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PLASMA CLUSTERIN CONCENTRATION IS ASSOCIATED WITH LONGITUDINAL BRAIN ATROPHY IN MILD COGNITIVE IMPAIRMENT

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Abstract

Recent genetic and proteomic studies demonstrate that clusterin/apolipoprotein-J is associated with risk, pathology, and progression of Alzheimer's disease (AD). Our main aim was to examine associations between plasma clusterin concentration and longitudinal changes in brain volume in normal aging and mild cognitive impairment (MCI). A secondary objective was to examine associations between peripheral concentration of clusterin and its concentration in the brain within regions that undergo neuropathological changes in AD. Non-demented individuals (N = 139; mean baseline age 70.5 years) received annual volumetric MRI (912 MRI scans in total) over a mean six-year interval. Sixteen participants (92 MRI scans in total) were diagnosed during the course of the study with amnestic MCI. Clusterin concentration was assayed by ELISA in plasma samples collected within a year of the baseline MRI. Mixed effects regression models investigated whether plasma clusterin concentration was associated with rates of brain atrophy for control and MCI groups and whether these associations differed between groups. In a separate autopsy sample of individuals with AD (N=17) and healthy controls (N=4), we examined the association between antemortem clusterin concentration in plasma and postmortem levels in the superior temporal gyrus, hippocampus and cerebellum. The associations of plasma clusterin concentration with rates of change in brain volume were significantly different between MCI and control groups in several volumes including whole brain, ventricular CSF, temporal gray matter as well as parahippocampal, superior temporal and cingulate gyri. Within the MCI but not control group, higher baseline concentration of plasma clusterin was associated with slower rates of brain atrophy

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in these regions. In the combined autopsy sample of AD and control cases, representing a range of severity in AD pathology, we observed a significant association between clusterin concentration in the plasma and that in the superior temporal gyrus. Our findings suggest that clusterin, a plasma protein with roles in amyloid clearance, complement inhibition and apoptosis, is associated with rate of brain atrophy in MCI. Furthermore, peripheral concentration of clusterin also appears to reflect its concentration within brain regions vulnerable to AD pathology. These findings in combination suggest an influence of this multi-functional protein on early stages of progression in AD pathology.

Keywords

clusterin; mild cognitive impairment (MCI); Alzheimer's disease (26); plasma; atrophy; biomarker

Introduction

The identification of peripheral biomarkers associated with core pathological features of Alzheimer's disease (AD) may accelerate the development of disease-modifying treatments (Lyketsos et al., 2008). In pre-symptomatic subjects at risk for subsequent development of AD, blood-based analytes reflecting neuropathology may help in effective targeting of treatments in those most likely to benefit from early intervention (Song et al., 2009). Similarly, peripheral markers of disease pathology in incipient stages of AD may be useful as surrogate end points in clinical trials of novel therapies (Cummings et al., 2007). However, the standard paradigm for biomarker discovery in AD is based upon identification of signals that provide binary discrimination between groups, i.e. AD or mild cognitive impairment (MCI) versus age-matched controls. Given that a significant proportion of elderly control subjects without cognitive impairment already harbor AD pathology, such as Aβ deposition in the brain (Savva et al., 2009), it is unlikely that this strategy will yield biomarkers accurately reflecting early neuropathology. Moreover, this approach also ignores the considerable heterogeneity in the rate of disease progression in patients with established AD (Kraemer et al., 1994).

To overcome some of these limitations, we have recently used proteomic analyses to identify plasma proteins primarily on the basis of their association with established neuroimaging endophenotypes of AD pathology such as brain atrophy and amyloid deposition. Using this approach, we found that higher plasma concentration of clusterin, also known as apolipoprotein-J (apoJ), is associated with greater severity and faster rate of clinical progression in patients with AD (Thambisetty et al., 2010). Our finding of an association between plasma clusterin concentration and disease severity in AD was recently replicated in an independent study by Schrijver and colleagues (Schrijvers et al., 2011). Together with recent genome wide association studies (GWAS) demonstrating associations between polymorphic variation in the clusterin gene (CLU) and risk of AD (Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010), these findings suggest a potential role for both the CLU gene and clusterin protein in AD pathogenesis. We also recently reported that specific brain regions show accelerated longitudinal tissue loss in individuals with mild cognitive impairment (MCI) (Driscoll et al., 2009). In the current study, our aim was to investigate whether plasma clusterin concentration is related to these longitudinal changes in brain volume in MCI as well as normal individuals. We addressed this question in the neuroimaging cohort of the Baltimore Longitudinal Study of Aging (BLSA), taking advantage of annual volumetric MRI assessments in this group of healthy control and MCI individuals. This rich longitudinal dataset acquired over a mean six year interval in each individual contained more than 900 MRI observations in total. We asked two main questions in this neuroimaging study:

1. Are there inter-group (MCI versus control) differences in the association between baseline plasma clusterin concentration and longitudinal changes in brain volumes?
2. Within each group (MCI and control separately), are there significant associations between baseline plasma clusterin concentration and rates of change in brain volumes?

Subsequently, in an independent analysis of autopsy samples from patients with AD and healthy control individuals, we asked whether plasma clusterin concentration was also related to its concentration within regions vulnerable to AD pathology.

