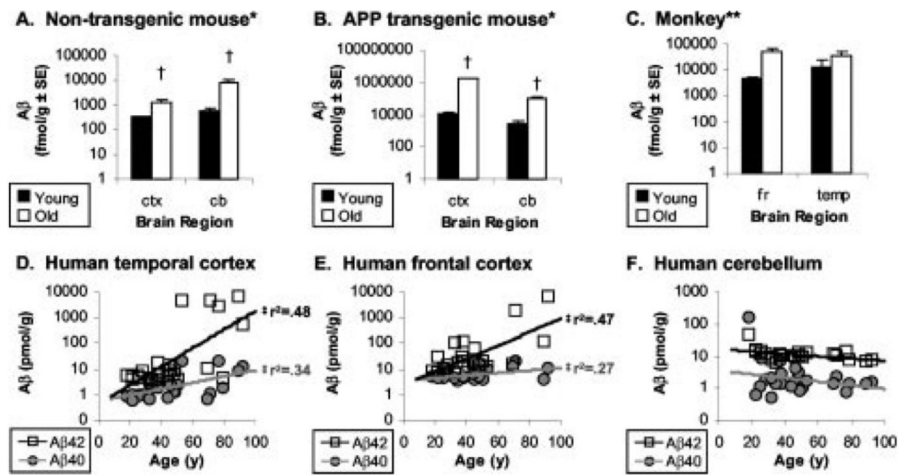


Figure 1:

β -Amyloid peptides in cerebral cortex increase with age in mice (A,B), monkey (C) and cognitively normal humans (D,E), but not cerebellum (F). Reprinted from Fukumoto et al. 2004 [13]. No human brain had NFTs; 3 brains had “diffuse cortical amyloid deposits without cortical NFT”. The $A\beta$ was extracted by formic acid from centrifuged pellets of an isotonic homogenate in Tris buffer with 0.1% Triton X-100; ELISA. The Tris-soluble fraction was not analyzed.

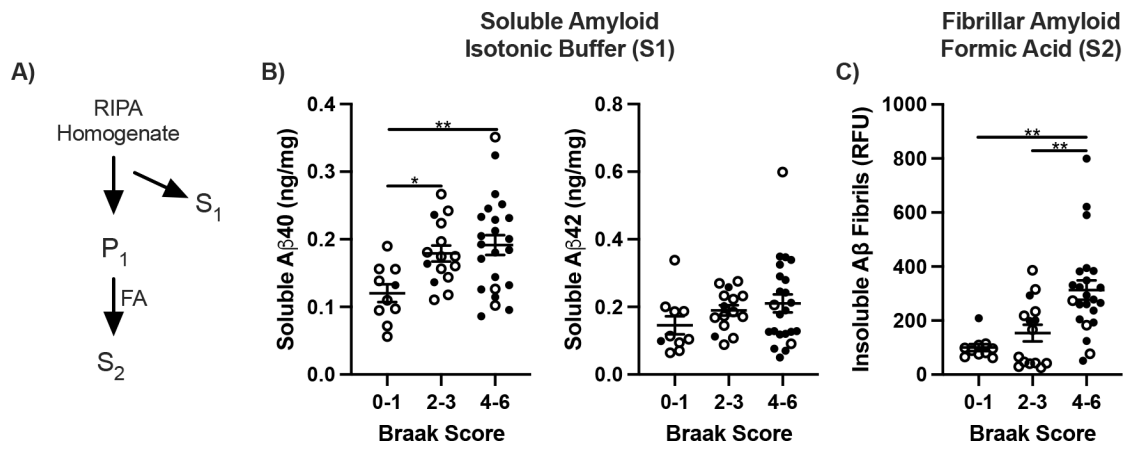


Figure 2: β -Amyloid in frontal cortex by Braak. **A)** Diagram of amyloid extraction and fractionation. S1, RIPA soluble amyloid; P1, insoluble pellet. S2, formic acid solubilized (FA). **B)** Values of A β 40 and A β 42 ng/mg per brain tissue in S1 calculated from internal standard curves and **B)** Fibrils as relative fluorescent units. One way ANOVA; * $p < 0.05$, ** $p < 0.01$.

