Original Article

The Association of Serum Clusterin Levels and *Clusterin* rs11136000 Polymorphisms with Alzheimer Disease in a Turkish Cohort

Gamze Guven, Ebru Ozer, Basar Bilgic[1], Hasmet Hanagasi[1], Hakan Gurvit[1], Ebba Lohmann[2,3], Nihan Erginel-Unaltuna

Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, TR, [1]Department of Neurology, Istanbul Faculty of Medicine, Behavioural Neurology and Movement Disorders Unit, Istanbul University, Istanbul, TR, [2]Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, GER, [3]DZNE, German Center for Neurodegenerative Diseases, Tübingen, GER

Abstract

Objectives: Several large-scale genome association studies have shown that variants in the "*Clusterin"'* (*CLU*) gene are important risk factors for Alzheimer's disease (AD). It has also been shown that plasma CLU levels were elevated in patients with AD and associated with disease severity and progression. In this study, we aimed to investigate whether the *CLU* rs11136000 polymorphism was associated with AD in our cohort of Turkish patients. We also evaluated the association of serum CLU levels and rs11136000 genotypes between patients and controls. **Materials and Methods:** Genotyping was performed in 327 patients who were diagnosed as having AD (mean age: 67.2 ± 10.8 years) and 344 controls (mean age: 57.7 ± 13.1 years). The rs11136000 genotypes were determined using quantitative real-time polymerase chain reaction with hydrolysis probes. Serum CLU levels were assessed in 25 patients with AD and 10 controls using enzyme-linked immunosorbent assay. **Results:** Our results showed no significant difference in genotype and allele frequencies of CLU rs11136000 polymorphisms between patients with AD and controls. Serum CLU levels in patients with AD did not differ from those of the controls. Furthermore, serum CLU levels showed no major difference between carriers of CC and TT + CT genotypes in the controls and patients with AD. **Conclusion:** Our results suggest that the *CLU* rs11136000 polymorphism is not associated with AD in our Turkish patients, and rs11136000 genotypes may not have an effect on serum CLU levels.

Keywords: *Alzheimer's disease, Clusterin, polymorphism, serum, Turkey*

Submitted: 25-Oct-2019
Revised: 10-Apr-2020
Accepted: 11-Apr-2020
Published: 16-Sep-2020

Introduction

Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disease characterized by decreased daily living activities and impaired cognitive abilities. Various factors have been identified in the pathogenesis of the disease including genetic factors. Numerous large-scale genome-wide association studies have identified several susceptibility genes associated with AD.[1] Since the discovery of the cholesterol-related gene "*Apolipoprotein E*" (*APOE*) epsilon 4 (ε4) allele, as the major risk variant for AD, it has been assumed that other genes involved in the metabolism of cholesterol may also be risk factors for the AD. Among these genes, "*Clusterin"* (*CLU*), also known as "*Apolipoprotein J,"* has been strongly associated with an increased risk of AD.

CLU is a lipoprotein and multifunctional chaperone protein expressed in almost all mammalian tissues most abundantly in the brain.[2] CLU specifically binds soluble amyloid β (Aβ) in cerebrospinal fluid (CSF) and is involved in the transport of Aβ across the blood–brain barrier.[3-5] It is also involved in the aggregation, deposition, and toxicity of Aβ.[6] Studies have shown that CLU protein levels in CSF and plasma were increased in patients with AD,[7-10] and plasma CLU levels were associated with disease severity and rapid cognitive decline.[11]

Address for correspondence: Dr. Gamze Guven, Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, TR.
E-mail: gguven@istanbul.edu.tr

Access this article online

Quick Response Code:

Website: www.nsnjournal.org

DOI: 10.4103/NSN.NSN_46_20

How to cite this article: Guven G, Ozer E, Bilgic B, Hanagasi H, Gurvit H, Lohmann E, *et al.* The association of serum clusterin levels and *Clusterin* rs11136000 polymorphisms with Alzheimer disease in a Turkish cohort. Neurol Sci Neurophysiol 2020;37:134-40.

