Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Analysis of Orthogonal Polynomial Contrasts and Cox Proportional Hazard Regression on the Association of Plasma Amylin Concentration With Risk of Alzheimer Disease

Multivariate COX regression	Hazard Ratio (95% CI)	P values
All participants (n =3020)		
X	64.14 (0.36, >100)	0.12
X^2	0.04 (<0.01, 3.58)	0.16
X^3	2.58 (0.59, 11.3)	0.21
X^4	0.91 (0.78, 1.07)	0.27
Male (n =1384)		
X	>100 (4.25, >100)	0.02
X^2	< 0.01 (<0.01, 0.31)	0.03
X^3	28.51 (1.29, >100)	0.03
X^4	0.70 (0.50, 1.00)	0.05
Female (n =1636)		
X	2.50 (0.01, >100)	0.76
X^2	0.68 (<0.01, >100)	0.88
X^3	1.07 (0.21, 5.50)	0.93
X^4	1.00 (0.84, 1.19)	0.99

The analysis of orthogonal polynomial contrasts and cox model (Proportional hazards regression) were conducted to explore a nonlinear association between plasma amylin and AD risk in all participants (n = 3020), and in males (n = 1384) vs. in females (n = 1636). Four single degree of freedom orthogonal polynomial contrasts of plasma amylin were generated: the log (base 10) transform of plasma amylin: X = log10 (amylin); the quadratic transform of log10 (amylin): X^2, the cubic of log10 (amylin): X^3, and the quartic of log10 (amylin): X^4 and used in cox models on the incidence of new AD cases after adjustment for confounders including age, sex, and eduction.

eTable 2. General Characterization of the Study Sample Based on Plasma Amylin Concentration Quintiles

Demographics and medical conditions	**Quintile 1 < 4.2 n=609**	**Quintile 2 4.2-5.9 n=616**	**Quintile 3 6.0-9.4 n=617**	**Quintile 4 9.5-27.0 n=607**	**Quintile 5 ≥ 27.0 n=612**	**Df**	**F**	**P**
Age7 (years), mean ± SD	61.4 ± 9.4	61.6 ± 9.3	60.2 ± 9.8	61.1 ± 9.5	60.8 ± 9.4	4	2.05	0.09
Education (years), mean ± SD	13.9 ± 2.5	14.1 ± 2.5	14.0 ± 2.7	14.2 ± 2.7	14.4 ± 2.6	4	2.31	0.06
Female, n (% of total n)	342 (55.8)	332 (54.2)	318 (51.9)	343 (56.1)	318 (52)	4	4.07	0.40
BMI (kg/m^2), mean ± SD	27.6 ± 5	28.2 ± 5.2	28.7 ± 5.8	28.4 ± 5.6	27.7 ± 4.9	4	4.99	0.001
WAIST (inch), mean ± SD	38.8 ± 5.4	39.3 ± 5.4	39.9 ± 5.8	39.5 ± 5.6	38.8 ± 5.4	4	4.31	0.002
HIP (inch), mean ± SD	41.1 ± 4.0	41.5 ± 4.3	41.7 ± 4.4	41.5 ± 4.0	41.2 ± 3.9	4	2.01	0.09
SBP (mmHg), mean ± SD	126.0 ± 18.6	127.8 ± 18	126.2 ± 19.8	128.1 ± 19.2	126.5 ± 18.1	4	1.57	0.18
DBP (mmHg), mean ± SD	72.9 ± 9.4	74.1 ± 9.7	74.4 ± 9.6	74.6 ± 10.2	74.0 ± 9.6	4	2.88	0.02
Diabetes, n (% of total n)	71 (11.6)	67 (10.9)	73 (11.9)	70 (11.5)	62 (10.1)	4	1.22	0.88
CVD, n (% of total n)	68 (12.9)	63 (11.8)	62 (11.9)	50 (9.5)	57 (10.5)	4	3.64	0.46
Glucose (mg/dL), mean ± SD	102.7 ± 23.8	103.9 ± 25.1	105.2 ± 27.6	105.2 ± 27.6	103.8 ± 27.9	4	0.95	0.44
Total cholesterol (mg/dL), mean ± SD	198.6 ± 35.8	203.2 ± 37.2	201.4 ± 37.9	199.4 ± 36.6	199.8 ± 36.2	4	1.55	0.19
Triglyceride (mg/dL), mean ± SD	121.8 ± 71.9	142.3 ± 78.7	144.6 ± 98.1	138.5 ± 93.6	137.7 ± 96.7	4	6.28	<0.001
LDL (mg/dL), mean ± SD	117.6 ± 31.5	122.2±32.9	120.8 ± 35.3	118.7 ± 31.6	120.0 ± 32.2	4	1.80	0.13

