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Positive Association between Plasma Amylin and Cognition in a Homebound Elderly Population

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Abstract

Our recent study reported that amylin, a pancreatic peptide that readily crosses the blood-brain barrier, improves learning and memory in Alzheimer's disease mouse models. However, the relationship between peripheral amylin and cognition in humans is unknown. In this follow-up study, using a cross-sectional, homebound elderly population, improvement in cognitive function with increasing quartiles of plasma amylin was suggested by positive association with verbal memory ($p = 0.0002$) and visuoconstruction tasks ($p = 0.004$), and inverse association with timed measures of attention ($p < 0.0001$) and executive function ($p = 0.04$). After adjusting for demographic information, apolipoprotein E4 allele, diabetes, stroke, kidney function, and lipid profile, $\log_{10}$ of plasma amylin remained associated with these cognitive domains. In contrast, plasma amyloid-β peptide was not associated with these specific cognitive domains. Our study suggests that peripheral amylin may be protective for cognitive decline, especially in the domains affected by Alzheimer's disease.

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SUPPLEMENTARY MATERIAL

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Keywords

Amylin; cognition; memory; visuospatial and executive function

INTRODUCTION

Amylin is a gut-brain axis hormone with 37 amino acids produced and secreted by the pancreas. Amylin easily crosses the blood-brain barrier (BBB) [1, 2] and mediates important brain functions including inhibition of appetite by acting on area postremal [3], relaxation of cerebrovascular structure [4, 5], and perhaps enhancement of neural regeneration [6]. It is co-secreted with insulin and plays an important role in regulating glucose metabolism [3]. Amylin shares several features with amyloid-β peptide (Aβ, one hallmark component of brain Alzheimer's disease (AD) pathology), including similar secondary structure [7], binding to the same amylin receptor [8], and being degraded by the same protease, insulin degrading enzyme [9, 10]. Using AD mouse models, our study [11] and Adler et al.'s study [12] found that peripheral injection of amylin or its clinical analog pramlintide improve learning and memory in these mice. Patients with amnestic MCI or AD have lower concentrations of plasma amylin than those with normal cognitive function [12]. Thus high levels of Aβ in the AD brain could compete for and interfere with amylin binding to its receptor in the brain [3].

Our preclinical study demonstrates that amylin-type peptides enhance the removal of Aβ from the brain into blood reducing AD pathology and improving cognitive function [11]. Of note, both amylin and Aβ can aggregate and form amyloids under pathological conditions like type 2 diabetes and AD [13, 14]. All of these research findings prompted us to study whether naturally occurring amylin in blood would be associated with cognition in humans. In this study, using a large homebound elderly population, we examined the cross-sectional relationship between plasma amylin and cognition, and explored the relationship in different disease conditions, especially diabetes.

METHODS

Study population and recruitment

We studied a group of 1,106 subjects from a population-based study, the Nutrition, Aging and Memory in the Elderly (NAME) study, all of whom had been assessed for plasma amylin levels [15]. From this group, 146 subjects were excluded because they did not have plasma samples available for use in this study. Subjects included homebound elderly clients who were enrolled in one of four homecare agencies in the Boston area between 2002 and 2007. Anyone receiving homecare services was registered with one of these agencies if he/she lived in the city of Boston, had an annual income <$18,890 and needed homecare services. All homebound elders aged 60 and older at each of the four agencies were invited to participate in the study. All enrolled subjects gave informed consent. The protocol and consent form were approved by the Institutional Review Boards of Tufts University-New England Medical Center and Boston University School of Medicine.

Cognitive function

Research assistants, trained by a board certified neuropsychologist, administered the cognitive tests. Cognition was assessed using a two-phase approach: 1) The population was screened for severe cognitive impairment using the Mini-Mental State Examination (MMSE) [16] and for estimated verbal IQ or poor literacy using the North American Adult Reading Test [17]. Those with MMSE < 10 or verbal IQ < 75 were not eligible to continue in the study. 2) Eligible subjects were subsequently examined using the following neuropsychological battery.

Verbal fluency (controlled oral word association test)

Total number of words generated beginning with a specific letter over 60 seconds, with three trials, each with a different letter. This test of phonemic generativity is usually viewed as a measure of executive functioning related to language ability (i.e., lexical access).

WAIS-III digit span test

Both digits forward and digits backward were performed, and the total raw score was recorded. This test was used to evaluate attention/concentration (forward span), and working memory, which is another component of executive function (backward span).

