Platelet Amyloid Precursor Protein Processing: A Biomarker for Alzheimer's Disease.

Roger N. Rosenberg, MD
Alzheimer's Disease Center
University of Texas Southwestern Medical Center at Dallas

Disclosures:

1. NIH/NIA P30AG12300-16 Alzheimer's Disease Center Grant
2. National Alzheimer's Coordinating Center Grant U5-01AG16976
3. The Rudman Foundation, Dallas, TX
4. U.S. Patent "Amyloid β Gene Vaccines"
   Assigned to the Regents of the University of Texas System; January 2009; Roger N. Rosenberg, MD, Inventor
5. Editor, Archives of Neurology, Editorial Board, JAMA

Altered Amyloid Protein Processing in Platelets of Patients With Alzheimer Disease

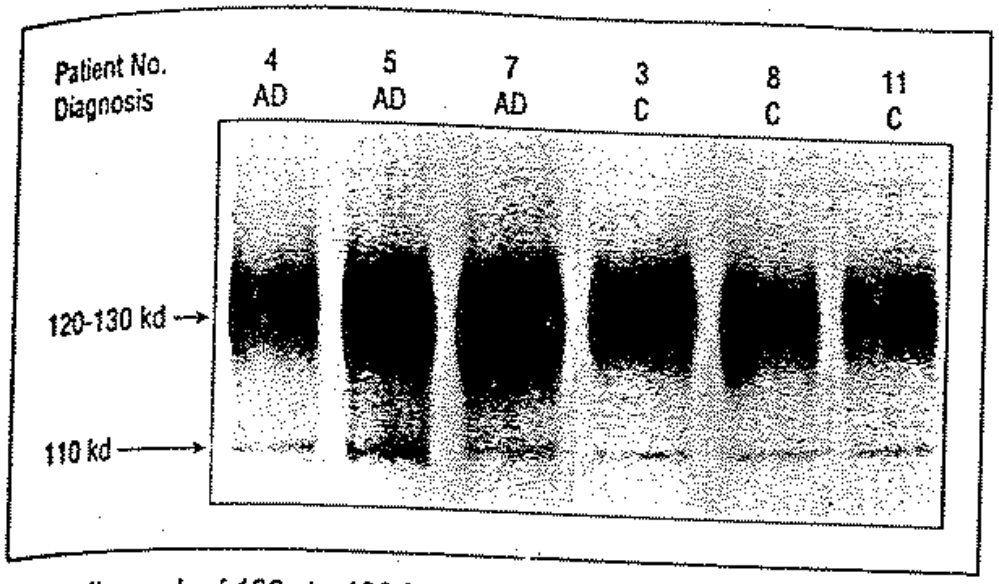
Roger N. Rosenberg, MD; Fred Baskin, PhD; Jennifer A. Fosmire, MD; Rick Risser, MS; Perrie Adams, PhD; Doris Svetlik, MS, RN; Lawrence S. Honig, MD, PhD; C. Munro Cullum, PhD; Myron F. Weiner, MD

The mean ratio of the 120- to 130-kd APP isoform to the 110-kd APP isoform in the patients with AD was significantly lower than that of the control subjects (5.98 vs 7.64; *P*=.03 [method 1] and 5.98 vs 7.92; *P*=.01 [method 2]) after adjusting for age and the increased incidence of *ApoE4* in patients with AD. The lower APP ratios were also associated with increased age and with the presence of an *ApoE4* allele

Arch Neurol. 1997;54(2):139-144

Altered Amyloid Protein Processing in Platelets of Patients With Alzheimer Disease

R.N. Rosenberg, et.al



Autoradiograph of 120- to 130-kd amyloid precursor protein (APP) and 110-kd APP from 3 patients with Alzheimer disease (AD) and from 3 control subjects (C), numbered as in Table 1 and visualized with enhanced chemiluminescence (method 1) after binding m22C11 antibody. Whereas a trend of reduced APP isoform density ratios is seen in these 3 patients with AD, quantitations of data from multiple fractionations were necessary to establish AD-related reduced APP ratios.

Arch Neurol. 1997;54(2):139-144

Altered Amyloid Protein Processing in Platelets of Patients With Alzheimer Disease

R.N. Rosenberg, et.al

Table 2. Observed APP Isoform Ratios in Patients With AD and in Control Subjects With and Without an *ApoE4* Allele*

	Ratio of 120- to 130-kd APP to 110-kd APP				
	Patients With AD		Control Subjects		
Method†	No.	Mean±SE	No.	Mean±SE	P‡
	All AD vs All Control				
1	15	5.61±0.41§	19	7.72±0.57§	.005
2	15	5.70±0.38§	18	8.01±0.55§	.002
	All With *ApoE4* vs All Without *ApoE4*				
1	21	6.24±0.55§	13	7.67±0.53§	.09
2	20	6.43±0.55§	13	7.78±0.48§	.1
	AD With *ApoE4* vs Control With *ApoE4*				
1	12	5.57±0.51	9	7.14±1.04	.2
2	12	5.68±0.48	8	7.54±1.10	.16
	AD Without *ApoE4* vs Control Without *ApoE4*				
1	3	5.77±0.38	10	8.24±0.57	.05
2	3	5.77±0.13	10	8.38±0.48	<.001

**APP indicates amyloid precursor protein; AD, Alzheimer disease; and* ApoE4, *apolipoprotein E4.*

†See Table 1 for explanation of methods.