In this study, our aim was to investigate the association between AD and the intronic *CLU* rs11136000 (NC_000008.11:g.27607002T>C)polymorphism, which has been previously shown to have a significant association with AD, in a cohort of Turkish patients. To evaluate the effects of this polymorphism on peripheral CLU levels, we compared the serum CLU levels among rs11136000 genotype carriers.

Materials and Methods

Patients and controls

The study population comprised 327 patients who were diagnosed as having AD and 344 controls with no history of any major neurologic, psychiatric, or systemic disease. All participants underwent detailed clinical and neuropsychological examinations, neuroimaging in most cases. The diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's disease.[12] The Mini-Mental State Examination test (MMSE) and Geriatric Depression Scale (GDS) were used to evaluate the global cognitive status and the presence of depressive symptoms in participants.

The study was approved by the Ethics Committee of the university (Date: October, 17, 2016, No: 1209). A neurologist took the necessary clinical information after obtaining informed consent from the patients or their legally authorized representatives.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes according to the standard procedures. Genotyping of *CLU* and *APOE* was performed using real-time polymerase chain reaction with labeled hydrolysis probes. Ten milliliters of reaction mixture consisted of 50 ng genomic DNA, 10 μM of each primer, 1 μM of each probe, and 5 μL of ×2 LC480 Probes Master. Amplification was performed using a LightCycler 480 real-time PCR instrument (Roche Diagnostics, Germany). Cycling conditions were 2 min at 50°C; 10 min at 95°C; 45 cycles of 95°C for 10 s; 56°C for 30 s; and 72°C for 1 s. The endpoint analysis was assessed using the LightCycler 480 genotyping software.

Measurement of serum clusterin levels

Serum samples were collected for the measurement of CLU levels. Only 35 individuals were available for serum sampling; therefore, CLU levels were measured in this randomly selected group. Peripheral blood samples were collected, centrifuged at 1400 g for 10 min, and serum samples were stored at −80°C. CLU levels in serum samples were determined using a Human CLU ELISA kit (BioVendor, Heidelberg, Germany) in duplicate according to the manufacturer's protocol.

Statistical analysis

Genotype and allelic distributions were compared using Pearson's Chi-square test. The Hardy–Weinberg equilibrium (HWE) was computed to the expected genotype distribution. The independent *t*-test was used to compare continuous variables and expressed as means and standard deviation, whereas categorical variables were compared using the Chi-square test. Maximum likelihood estimates of odds ratios (OR) and associated 95% confidence intervals (CIs) were calculated for dominant, recessive, and overdominant comparison models through logistic regression analysis adjusted for age, sex, and *APOE* ε4 carrier status (carrier and noncarrier) as covariates. The association between rs11136000 genotypes and lipid parameters was determined through univariate analysis of variance using age, sex, and *APOE* ε4 carrier status as covariates. Correlations between serum CLU levels and demographic and biochemical characteristics were tested using Spearman's rho. All statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 software (IBM Corp, Armok, NY, USA). The results were considered statistically significant with $P < 0.05$.

Results

Demographic and biochemical characteristics of the study group

The clinical data and lipid parameters of all patients and controls are summarized in Table 1. As shown in the Table 1, *APOE* ε4 allele carriers were significantly more frequent ($P < 0.001$) in the AD group. In addition, the total MMSE score was found to be significantly decreased, as expected in patients with AD. Furthermore, the GDS scores of the patient group were significantly increased.

There was also a statistically significant difference ($P < 0.001$) in terms of age between the AD (mean age: 67.2 ± 10.8 years) and controls (mean age: 57.7 ± 13.1 years). Lipid parameters showed no statistically significant difference between the two groups.