WMS-III Word List Learning (WLL)

The task consisted of four learning trials of a 12 word list with an immediate recall score computed by summing the number of correct items recalled across all 4 trials. After a 30-minute delay, the subject was asked to recall the same list of words again, with the total correct items recorded to compute a delayed recall score. A percent retention score was calculated by dividing the number of words recalled on delay. These scores were used as measures of verbal learning and memory.

WMS-III Logical Memory (LM)

Two stories (A and B) were read aloud to the subject; the subject was then asked to repeat after each story, with all correctly recalled items totaled for an immediate recall score. After 30 minutes, the subject was asked to repeat both stories, total items correctly recalled comprised a delayed recall score. The ratio of the delayed recall score over the immediate recall score was used to calculate percent retention. These tests measured the different aspect of memory from WLL.

Trailmaking A

This test of visuomotor attention and processing speed requires participants to connect circles with numbers in them scattered across a page as quickly as possible. The time to completion is recorded, with a cap time of 301 seconds.

Trailmaking B

The subject was asked to draw a line connecting alternating letters and numbers in consecutive order. Time to completion was recorded, with cap time of 301 seconds.

Trailmaking A time to completion was then subtracted from Trailmaking B total time to account for psychomotor speed and provide a more direct measure of executive function.

WAIS-III Block design

Subjects assembled red and white blocks to match a pictured design, with points assigned for each correctly replicated design and added together to compute a total score as a presumed measure of visuospatial skills.

Measurements

Plasma amylin and Aβ—Blood draw was conducted after 12 hours of fasting. Blood samples were centrifuged immediately to isolate plasma. We used an ELISA assay to measure amylin concentration in plasma according to the manufacturer's instructions (Cat: EZHA-52K, LINCO Research, St. Charles, Missouri). All samples were assayed in duplicate and then averaged to give final values.

To measure Aβ, sandwich Aβ ELISA was used, as described previously [18]. Briefly, plates were coated with 2G3 (anti-$A\beta_{40}$) and 21F12 (anti-$A\beta_{42}$) antibodies overnight at 4°C. Samples were then loaded and incubated overnight at 4°C followed by incubation with a biotinylated monoclonal anti-N terminus Aβ antibody (3D6B) for 2 hours. Finally, streptavidin-conjugated alkaline phosphatase (Promega, USA) was added and incubated, and the signal was amplified by adding alkaline phosphatase fluorescent substrate (Promega, USA), which was then measured.

ApoE genotyping—A 244 bp fragment of the ApoE gene including the two polymorphic sites was amplified by PCR using a robotic Thermal Cycler (ABI 877, Perkin-Elmer/Applied Biosystems), using oligonucleotide primers F4 (5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′) and F6 (5′-TAAGCTTGGCACGGCTGTCCAAGGA-3′). The PCR products were digested with 5 units of Hha I and the fragments separated by electrophoresis on 8% polyacrylamide non-denaturing gel. The specific allelic fragments were: E2; E3; and E4. ApoE4 was defined by E4/4, E3/4, or E2/4.

Other blood tests—Serum lipid profile including cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and serum creatinine were measured by the clinical laboratory according to the standard protocols at Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA), Tufts University.

Other clinical evaluation

Weight and height were measured twice using standardized instruments, and the average of two measurements was used to calculate body mass index (BMI, kg/m^2). Diabetes was defined as the use of anti-diabetic medication or fasting glucose greater than 126 mg/dl, parameters widely used in population-based studies [19]. History of stroke was self-reported.

Statistical analysis

Statistical analysis was performed using SAS (version 9.1). Subjects were divided into quartiles according to concentration of plasma amylin. Continuous variables were presented as mean SD ± and compared using ANOVA test. The Chi-Square test was used to compare proportions for binary endpoints. Amylin (Log Amylin), insulin (Log Insulin), $A\beta_{42}$ (Log Aβ42) and $A\beta_{40}$ (Log Aβ40) were transformed to $\log_{10}$ for multivariate regression due to skewed distributions. Multivariate linear regression was used to examine associations between Log Amylin or Log Aβ40 or Log Aβ42 and tests of the various cognitive domains while adjusting for confounders. For all analyses, the two-sided significance level of 0.05 was used.