Materials and Methods

Participants

The neuroimaging study included 139 individuals (ages 57–87 years) from the neuroimaging substudy of the Baltimore Longitudinal Study of Aging (BLSA) who were free of a clinical diagnosis of dementia at the baseline evaluation and received annual brain MRI scans over a mean follow up interval of six-years. Sixteen participants were diagnosed with MCI during the course of the study, of whom seven went on to develop dementia. The diagnosis of MCI was made according to Petersen criteria (Petersen, 2004) during a consensus case conference. During each annual neuroimaging visit, participants completed a battery of twelve neuropsychological tests evaluating six cognitive domains. Memory was assessed using the California Verbal Learning Test (CVLT) and Benton Visual Retention Test (BVRT). Word knowledge and verbal ability were measured using Primary Mental Abilities Vocabulary (PMA). Verbal fluency was assessed by Letter (i.e. FAS) and Category fluency tests. Attention and working memory were measured by the Digit Span Test of the Wechsler Adult Intelligence Scale-Revised, and the Trail Making Test. Digits Backward, Trails B, and Verbal Fluency (categories and letters) assessed executive function. The Card Rotations Test assessed visuospatial function. Data from time points at and subsequent to the diagnosis of dementia (using DSM III criteria) were excluded from the analysis. Exclusion criteria at the time of entry into the neuroimaging study included clinically significant stroke, severe cardiovascular or pulmonary disease as well as metastatic cancer. The results reported here are derived from analyses of longitudinal MRI data obtained from more than 900 observations in the two groups of participants (MCI=92 and control=820 observations). This study was approved by the local institutional review boards and all participants gave written informed consent prior to each assessment.

In the autopsy study, post-mortem brain tissue from seventeen AD patients and four healthy subjects was examined. Cognitive function of these individuals had been assessed during life annually using the Mini-Mental State Examination (MMSE) (Folstein et al., 1983). AD patients fulfilled the criteria for probable AD based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) and DSM-IV-TR guidelines (McKhann et al., 1984). Following death, definitive diagnosis was made by histopathological staining of brain tissue sections. Tissue samples were obtained from the superior temporal gyrus (STG), hippocampus (HIP) and cerebellum (CER), randomly from either the left or right hemisphere of the brain. The experiments were undertaken with the understanding and written consent of each subject prior to death and/or their family members and in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). The studies described from the autopsy sample cohort were approved by the King's College London Ethics Committee.

MRI acquisition and analysis

MRI scans were obtained from a GE Signa 1.5T scanner (Milwaukee, WI) using a high-resolution spoiled-grass axial series (repetition time=35 msec, echo time=5 msec, field of view = 24cm, flip angle = 45°, matrix =256 × 256).. Image processing procedures have been previously validated and described. 11,22,23 Briefly, images are corrected for head tilt and rotation, and reformatted parallel to the anterior-posterior commissure plane. Extracranial tissue is removed using a semiautomated procedure followed by manual editing. Next, images are segmented into white matter (WM), gray matter (GM), and CSF. The final step involves stereotaxic normalization and tissue quantitation for specific regions of interest. A template-based deformation approach is employed, using the ICBM standard MRI (Montreal Neurologic Institute) as the template and a hierarchical elastic matching algorithm for deformation and regions of interest determination (Shen and Davatzikos, 2002). All images are normalized individually to the same template. Voxel-based analysis utilizes our RAVENS approach (regional analysis of volumes examined in normalized space) (Goldszal et al., 1998), whereby local values of tissue density maps (one for GM, one for WM, and one for CSF) reflect the amount of respective tissue in the vicinity of a voxel. Tissue densities are mathematical quantities measuring local tissue volumes and do not reflect any microstructural physical density of brain tissue. Intracranial volume (ICV) is determined using the template warping algorithm modified for head image registration. First, the ICV in the template is manually and carefully delineated by an expert. Then, the template with its ICV mask is warped to the space of each individual head. Finally, the warped ICV mask of the template is used to directly extract the ICV of the individual. ICV at initial imaging evaluation is used for statistical adjustment to avoid potential biases of brain atrophy and partial volume effects on estimation of ICV (Pengas et al., 2009).

Plasma samples and clusterin ELISA assay

In the neuroimaging study, the average interval between plasma samples collected for clusterin assays and the baseline MRI scans was 22 days. Baseline MRI scan was defined as the first study that had a concurrent (within one year) plasma sample available from a participant. Samples were collected from study participants after overnight fasting and were stored at −80 degrees centigrade until analysis. In the autopsy study, the mean interval between the collection of the plasma samples and autopsy was 559 days with a mean postmortem delay of 1.1 days. Plasma concentration of clusterin was assayed by a commercially available sandwich ELISA kit (Human Clusterin ELISA, RD194034200R, Biovendor Laboratory Medicine Inc, Modric, Czech Republic). All samples were run in duplicate. Coefficients of variation of the ELISA assays were 3.1% in the neuroimaging and 3.8% in the autopsy studies respectively

Clusterin protein concentration in brain tissue (autopsy study)

Frozen brain sections (7 μm thick) were lysed in a five-fold volume of urea / thiourea buffer; 7M urea (Sigma, 57136), 2M thiourea (Sigma, 62566), 4% CHAPS (Sigma, 75621033), 2% DTT (Sigma, 110187), protease inhibitors (1tablet/10ml; Roche, 04693159001). Homogenization was carried out in a 3ml tissue grinder (Jensons), and the lysate spun at 15000g for 10 minutes. The supernatant was retained and the total protein concentration quantified with a Bradford assay. One-dimensional (SDS-PAGE) of proteins was carried out with the NuPAGE® Electrophoresis System as described above, using 4–12% Bis-Tris, 26W Novex® Pre-Cast Gels (WG1403BOX). Samples were prepared with a final concentration of 1μg/μl and 15ul volumes were run in duplicates together with a reference sample on each blot. Membranes were probed with mouse monoclonal antibody to clusterin (Millipore, 05354, 1:1000) and detected with a secondary antibody (Alexa Fluor goat anti-mouse 680, Invitrogen, A21057, 1:6000). Blots were further probed with an antibody against a housekeeping protein, VCP (vasolin-containing protein, Mr 97kDa) (Abcam,

ab11433, 1:2000) and normalized against it to correct for protein loading and transfer efficiency. Sample intensities were expressed as a ratio to the reference sample, adjusted to the intensity of VCP and the results from duplicate runs averaged.