HDL(mg/dL), mean ± SD	56.8 ± 16.6	53.0 ± 16.0	52.9 ± 17.2	53.6 ± 17.6	53.1 ± 17.2	4	5.90	<0.001
Creatinine (mg/dL), mean ± SD	0.8 ± 0.2	0.9 ± 0.4	0.9 ± 0.4	0.9 ± 0.3	0.9 ± 0.2	4	1.85	0.12
CRP (mg/dL), mean ± SD	3.7 ± 4.7	4.7 ± 6.9	4.4 ± 6.8	4.1 ± 5.3	4.5 ± 12	4	1.52	0.20
ApoE4, n (% of total n)	125 (20.4)	130 (21.2)	145 (23.7)	113 (18.5)	121 (19.8)	4	5.53	0.24

BMI = Body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; CVD = cardiovascular disease; LDL = low density lipoprotein; HDL = high density lipoprotein; CRP = C-reactive protein; ApoE4 = apolipoprotein E4.
3061 subjects were divided based on plasma amylin quintiles. Mean ± SD with one-way ANOVA was used to test differences in the variables among amylin quintiles, median (Q1, Q3) with Kruskal-Wallis test was applied when a concentration distribution was skewed. And Chi-square (χ^2) test was used to compare counts, n/total (%). P values for statistical significance are shown for the comparisons.

eTable 3. Association of Plasma Amylin Concentration Quintiles With Alzheimer Disease

	Amylin, pM							
Cognitive incidence	**Quintile 1 < 4.2 n = 609**	**Quintile 2 4.2-5.9 n = 616**	**Quintile 3 6.0-9.4 n = 617**	**Quintile 4 9.5-26.9 n = 607**	**Quintile 5 ≥ 27.0 n = 612**	**Df**	**F**	**P**
Dementia incidence, n (% of total n)	40 (6.6)	38 (6.2)	42 (6.8)	42 (6.9)	48 (7.8)	4	1.10	0.90
AD incidence, n (% of total n)	30 (4.9)	32 (5.2)	33 (5.3)	36 (5.9)	38 (6.2)	4	1.10	0.90

AD = Alzheimer's disease

Participants who had plasma amylin measurements were used to divide into quintiles. Fisher exact test was used for the prevalence comparisons, and log-rank test was used for the incidence comparisons of dementia and AD.

eTable 4. Brain Volume Comparisons Among Patients With a Low, High, or Extremely High Plasma Amylin Concentration

Brain volumes	Amylin < 75 pM n = 1938	Amylin ≥ 75 to < 2800 pM n = 152	Amylin ≥ 2800 pM n = 58	Df	F	P
Gray/TCBV %, mean±SD	41.4 ± 1.9	41.7±1.7	41.5±1.9	2	3.30	0.04
TBV/TCBV %, mean±SD	13.6±0.8	13.8±0.7	13.6±0.7	2	4.14	0.02
TBV Gray/TCBV %, mean±SD	8.4±0.5	8.5±0.5	8.4±0.5	2	5.74	0.003
HBV/TCBV %, mean±SD	0.47±0.04	0.47±0.04	0.48±0.05	2	3.29	0.04
WMHI, median (Q1, Q3)	1.07 (0.48, 2.19)	0.82 (0.42, 1.56)	1.14 (0.58, 2.50)	2	3.85	0.15

TCBV = total cerebral brain volume; FBV = frontal lobe brain volume; TBV = temporal lobe brain volume; HPV = hippocampal volume; WMHI = white matter hyperintensities volume. 2148 participants who had plasma amylin measurements and a brain MRI were used to divide into quintiles (A) and into three cut-off based groups (B). Mean ± SD with one-way ANOVA was used to test brain volume differences among amylin quintiles, median (Q1, Q3) with Kruskal-Wallis test was applied to WMHI due to its skewed distribution. P values adjusted by using conventional Bonferroni correction ($p < 0.004$) were used.