RESULTS

Study population

For this study analysis, 1,106 subjects with measured levels of plasma amylin from the completed NAME study were used. The average age (mean ± SD) of this study sample was 75.0 ± 8.0 years old, and 76% were female. The population was multi-ethnic with 61% Caucasian, 35% African-American, and 4% other ethnicities. Sixty-seven percent had high school education or above and 24% carried at least one ApoE4 allele. We measured the concentrations of amylin, insulin, $A\beta_{1–42}$, and $A\beta_{1–40}$ in plasma. For amylin (pM/L): median = 22.3, Q1 = 11.8, Q3 = 40.0; for insulin (pM/L), median = 80.5, Q1 = 49.3, Q3 = 139.6; for $A\beta_{1–42}$ (pg/ml): median = 17.4, Q1 = 11.8, Q3 = 22.6, and for $A\beta_{1–40}$ (pg/ml): median = 133.4, Q1 = 99.5, Q3 = 172.7.

Subjects with different concentrations of plasma amylin were divided into four quartiles (Table 1). Across the four quartiles of amylin, there were no demographic differences in age, gender, ethnicity, or education, nor were there differences in the frequency of ApoE4 allele. While there was no difference in stroke history among the four amylin quartiles, the average of BMI ($p < 0.0001$) and creatinine ($p < 0.0001$), and the rate of diabetes ($p < 0.05$) increased with increasing quartile of amylin. Cholesterol and LDL levels had a positive relationship with increasing 1st to 3rd quartile of amylin, but the levels were lower at 4th quartile of amylin, indicating a nonlinear relationship. HDL concentration was inversely associated with increasing quartile of amylin ($p < 0.0001$).

The relationships between plasma amylin and cognition

Table 2 summarizes performance on the measures of cognitive functioning in each domain across the four plasma amylin quartiles. Increasing quartiles of plasma amylin were positively associated with word learning list (WLL) delayed recall recall (Mean±SD: Q1 = 3.4±2.7; Q2 = 3.2±2.7; Q3 = 4.1±2.9; Q4 = 3.8±2.8, $p = 0.002$), delayed logical memory (LM) (Mean±SD: Q1 = 17.4±10.2; Q2 = 16.8±9.7; Q3 = 19.9±9.6; Q4 = 19.4±9.3, $p = 0.0002$) and Block design (Mean±SD: Q1 = 18.7 ±8.9; Q2 = 19.3±8.8; Q3 = 20.6±8.8; Q4 = 21.5± 8.7, $p = 0.004$), and inversely associated with Trailmaking A (Mean±SD: Q1 = 98.8±66.6; Q2 = 93.7±70.3; Q3 = 78.2±45.9; Q4 = 78.7±53.0, $p < 0.0001$) and Trailmaking B (Mean±SD: Q1 = 223.0±80.4; Q2 = 215.1±84.1; Q3 = 205.0± 83.7; Q4 = 204.1±86.1, $p = 0.04$). In contrast, there were no differences in MMSE, verbal fluency, and digit span across

the quartiles of plasma amylin. There were also no differences in depression and activities of daily life across the four quartiles (data not shown).

Multivariate regression analyses of the relationship between plasma amylin and cognitive function

Next, amylin was transformed to $\log_{10}$ for multivariate linear regression due to its skewed distribution. Using multivariate regression (Table 3), $\log_{10}$ of amylin remained to be associated with LM delayed recall (β= +0.492, SE = 0.248, $p = 0.05$), Trailmaking A (β = −6.077, SE = 1.541, $p < 0.0001$), Trailmaking B (β = −4.243, SE = 2.075, $p = 0.04$), and Block design (β = +0.634, SE = 0.230, $p = 0.006$), but not WLL delayed recall, as an outcome after adjusting for age, gender, ethnicity, education and ApoE4 (Model I). After adding BMI, diabetes, stroke, creatinine, and the lipid profile including cholesterol, LDL, and HDL into the model, $\log_{10}$ of amylin remained to be associated with LM delayed recall (β = +0.620, SE = 0.277, $p = 0.03$), Trailmaking A (β= −4.810, SE = 1.653, $p = 0.004$), and Block design (β =+0.520, SE = 0.258, $p = 0.04$) (Model II), but Trailmaking B (β = −3.462, SE = 2.335, $p = 0.14$) only showed a trend toward significance.

In contrast to plasma amylin, $\log_{10}$ of plasma $A\beta_{1–42}$ or $A\beta_{1–40}$ or insulin was not associated with scores of specific memory, visuospatial, and executive tests in the multivariate regression analyses (Supplementary Table 1). A marginal association between plasma $A\beta_{1–42}$, but not $A\beta_{1–40}$ or insulin, and MMSE scores was observed.