‡By the Student t *test.*

§These comparisons are not adjusted for the difference in incidence of the ApoE4 *allele in the patients with AD and control subjects or for the incidence of AD in the patients with the* ApoE4 *allele and those without the* ApoE4 *allele.*

Arch Neurol. 1997;54(2):139-144

PLATELET APP ISOFORM RATIOS CORRELATE WITH DECLINING COGNITION IN AD

F. Baskin, PhD, R. N. Rosenberg, MD, L. Iyer, PhD, L. Hynan, PhD and C. M. Cullum, PhD

BACKGROUND: Platelets and neurons both contain large quantities of two carboxyl-truncated 120 to 130 and 110 kDa Alzheimer amyloid precursor proteins (APPs). Platelets taken from patients with AD have been reported to contain a reduced ratio of these APPs.
OBJECTIVE: To further study the AD specificity of reduced platelet APP ratios and to determine whether, after 3 years, cognitive losses in AD are accompanied by similarly reduced platelet APP ratios.
METHODS: To test the AD specificity of reduced platelet APP ratios, we quantitated these APPs in eight patients with PD and six patients with hemorrhagic stroke (HS). To determine whether further cognitive losses correlate with platelet APP ratio reductions in patients with AD, the authors re-examined platelet APPs and Mini-Mental State Examination (MMSE) scores of 10 patients with AD and 11 controls, who were tested 3 years ago. APP ratios were determined by the average of six assays using Western blotting with m22C11 monoclonal antibody, enhanced chemoluminescence, and digital scanning of autoradiographs.
RESULTS: APP ratios were normal in the patients with PD and HS, further supporting the AD specificity of this assay. After 3 years, the MMSE scores and APP ratios of our control subjects changed by <4%. However, the average MMSE scores of our patients with AD declined from 16.4 to 8.3, and their average 120 to 130/110 kDa APP ratios declined from 5.8 to 3.6. The difference between AD and control APP ratios, with no overlap, is significant and the correlation between the 3-year decline in AD MMSE scores and reduced APP ratios (r = 0.69) was significant.
CONCLUSIONS: Although the number of subjects analyzed was limited, reduced platelet APP ratios appear to be a specific biological marker of AD and a biological index of the severity of cognitive loss in AD.
Key words: Mini-Mental State Examination—Alzheimer amyloid precursor protein—Platelets.

Neurology 2000;54:1907-1909

PLATELET APP ISOFORM RATIOS CORRELATE WITH DECLINING COGNITION IN AD

F. Baskin, PhD, R. N. Rosenberg, MD, L. Iyer, PhD, L. Hynan, PhD and C. M. Cullum, PhD

Group	n	MMSE ± SE	p Value	R ± SE	p Value
AD–1999	10	8.30 ± 2.13	—	3.57 ± 0.47	—
AD–1996	10	16.40 ± 1.56	0.001[c]	5.83 ± 0.51	<0.001[e]
Control–1999	11	28.91 ± 0.25	<0.001[a]	8.31 ± 0.27	<0.001[b]
Control–1996	11	29.64 ± 0.20	0.012[d]	8.09 ± 0.61	0.573[f]
PD	8	29.50 ± 0.26	<0.001[a]	9.28 ± 0.85	<0.001[b]

[a] ANOVA $F(3/31) = 71.92$, $p < 0.001$. *p* Values are for multiple comparison (Tamhane) tests with the mean AD–1999 MMSE score.

[b] ANOVA $F(3/31) = 50.03$, $p < 0.001$. *p* Values are for multiple comparison (Bonferroni) tests with the mean AD–1999 platelet APP ratio.

[c] Paired samples *t*-test, $t = 4.93$ for AD–1999 versus AD–1996.

[d] Paired samples *t*-test, $t = 3.07$ for Control–1999 versus Control–1996.

[e] Paired samples *t*-test, $t = 6.98$ for AD–1999 versus AD–1996.

