Blood-Based Biomarkers

Plasma clusterin levels and risk of dementia, Alzheimer's disease, and stroke

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Abstract

Introduction: Genetic variation in the clusterin gene has been associated with Alzheimer Disease (AD), and the clusterin protein is thought to play a mechanistic role. We explored the associations of clusterin plasma levels with incident dementia, AD, and stroke.

Methods: Plasma clusterin was assessed in 1532 nondemented participants from the Framingham Study Offspring cohort between 1998 and 2001 (mean age, 69 ± 6; 53% women). We related clusterin levels to risk of incident dementia, AD, and stroke using Cox-proportional hazards models and examined potential interactions.

Results: A significant interaction of plasma clusterin levels with age was observed. Clusterin was significantly associated with increased risk of dementia among elderly persons (>80 years; hazard ratio [HR], 95% confidence interval = 6.25, 1.64–23.89; P = .007) and with decreased risk of dementia (HR = 0.53, 0.32–0.88; P = .013) and stroke (HR = 0.78, 0.63–0.97; P = .029) among younger participants.

Discussion: The association between plasma clusterin levels and risk of dementia and stroke may be modified by age or an age-related factor.

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Keywords: Epidemiology; Plasma clusterin; Dementia; Alzheimer's disease; Stroke; Risk factors

1. Introduction

Genetic variation within the clusterin (*CLU*, also previously called apolipoprotein J, ApoJ) gene has been associated with risk of Alzheimer's disease (AD) in multiple independent genome-wide association studies of diverse ethnic groups [1]. In addition, among healthy participants in the Baltimore Longitudinal Study of Aging, carriers of the protective *CLU* allele showed slower rates of decline in memory performance relative to carriers of

the risk allele [2]. However, our knowledge is very limited regarding the mechanisms through which genetic variation in the clusterin gene modifies the risk of AD. Some evidence suggests that the clusterin protective variant is associated with elevated gene expression and higher plasma clusterin levels [3–6]. In turn, there is substantial evidence suggesting neuroprotective roles for clusterin in AD pathogenesis. For instance, clusterin acts as a chaperone to alter amyloid beta (aβ) aggregation and toxicity, it has a role in aβ clearance as well as in lipid metabolism in the brain, and it modulates inflammation and inhibits apoptosis [7–9]. In contrast, clusterin mRNA and protein levels have been shown to be higher in AD [5,6,10,11] and mild cognitive impairment [11,12] in some but not all [4,6,13] studies. Clusterin

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protein levels in plasma are also increased with increasing severity of the disease [6,10] as well as in persons showing a more rapid decline [6]. Even in nondemented individuals, clusterin levels are negatively correlated with performance on tests of global cognition and attention/processing speed [12], with greater brain atrophy [6,12] and with a greater decline in white-matter volume [12]. These findings have led to the hypothesis that clusterin is elevated in response to brain pathology to exert its neuroprotective effect. In contrast, in vitro studies suggest that clusterin can promote amyloid aggregation and toxicity when the clusterin/aβ ratio is low [14,15]. Furthermore, in a longitudinal study of 241 old individuals, clusterin levels in the cerebrospinal fluid (CSF) have been related to a greater entorhinal cortex atrophy rate, and this relationship was observed only among CSF aβ1-42-positive individuals but not among CSF aβ1-42-negative individuals [16]. We postulate that one reason for these apparently contradictory findings may be variation in the role of circulating clusterin with increasing age, inflammation, and amyloid pathology.

It is not clear whether clusterin levels in plasma can serve as an early predictor of dementia and AD among cognitively healthy individuals. The discovery of a well-established peripheral AD biomarker that is easily accessible and cost effective is of great importance because AD incidence increases as the population ages, and the ability to identify high-risk populations is thought to be crucial for the success of current and future therapies. We tested the hypothesis that elevated plasma levels of clusterin are associated with a higher risk of new-onset dementia and AD in cognitively healthy participants. We then examined whether age and sex modify this association. An interaction of clusterin levels with plasma aβ was also tested, as the impact of clusterin may depend on aβ burden [16]. Furthermore, because clusterin has been shown to play a significant role in inflammation and immune responses [17], we assessed whether its relationships with dementia/AD risk differ in individuals with low compared to high serum C-reactive protein (CRP) levels. Finally, we have explored the relationship between clusterin levels and the risk for stroke in the current analysis, as both stroke and dementia share common risk factors and etiologies [18]. Moreover, clusterin also has been shown to alter the risk of cardiovascular and metabolic diseases [19].