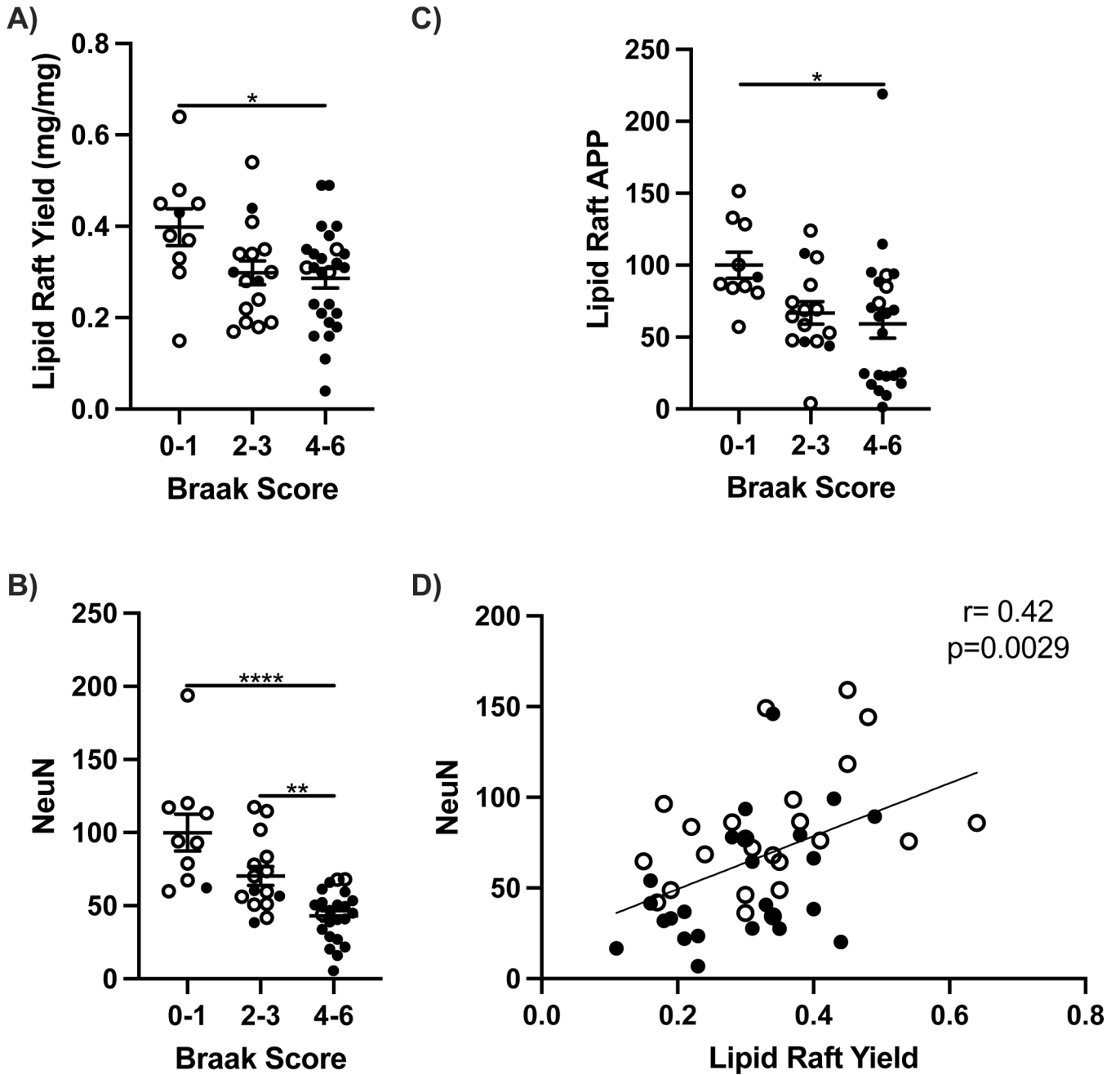


Figure 3: Lipid raft (LR) yield, amyloid precursor protein (APP) and NeuN by Braak score in frontal cortex and cerebellum of 65+ year old cognitively normal (open circles) and demented (closed circles). **A)** LR yield per mg tissue, **B)** APP, **C)** NeuN and **D)** coplot of NeuN and LR yield. One way ANOVA; * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Spearman correlation; ** $p < 0.05$.

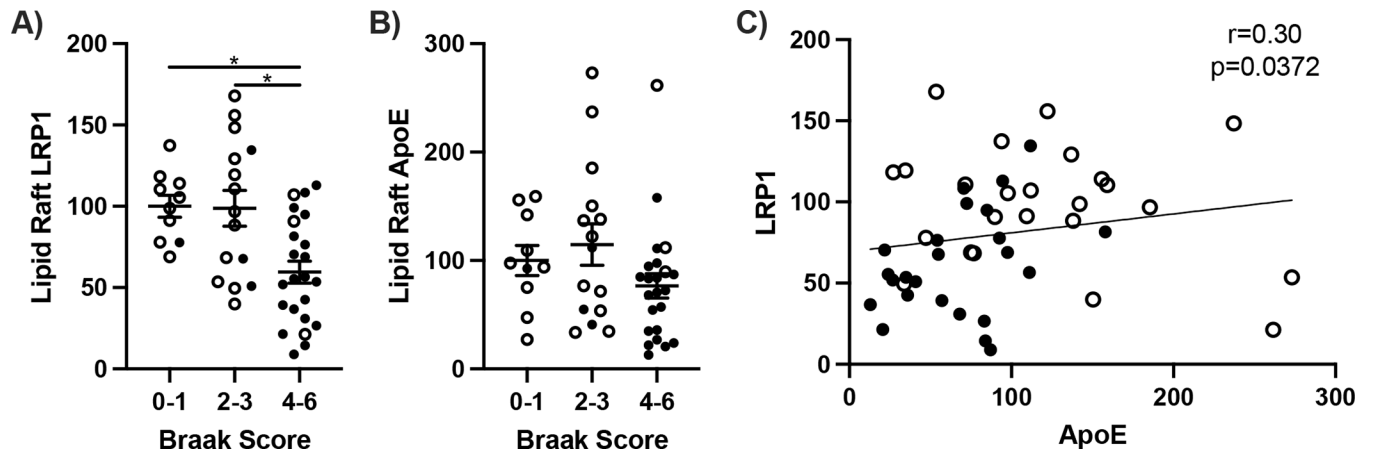


Figure 4:
 β -Amyloid clearance proteins in lipid rafts. Lipid raft A) LRP1, B) ApoE, and C) coplot of LRP1 and ApoE from frontal cortex. One way ANOVA; * $p<0.05$. Spearman correlation; * $p<0.05$.