Plasma clusterin concentration and longitudinal changes in brain volumes (neuroimaging study)

Our primary goal was to test whether MCI and control groups showed different relationships between baseline plasma concentration of clusterin and longitudinal changes in brain volume. Next, we investigated within-group (MCI and control separately) associations between baseline plasma clusterin concentration and rates of change in brain volumes.

Linear mixed effects models were used for longitudinal analyses of associations between plasma clusterin concentration and rates of change in brain volumes (Hartley and Rao, 1967; Shen and Davatzikos, 2002). The models were fit using PROC MIXED in SAS 9.1 (SAS Institute, Cary, NC) software. All the analyses were age and sex adjusted. Regional brain volumes were the dependent variables. We first estimated the rates of change for the control and MCI groups as well as inter-group differences in rates of change in brain volumes as reported previously in a larger sample (Driscoll et al., 2009) in this subset of participants with plasma clusterin concentration data. We included in the fixed effects part of the model, intracranial volume, sex, baseline age (age0), diagnosis (mci vs normal), time from baseline in years (time) and interaction between time and sex, age0, and diagnosis.

To test group differences in the associations between baseline clusterin concentration and rates of change in brain volumes, we included the following additional covariates in this model: baseline plasma clusterin concentration, diagnosis*clusterin concentration, clusterin concentration*time and diagnosis*clusterin concentration*time. In regions where the 3-way interaction term diagnosis*clusterin concentration*time was non-significant, the term was dropped from the model. This allowed us to examine the overall effect of baseline plasma clusterin concentration on rates of change in brain volumes. The random effects part of the model included intercept and time, allowing the intercept and slope to vary among individual subjects. The results are reported as standardized regression coefficients.

Results

Sample characteristics

In the neuroimaging study, participants diagnosed with MCI were significantly older than cognitively healthy controls and also had lower MMSE scores at baseline. Baseline performance scores on other cognitive tests in the two groups are shown in supplementary table-1. As expected, the MCI group performed worse than the control group on many of the cognitive tests. The groups did not differ significantly in sex, duration of follow-up, or number of years of education (Table-1A). The duration of follow up and the total number of visits for individuals in the MCI group was slightly lower (not statistically significant) than the control group as data from time points at and subsequent to a diagnosis of dementia were excluded from the analyses. The sample characteristics of AD and control subjects included in the autopsy study are shown in table-1B.

Plasma clusterin concentration (neuroimaging study)

There was a trend for higher plasma clusterin concentration in the MCI (134.5±42.0 μg/ml) compared to the control (117.4±44.8 μg/ml) group. However this difference did not reach statistical significance (p=0.15).

There was no significant effect of age on plasma clusterin concentration in either the MCI (Spearman correlation rho = −0.22, P-value = 0.42). or control (Spearman correlation rho = 0.02, P-value = 0.79) groups.

Differences in rates of change in brain volumes between MCI and control groups (neuroimaging study)

Consistent with our prior report in the full sample (Driscoll et al., 2009), we observed significantly greater declines over time in MCI compared with controls in several global (whole brain (p=0.02), total gray matter(p=0.04)), lobar (frontal and temporal gray matter (p=0.03 and 0.0002 respectively)) and regional ((superior, middle and medial frontal gyri (p=0.002, 0.01 and 0.04 respectively), orbitofrontal gyrus (p=0.006), middle temporal gyrus (0.002), hippocampus (p=0.02), entorhinal cortex, (p=0.04) inferior occipital gyrus (p=0.03) and insula (p=0.0001)) brain volumes. The MCI group also showed a greater increase in total ventricular CSF (p=0.02) over time.

Inter-group (MCI versus control) differences in the associations between baseline plasma clusterin concentration and longitudinal changes in brain volume (neuroimaging study)

There were significant diagnosis by baseline clusterin concentration by time interactions for a number of brain measurements. These 3-way interactions indicated that the associations between baseline plasma clusterin concentrations and rates of change in brain volumes differed between MCI and control groups in several global and lobar volumes including whole brain (p=0.005), vCSF (p=0.03), temporal gray matter (p=0.05), and total and temporal white matter (p=0.04, 0.02 respectively). Similar results also were observed in many individual regions including superior parietal lobule (p=0.03), superior temporal gyrus (p=0.02), parahippocampal gyrus (p=0.02) and the cingulate gyrus (0.04) (figure-1 and supplementary table-2).

Within group (MCI and control separately) associations between baseline plasma clusterin concentration and longitudinal changes in brain volume (neuroimaging study)

Associations between baseline clusterin concentrations and rates of change in brain volumes within each group were next investigated in regions showing significant between-group differences in associations. We observed significant associations between plasma clusterin concentration and longitudinal changes in brain volume within the MCI group but not the control group in several regions while there were few significant associations within the control group (figure-1 and supplementary table-2). The direction of association between baseline concentration of plasma clusterin and slope of change in brain volumes in subjects with MCI indicated that higher clusterin concentration is associated with lower rates of brain atrophy in this group of at-risk individuals.