Allele and genotype frequencies of the *Clusterin* rs11136000 polymorphism

For allele and genotype analyses, patients with AD were divided into two groups according to age of onset: late-onset AD (LOAD; age of onset ≥65 years, mean age: 76.5 ± 4.9 years, $n = 154$) and early-onset AD (EOAD; age of onset <65 years, mean age:

58.9 ± 7.5 years, *n* = 173). The AD groups were compared with their age matched controls. The demographic and clinical characteristics of these groups are given in Supplementary Table S1. The allele and genotype frequencies were compared between LOAD, EOAD, and all patients with AD versus controls age ≥70 years, controls age <70 years, and all controls, respectively. The distribution of genotypes was in HWE in all groups. The allele and genotype distributions of *CLU* rs11136000 polymorphisms in patients and controls within each study group are shown in Table 2. There was no statistically significant difference in allele and genotype frequencies in any of the groups. We also tested the association of *CLU* rs11136000 genotypes

Table 1: Descriptive characteristics and lipid parameters of patients and controls

	Patients (*n*=327)	Controls (*n*=344)	*P*
Age at recruitment, years	67.2±10.8	57.7±13.1	<0.001
Age of onset, years	63±10.6		
Sex, *n* (%)			
Male	132 (40.4)	158 (45.9)	0.146
Female	195 (59.6)	186 (54.1)	
MMSE score	18.1±7.7 (*n*=240)	29.06±1.3 (*n*=50)	<0.001
GDS	10.9±6.4 (*n*=97)	6.9±5.3 (*n*=26)	0.004
APOE ε4 status, *n* (%)			
ε4 carrier	125 (38.7)	47 (13.8)	<0.001
ε4 noncarrier	198 (61.3)	293 (86.2)	
Triglyceride (mg/dL)	150.6±73.3 (*n*=195)	139.8±65.7 (*n*=55)	0.325
Total cholesterol (mg/dL)	210.9±48.6 (*n*=199)	207.7±47 (*n*=55)	0.666
LDL (mg/dL)	131.6±42.2 (*n*=159)	134.1±41.6 (*n*=49)	0.723
HDL (mg/dL)	53.9±15.9 (*n*=198)	51.3±16.9 (*n*=54)	0.292
VLDL (mg/dL)	27.04±11.1 (*n*=149)	25.7±9.0 (*n*=37)	0.481

Continuous variables are presented as mean±SD and dichotomous variables as percentage. A *t*-test was used for comparison of means and Chi-square test for percentages. GDS: Geriatric depression scale, MMSE: Mini-Mental State Examination, *n*: Number of individuals, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very LDL, APOE: Apolipoprotein E

Table 2: Genotype and allele frequencies of *CLU* rs11136000 polymorphism in patients with Alzheimer's disease and controls in different groups

Group	CLU rs11136000	Patients (%)	Controls (%)	*P*	Genetic models	*P*†	OR (95%CI)
All subjects	Genotype						
	CC	141 (43.5)	156 (45.7)	0.831*	Dominant	0.65	1.08 (0.768-1.527)
	CT	142 (43.8)	142 (41.6)		Recessive	0.797	1.069 (0.643-1.779)
	TT	41 (12.7)	43 (12.6)		Over-dominant	0.783	1.05 (0.744-1.482)
	Allele						
	C	424 (65.4)	454 (66.6)	0.66			1.05 (0.83-1.32)
	T	224 (34.6)	228 (33.4)				
≥65 years	Genotype						
	CC	68 (44.4)	30 (40)	0.787*	Dominant	0.492	1.23 (0.677-2.247)
	CT	66 (43.1)	34 (45.3)		Recessive	0.699	1.18 (0.505-2.770)
	TT	19 (12.4)	11 (14.7)		Overdominant	0.678	1.33 (0.628-2.043)
	Allele						
	C	202 (66)	94 (62.7)	0.703			1.15 (0.77-1.73)
	T	104 (34)	56 (37.3)				
<65 years	Genotype						
	CC	73 (42.7)	126 (47.4)	0.630*	Dominant	0.463	1.174 (0.765-1.800)
	CT	76 (44.4)	108 (40.6)		Recessive	0.595	1.189 (0.628-2.248)
	TT	22 (12.9)	32 (12)		Overdominant	0.706	1.086 (0.707-1.669)
	Allele						
	C	222 (64.9)	360 (67.7)	0.399			0.88 (0.663-1.177)
	T	120 (35.1)	172 (32.3)				