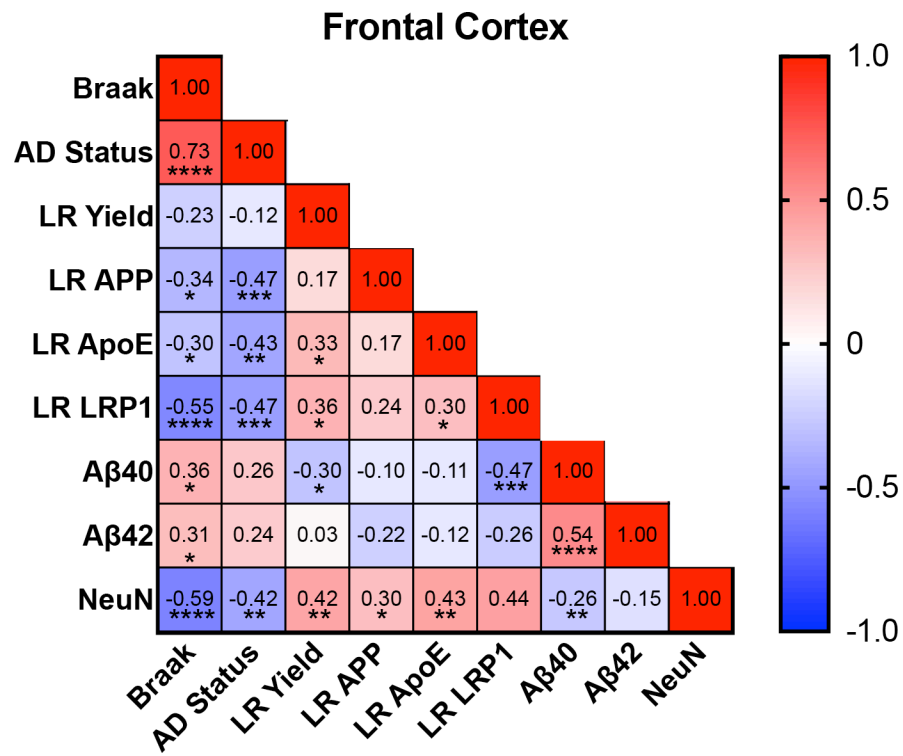


Figure 5: Correlation matrices examining relationships between soluble amyloid, LR, and amyloid clearance for frontal cortex. Spearman correlation; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