Associations between clusterin protein concentration in plasma and brain (autopsy study)

As we have found an association between plasma clusterin levels and rates of atrophy in people at risk of dementia we wanted to explore whether plasma clusterin was a marker of brain clusterin levels. In these experiments, we used an independent set of autopsy samples derived from both AD cases and healthy controls. These individuals represented a continuum of neuropathological changes i.e. Braak stages 1 to 3 in controls and 3 to 6 in AD patients (Braak et al., 1998) (table-1B). We observed a significant association between plasma concentration of clusterin and that in the superior temporal gyrus (Pearson correlation coefficient R=0.45, p=0.04) but not in the other regions examined (hippocampus; (Pearson correlation coefficient R= 0.26, p=0.25 and cerebellum; Pearson correlation coefficient R= −0.152, p=0.51). In order to confirm that the significant association between brain and plasma clusterin concentrations in the superior temporal gyrus was not driven by

the control group, we also examined this association within the AD group alone and found a similar positive association (Pearson correlation coefficient R=0.47, p=0.05).

Discussion

We investigated whether plasma clusterin concentration predicts subsequent rates of change in brain volumes in MCI and control individuals. To test whether peripheral clusterin concentration was related to progression of early pathological changes in incipient stages of the disease, we first determined whether associations between plasma clusterin concentration at baseline and longitudinal changes in brain volumes differed between MCI and cognitively normal participants from the neuroimaging substudy of the BLSA. We then further defined these group differences in associations by investigating associations between plasma clusterin concentration and rates of change in brain volumes within each group. Finally, we examined whether there was an association between peripheral concentration of clusterin and that in brain tissue samples in regions vulnerable to AD pathology.

We observed widespread and significant differences in the association of plasma clusterin concentration with rates of brain atrophy between cognitively normal and MCI groups for the same cohort of BLSA participants in whom we recently reported accelerated tissue loss in MCI compared to normal individuals (Driscoll et al., 2009).

Significant differences in associations occurred for several global, lobar and regional brain volumes. In most of these regions, associations between baseline clusterin concentration and subsequent rates of brain atrophy reached significance within the MCI (but not control) group. The direction of this effect suggests that higher concentrations of plasma clusterin are associated with lower rates of brain atrophy in the MCI individuals. In addition to the associations with global rates of atrophy (whole brain and vCSF), higher plasma clusterin concentration was related to lower rate of atrophy in temporal gray matter in the MCI group. Other regional brain volumes showing similar associations in MCI included those vulnerable to pathological changes in AD, including superior temporal, parahippocampal and cingulate gyri (figure-1).

By showing that peripheral concentration of clusterin is associated with changes in brain volumes over time in individuals with MCI, our current observations provide further evidence linking clusterin with pathological processes intrinsic to AD. While we have not addressed the precise mechanisms underlying this association, several lines of evidence suggest biological roles for clusterin in pathways relevant to AD pathogenesis, including amyloid clearance, complement modulation and apoptosis (DeMattos et al., 2004; DeMattos et al., 2002; French et al., 1994; Nuutinen et al., 2009; Tschopp and French, 1994). Our current results also suggest that clusterin is elevated in the process of AD, perhaps as a protective mechanism. The fact that higher clusterin levels predict slower rates of atrophy in MCI may reflect its role in enhancing amyloid clearance and/or modulating neuroinflammation during disease progression, such as in individuals with MCI. Consistent with this hypothesis, a previous post-mortem study suggested a neuroprotective role for clusterin in brain regions vulnerable to AD pathology (Giannakopoulos et al., 1998). Previous reports on differences in clusterin concentration in the CSF between AD and controls have been inconclusive (Lidstrom et al., 2001; Sihlbom et al., 2008). More recently, Schrijvers and colleagues reported a significant association between higher plasma clusterin concentration and disease severity in AD as well as an increase relative to healthy controls and suggest that increased expression of clusterin in AD reflects a neuroprotective response.

These findings extend our recent observations on the association between plasma clusterin concentration and endophenotypes of disease pathology in patients with established AD

(Thambisetty et al., 2010). In this previous cross-sectional investigation, we found that higher concentrations of plasma clusterin were associated with greater atrophy of the entorhinal cortex in AD. In the light of these previous results, we predicted that we would observe a similar direction of effect in this longitudinal analysis of MCI and control subjects. However, contrary to our expectation, we found a significant association between increased plasma clusterin concentration and decreased rate of brain atrophy in MCI subjects. One plausible explanation might be that in individuals with MCI, elevated plasma clusterin is associated with decreased rate of atrophy as would be expected for a protective mechanism. However, in individuals with established AD, the association of plasma clusterin with pathology may reflect the eventual failure of such protective mechanisms in the setting of genetic risk factors and/or adverse environmental influences. This model would predict that in individuals with established AD, increased plasma clusterin concentration and its association with greater extent of brain atrophy might be indicative of a more ‘aggressive’ disease course. Indeed, in our previous cross-sectional study, we observed that higher baseline plasma clusterin concentration in patients with established AD was associated with a faster rate of clinical progression (Thambisetty et al., 2010). While this hypothesis is in line with experimental evidence suggesting a protective effect of clusterin in pathological processes in AD (Calero et al., 2000), we acknowledge that alternative models to explain our observations are possible. The potential role of clusterin in pathological processes distinct from brain atrophy was highlighted by a recent study where the Alzheimer risk variant of the clusterin gene (CLU) was associated with lower white matter integrity in young healthy adults (Braskie et al., 2011). Future studies combining analyses of genetic variation in the CLU gene with clusterin protein concentration in relation to pathological processes in at-risk individuals are likely to be revealing of the precise role of clusterin in Alzheimer’s biology.