eTable 5: Characterization of Brain Volume Based on Plasma Amylin Concentration Quintiles

Brain volumes, mean ± SD	**Quintile 1 < 4.2 n = 420**	**Quintile 2 4.2-5.9 n = 440**	**Quintile 3 6.0-9.4 n = 423**	**Quintile 4 9.5-26.9 n = 435**	**Quintile 5 ≥ 27.0 n = 430**	**Df**	**F**	**P**
TCBV	1408.8 ± 144.3	1407.6 ± 136.4	1416 ± 140.7	1418.1 ± 140.4	1419 ± 141.7	4	0.62	0.65
Gray/TCBV %	41.3 ± 1.8	41.3 ± 1.8	41.4 ± 1.9	41.4 ± 2.0	41.6 ± 1.8	4	1.60	0.17
White/TCBV %	34.4 ± 2.5	34.5 ± 2.5	34.6 ± 2.5	34.4 ± 2.5	34.4 ± 2.4	4	0.48	0.75
FBV/TCBV %	23.6 ± 1.2	23.6 ± 1.3	23.7 ± 1.3	23.7 ± 1.4	23.6 ± 1.3	4	0.87	0.48
FBV Gray/TCBV %	11.9 ± 0.7	11.9 ± 0.7	12.0 ± 0.8	12.0 ± 0.8	12.0 ± 0.7	4	1.28	0.27
FBV White/TCBV %	11.7 ± 1.0	11.7 ± 1.0	11.7 ± 1.0	11.7 ± 1.0	11.6 ± 1.0	4	0.90	0.46
PBV/TCBV %	13.0 ± 0.7	13.0 ± 0.7	13.0 ± 0.8	13.0 ± 0.7	13.0 ± 0.8	4	0.22	0.93
PBV Gray/TCBV %	6.7 ± 0.4	6.7 ± 0.4	6.7 ± 0.5	6.7 ± 0.4	6.7 ± 0.4	4	0.48	0.75
PBV White/TCBV %	6.4 ± 0.5	6.3 ± 0.6	6.4 ± 0.6	6.3 ± 0.5	6.3 ± 0.5	4	1.17	0.32
TBV/TCBV %	13.5 ± 0.8	13.7 ± 0.8	13.6 ± 0.8	13.6 ± 0.8	13.7 ± 0.8	4	2.39	0.05
TBV Gray/TCBV %	8.3 ± 0.5	8.4 ± 0.5	8.4 ± 0.5	8.4 ± 0.5	8.4 ± 0.5	4	2.34	0.05
TBV White/TCBV %	5.2 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	4	1.11	0.34
HBV/TCBV %	0.47 ± 0.04	0.47 ± 0.04	0.47 ± 0.04	0.47 ± 0.04	0.47 ± 0.05	4	0.76	0.55
WMHI	1.14 (0.54, 2.33)	1.21 (0.53, 2.42)	0.93 (0.43, 1.97)	1.05 (0.47, 2.09)	0.95 (0.47, 2.08)	4	14.02	0.007