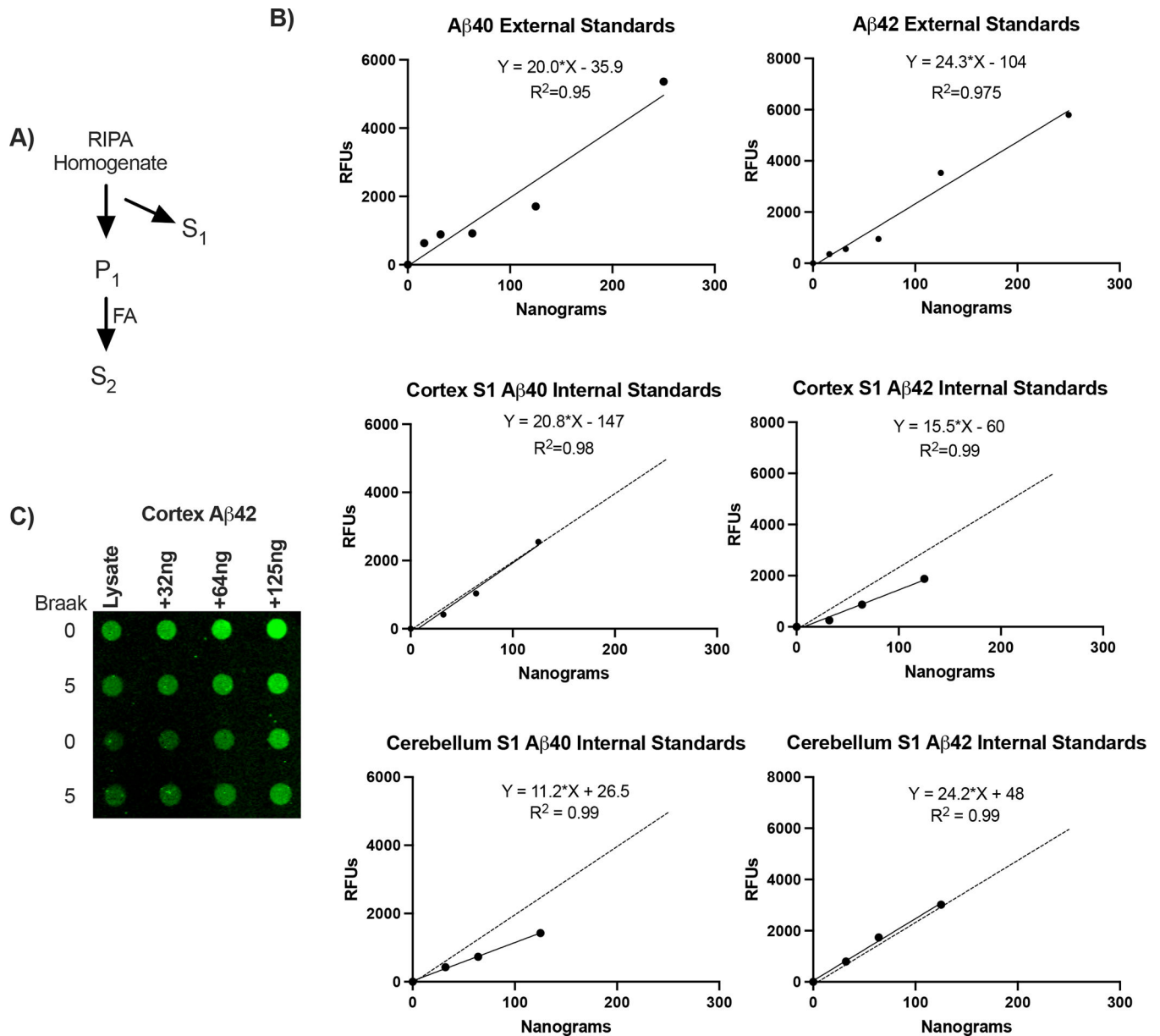


Figure 6:
 β-Amyloid peptide standard curves. **A)** Diagram of amyloid extraction and fractionation. S1, RIPA soluble amyloid; P1, insoluble pellet. S2, formic acid solubilized (FA). **B)** Standard curves for reagent Aβ40 and Aβ42 are linear for 100-fold range of relative fluorescence units (N, 6–8 per curve). **C)** Representative dot blot for internal standard curves of Aβ42 added to S1 lysates of cortex.

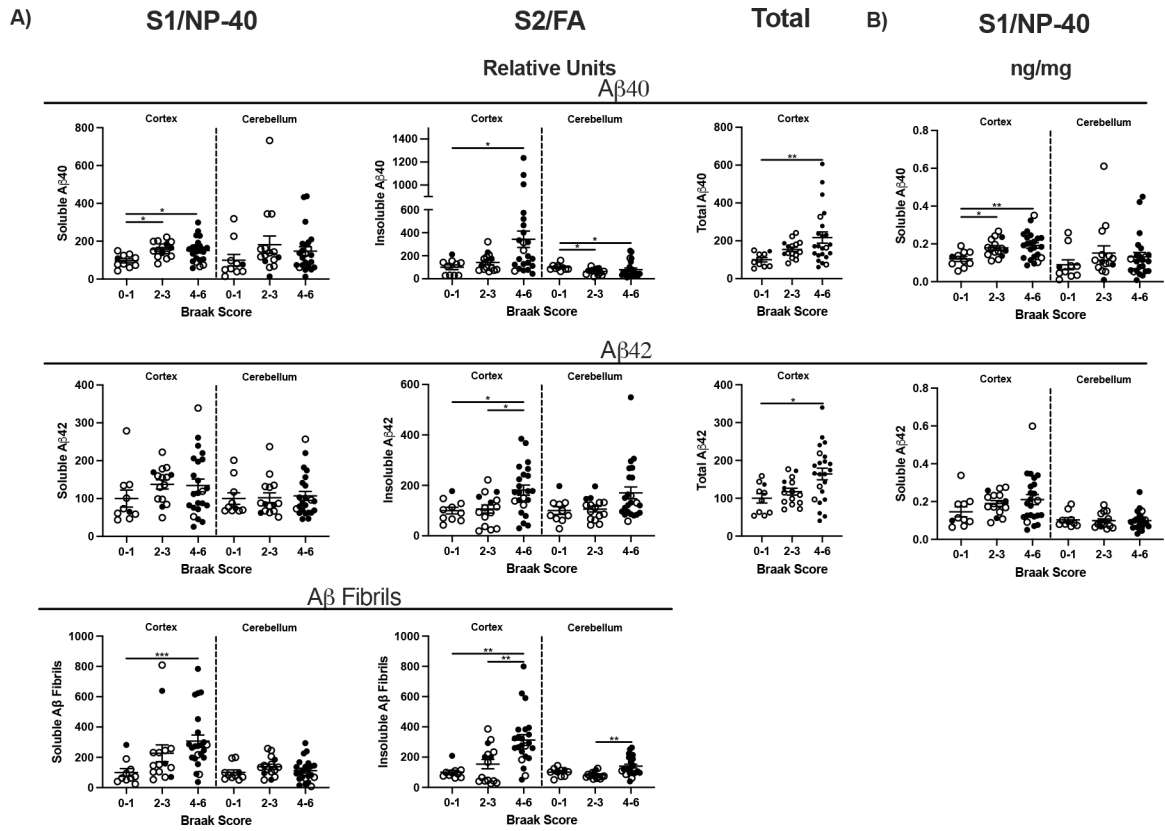


Figure 7:
 β -Amyloid in frontal cortex and cerebellum by Braak. **A)** β -Amyloid levels S1, S2, and fibrils shown as relative fluorescence units. **B)** Transformed values of A β 40 and A β 42 ng/mg brain tissue in S1 and S2, calculated from internal standard curves. One way ANOVA; *p<0.05, **p<0.01.