Finally, in order to test whether plasma clusterin concentration reflects its role in the brain in response to AD pathology, we also asked whether peripheral concentration of clusterin was associated with its concentration in brain regions vulnerable to such pathological changes. By demonstrating a significant association between plasma concentration of clusterin and its concentration within the superior temporal gyrus, a region especially vulnerable to AD pathology (Whitwell et al.), we suggest that peripheral clusterin levels do reflect its biological role in brain regions undergoing pathological changes in AD. The precise mechanisms underlying this observation however remain to be identified as it is unclear whether changes in plasma clusterin levels are a consequence of early brain pathology in incipient AD or precede these changes and are a systemic peripheral signature in response to physiological stress such as oxidative damage. It is worth noting in this context that a variety of perturbations including changes in temperature, pH, oxidative stress and protease degradation can provoke extracellular chaperone responses from proteins like clusterin (Wyatt et al., 2011) (Naiki and Nagai, 2009).

The strengths of this study include the longitudinal design incorporating data points from a large number (>900 MRI observations) of serial assessments, relatively long follow-up interval and extensive characterization of the study sample. However, some caveats must be considered in the interpretation of our results. Since our principal aim was to undertake an exploratory analysis of the association between plasma clusterin concentration and longitudinal changes in brain volumes in MCI and control individuals, we have chosen to present results from all brain regions examined without correction for multiple comparisons. Furthermore, as the outcome variables in these analyses were measures of changes in brain volumes that are themselves highly correlated with each other, correcting for multiple comparisons in this scenario is especially problematic. While our approach might increase the risk of type-I error, we believe that it now allows for further directed analyses of the relationship between clusterin and AD-related changes in specific brain regions as well as

testing *a priori* hypotheses on the putative role of clusterin as a biological modifier of such changes.

In summary, our results substantially extend recent studies that have found an association between plasma clusterin concentration and measures of disease severity and progression in patients with AD. By demonstrating an association between plasma clusterin concentration and longitudinal changes in regional brain volumes in at-risk older individuals, they add to the mounting evidence linking this multi-functional lipoprotein with pathological mechanisms in Alzheimer's disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

Braak H, Braak E, Bohl J, Bratzke H. Evolution of Alzheimer's disease related cortical lesions. J Neural Transm Suppl. 1998; 54:97–106. [PubMed: 9850918]

Braskie MN, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI, Martin NG, Wright MJ, Ringman JM, Toga AW, Thompson PM. Common Alzheimer's Disease Risk7 Variant Within the CLU Gene Affects White Matter Microstructure in Young Adults. J Neurosci. 2011; 31:6764–6770. [PubMed: 21543606]

Calero M, Rostagno A, Matsubara E, Zlokovic B, Frangione B, Ghiso J. Apolipoprotein J (clusterin) and Alzheimer's disease. Microscopy Research and Technique. 2000; 50:305–315. [PubMed: 10936885]

Cummings JL, Doody R, Clark C. Disease-modifying therapies for Alzheimer disease: challenges to early intervention. Neurology. 2007; 69:1622–1634. [PubMed: 17938373]

DeMattos RB, Cirrito JR, Parsadanian M, May PC, O'Dell MA, Taylor JW, Harmony JA, Aronow BJ, Bales KR, Paul SM, Holtzman DM. ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism in vivo. Neuron. 2004; 41:193–202. [PubMed: 14741101]

DeMattos RB, O'Dell MA, Parsadanian M, Taylor JW, Harmony JA, Bales KR, Paul SM, Aronow BJ, Holtzman DM. Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A. 2002; 99:10843–10848. [PubMed: 12145324]

Driscoll I, Davatzikos C, An Y, Wu X, Shen D, Kraut M, Resnick SM. Longitudinal pattern of regional brain volume change differentiates normal aging from MCI. Neurology. 2009; 72:1906–1913. [PubMed: 19487648]

Folstein MF, Robins LN, Helzer JE. THE MINI-MENTAL STATE EXAMINATION. Archives of General Psychiatry. 1983; 40:812–812. [PubMed: 6860082]

French LE, Wohlwend A, Sappino AP, Tschopp J, Schifferli JA. Human clusterin gene expression is confined to surviving cells during in vitro programmed cell death. J Clin Invest. 1994; 93:877–884. [PubMed: 8113419]

Giannakopoulos P, Kovari E, French LE, Viard I, Hof PR, Bouras C. Possible neuroprotective role of clusterin in Alzheimer's disease: A quantitative immunocytochemical study. Acta Neuropathologica. 1998; 95:387–394. [PubMed: 9560017]

Goldszal AF, Davatzikos C, Pham DL, Yan MX, Bryan RN, Resnick SM. An image-processing system for qualitative and quantitative volumetric analysis of brain images. J Comput Assist Tomogr. 1998; 22:827–837. [PubMed: 9754125]

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schurmann B, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Hull M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet. 2009; 41:1088–1093. [PubMed: 19734902]