The relationship between plasma amylin and cognitive function in different stratified groups

To further understand the underlying mechanism between peripheral amylin and brain functions, we stratified the subjects in each amylin quartile according to presence/absence of ApoE4 allele (Table 4), diabetes (Table 5), and history of stroke (Table 6). Although most associations between plasma amylin and cognitive function held up or tended to hold up in the stratified subgroups, some associations notably disappeared. For example, the block design effect was significant only in those who did not have diabetes (Table 5). When stroke history was considered, the relationships between increasing amylin quartiles and all cognitive functions disappeared (Table 6). Among those who were ApoE4 non-carriers, or those who did not have diabetes, or those who did not have history of stroke, the relationship between increasing quartile of plasma amylin and each cognitive domain function was strengthened compared to the total study sample. In contrast, among those ApoE4 carriers, or among those who had diabetes, the relationship between increasing quartile of plasma amylin and cognitive functions were weaker although they could be explained by the smaller numbers across amylin quartiles.

DISCUSSION

Episodic memory decline is a signature feature of early stage of AD, and psychomotor speed, attention, visuospatial skills, and executive dysfunction are often present in a later stage of AD [20]. Much milder decline in these cognitive areas can also occur in asymptomatic middle to older aging process. Our study clearly showed a positive

relationship between plasma amylin levels and these cognitive domains, suggesting a beneficial effect of this pancreatic peptide to brain function.

The positive relationship between plasma amylin and cognition was probably meaningful. Our recent study shows that intraperitoneal injection (i.p) of amylin or pramlintide removes Aβ from the brain into blood and reduces cognitive impairment in AD animal models [11]. Independently, Adler et al. used the pramlintide pump to treat another AD mouse model, SAMP8, which does not have typical amyloid plaques but presents with increased Aβ and other AD pathology [12]. Their study found that the pramlintide treatment improves performance in the novel object recognition task in these mice, and demonstrated that the pramlintide-treated mice had increased expression of the synaptic marker synapsin I and the kinase cyclin-dependent kinase-5 in the hippocampus, as well as decreased oxidative stress and inflammatory markers in the hippocampus. Additionally, amylin likely improves glucose metabolism in the brain since it readily crosses the BBB [21], relaxes cerebrovascular structures thereby increasing blood supply to the brain [4, 5], and is shown to enhance neural regeneration [6]. Several studies have shown that monomeric amylin and its analogs inhibit the formation of Aβ aggregation, a key element in the AD pathogenesis [22–26]. These results taken together could account for its positive association with cognition in elderly adults in this study.

Plasma amylin was positively associated with specific cognitive domain including memory, visuospatial ability and executive function (Tables 2 and 3). Although plasma $A\beta_{1–42}$ was marginally associated with general cognition assessed by MMSE scores, neither Aβ peptide nor insulin was associated with test scores of specific cognitive domains (Supplementary Table 1). As peripheral Aβ and insulin barely cross through the BBB and thus their plasma levels do not reflect the levels of Aβ and insulin in the brain [27], it is reasonable that Aβ and insulin in plasma are not associated with brain functions including cognition. In contrast, plasma amylin is a more accurate surrogate measure of amylin in the brain, since amylin readily crosses the BBB [1, 2]. Thus peripheral amylin, but not peripheral Aβ or insulin, is association with cognition. Additionally, despite amylin and Aβ's binding to the same amylin receptor [8], amylin increases intracellular cAMP, an important secondary messenger for learning and memory, but Aβ does not do so [28]. It is possible that abundant Aβ in the AD brain interferes with the ability of amylin to bind to its receptor, hindering normal amylin functions in the brain [3].

We found that a high concentration of plasma amylin was associated with obesity and type 2 diabetes (Table 1), consistent with findings from other studies [29–31]. It is worth noting that amylin amyloids are harmful in the pancreas of type 2 diabetes [14] and probably in the brain of AD [32]. However, our data also suggest that soluble amylin in plasma may be a protective factor against cognitive decline, especially in the memory domain, even in the presence of diabetes although plasma amylin was not associated with block design scores (Table 5). Since we immediately centrifuged the blood samples after collection, the plasma amylin peptides measured in this study were soluble and should not contain large aggregates, although the existence of amylin oligomers might exist.