Dominant: (TT and CT vs. CC), recessive: (TT vs. CC and CT), overdominant: (CT vs.CC and TT), **P* value was calculated using the Chi-square test for genotype distributions, †*P* value with adjustment of age, sex, and *APOE* ε4 status. OR: Odds ratio, CI: Confidence interval, *APOE*: Apolipoprotein E

using dominant, recessive, and overdominant models, which were adjusted for age, sex, and *APOE* ε4 carrier status [Table 2]. However, no significant AD risk was observed for *CLU* rs11136000 genotypes in different genetic models.

Association of *Clusterin* rs11136000 polymorphism with lipid parameters

Table 3 shows the estimated marginal mean values of the lipid parameters according to rs11136000 genotypes among the patients and controls adjusted for age, sex, and *APOE* ε4 carrier status. There were no statistically significant associations between the *CLU* rs11136000 genotypes and lipid parameters.

Serum Clusterin levels

Serum CLU levels were available for 25 patients with AD and 10 controls. The demographic and clinical characteristics of these patients and controls are given in Supplementary Table S2. As shown in Figure 1, the mean serum CLU level in patients with AD was 59.4 μg/mL (range: 43.2–85.5), which was similar to that of the controls (mean: 61.9 μg/mL, range: 46.1–84.2 μg/mL).

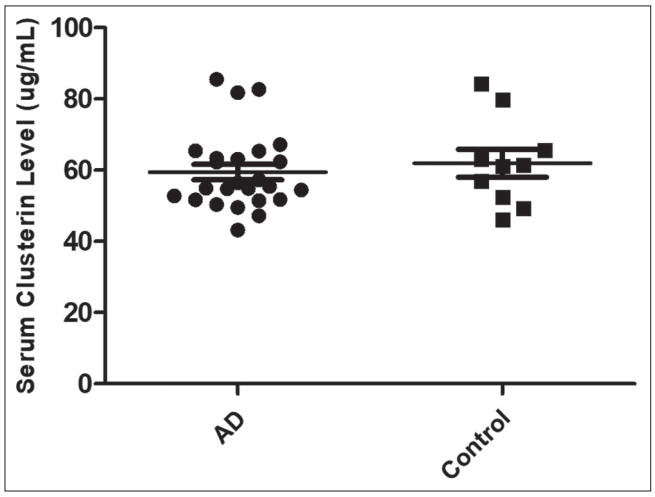

Figure 1: Serum clusterin levels in patients with Alzheimer's disease and controls. The mean is indicated together with the standard deviation (Alzheimer's disease, *n* = 23; Controls, *n* = 10)

To assess whether serum CLU levels were influenced by *CLU* rs11136000 genotypes, we examined CLU levels in the carriers of CC versus TT + CT genotypes in controls and patients with AD [Figure 2a and b]. In patients with AD, the CC carriers (mean: 57.5 μg/mL, range: 47.2–67.5 μg/mL n=10) tended to have lower serum CLU levels compared with TT + CT carriers (mean: 60.7 μg/mL, range: 43.2–85.5 μg/mL n=15). Likewise, in the control group, serum CLU levels of CC carriers (mean: 57.1 μg/mL, range: 49.3–65.7 μg/mL, *n* = 4) were lower than the levels for TT + CT carriers (mean: 65.1 μg/mL, range: 46.1–84.2 μg/mL, *n* = 6) carriers. Furthermore, the presence of the *APOE* ε4 alleles had no effect on serum CLU levels. The correlation analysis showed that there was no significant correlation between serum CLU levels and age, age at onset, MMSE, GDS, and lipid parameters.