2. Methods

2.1. Study sample

The FHS is a longitudinal community-based cohort study that was initiated in 1948 with the enrollment of 5209 participants aged 28–62 years. In 1971, offspring of the first generation participants and spouses of these offspring were enrolled as the Offspring cohort (n = 5124, age = 12–58 years). Since their recruitment, participants
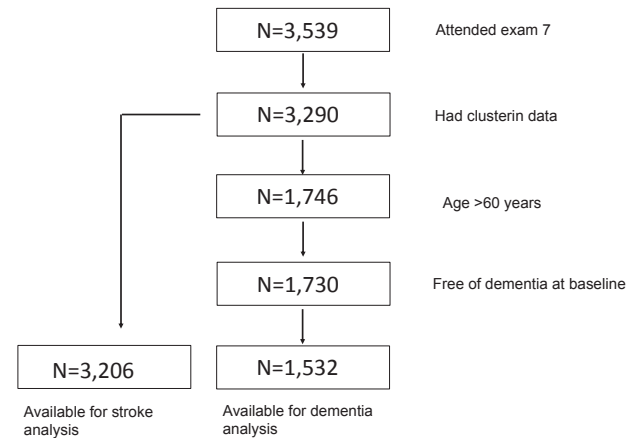

Fig. 1. Flow diagram of study participants.

from the Offspring cohort have had 9 serial examinations including standardized interviews, physician examinations, and laboratory testing [20].

The study design is described in Fig. 1. Clusterin was measured in blood drawn from Offspring participants at the 7th offspring examination between 1998 and 2001, and of 3539 who attended that examination, 3290 had blood samples available for plasma clusterin assay. We excluded persons age <60 years because *CLU* has been associated only with late-onset dementia, and persons with prevalent dementia at the 7th Offspring examination. Therefore, 1730 participants remained, of whom, 1532 also had further follow-up information on cognitive status for at least 1 year and thus constitute our study sample. For analyses of stroke outcome, we did not exclude younger participants, so after excluding 84 participants with prevalent stroke, 3206 of the 3290 persons with plasma clusterin data were available for analysis.

2.2. Laboratory measurements of clusterin, aβ, and CRP

Whole blood was collected in fasting state using a 21-gage needle. For serum preparation, blood was collected into red top tubes and was allowed to clot in a vertical position at room temperature for 30 minutes before centrifugation. For plasma preparation, blood was collected to EDTA-treated tubes and gently inverted 5–10 times. All tubes underwent centrifugation at 3000 rpm/1850 g for 30 minutes at 4°C. Serum and plasma were then apportioned into 0.5-mL aliquots and stored at −80°C.

All samples were analyzed for beta-amyloid levels at the Department of Molecular Pharmacology and Experimental Therapeutics of the Mayo Clinic, Jacksonville, FL. Quantification of aβ in plasma was performed using INNO-BIA assays (Innogenetics, Ghent, Belgium), which is a multiplex microsphere-based Luminex xMAP technique. Intra-assay coefficients of variations (CVs) for aβ$_{1-40}$ and aβ$_{1-42}$ were 3.2% and 2.6%, and inter-assay CVs were 10.5% and 7.6%, respectively. Plasma clusterin

levels were assessed as part of the Systems Approach to Biomarker Research (SABRe) project 2 panel 1 using a Luminex xMAP assay. The Multiplex assay had five protein targets (Clusterin, Apo-A1, Apo-B100, Lp(a), and CRP) and used a plasma dilution of 1/10,000. The intra-assay %CV for the clusterin measurements was 3.95%, and Inter-assay % CV of 8.4%. The assay had a lower limit of quantitation of 1.5 and upper limit of quantitation (ULOQ) of 2.67×10^4, with a seven-point calibration curve. Two quality control (QC) samples were prepared to include five protein targets in the panel, one a high concentration (QC1) and the other a low concentration (QC2). To determine the plate-to-plate and day-to-day performance of each assay plate, each 96 well assay plate included (1) the standards in triplicate, (2) the QC1 and QC2 in quadruplicate, and (3) 32 individual plasma samples in duplicate. The acceptable range for each FHS assay plate was determined to be the mean $+/- 3SD$. If the QC1 or QC2 value of any assay plate was out of the range, the plate was repeated.