The ApoE4 allele is a genetic risk factor for AD, and our study showed that the association between plasma amylin and specific cognitive functions was attenuated and became insignificant statistically in the presence of ApoE4 (Table 4). ApoE4 influences the deposition of $A\beta_{1–40}$ in the cerebrovasculature of the AD brain [33]. Our recent study found that amylin was associated with Aβ in plasma. However, in the presence of ApoE4, the association between amylin and $A\beta_{1–40}$ disappeared [34]. ApoE4 may either block or attenuate amylin's effect on blood vessels in the brain that leads to cognitive decline. Decreased cerebral blood flow, and impaired vascular clearance of Aβ from brain are all thought to contribute to AD pathogenesis [35].

Currently there are only few medications prescribed that delay memory decline in AD and their effects are moderate. More strikingly, there are no available treatments for visuospatial and executive dysfunction in dementia, in cerebrovascular diseases, or in normal aging. The current study suggests that amylin, natural or synthetic, may provide a new avenue for treatment of memory, psychomotor speed, visuospatial, and executive dysfunction in humans. Although amylin's self-aggregation feature may be an obstacle for drug development for AD, unlike human amylin pramlintide, an amylin analog, does not have any tendency to aggregate, but keep the potency of amylin. Pramlintide is a drug in clinical use for diabetes with a favorable safety profile [36, 37] and may be beneficial for AD. Thus a double blind, placebo controlled clinical trial should be considered for the repurpose use of pramlintide for AD. It is noteworthy that not all diabetes medications are beneficial for AD. For example, a recent study suggests that metformin use may increase the risk of AD development [38], while metformin is found to lower serum amylin concentrations in patients with type 2 diabetes [39].

The limitations of this study are: 1) this is a cross-sectional study, and we cannot conclude a protective relationship between a high concentration of amylin in plasma and attenuated cognitive decline; and 2) this analysis does not include AD diagnosis, neuroimaging, or autopsy measure, so the relationship between plasma amylin and brain structures or pathology is unknown. Nevertheless, our results, in addition to those of other studies, suggest that a longitudinal study is needed to study whether amylin is beneficial for preserving cognitive function in the elderly and for preventing development of AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Comparisons of demographic information, vascular diseases and lipid profiles across amylin quartiles

Amylin quartiles	**Quartile 1** ***n*** **= 276**	**Quartile 2** ***n*** **= 277**	**Quartile 3** ***n*** **= 277**	**Quartile 4** ***n*** **= 276**
Age, year, mean±SD	75.5±8.7	75.0±8.4	75.1±8.5	74.2±8.5
Female, *n*/total (%)	213/276 (77%)	204/277 (74%)	214/277 (77%)	212/276 (77%)
African Americans, *n*/total (%)	108/275 (39%)	95/276 (34%)	95/275 (35%)	90/275 (33%)
High School Graduate and above, *n*/total (%)	170/275 (62%)	174/276 (63%)	185/274 (68%)	180/275 (65%)
Body mass index, Mean±SD**	30.0±8.4	31.4±9.0	32.0±7.6	32.9±9.1
ApoE4 carriers, *n*/total (%)	63/272 (23%)	61/275 (22%)	68/275 (25%)	65/271 (24%)
Vascular Diseases				
Diabetes, *n*/total (%)*	97/265 (37%)	87/269 (32%)	92/268 (34%)	117/265 (44%)
Stroke, *n*/total (%)	54/265 (20%)	61/272 (22%)	53/270 (20%)	50/268 (19%)
Creatinine, mg/dL, mean±SD***	0.90±0.68	0.93±0.92	1.05±0.74	1.39±1.53
Lipid Profile				
Cholesterol, mg/dL, Mean±SD**	183.8 ± 46.3	183.8 ± 41.3	192.1±43.9	179.0±40.2
LDL, mg/dL, mean ± SD**	106.7±38.1	108.1±35.1	112.4±35.6	100.9±33.5
HDL, mg/dL, mean ± SD***	53.6 ± 16.6	49.3 ± 13.7	49.2 ± 14.7	46.6 ± 13.4

Mean±SD with ANOVA test or n/total with Chi-Square test is used to describe the distributions and comparisons of age, diseases, kidney function assessed by the measurement of creatinine and the lipid biomarkers across the amylin quartiles.