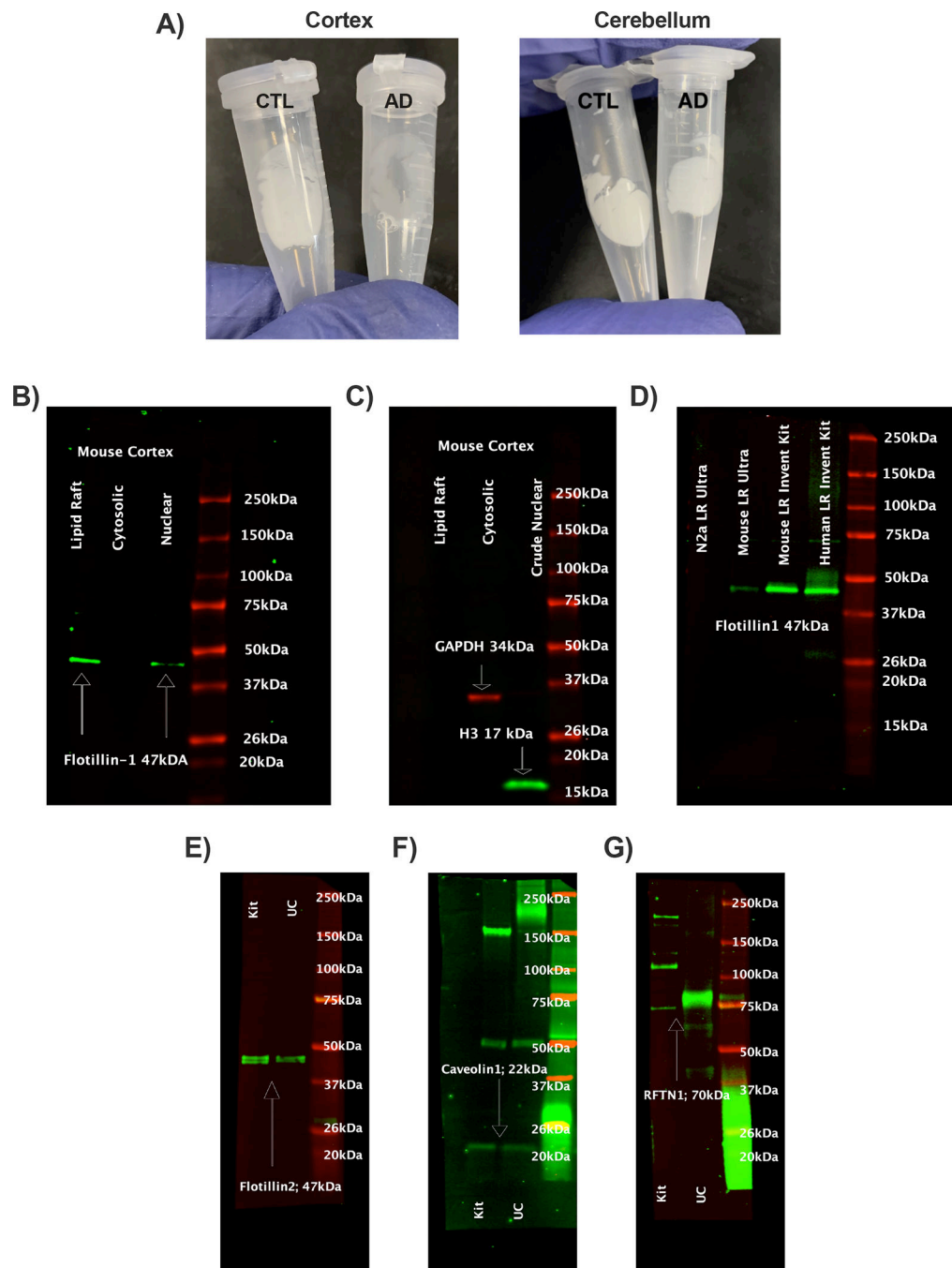


Figure 8: Validation of lipid rafts. **A)** Representative comparison of isolated human lipid rafts from cognitively normal and Alzheimer’s disease frontal cortex and cerebellum derived from Invent Biotechnologies’ kit (LR-039). Lipid raft, cytosolic, and nuclear fractions from the same extraction were probed for **B)** Flotillin1, a lipid raft marker, **C)** Histone 3 (nuclear), and GAPDH (cytosolic). **D)** LRs were compared with ultracentrifugation (UC) methods from mouse cortex and neuronal cells from and cortex for Flotillin1. Additional validations

comparing kit and UC rafts for presence of **D)** Flotillin 2, **E)** Caveolin 1, and **F)** Lipid Raft Linker 1 (RFTN1).