Discussion

In the first step of our study, we investigated whether the *CLU* rs11136000 polymorphism was associated with the presence of AD in our cohort of Turkish patients with AD. In addition, we examined the distribution of rs11136000 genotypes in LOAD and EOAD groups and their age-matched controls.

Two different large-scale genome-wide association studies have shown that the *CLU* rs11136000 intronic polymorphism is significantly associated with LOAD in Caucasian populations.[13,14] Both studies suggested that the T allele of rs11136000 had a protective role, and carriers of this allele had a reduced risk of AD (OR <1), whereas the C allele, which is carried by ~88% of Caucasians,[13,14] was considered the risk allele and conferred a 1.16-fold increased risk for developing AD.[15] Thereafter, several studies investigating the association between the *CLU* rs11136000 polymorphism and AD were conducted in numerous populations, including Caucasian, Asian, African, and Hispanic.[1] However, the results of these studies were inconsistent, indicating that

Table 3: Estimated marginal mean values±standard deviation of lipid parameters according to *CLU* rs11136000 genotypes

	Patients			Controls		
	CC	CT+TT	*P*	CC	CT + TT	*P*
HDL	56.18±1.7 (*n*=81)	52.1±1.4 (*n*=113)	0.070	51.99±3.5 (*n*=22)	50.55±3.04 (*n*=29)	0.759
Triglyceride	153.31±8.4 (*n*=78)	149.6±6.9 (*n*=113)	0.735	143.07±13.9 (*n*=22)	141.40±11.9 (*n*=30)	0.929
VLDL	26.87±1.4 (*n*=60)	27.08±1.2 (*n*=87)	0.909	23.46±2.4 (*n*=14)	27.73±1.99 (*n*=20)	0.189
Cholesterol	215.65±5.2 (*n*=81)	207.89±4.4 (*n*=114)	0.259	194.31±9.8 (*n*=22)	213.95±8.37 (*n*=30)	0.140
LDL	134.01±5.0 (*n*=64)	130.52±4.2 (*n*=92)	0.594	121.96±9.7 (*n*=19)	138.84±8.07 (*n*=27)	0.199

P values were obtained by univariate analysis of variance for comparisons among *CLU* rs11136000 genotypes. Data are shown as mean±SE. Values were adjusted for age, gender, and *APOE* ε4 status. SE: Standard deviation, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very LDL, APOE: Apolipoprotein E

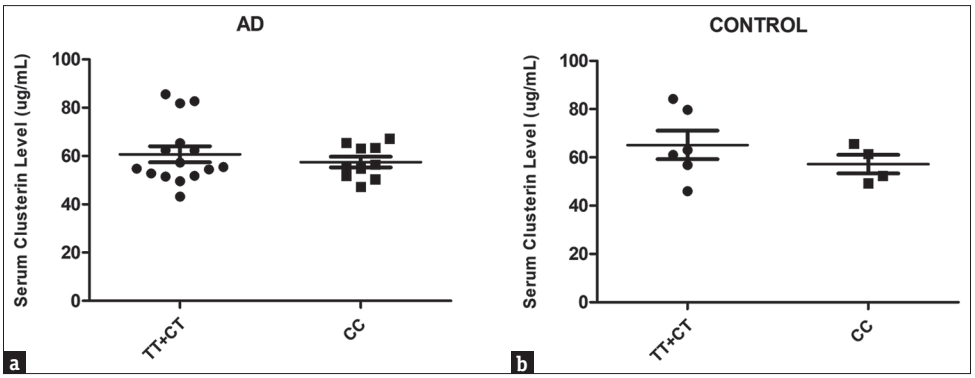

Figure 2: Serum clusterin levels based on *Clusterin* rs11136000 genotypes. (a) Alzheimer's disease (CC *n* = 10, TT + CT *n* = 15). (b) Controls (CC *n* = 4, TT + CT *n* = 6). The mean is indicated together with the standard deviation