*$p < 0.05$,

**$p < 0.001$,

***$p < 0.0001$ for the statistical significance are shown. LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 2

Comparisons of functions in cognitive domains across amylin quartiles

Amylin quartiles	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p* values
General Cognition					
MMSE Scores, mean±SD	24.7±4.0	25.1±3.3	25.5±3.3	25.3±3.3	0.15
Language					
Verbal fluency, mean±SD	26.9±16.3	26.4±12.9	28.8±12.6	27.8±12.0	0.17
Attention and Concentration					
Digit span, mean±SD	13.6±3.8	13.5±3.7	14.1±3.6	13.9±3.8	0.25
Memory					
WLL delayed recall, mean±SD	3.4±2.7	3.2±2.7	4.1±2.9	3.8±2.8	0.002
LM delayed recall, mean±SD	17.4±10.2	16.8±9.7	19.9±9.6	19.4±9.3	0.0002
Visuospatial and Executive Function					
Trailmaking A, mean±SD	98.8±66.6	93.7±70.3	78.2±45.9	78.7±53.0	<0.0001
Trailmaking B, mean±SD	223.0±80.4	215.1±84.1	205.0±83.7	204.1±86.1	0.04
Block design, mean±SD	18.7±8.9	19.3±8.8	20.6±8.8	21.5±8.7	0.004

Mean±SD with ANOVA test is used to describe the distributions and comparisons of test scores in each cognitive domain across the amylin quartiles. *P* values for the statistical significance are shown. MMSE, Mini-Mental State Exam; WLL, Word learning list; LM, Logical memory.

Table 3

Effects of plasma amylin on cognitive tests in multivariate regression analyses

Outcomes	Log_{10} Amylin			
	Model I Adjusting for age, gender, ethnicity, school, and ApoE4		Model II Model I plus BMI, diabetes, stroke, kidney function, and lipid profile	
	Estimate β (SE)	*p* value	Estimate β (SE)	*p* value
MMSE Scores	+0.111 (0.086)	0.20	+0.143 (0.096)	0.14
Verbal fluency	+0.212 (0.318)	0.50	+0.336 (0.359)	0.35
Digit span	+0.019 (0.095)	0.84	+0.081 (0.108)	0.45
WLL delayed recall	+0.032 (0.072)	0.66	+0.063 (0.083)	0.45
LM delayed recall	+0.492 (0.248)	0.05	+0.620 (0.277)	0.03
Trailmaking A	−6.077 (1.541)	<0.0001	−4.810 (1.653)	0.004
Trailmaking B	−4.243 (2.075)	0.04	−3.462 (2.335)	0.14
Block design	+0.634 (0.230)	0.006	+0.520 (0.258)	0.04

Plasma amylin was transformed to log_{10} (Log_{10} Amylin) as a determining factor. The lipid profile includes cholesterol, LDL, and HDL. MMSE, Mini-Mental State Exam; WLL, Word learning list; LM, Logical memory.

Table 4

Comparisons of cognitive function across amylin quartiles in the absence and presence of ApoE4 allele

	Amylin quartiles				
ApoE4 non-carriers	**Quartile 1 *n*=209**	**Quartile 2 *n*= 214**	**Quartile 3 *n*= 207**	**Quartile 4 *n*= 206**	***p* values**
WLL delayed recall, mean±SD	3.3±2.7	3.3±2.7	4.1±3.0	3.8 ±2.9	0.01
LM delayed recall, mean±SD	17.4±10.3	17.2±9.6	20.2±9.9	19.6±8.8	0.002
Trailmaking A, mean±SD	97.2±66.2	94.4±71.9	76.8±44.1	76.8±50.6	0.0007
Trailmaking B, mean±SD	222.8±79.6	213.7±83.3	204.5±83.8	200.5±85.6	0.05
Block design, mean±SD	18.9±8.8	19.8±8.9	20.6±9.0	22.3±10.0	0.005
Amylin Quartiles					
ApoE4 carriers	*n*=63	*n*=61	*n*=68	*n*=65	
WLL delayed recall, mean±SD	3.6±2.8	2.8±2.5	3.7±2.6	3.7±2.7	0.17
LM delayed recall, mean±SD	16.8±9.6	15.6±10.1	19.1±8.6	18.5±10.8	0.11
Trailmaking A, mean±SD	105.4±69.2	92.1±66.0	83.1±50.3	85.4±61.4	0.06
Trailmaking B, mean±SD	227.7±82.1	220.1±88.4	210.3±82.7	213.8±89.2	0.62
Block design, mean±SD	18.1±9.4	17.5±8.2	20.8±8.4	18.7±8.1	0.09

Subjects are divided into ApoE4 non-carriers and ApoE4 carriers in each amylin quartile. Mean±SD with ANOVA test is used to describe the distributions and comparisons of test scores in each cognitive domain across the amylin quartiles in the absence or in the presence of ApoE4. *P* values for the statistical significance are shown. WLL, Word learning list; LM, Logical memory.