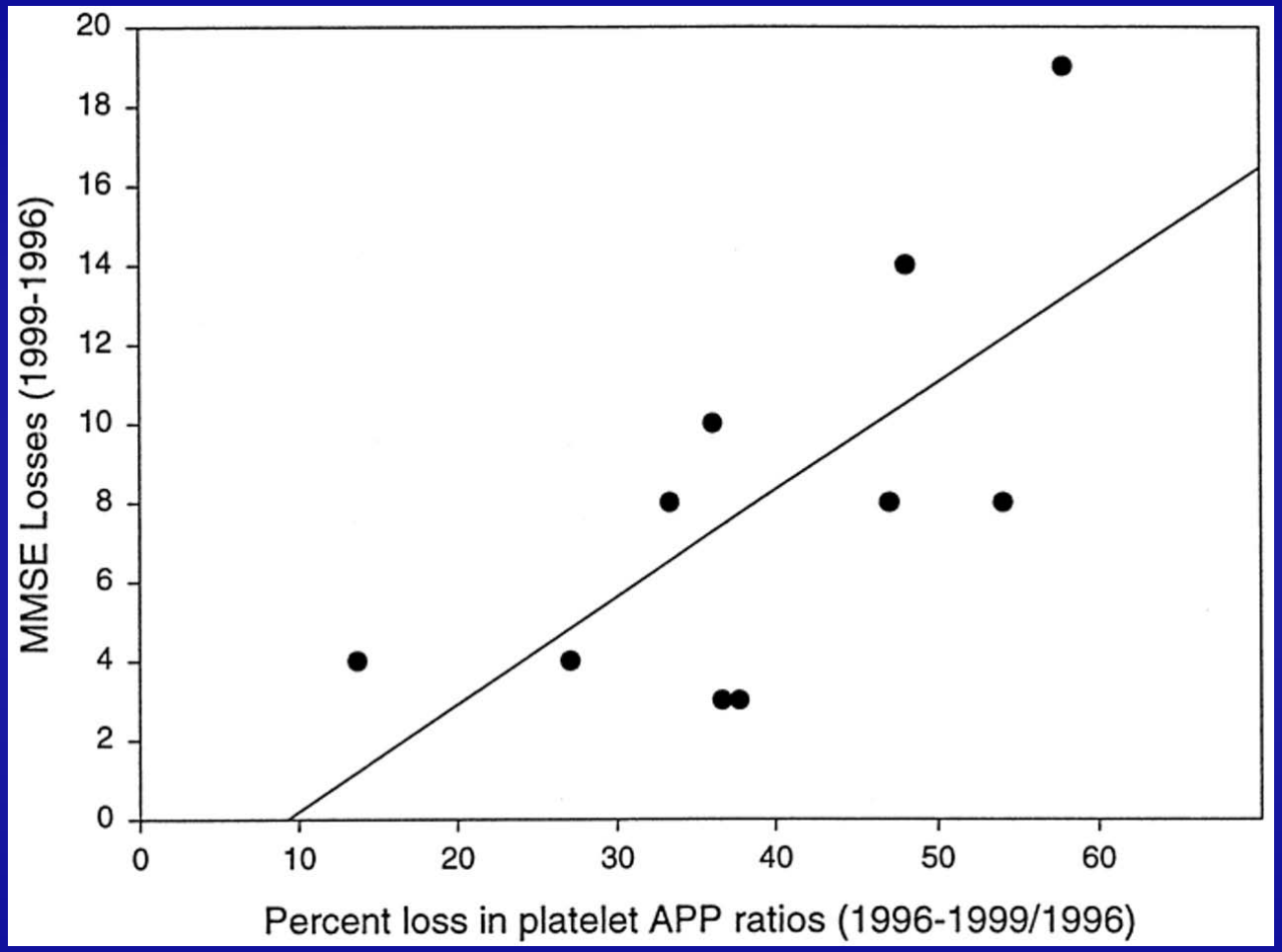
[f] Paired samples *t*-test, $t = -0.58$ for Control–1999 versus Control–1996.

ANOVA = analysis of variance.

Neurology 2000;54:1907-1909

PLATELET APP ISOFORM RATIOS CORRELATE WITH DECLINING COGNITION IN AD

F. Baskin, et al.



Proportional losses in platelet Alzheimer amyloid precursor protein (APP) ratios and Mini-Mental State Examination (MMSE) scores between 1996 and 1999 for 10 AD patients

Neurology 2000;54:1907-1909

Correlation Of Statin-increased Platelet APP Ratios And Reduced Blood Lipids In AD Patients

F. Baskin, PhD, R. N. Rosenberg, MD, X. Fang, MD, L. S. Hynan, PhD, C. B. Moore, MA, M. Weiner, MD and G. L. Vega, PhD

Platelets, like neurons, contain 120- to 130- and 110-kd amyloid precursor proteins (APPs). Their ratio is reduced in AD, further reductions correlating with reduced Mini-Mental Status Examination scores [$r(11) = 0.69$, $p < 0.05$]. As statins alter APP processing, platelet APPs were analyzed in patients with AD given anticholesterol drugs for 6 weeks. APP ratios increased [$t(37) = -3.888$, $p = 0.0004$], proportionally with reduced cholesterol [$r(36) = -0.45$, $p = 0.005$]. Longer trials may reveal slowed cognitive loss, validating this index.