High-sensitivity CRP levels were determined in serum by Dade Behring BN100 nephelometer, and the average interassay coefficients of variation was 2.2%.

2.3. Ascertainment of dementia and AD

All FHS participants are under ongoing continuous surveillance for onset of cognitive impairment and clinical dementia. We have previously outlined our screening and surveillance methods for the development of dementia. Dementia was diagnosed according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* [21], and AD was diagnosed based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) for definite, probable, or possible AD [22].

2.4. Definition of covariates

Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications; diabetes mellitus was defined as fasting glucose ≥7 mmol/L or use of an antidiabetic therapy; body mass index was defined as weight (in kilograms) divided by the square of height (in meters). Current smoking was defined by having smoked at least one cigarette per day for the previous year. Total and high-density lipoprotein (HDL) cholesterol were measured after an overnight fast. *APOE* genotype was assessed as previously described [23] and was defined according to whether the participant had at least one or more ε4 alleles (yes vs. no).

2.5. Statistical analyses

Age-adjusted and sex-adjusted linear regression models were constructed to estimate differences in each clinical measure per standard deviation of plasma clusterin levels. Cox regression models were used to evaluate the association of plasma clusterin levels with incident dementia, AD, and stroke. We tested for interaction of clusterin levels with age, sex, and with two circulating biomarkers, plasma aβ and serum CRP levels, in determining the risk of each outcome by including these interaction terms in the Cox regression models. If a significant interaction was found, we ran a stratified model. Age was stratified into 10-year age groups for dementia and AD, and into two groups of above and below 80 years for stroke. All primary analyses were first adjusted for age and sex, the dementia and AD outcomes were examined after additional adjustment for interim strokes. In model 2, we further adjusted for hypertension, current smoking, diabetes, body mass index, and *APOE* ε4 genotype status. In a secondary analysis, we tested whether CRP modified the association of plasma clusterin levels with dementia risk. The association of clusterin and dementia risk was evaluated among persons with CRP levels in the bottom two tertiles and in the top tertile, in each age group. All statistical analyses were done using SAS, v.9.4 (Cary, NC). A two-sided P value $<.05$ was considered statistically significant, with the exception of interaction assessment in which case P value $<.10$ was considered statistically significant, owing to low power of the test.

3. Results

Clinical and demographic characteristics of the study participants at the time of clusterin measurement are listed in Table 1; their mean age was 69 ± 6 years and 806 (53%) were women, mean plasma clusterin was 53 ± 13 μg/mL. The mean duration of follow-up for dementia and AD was 8 ± 3 years. The mean duration of follow-up was shorter among the older participants (8 ± 3 years for baseline age stratum 60–69 years, 7 ± 3 years at ages 70–79 years and 6 ± 4 years at ages 80–89 years). During the follow-up period, 76 of 1528 participants developed all-cause dementia (58 AD). The associations of plasma clusterin levels with the baseline characteristics are listed in Table 1. Clusterin levels were significantly higher in women, were inversely associated with age, and were positively associated with higher total and HDL cholesterol. Of the 3206 participants available for the stroke analysis, 107 developed a stroke during the 9 ± 2 years of follow-up.

Overall, clusterin levels were not associated with risk of dementia or AD. Higher plasma clusterin levels were associated with a decreased risk of stroke after additional adjustment for vascular risk factors (Table 2). We found significant interactions of age with clusterin levels in determining the risk of dementia and AD ($P = .006$ and $P = .012$ for dementia and AD, respectively). Age-specific analyses revealed opposite directions of the relations of clusterin levels to risk of dementia and AD: in

Table 1
Baseline characteristics of study sample and association with plasma clusterin levels (N = 1532)