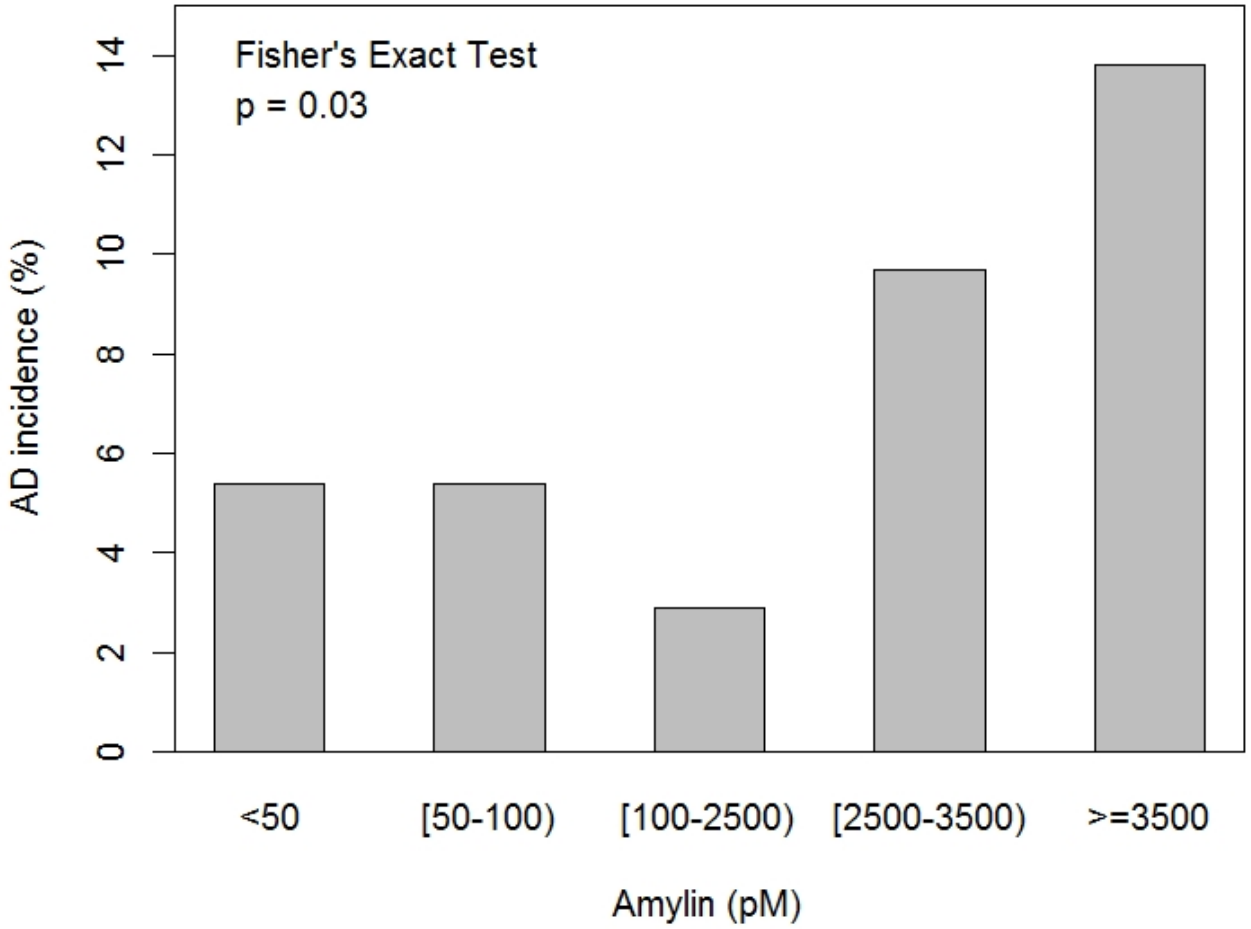
TCBV = total cerebral brain volume; FBV = frontal lobe brain volume; TBV = temporal lobe brain volume; HPV = hippocampal volume; WMHI = white matter hyperintensities volume. 2148 participants who had plasma amylin measurements and a brain MRI were used to divide into quintiles (A) and into three cut-off based groups (B). Mean ± SD with one-way ANOVA was used to test brain volume differences among amylin quintiles, median (Q1, Q3) with Kruskal-Wallis test was applied to WMHI due to its skewed distribution. P values adjusted by using conventional Bonferroni correction ($p < 0.004$) were used.

eTable 6: General Linear Regression Analyses on the Association of Plasma Amylin Concentration With Brain Volume

Amylin concentrations	TBV gray matter/TCBV% n = 2148		Logarithm of WMHI/TCBV % n = 2148	
	β Estimate ± SE	p values	β Estimate ± SE	p values
Q1	Reference	-	Reference	-
Q2	0.06 ± 0.04	0.11	0.39 ± 0.57	0.49
Q3	0.02 ± 0.04	0.64	-0.53 ± 0.58	0.36
Q4	0.04 ± 0.04	0.36	-0.56 ± 0.57	0.33
Q5	0.10 ± 0.04	0.007	-1.11 ± 0.57	0.05