Neurology 2003;60:2006-2007

Correlation Of Statin-increased Platelet APP Ratios and Reduced Blood Lipids In AD Patients

F. Baskin, et al.

Comparison of mean platelet amyloid precursor protein ratio changes (delta-R) after 6 weeks and total cholesterol reductions ± SE in lovastatin -, pravastatin -, simvastatin -, and niacin-treated patient groups

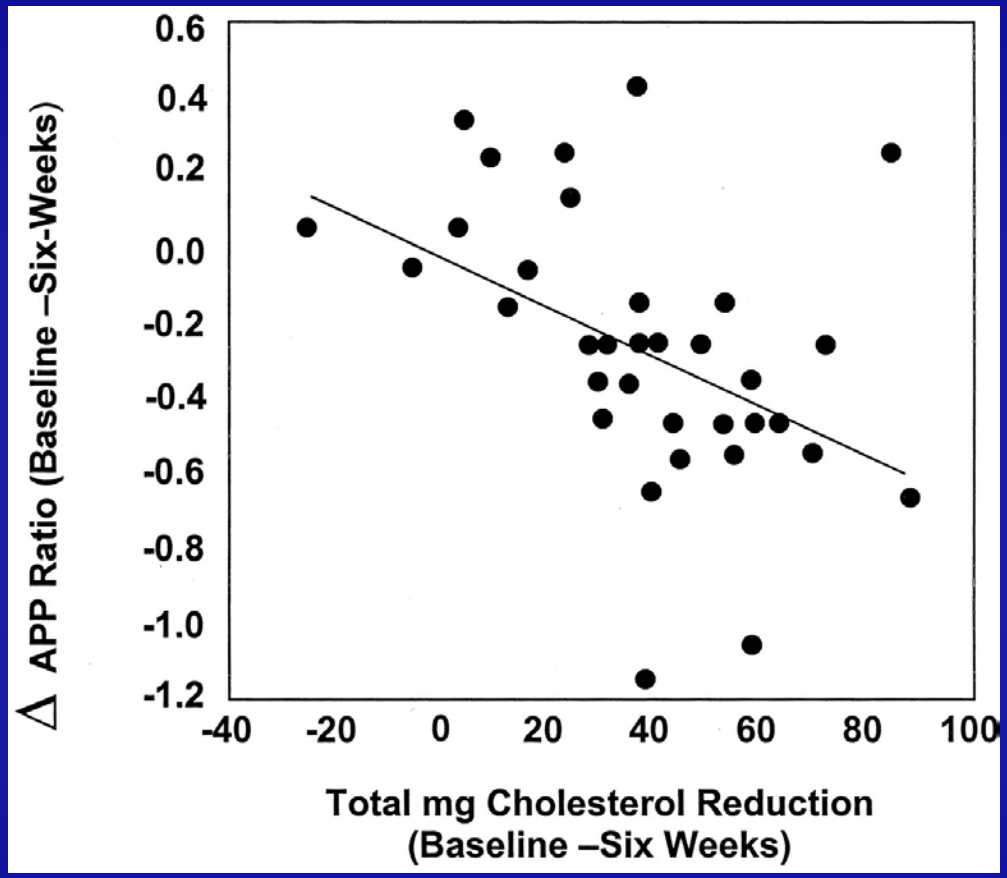
		Drop in total cholesterol (mg/dL)		Change in APP ratio		Correlation* between mg drop in total cholesterol and change in APP ratio	
Drug	*n*	Mean	SD	Mean	SD	*R*	*p* Value
Lovastatin	9	35.1	19.6	-0.11	0.33		
Niacin	10	28.8	31.3	-0.12	0.35		
Pravastatin	9	36.7	13.0	-0.31	0.35		
Simvastatin	10	58.6	15.9	-0.33	0.35		
Overall	38	40.00	23.56	-0.22	0.35	-0.45	0.005

**Pearson product moment correlation coefficient*
APP = amyloid precursor protein

Neurology 2003;60:2006-2007

Correlation Of Statin-increased Platelet APP Ratios and Reduced Blood Lipids In AD Patients

F. Baskin, et al.



Increases in AD platelet amyloid precursor protein (APP) ratios are proportional to statin-reduced blood lipid levels. Proportional increases in platelet APP ratios (Y-axis) and decreases in blood cholesterol levels (X-axis) for 38 patients with probable AD after 6 weeks of taking a cholesterol-reducing agent.

Neurology 2003;60:2006-2007

PLATELET AMYLOID PRECURSOR PROTEIN PROCESSING: A BIO-MARKER FOR ALZHEIMER'S DISEASE

Kun Tang[a], Linda S. Hynan[b], Fred Baskin[a], and Roger N. Rosenberg[a]

[a] Department of Neurology, University of Texas Southwestern Medical Center at Dallas, United States

[b] Center for Biostatistics and Clinic Science and the Alzheimer's Disease Center, University of Texas Southwestern Medical Center at Dallas, United States

The amyloid precursor protein (APP) in brain is processed either by an amyloidogenic pathway by β-secretase and γ-secretase to yield Aβ (β-amyloid 4 kDa) peptide or by α-secretase within the β-amyloid domain to yield non-amyloidogenic products. We have studied blood platelet levels of a 22-kDa fragment containing the Aβ (β-amyloid 4 kDa) peptide, β-secretase (BACE1), α-secretase (ADAM10), and APP isoform ratios of the 120–130 kDa to 110 kDa peptides from 31 Alzheimer's disease (AD) patients and 10 age-matched healthy control subjects. We found increased levels of Aβ4, increased activation of β-secretase (BACE1), decreased activation of α-secretase (ADAM10) and decreased APP ratios in AD patients compared to normal control subjects. These observations indicate that the blood platelet APP is processed by the same amyloidogenic and non-amyloidogenic pathways as utilized in brain and that APP processing in AD patients is altered compared to control subjects and may be a useful bio-marker for the diagnosis of AD, the progression of disease and for monitoring drug responses in clinical trials.