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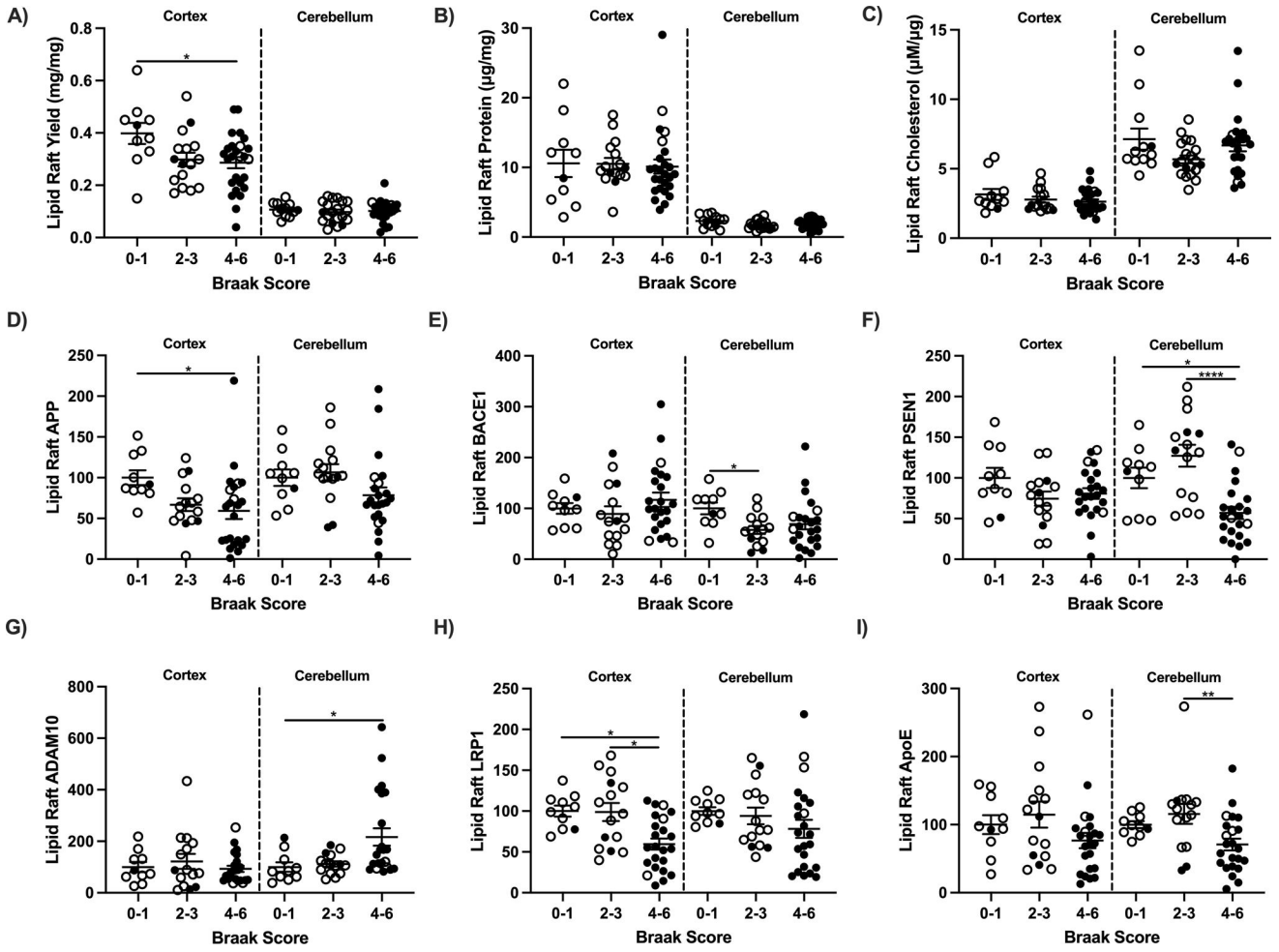


Figure 9: Lipid raft (LR) composition, amyloid precursor protein (APP) and amyloid processing enzymes by Braak score in frontal cortex and cerebellum of 65+ year old cognitively normal (open circles) and demented (closed circles). **A)** LR yield per mg tissue, **B)** total protein, **C)** cholesterol, **D)** APP, **E)** BACE1, **F)** PSEN1, **G)** ADAM10, **H)** LRP1, and **I)** ApoE. One way ANOVA; * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