the risk associated with the rs11136000 polymorphism might vary among the populations of different ethnic and geographic origins. A recent meta-analysis of 74,248 samples confirmed the association of the T allele with reduced AD risk in Caucasians but not in a Chinese population (OR = 0.864, 95% CI: 0.842–0.888; $P < 0.001$).[16] The T allele frequency varies between 0.33 and 0.43 in Caucasian populations. Consistent with these findings, in our study, the T allele frequency was found as 0.34 in the overall study group. Although the T allele frequency in our study was similar to that in other studies, we found no statistically significant difference in allelic and genotypic distributions between the groups. Our results were consistent with the two previous studies performed in Turkish patients with AD, which also found no significant associations.[17,18] However, it should not be ruled out because these studies were conducted in small-scale study groups, and therefore, further studies in larger cohorts are necessary to confirm the results reported in the Turkish cohort.

In addition, in our study, the relationship between the *CLU* rs11136000 polymorphism and different lipid parameters was also investigated, but no significant association was found. Apart from our study, only one other research group has investigated the association between rs11136000 genotypes and lipid parameters.[19] Aghajanpour-Mir *et al*. showed that the CC genotype of the rs11136000 polymorphism was associated with decreased high-density lipoprotein (HDL) cholesterol levels in Iranian patients with mild cognitive impairment (MCI) compared with controls and proposed that low HDL levels in CC carriers could increase the risk of developing MCI through altering glucose metabolism.[19] It has been known that higher levels of HDL are associated with decreased AD risk[20] and low levels are associated with increased risk.[21,22] However, we found unexpectedly high HDL levels in patients with AD with the CC risk genotype. The association between HDL levels and rs11136000 genotypes needs to be confirmed by further replicative studies because this finding was statistically nonsignificant.

Another goal of this study was to investigate the relationship between serum CLU levels and the presence of AD. High plasma CLU levels in patients with AD were reported to be associated with brain atrophy, disease severity, and rapid clinical progression.[11] Subsequently, several studies have shown elevated levels of plasma CLU both in patients with MCI[10,23-25] and AD.[8-10] These results suggest to the authors that high levels of plasma CLU in patients with AD and MCI may reflect a protective response to the neurodegenerative process.[8,10,26] By contrast, Schürmann *et al*.,[27] Silajdžić *et al*.[28] and Jongbloed *et al*.[24] found that plasma CLU levels were not significantly changed in patients with AD. Moreover, Ijsselstijn *et al*.[29] showed no statistically significant difference in serum CLU levels between presymptomatic patients with AD and controls, suggesting that serum CLU was not an early, presymptomatic biomarker for AD. Consistently, in our study, serum CLU levels of patients with AD were not different from those of controls. Due to the discrepancies between studies concerning plasma or serum CLU levels, additional studies measuring CLU levels in serum/plasma together with CSF will be needed to elucidate the exact role of CLU in the pathogenesis of AD.

Our study also investigated whether serum CLU levels were influenced by the rs11136000 genotypes. We observed no major effect of the rs11136000 genotypes on serum CLU levels either in patients with AD or in controls. In agreement with our data, Thambisetty *et al*.[11] showed that rs11136000 genotypes did not influence plasma CLU levels in patients with AD and controls. Furthermore, Cai *et al*.[25] found no significant association of rs11136000 genotypes with plasma CLU levels in patients with MCI. However, there are also some studies that showed significant associations. In the study by Schürmann *et al*.,[27] lower levels of plasma

CLU level were observed in the CC carriers among healthy controls, whereas no statistically significant difference was found in patients with AD. On the contrary, Mullan *et al*.[10] showed that plasma CLU levels were lower in TT genotype carriers in the control group. The conflicting results from different studies may be due to the fact that association between CLU expression and AD is independent of genetic variation in the *CLU* gene[11] or rs11136000 may not be the polymorphism that actually causes expression changes in the CLU protein.[9,25] However, we cannot exclude the effect of other *CLU* polymorphisms on CLU expression because this study only deals with the rs11136000 polymorphism. Different studies with a larger sample sizes should be performed to clarify the potential effects of the rs11136000 polymorphism on CLU expression levels.