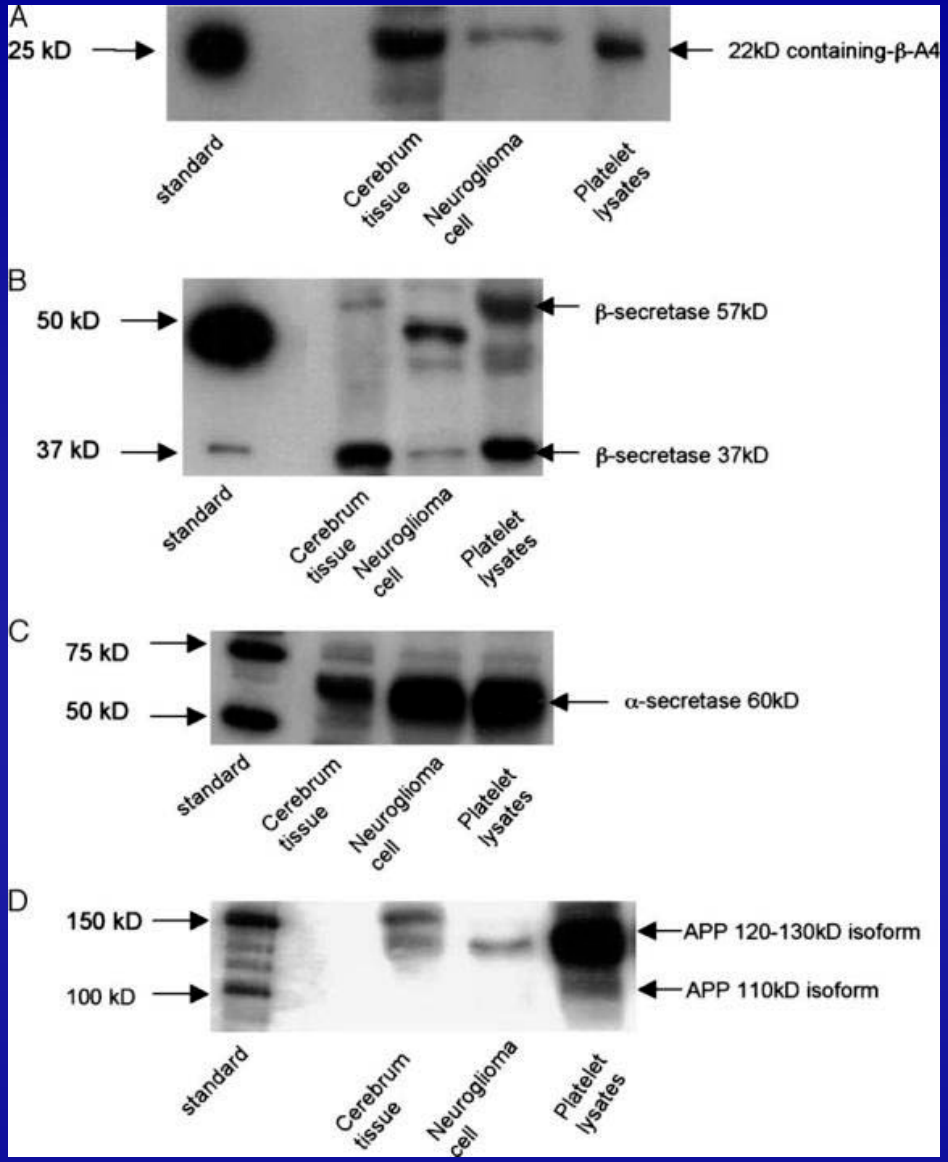
Keywords: Alzheimer's disease, Platelets, β-Amyloid, β-Secretase, α-Secretase, APP

J Neurol Sci. 2006, Jan 15, 240 (1-2) 53-58

PMCID: PMC1479090 ; NIHMSID: NIHMS10428

Platelet Amyloid Precursor Protein Processing: A Bio-marker For Alzheimer's Disease

K. Tang, et al.



A) Positive control samples and platelet lysates detected with pAb β-amyloid. (B) Positive control samples and platelet lysates detected with pAb BACE1. (C) Positive control samples and platelet lysates detected with pAb ADAM10. (D) Positive control samples and platelet lysates detected with mAb m22C11.