Characteristics	Value	Difference in plasma clusterin levels (per 100,000 μg/mL)	
		β ± SE	P value
Age in y	68.6 ± 5.7	−1.52 ± 0.59	**.01**
Women	806 (52.6)	49.2 ± 6.66	**<.0001**
Height, in	65.5 ± 3.7	−0.55 ± 1.40	.69
Waist hip ratio	0.97 ± 0.07	47.5 ± 49.4	.34
Body mass index in kg/m²,*	27.5 (24.7, 30.9)	−19.9 ± 19.8	.32
Total cholesterol in mg/dL	199 (37)	0.41 ± 0.095	**<.0001**
HDL cholesterol in mg/dL*	50 (40, 63)	23.9 ± 11.8	**.04**
Systolic blood pressure, mmHg	132 ± 19	0.13 ± 0.18	.45
Prevalent hypertension†	880 (58)	−0.38 ± 6.85	.96
Hypertension treatment	660 (43)	−4.81 ± 6.80	.48
Current smoking	123 (8)	7.03 ± 12.3	.57
Prevalent diabetes‡	246 (16)	−9.98 ± 9.16	.28
APOE ε4 genotype	341 (23)	−3.71 ± 8.01	.64
Prevalent cardiovascular disease	284 (19)	−2.05 ± 8.86	.82
Prevalent atrial fibrillation	93 (6)	−4.60 ± 14.1	.74
Prevalent stroke	41 (3)	2.38 ± 20.8	.91
Education			
No high-school degree	93 (6)	−6.03 ± 14.9	.69
High-school degree	501 (33)	−14.4 ± 8.52	.09
Some college	440 (29)	1.35 ± 8.78	.88
College graduate	466 (31)	Referent	—
HOMA-IR*	2.8 (2.0–4.2)	−6.42 ± 5.66	.26

NOTE. Values in bold indicate P ≤.05.

NOTE. Values are n (%) or mean ± SD unless otherwise specified.

NOTE. All models are adjusted for age and sex.

NOTE. Differences in plasma levels are for 1 unit increase or for yes versus no in characteristics.

*Values are median (interquartile range) and β is for increase in 1 unit log transformation.

†Defined as either systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or the use of antihypertensive medication.

‡Defined as a recorded random blood glucose level ≥200 mg/dL (11.1 mmol/L), a previous diagnosis of diabetes mellitus, or the use of insulin or a hypoglycemic agent.

persons aged 60 to 69 years, each 1 SD increment in plasma clusterin levels was associated with a 59% (P = .005) and 52% (P = .038) lower risk of all-cause dementia and AD, respectively, after adjustment for other risk factors. In the 70-to-79-year age group, the hazard ratios were close to 1, and the associations were not statistically significant. In the oldest age group (ages, 80–89 years), we were not able to assess this relationship while controlling for vascular risk factors because the numbers were too low; however, we found that each SD increase in plasma clusterin levels was associated with about 6 times the risk of dementia (P = .007) and 5 times the risk of AD (P = .011), after adjusting for age and sex (Table 3).

Age did not significantly modify the association between clusterin levels and the risk of stroke and the associations within each of the individual 10-year age groups (60–69 years, 70–79 years) were not statistically significant, likely

Table 2
Hazard ratios (95% confidence interval [CI]) associated with 1 SD increase in clusterin plasma levels

Outcome	Model 1		Model 2	
	HR (95% CI)	P value	HR (95% CI)	P value
All-cause dementia*	1.00 (0.79–1.27)	.978	1.00 (0.79–1.29)	.943
Alzheimer's disease*	1.03 (0.79–1.35)	.831	1.04 (0.79–1.37)	.796
Stroke	0.82 (0.67–1.02)	.075	0.79 (0.64–0.99)	**.042**

NOTE. Values in bold indicate P ≤.05.

NOTE. Model 1: Adjusted for age and sex.

NOTE. Model 2: Adjusted in addition for hypertension, smoking, prevalent diabetes, body mass index and APOE ε4 genotype status.

*Models are also adjusted for prevalent stroke.

due to the small numbers in each subgroup. However, we did observe that higher clusterin levels were associated with a lower risk of stroke in participants <80 years (HR = 0.75 [0.60–0.95]; P = .017). In those who were 80 years old or more, this association was in the opposite direction although it was not statistically significant (HR = 1.22 [0.48–3.06]; Table 3).