Hartley HO, Rao JN. Maximum-likelihood estimation for the mixed analysis of variance model. Biometrika. 1967; 54:93–108. [PubMed: 6049561]

Kraemer HC, Tinklenberg J, Yesavage JA. HOW FAR VS HOW FAST IN ALZHEIMERS-DISEASE - THE QUESTION REVISITED. Archives of Neurology. 1994; 51:275–279. [PubMed: 8129639]

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fievet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanche H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M, Amouyel P. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet. 2009; 41:1094–1099. [PubMed: 19734903]

Lidstrom AM, Hesse C, Rosengren L, Fredman P, Davidsson P, Blennow K. Normal levels of clusterin in cerebrospinal fluid in Alzheimer's disease, and no change after acute ischemic stroke. J Alzheimers Dis. 2001; 3:435–442. [PubMed: 12214028]

Lyketsos CG, Szekely CA, Mielke MM, Rosenberg PB, Zandi PP. Developing new treatments for Alzheimer's disease: the who, what, when, and how of biomarker-guided therapies. Int Psychogeriatr. 2008; 20:871–889. [PubMed: 18498669]

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. CLINICAL-DIAGNOSIS OF ALZHEIMERS-DISEASE - REPORT OF THE NINCDS- ADRDA WORK GROUP UNDER THE AUSPICES OF DEPARTMENTOF- HEALTH-AND-HUMAN-SERVICES TASK-FORCE ON ALZHEIMERSDISEASE. Neurology. 1984; 34:939–944. [PubMed: 6610841]

Naiki H, Nagai Y. Molecular Pathogenesis of Protein Misfolding Diseases: Pathological Molecular Environments Versus Quality Control Systems Against Misfolded Proteins. Journal of Biochemistry. 2009; 146:751–756. [PubMed: 19643812]

Nuutinen T, Suuronen T, Kauppinen A, Salminen A. Clusterin: a forgotten player in Alzheimer's disease. Brain Res Rev. 2009; 61:89–104. [PubMed: 19651157]

Pengas G, Pereira JM, Williams GB, Nestor PJ. Comparative reliability of total intracranial volume estimation methods and the influence of atrophy in a longitudinal semantic dementia cohort. J Neuroimaging. 2009; 19:37–46. [PubMed: 18494772]

Petersen RC. Mild cognitive impairment as a diagnostic entity. Journal of Internal Medicine. 2004; 256:183–194. [PubMed: 15324362]

Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age, neuropathology, and dementia. N Engl J Med. 2009; 360:2302–2309. [PubMed: 19474427]

Schrijvers EM, Koudstaal PJ, Hofman A, Breteler MM. Plasma clusterin and the risk of Alzheimer disease. JAMA. 2011; 305:1322–1326. [PubMed: 21467285]

Seshadri S, Fitzpatrick AL, Ikram MA, Consortia CGE. Genome-wide Association Study of Genetic Loci and Alzheimer Disease Reply. Jama-Journal of the American Medical Association. 2010; 304:858–858.

Shen D, Davatzikos C. HAMMER: hierarchical attribute matching mechanism for elastic registration. IEEE Trans Med Imaging. 2002; 21:1421–1439. [PubMed: 12575879]

Sihlbom C, Davidsson P, Sjogren M, Wahlund LO, Nilsson CL. Structural and quantitative comparison of cerebrospinal fluid glycoproteins in Alzheimer's disease patients and healthy individuals. Neurochem Res. 2008; 33:1332–1340. [PubMed: 18288611]

Song F, Poljak A, Smythe GA, Sachdev P. Plasma biomarkers for mild cognitive impairment and Alzheimer's disease. Brain Res Rev. 2009; 61:69–80. [PubMed: 19464319]

Thambisetty M, Simmons A, Velayudhan L, Hye A, Campbell J, Zhang Y, Wahlund LO, Westman E, Kinsey A, Guntert A, Proitsi P, Powell J, Causevic M, Killick R, Lunnon K, Lynham S, Broadstock M, Choudhry F, Howlett DR, Williams RJ, Sharp SI, Mitchelmore C, Tunnard C, Leung R, Foy C, O'Brien D, Breen G, Furney SJ, Ward M, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Hodges A, Murphy DGM, Parkins S, Richardson JC, Resnick SM, Ferrucci L, Wong DF, Zhou Y, Muehlboeck S, Evans A, Francis PT, Spenger C, Lovestone S. Association of Plasma Clusterin Concentration With Severity, Pathology, and Progression in Alzheimer Disease. Archives of General Psychiatry. 2010; 67:739–748. [PubMed: 20603455]

Tschopp J, French LE. Clusterin: modulation of complement function. Clin Exp Immunol. 1994; 972(Suppl):11–14. [PubMed: 8070141]