Conclusion

Our results suggest that the *CLU* rs11136000 polymorphism may not be associated with AD risk in our cohort of Turkish patients, and rs11136000 genotypes may not have an effect on serum CLU levels. However, the relatively small sample size is a limitation of our study, which means that the results of this study should be interpreted carefully.

Acknowledgments

The authors thank all the patients and their families. Meltem Pak helped with obtaining the samples.

Financial support and sponsorship

This work was supported by the Research Fund of Istanbul University (Project No: TAB-2017-23218).

Conflicts of interest

There are no conflicts of interest.

References

1. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: The AlzGene database. Nat Genet 2007;39:17-23.
2. De Silva HV, Harmony JA, Stuart WD, Gil CM, Robbins J. Apolipoprotein J: Structure and tissue distribution. Biochemistry 1990;29:5380-9.
3. Ghiso J, Matsubara E, Koudinov A, Choi-Miura NH, Tomita M, Wisniewski T, *et al*. The cerebrospinal-fluid soluble form of Alzheimer's amyloid beta is complexed to SP-40,40 (apolipoprotein J), an inhibitor of the complement membrane-attack complex. Biochem J 1993;293(Pt 1):27-30.
4. Golabek A, Marques MA, Lalowski M, Wisniewski T. Amyloid beta binding proteins *in vitro* and in normal human cerebrospinal fluid. Neurosci Lett 1995;191:79-82.
5. Bell RD, Sagare AP, Friedman AE, Bedi GS, Holtzman DM, Deane R, *et al*. Transport pathways for clearance of human Alzheimer's amyloid β- peptide and apolipoproteins E and J in the mouse central nervous system. J Cereb Blood Flow Metab 2007;27:909-18.
6. Yerbury JJ, Poon S, Meehan S, Thompson B, Kumita JR, Dobson CM, *et al*. The extracellular chaperone clusterin influences amyloid formation and toxicity by interacting with prefibrillar structures. FASEB J 2007;21:2312-22.
7. Nilselid AM, Davidsson P, Nägga K, Andreasen N, Fredman P, Blennow K. Clusterin in cerebrospinal fluid: Analysis of carbohydrates and quantification of native and glycosylated forms. Neurochem Int 2006;48:718-28.
8. Schrijvers EM, Koudstaal PJ, Hofman A, Breteler MM. Plasma clusterin and the risk of Alzheimer disease. JAMA 2011;305:1322-6.
9. Xing YY, Yu JT, Cuic WZ, Zhonga XL, Wua ZC, Zhanga Q, *et al*. Blood clusterin levels, rs9331888 polymorphism, and the risk of Alzheimer's disease. J Alzheimer Dis 2012;29:515-9.
10. Mullan GM, McEneny J, Fuchs M, McMaster C, Todd S, McGuinness B, *et al*. Plasma clusterin levels and the rs11136000 genotype in individuals with mild cognitive impairment and Alzheimer's disease. Curr Alzheimer Res 2013;10:973-8.
11. Thambisetty M, Simmons A, Velayudhan L, Hye A, Campbell J, Zhang Y, *et al*. Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. Arch Gen Psychiatry 2010;67:739-48.
12. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr., Kawas CH, *et al*. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer Dement 2011;7:263-9.
13. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, *et al*. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009;41:1088-93.
14. Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, *et al*. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009;41:1094-9.
15. Bertram L, Tanzi RE. Alzheimer disease: New light on an old CLU. Nat Rev Neurol 2010;6:11-3.
16. Han Z, Qu J, Zhao J, Zou X. Analyzing 74,248 samples confirms the association between CLU rs11136000 polymorphism and Alzheimer's disease in Caucasian but not Chinese population. Sci Rep 2018;8:11062.
17. Sen A, Arslan M, Erdal M, İzci Ay Ö, Görücü YŞ, Kurt E, *et al*. Lack of associations between CLU and PICALM gene polymorphisms and Alzheimer's disease in a Turkish population. Ideggyogy Sz 2015;68:113-20.
18. Alaylıoğlu M, Gezen-Ak D, Dursun E, Bilgiç B, Hanağası H, Ertan T, *et al*. The Association between clusterin and APOE polymorphisms and late-ons *et al.* Zheimer disease in a Turkish cohort. J Geriatr Psychiatry Neurol 2016;29:221-6.
19. Aghajanpour-Mir M, Amjadi-Moheb F, Dadkhah T, Hosseini SR, Ghadami E, Assadollahi E, *et al*. Informative combination of CLU rs11136000, serum HDL levels, diabetes, and age as a new piece of puzzle-picture of predictive medicine for cognitive disorders. Mol Biol Rep 2019;46:1033-41.
20. Bonarek M, Barberger-Gateau P, Letenneur L, Deschamps V, Iron A, Dubroca B, *et al*. Relationships between cholesterol, apolipoprotein E polymorphism and dementia: A cross-sectional analysis from the PAQUID study. Neuroepidemiology 2000;19:141-8.
21. van Exel E, de Craen AJ, Gussekloo J, Houx P,