No modifying effect of either sex or aβ levels on the risk of any outcome was observed. Among the 60-to-69-year age group, CRP levels significantly modified the association of clusterin and incident dementia (P for interaction = .057). In this subsample, increased clusterin levels were associated with decreased dementia risk only in those with CRP levels in the bottom 2 tertiles (below 4.25 mg/l; HR = 0.41 [0.25–0.68] vs. 0.99 [0.52–1.89] in those with CRP ≥4.25). There was no interaction in the 70-to-79-year age group, and the numbers among the 80-to-89-year age group were too small to test for interaction. Mean CRP levels in the 60-to-69-year, 70-to-79-year, and 80-to-89-year age groups were 4.5 ± 6.6, 5.1 ± 8.2 and 5.3 ± 7.8 mg/l, respectively.

4. Discussion

In our community-based study, age modified the association between plasma clusterin levels and incident dementia and AD such that higher clusterin levels were protective in younger persons but were associated with a higher risk in the older participants. Higher clusterin levels were also associated with reduced risk of stroke in younger persons.

In accordance with our findings, there were no associations overall between plasma clusterin levels and risk of incident AD in an earlier report from the Rotterdam study [10,24]. In the latter, an additional cross-sectional analysis was conducted which demonstrated an association of higher plasma clusterin with prevalent AD and with greater severity of the disease. Other cross-sectional studies support these findings [6,11] and further suggest that the CLU genetic variant only influences plasma clusterin levels in cognitively intact individuals, whereas levels are

Table 3
Hazard ratios (95% confidence interval [CI]) associated with 1 SD increase in clusterin plasma levels stratified by age

	Age	Cases/N	Model 1		Model 2	
			HR (95% CI)	*P* value	HR (95% CI)	*P* value
All-cause dementia*	60–69	16/937	0.53 (0.32–0.88)	**.013**	0.41 (0.22–0.76)	**.005**
	70–79	52/555	1.05 (0.78–1.42)	.730	1.10 (0.82–1.48)	.539
	80–89	8/40	6.25 (1.64–23.89)	**.007**	55.5 (2.73–1130.87)	**.009**
Alzheimer's disease*	60–69	12/937	0.59 (0.33–1.07)	.080	0.48 (0.24–0.96)	**.038**
	70–79	39/555	1.03 (0.73–1.45)	.883	1.11 (0.79–1.57)	.548
	80–89	7/40	5.19 (1.45–18.62)	**.011**	—	—
Stroke	<80	102/3156	0.78 (0.63–0.97)	**.029**	0.75 (0.60–0.95)	**.017**
	≥80	5/50	1.73 (0.75–3.96)	.197	1.22 (0.48–3.06)	.678

NOTE. Values in bold indicate *P* ≤.05.
NOTE. Model 1: Adjusted for age and sex.
NOTE. Model 2: Adjusted in addition for hypertension, smoking, prevalent diabetes, body mass index, and *APOE* ε4 genotype status.
*Models are also adjusted for prevalent stroke.

increased in mild cognitive impairment and AD cases regardless of the genotype [11]. Together, these findings suggest that plasma clusterin levels are elevated only late in the development of the disease and do not precede it. Whereas some data suggest that even among dementia-free individuals, clusterin levels in the plasma may be related to AD endophenotypes such as cognitive performance and MRI measures of white-matter brain injury, these studies were in older cohorts who are more likely to have clinically undetected AD pathology [6,12,25]. Keeping this in mind, our findings of a positive association between clusterin levels and risk of dementia and AD in individuals >80 years may be explained as an elevation of clusterin levels in response to brain injury, which in turn may be associated with a higher risk of dementia and AD. This hypothesis is also strengthened by the fact that the mean duration of follow-up for this age group was shorter; thus, it is more likely that early pathologic brain changes preceding the clinical diagnosis of dementia might already be present when plasma clusterin was measured. In line with these findings, previous literature shows that clusterin may have a proamyloidogenic effect when aβ protein, the clusterin substrate, is present at a very high molar excess [14,15]. Indeed, overexposure of endothelial cells to aβ has been shown to induce morphologic and biochemical alterations which affect blood-brain barrier permeability [26], and as a result, an accumulation of amyloid deposition in the brain and simultaneously, elevated clusterin levels in the periphery may be apparent [27,28], although other mechanisms may also be involved. This hypothesis has been recently reinforced in a cohort study of 241 nondemented elderly, where elevated CSF clusterin levels have been associated with a greater entorhinal cortex volume loss only when CSF aβ levels were low (indicating increased intracranial deposition) [16]. We were unable to estimate this ratio in the CSF or brain and did not observe a modifying effect of plasma aβ on the relationship of clusterin levels and dementia risk possibly due to poor correlation of aβ plasma and brain/CSF levels.