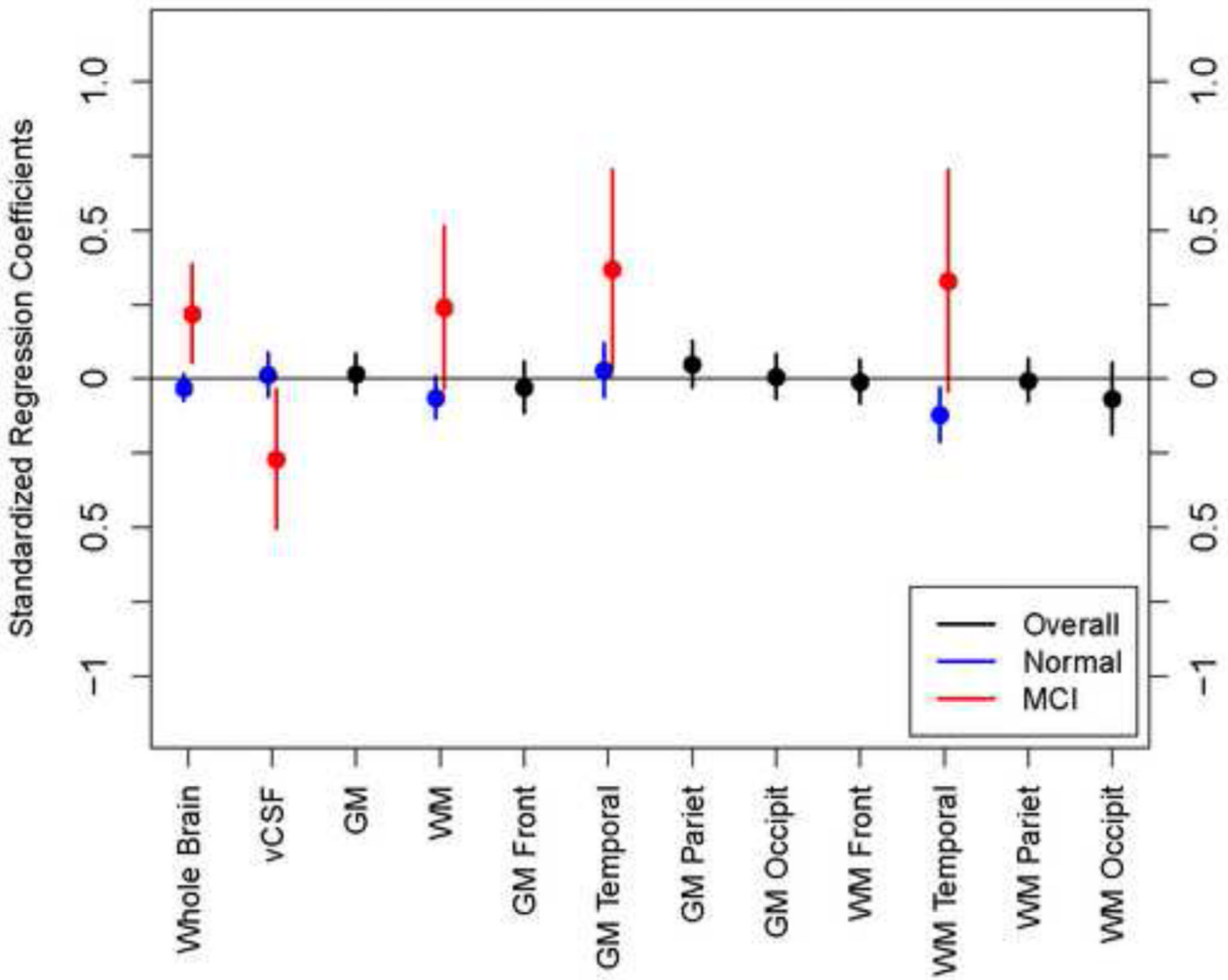
Whitwell JL, Jack Jr CR Jr, Przybelski SA, Parisi JE, Senjem ML, Boeve BF, Knopman DS, Petersen RC, Dickson DW, Josephs KA. Temporoparietal atrophy: A marker of AD pathology independent of clinical diagnosis. Neurobiology of Aging. In Press, Corrected Proof.

Wyatt AR, Yerbury JJ, Berghofer P, Greguric I, Katsifis A, Dobson CM, Wilson MR. Clusterin facilitates in vivo clearance of extracellular misfolded proteins. Cell Mol Life Sci. 2011

HIGHLIGHTS

- Plasma clusterin concentration is associated with rates of brain atrophy in MCI
- Plasma clusterin levels reflect its concentration in brain regions with AD pathology
- Peripheral concentration of clusterin reflects its role in early AD pathology