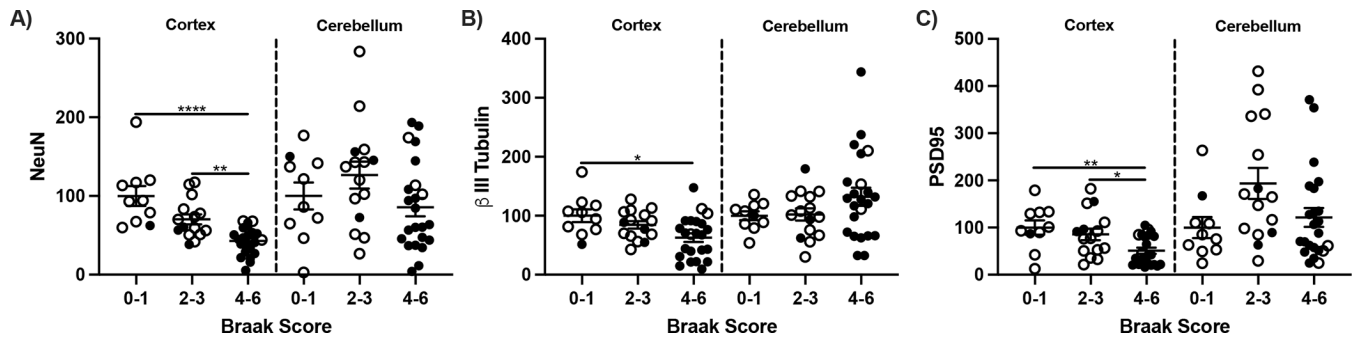


Figure 10: Neuronal markers in frontal cortex and cerebellum. **A)** NeuN, **B)** β III Tubulin, and **C)** PSD95 protein presented as relative fluorescent units. One way ANOVA One way ANOVA; *p<0.05, **p<0.01, ****p<0.0001.

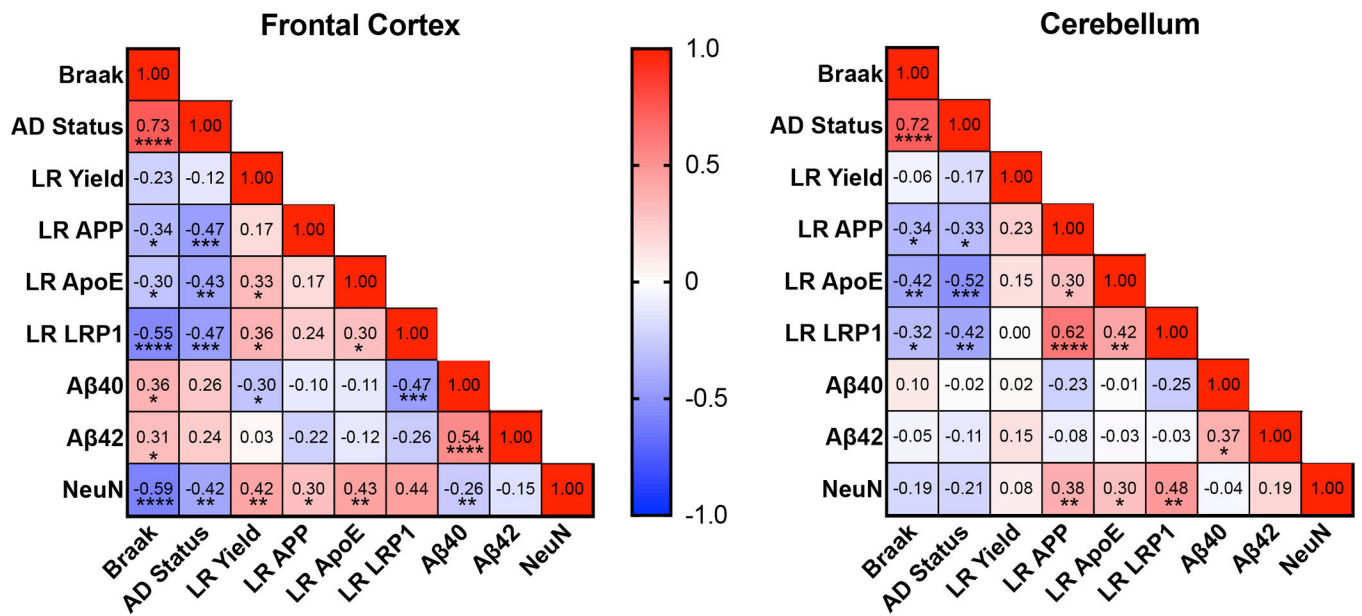


Figure 11: Correlation matrices examining relationships between soluble amyloid, LR, and amyloid clearance for frontal cortex and cerebellum. Aβ40, and -42 are transformed S1 values (Fig 7B). Spearman correlation; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Table 1:

Patient information for brain tissues by source.