J Neurol Sci. 2006, 240 (1-2) 53-58
PMCID: PMC1479090 ; NIHMSID: NIHMS10428

Platelet Amyloid Precursor Protein Processing: A Bio-marker For Alzheimer's Disease

K. Tang, et al.

Kruskal–Wallis test and Dunn multiple comparisons post hoc test

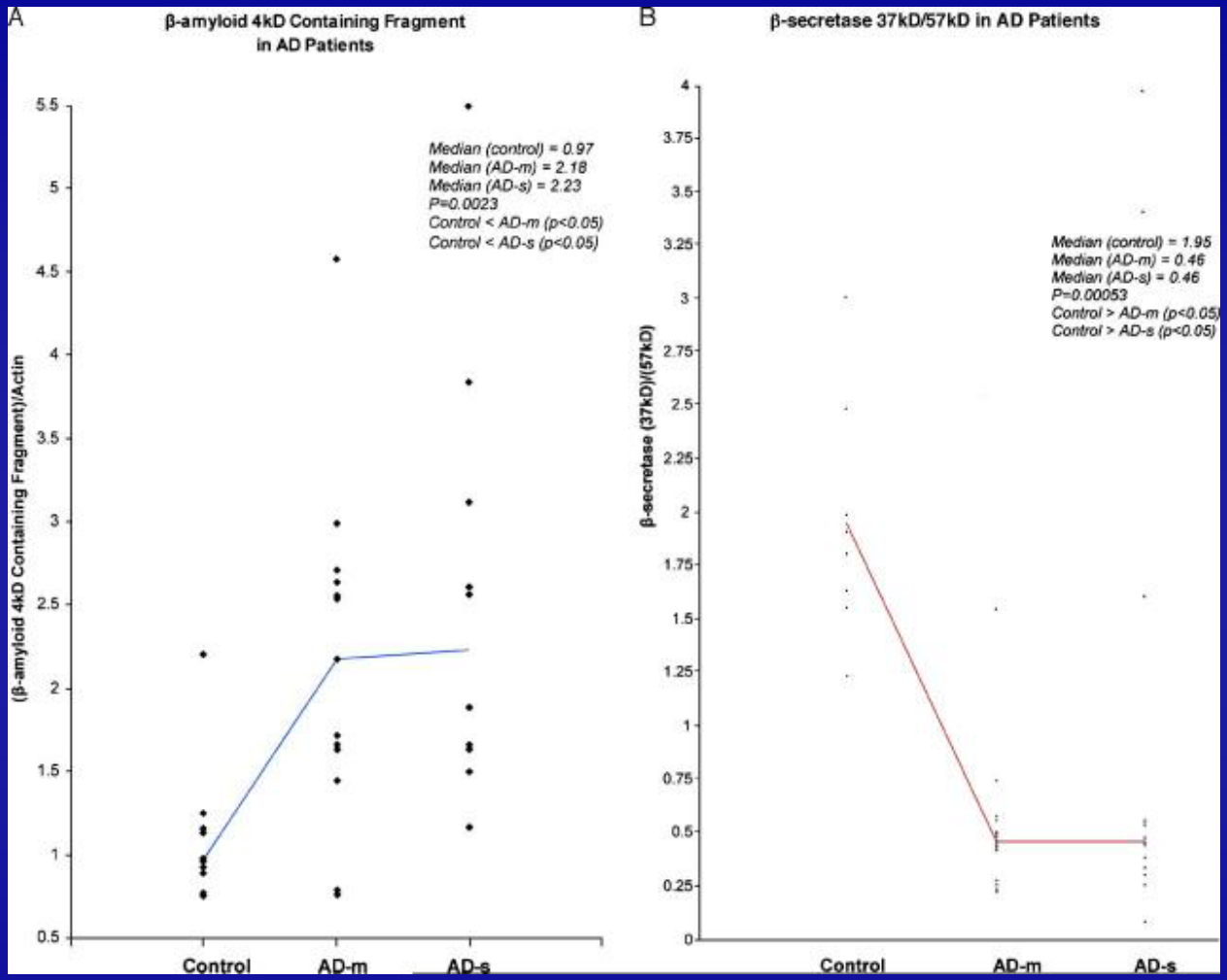
Ratios	Controls			AD-Mild			AD-severe			*Kruskal-Wallis test*
	N	Median	IQ range	N	Median	IQ range	N	Median	IQ range	*p*-value
β-AmyA4/actin	10	0.97	0.32	13	2.18	1.13	10	2.23	1.7	0.0023**
β-Secretase (37 kDa)/(57 kDa)	10	1.95	5.67	18	0.46	0.18	13	0.46	0.76	0.0005*
α-Secretase/actin	10	1.34	0.35	18	0.53	0.41	13	0.30	0.41	0.0014*
APP ratio	10	7.32	2.25	18	6.28	0.73	13	5.66	0.94	0.0003*

*Dunn multiple comparisons post hoc test: *Control>AD-m, p<0.05; Control>AD-s, p<0.05. **Control<AD-m, p<0.05; Control<AD-s, p<0.05.*