1A



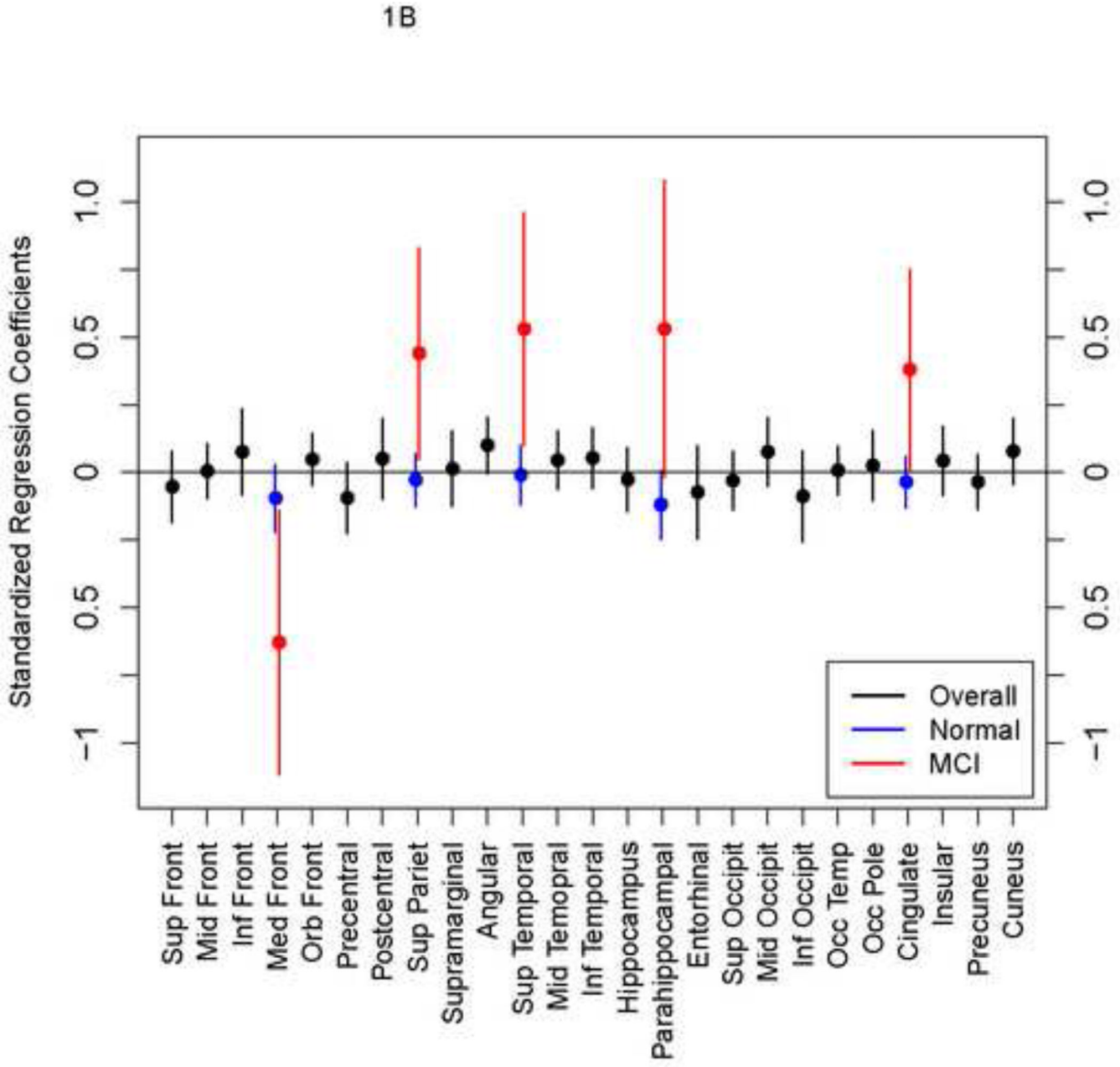


Figure-1.

Dot plots of estimated standardized regression coefficients showing the association of plasma clusterin concentration with rates of change in brain volumes with the corresponding 95% confidence intervals. Estimated regression coefficients for MCI (red dots) and healthy control (blue dots) groups are plotted separately for regions that show significant inter-group (MCI versus control) differences in the association between clusterin concentration and longitudinal changes in brain volumes. The single overall effects (pooled across control and MCI groups; black dots) are plotted for regions that did not show significant interg-roup differences in the association between clusterin concentration and longitudinal changes in brain volumes. If the 95% CI crosses the zero reference line, the effect is not statistically significant at p=0.05 level. For example, figure-1A shows a significant inter-group (MCI

versus control) difference in the association between baseline plasma clusterin concentration and longitudinal changes in the temporal gray matter volume. This difference is therefore shown in distinct colors of the estimated standardized regression coefficients i.e. control (blue) and MCI (red). Furthermore, in this region, the within-group (MCI and control separately) association between plasma clusterin concentration and longitudinal changes in temporal gray matter volume reached statistical significance only in the MCI group (i.e. the 95% CI does not cross the zero reference line) but not in the control group (i.e. the 95% CI crosses the zero reference line). The same figure shows a black dot representing the single overall effects of the association between clusterin concentration and rates of change in the parietal gray matter volume as there were no significant inter-group differences in the association in this region. Fig. 1A shows global and lobar brain volumes. Fig. 1B shows regional brain volumes.

Table-1

A. Sample characteristics of participants included in the neuroimaging study.								
	N	**Sex (male)**	**Age (baseline)**	**Number of visits**	**Interval (years)**	**Education (years)**	**Clusterin (μg/ml)**	**MMSE (baseline)**
Total sample	139	82	70.5 (7.7)	6.5 (2.5)	6.0 (2.5)	16.2 (2.8)	119.6 (44.7)	28.9 (1.5)
Control	123	73	69.6 (7.4)	6.7 (2.5)	6.1 (2.5)	16.3 (2.7)	117.4 (44.8)	29.1 (1.1)
MCI	16	9	77.0 (7.1)	5.6 (2.3)	5.1 (2.3)	15.6 (3.7)	134.5 (42.0)	27.5 (2.7)
p-value of difference		0.81	0.00	0.10	0.11	0.32	0.15	0.04

B. Sample characteristics of participants included in the autopsy study.								
GROUP	**N**	**Sex (male)**	**Age (baseline) (SD)**	**Age at death (SD)**	**Age when blood sample collected (SD)**	**Clusterin (μg/ml) (SD)**	**Average MMSE at baseline (SD)**	**Braak score (range)**
Total	21	5		87(6.43)	85 (6.39)	102.8 (22.6)	12 (10.93)	1–6
Control	4	2		86 (7.41)	81 (7.49)	88.0 (7.2)	28 (2.08)	1–3
AD	17	3		87 (6.42)	86 (6.36)	106.3 (23.7)	8 (8.68)	3–6