Table 5

Comparisons of cognitive function across amylin quartiles in the absence and presence of diabetes

	Amylin quartiles				
Non-diabetics	**Quartile 1 *n*=163**	**Quartile 2 *n*=182**	**Quartile 3 *n*=176**	**Quartile 4 *n*=148**	***p* values**
WLL delayed recall, mean±SD	3.7±2.9	3.2±2.7	4.1±2.9	3.8±2.9	0.02
LM delayed recall, mean±SD	18.2±10.2	16.9±10.0	20.7±9.4	20.3±8.8	0.001
Trailmaking A, mean±SD	94.3±63.3	89.2±67.2	75.9±43.1	74.1±49.4	0.0008
Trailmaking B, mean±SD	216.0±80.2	209.9±84.8	200.3±85.9	193.5±85.1	0.12
Block design, mean±SD	19.0±9.0	19.8±8.6	21.5±8.6	22.7±10.0	0.006
Amylin Quartiles					
Diabetics	*n*=97	*n*=87	*n*=92	*n*=117	
WLL delayed recall, mean±SD	2.9±2.4	3.1±2.6	4.1±2.9	3.8±2.9	0.02
LM delayed recall, mean±SD	15.8±9.8	16.6±9.2	18.9±9.7	18.4±9.7	0.09
Trailmaking A, mean±SD	102.6±68.7	100.2±73.0	82.0±51.4	82.4±54.9	0.04
Trailmaking B, mean±SD	232.1±80.5	225.9±81.7	212.7±79.4	212.3±87.3	0.23
Block design, mean±SD	18.4±9.0	18.5±9.2	19.2±9.0	20.2±9.2	0.40

Subjects are divided into those without and with diabetes in each amylin quartile. Mean ± SD with ANOVA test is used to describe the distributions and comparisons of test scores in each cognitive domain across the amylin quartiles in the absence or in the presence of diabetes. *P* values for the statistical significance are shown. WLL, Word learning list; LM, Logical memory.

Table 6

Comparisons of cognitive function across amylin quartiles in the absence and presence of stroke

	Amylin quartiles				
Non-stroke	**Quartile 1 *n*=211**	**Quartile 2 *n*= 211**	**Quartile 3 *n*=217**	**Quartile 4 *n*= 218**	***p* values**
WLL delayed recall, mean±SD	3.4±2.7	3.3±2.7	4.1±3.0	3.8±2.8	0.007
LM delayed recall, mean±SD	16.8±10.5	16.9±10.0	20.1±9.4	20.0±9.5	0.0003
Trailmaking A, mean±SD	98.1±65.3	88.6±65.9	75.0±43.3	75.8±52.1	<0.0001
Trailmaking B, mean±SD	219.6±82.0	208.0±86.6	200.0±83.1	200.7±86.1	0.07
Block design, mean±SD	18.9±8.9	19.8±8.7	21.0±8.5	21.6±9.8	0.03
Amylin quartiles					
Stroke history	*n*=4	*n*=61	*n*=53	*n*=50	
WLL delayed recall, mean±SD	3.5±2.9	2.9±2.5	3.9±2.6	3.6±2.8	0.29
LM delayed recall, mean±SD	18.9±9.3	16.9±8.6	19.5±10.0	19.0±8.2	0.39
Trailmaking A, mean±SD	97.7±65.9	101.4±73.1	90.8±54.5	87.6±56.1	0.65
Trailmaking B, mean±SD	230.8±73.7	231.8±73.1	222.3±83.2	212.9±86.6	0.76
Block design, mean±SD	18.0±9.1	18.3±8.9	19.1±9.7	21.4±9.9	0.22

Subjects are divided into those without and with a history of stroke in each amylin quartile. Mean ± SD with ANOVA test is used to describe the distributions and comparisons of test scores in each cognitive domain across the amylin quartiles in the absence or in the presence of stroke. *P* values for the statistical significance are shown. WLL, Word learning list; LM, Logical memory.