J Neurol Sci. 2006, 240 (1-2) 53-58
PMCID: PMC1479090 ; NIHMSID: NIHMS10428

Platelet Amyloid Precursor Protein Processing: A Bio-marker For Alzheimer's Disease

K. Tang et al.

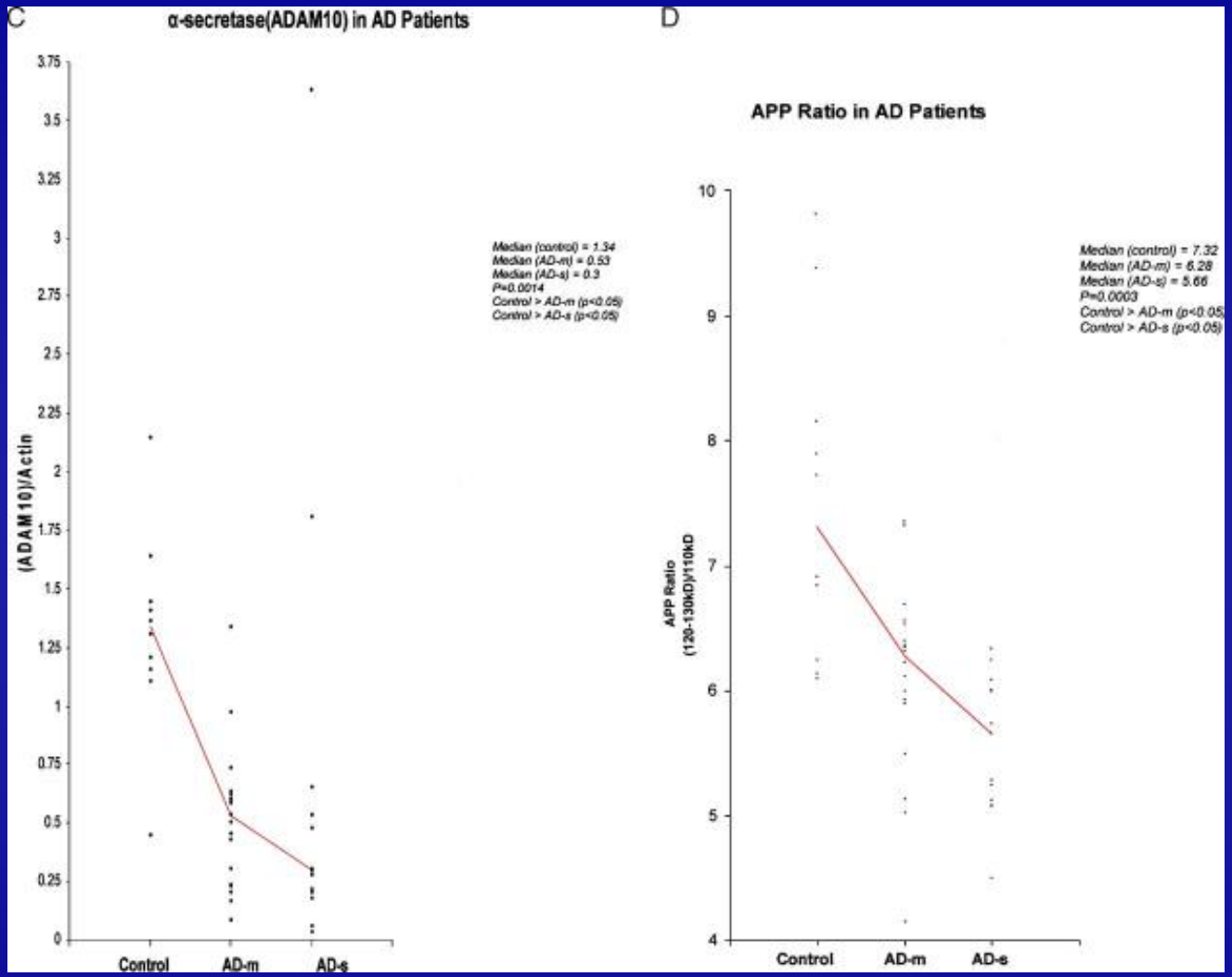


(A) β-Amyloid 4 kDa containing fragment 22 kDa/β-actin in control, AD-m and AD-s groups. (B) β-Secretase (37 kDa)/(57 kDa) by groups. Two points in control group (20.12, 26.69) and one point in AD-m (8.15) are not shown in this chart.