Evidence also exists supporting our opposing findings in the 60-to-69-year age category compared to those >80 years. Studies show that clusterin may have a protective effect on the brain possibly through its interaction with soluble aβ [29]. The latter, but not monomers or insoluble amyloid fibrils, may be responsible for synaptic dysfunction in the brains of AD patients and is considered the most neurotoxic form [30,31]. The clusterin-aβ complex formation significantly prevents aggregation and polymerization of soluble aβ and protects soluble aβ from proteolytic degradation, hence preventing synaptic dysfunction [29]. Another protective mechanism of clusterin is its role as a transporter of aβ peptides from the brain to the circulation across the blood-brain barrier [7,9].

Our findings suggest that CRP levels modify the association of clusterin and dementia risk, such that a protective effect of clusterin is present only when CRP levels are low. Emerging evidence suggests that systemic and central innate immune systems communicate closely, and several routes of communication have been demonstrated [32–34]. It is also increasingly recognized that CRP is a pathogenic factor in AD progress along with aβ oligomer initiation [31,35,36], and recent evidence suggests that CRP may regulate aβ formation [37]. The role of clusterin in these complex mechanisms needs to be further explored.

Our results also show that the link of clusterin to dementia and AD exists in a similar manner with stroke. Although this finding may point to other clusterin mechanisms that affect vascular injury, it is also possible that the same clusterin/aβ mechanisms underlie both neurodegeneration and vascular pathology. Indeed, amyloid deposition in the arteriolar wall has been shown to enhance vasoconstriction, and aβ is also cytotoxic to endothelial and smooth-muscle cells, conferring a predisposition to lobar hemorrhage in advanced age. Alternatively, vascular injury and parenchymal inflammation perpetuate the cycle of protein aggregation and oxidation in the brain, which may in turn lead to cognitive impairment and dementia [31].

Although in the present study, clusterin has been measured in plasma, evidence suggests that these levels may reflect those of brain regions which are vulnerable to AD pathology [25]. Furthermore, the involvement of clusterin in the transport of aβ from the brain to the periphery [27,28] indicates that its levels in the latter are important to the assessment of AD risk.

It is important to note that due to small numbers of incident events our results must be considered hypothesis generating and need to be replicated in larger studies. Yet, the fact that previous literature exists demonstrating both neuroprotective and neurotoxic effects of clusterin under different conditions, and the similar associations found in the current study for both dementia and stroke outcomes may indicate that our findings are real. In addition, owing to the small number of events, we were unable to assess the relationship between clusterin plasma levels and the type or severity of dementia or its progression and to explore cross-sectional associations. The strengths of this study are its community-based prospective design, the large sample of dementia-free participants with clusterin data who were followed-up and the careful surveillance for endpoints.

In conclusion, we suggest that plasma clusterin is associated with incident dementia, AD and stroke; however, age, or age-related factors, may interact with clusterin leading to contradictory associations among the young-old and the oldest-old. If validated in other studies, these findings may have several implications: plasma clusterin levels can potentially serve as predictors for dementia, AD, and stroke in specific pre-defined subpopulations and may allow for better clinical trial design. Moreover, identifying modifiers of these associations can substantially leverage our understanding of the mechanisms underlying dementia, AD, and stroke.

Acknowledgments

RESEARCH IN CONTEXT

1. Systematic review: We searched PubMed for reports of the association between clusterin plasma levels and dementia, Alzheimer's disease (AD), cognition, and stroke. Previous work has been done mostly using animal models, and the association with stroke has been rarely investigated. Cross-sectional studies in humans suggest that plasma clusterin levels are higher in persons with AD, cognitive impairment, or structural brain changes; however, in a prospective work, no associations with AD risk have been observed.

2. Interpretation: We are showing for the first time that age modifies the relationship of clusterin levels with incident dementia and AD. In accordance with findings from animal models, this may imply that factors associated with age, such as amyloid-beta-42 and low-grade inflammation, interact with clusterin to affect dementia risk.

3. Future directions: Future research should further explore the role of clusterin as an AD/stroke risk biomarker and as an active player in AD pathophysiology.

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