Age	Sex	AD Status	Braak	ApoE Allele	PMI (hours)	Frontal Cortex	Cerebellum	Race	MMSE	MMSE Interval (Months)	ADRC
99	Female	CTL	0	3,3	9	x	x	White	28	23.4	USC
91	Female	CTL	0	3,3	8.75	x	x	White	20	84	USC
85	Female	CTL	0	3,3	7	x	x	White	N/A	N/A	USC
95	Female	CTL	1	3,3	3.25	x	x	White	N/A	N/A	USC
87	Female	CTL	2	3,3	10.75	x	x	White	29	1	USC
95	Female	CTL	2	3,3	2.92	x	x	Hispanic	N/A	N/A	UCI
86	Female	CTL	3	3,3	6.17	x	x	White	30	35.5	UCI
90	Female	CTL	3	3,3	2.2	x	x	White	N/A	N/A	UCI
89	Female	CTL	3	3,3	3.58	x	N/A	White	24	5.9	UCI
94	Female	AD	4	3,3	10.5	x	x	Black	N/A	N/A	USC
81	Female	AD	5	3,3	7.5	x	x	Hispanic	N/A	N/A	USC
89	Female	AD	5	3,3	1.5	x	x	White	N/A	N/A	USC
66	Female	AD	6	3,3	17	x	x	White	N/A	N/A	USC
70	Female	CTL	1	3,4	4.17	N/A	x	White	28	20.1	UW
86	Female	CTL	3	3,4	9.33	N/A	x	White	29	12.3	UW
97	Female	CTL	3	3,4	0	N/A	x	N/A	26	6.6	UW
91	Female	CTL	3	3,4	11.2	x	x	N/A	23	10.1	UW
85	Female	CTL	3	3,4	4.2	N/A	x	White	29	37.9	UW
89	Female	CTL	3	3,4	3.5	N/A	x	White	27	28.7	UW
74	Female	CTL	3	3,4	4.5	N/A	x	White	30	44.3	UW
91	Female	CTL	3	3,4	3.33	x	x	White	27	42.5	UCI
87	Female	CTL	3	3,4	4.57	x	x	White	29	85.6	UCI
93	Female	CTL	5	3,4	4.15	x	x	White	30	81.6	UCI
81	Female	AD	5	3,4	5.5	x	x	White	N/A	N/A	USC
84	Female	AD	5	3,4	6	x	x	White	N/A	N/A	USC
80	Female	AD	5	3,4	19	x	x	White	N/A	N/A	USC
96	Female	AD	6	3,4	9	N/A	x	Black	N/A	N/A	USC

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Age	Sex	AD Status	Braak	ApoE Allele	PMI (hours)	Frontal Cortex	Cerebellum	Race	MMSE	MMSE Interval (Months)	ADRC
77	Female	AD	6	3,4	8.25	x	x	White	N/A	N/A	USC
87	Female	AD	6	3,4	4.5	N/A	x	N/A	N/A	N/A	USC
78	Female	AD	4	4,4	15.5	x	x	White	N/A	N/A	USC
81	Female	AD	5	4,4	4	x	x	White	N/A	N/A	USC
80	Female	AD	5	4,4	3.75	x	x	White	N/A	N/A	USC
76	Female	AD	5	4,4	5.25	x	x	White	N/A	N/A	USC
82	Male	CTL	0	3,3	9	x	x	White	28	N/A	USC
93	Male	CTL	0	3,3	12	x	x	White	30	1229.2	USC
93	Male	CTL	0	3,3	3.75	x	x	White	N/A	N/A	USC
76	Male	CTL	0	3,3	11.25	x	x	Hispanic	N/A	N/A	USC
86	Male	CTL	2	3,3	4.42	x	x	White	25	48.4	UCI
83	Male	CTL	2	3,3	3.18	x	x	White	N/A	N/A	UCI
86	Male	CTL	3	3,3	2.92	x	x	White	20	37	UCI
80	Male	CTL	3	3,3	4.05	x	x	White	26	95.1	UCI
97	Male	AD	3	3,3	5.25	x	x	Hispanic	N/A	N/A	USC
87	Male	AD	4	3,3	4.75	x	x	White	N/A	N/A	USC
76	Male	AD	5	3,3	9.75	x	x	Hispanic	N/A	N/A	USC
88	Male	AD	5	3,3	6.75	x	x	Hispanic	N/A	N/A	USC
92	Male	CTL	0	3,4	5.25	x	x	White	N/A	N/A	USC
87	Male	CTL	1	3,4	4	N/A	x	White	25	8.9	UW
90	Male	CTL	3	3,4	5.8	x	x	White	N/A	N/A	UCI
97	Male	CTL	4	3,4	5.83	x	x	White	26	7	UCI
87	Male	CTL	4	3,4	6.28	x	x	White	22	101.5	UCI
77	Male	AD	2	3,4	6	x	x	White	N/A	N/A	USC
81	Male	AD	3	3,4	7.25	x	x	Hispanic	N/A	N/A	USC
85	Male	AD	5	3,4	7.25	x	x	Hispanic	N/A	N/A	USC
72	Male	AD	6	3,4	9	x	x	White	N/A	N/A	USC
75	Male	AD	0	4,4	9.5	x	x	White	N/A	N/A	USC
88	Male	AD	5	4,4	5	x	x	White	N/A	N/A	USC

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Age	Sex	AD Status	Braak	ApoE Allele	PMI (hours)	Frontal Cortex	Cerebellum	Race	MMSE	MMSE Interval (Months)	ADRC
92	Male	AD	5	4,4	8.25	x	x	White	N/A	N/A	USC
70	Male	AD	5	4,4	3.5	x	x	White	N/A	N/A	USC
81	Male	AD	5	4,4	6.54	N/A	x	Asian	N/A	N/A	USC

ADRC; Alzheimer's Disease Research Center, MMSE; Mini-Mental State Exam, PMI; Postmortem Interval, UW; University of Washington, UCI; University of California Irvine, USC; University of Southern California. Column headings of ethnicity ('Race') are shown as recorded by source.