J Neurol Sci. 2006, 240 (1-2) 53-58
PMCID: PMC1479090 ; NIHMSID: NIHMS10428

Platelet Amyloid Precursor Protein Processing: A Bio-marker For Alzheimer's Disease

K. Tang et al.

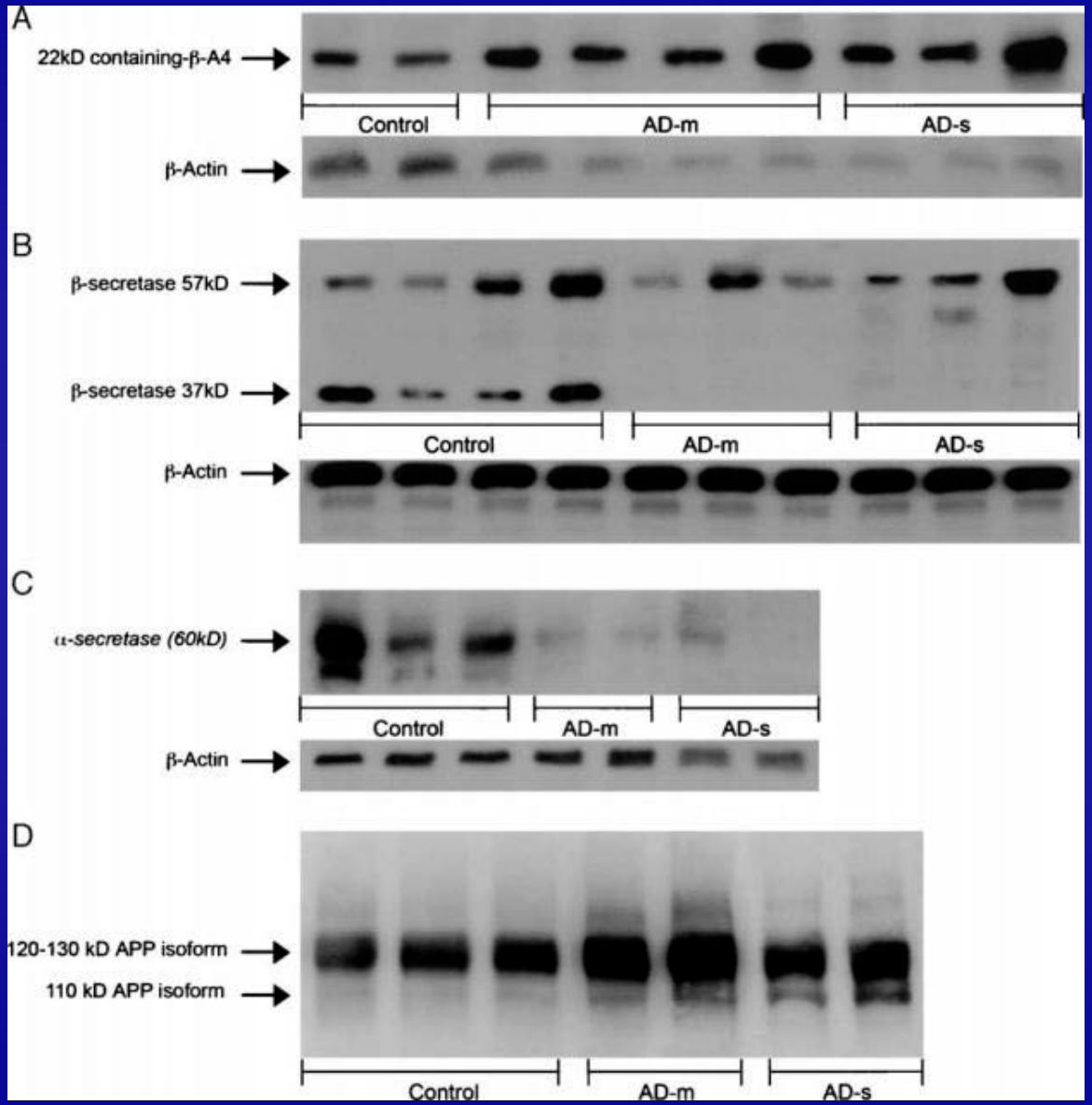


(C) α-Secretase (60 kDa)/β-actin for control, AD-m and AD-s groups. (D) APP ratio of (120–130 kDa) in control, AD-m, and AD-s groups.

J Neurol Sci. 2006, 240 (1-2) 53-58
PMCID: PMC1479090 ; NIHMSID: NIHMS10428

Platelet Amyloid Precursor Protein Processing: A Bio-marker For Alzheimer's Disease

K. Tang et al.



(A) β-Amyloid 4 kDa containing fragment 22 kDa with β-actin in Western blot. (B) β-Secretase (BACE1) 37 kDa (lower band), 57 kDa (upper band), with β-actin in Western blot. (C) Western blot of α-secretase (ADAM10) 60 kDa with β-actin in control, AD-m and AD-s groups. (D) Western blot of APP isoforms in control, AD-m and AD-s groups.

J Neurol Sci. 2006, 240 (1-2) 53-58
PMCID: PMC1479090 ; NIHMSID: NIHMS10428

Conclusions

1. Increased β-secretase and decreased α-secretase activities in AD platelets compared to normal control subjects.
2. Increased levels of Aβ are produced by platelets from AD patients compared to normal control subjects.
3. APP isoform ratios decreased in AD patients compared to normal control subjects.
4. Amyloidogenic pathway activation in AD patients is shown: increased β and decreased α-secretase activities in AD platelets compared to normal control subjects.
5. AD platelets may serve as a bio-marker to measure the biological progression of disease and to evaluate drug effects in clinical trials.