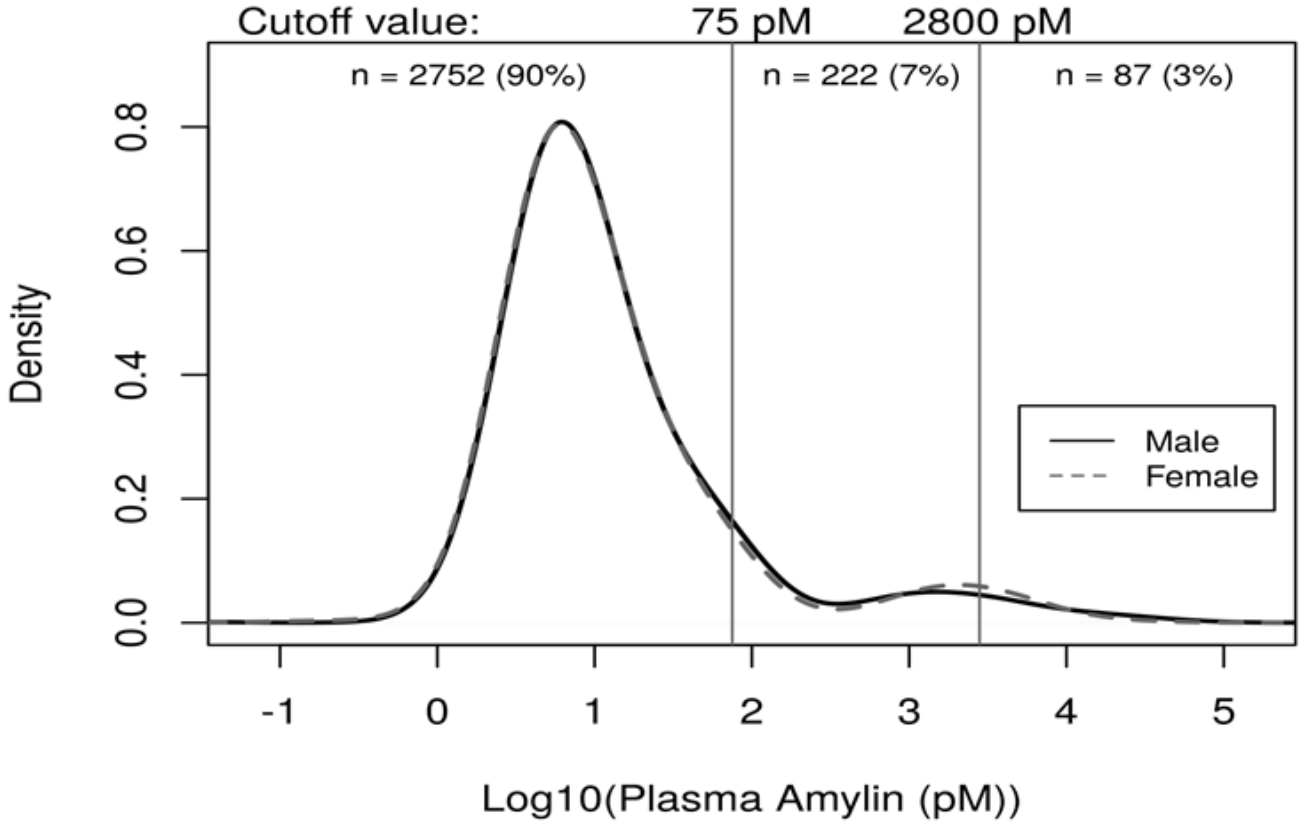
TCBV = total cerebral brain volume; TBV = Temporal lobe brain volume; WMHI = white matter hyperintensities.
General linear regression (GLM) was used to study the association between quintiles and brain volumes as an outcome. The models were adjusted for age, sex, education, smoking, BMI, diabetes, cardiovascular diseases, ApoE4 and HDL. P values adjusted by using conventional Bonferroni correction ($p < 0.004$) were used.

eFigure 1. Accumulative Alzheimer Disease Incidence Rates Based on Different Cutoffs for Plasma Amylin Concentration



Participants were divided into five subgroups: 1) < 50 (n = 2642), 2) 50-100 (n = 185), 3) 100-2500 (n = 138), 4) 2500-3500 (n = 31) and 5) ≥ 3500 (n = 65) pM. The AD incident rates among the five plasma amylin subgroups were compared by using Fisher's Exact Test (Y-axis) with a p value illustrated.

eFigure 2. Distribution of Plasma Amylin Concentrations Among the Population of the Framingham Heart Study



eFigure 2: Distribution of plasma amylin in the population of Framingham Heart Study
The data of plasma amylin were logarithmized (X-axis) and the distributions of particiapnts (numbers, Y-axis) in females and males were presented. The corresponding raw concentrations of plasma amylin for the cut-offs were also illustrated.