Bootsma-van der Wiel A, Macfarlane PW, *et al*. Association between high-density lipoprotein and cognitive impairment in the oldest old. Ann Neurol 2002;51:716-21.

22. Zuliani G, Cavalieri M, Galvani M, Volpato S, Cherubini A, Bandinelli S, *et al*. Relationship between low levels of high-density lipoprotein cholesterol and dementia in the elderly. The in chianti study. J Gerontol A Biol Sci Med Sci 2010;65:559-64.
23. Thambisetty M, An Y, Kinsey A, Koka D, Saleem M, Güntert A, *et al*. Plasma clusterin concentration is associated with longitudinal brain atrophy in mild cognitive impairment. Neuroimage 2012;59:212-7.
24. Jongbloed W, van Dijk KD, Mulder SD, van de Berg WD, Blankenstein MA, van der Flier W, *et al*. Clusterin levels in plasma predict cognitive decline and progression to Alzheimer's disease. J Alzheimers Dis 2015;46:1103-10.
25. Cai R, Han J, Sun J, Huang R, Tian S, Shen Y, *et al*. Plasma clusterin and the CLU Gene rs11136000 variant are associated with mild cognitive impairment in type 2 diabetic patients. Front Aging Neurosci 2016;8:179.
26. Nuutinen T, Suuronen T, Kauppinen A, Salminen A. Clusterin: A forgotten player in Alzheimer's disease. Brain Res Rev 2009;61:89-104.
27. Schürmann B, Wiese B, Bickel H, Weyerer S, Riedel-Heller SG, Pentzek M, *et al*. Association of the Alzheimer's disease clusterin risk allele with plasma clusterin concentration. J Alzheimers Dis 2011;25:421-4.
28. Silajdžić E, Minthon L, Björkqvist M, Hansson O. No diagnostic value of plasma clusterin in Alzheimer's disease. PLoS One 2012;7:e50237.
29. Ijsselstijn L, Dekker LJ, Koudstaal PJ, Hofman A, Sillevis Smitt PA, Breteler MM, *et al*. Serum clusterin levels are not increased in presymptomatic Alzheimer's disease. J Proteome Res 2011;10:2006-10.

Supplementary Table S1: Demographic and clinical characteristics of <65 years and ≥65 years groups

	<65 years			≥65 years		
	EOAD (AAO <65 years) (*n*=173)	**Controls (age <70 years) (*n*=268)**	***P***	**LOAD (AAO ≥65 years) (*n*=154)**	**Controls (age ≥70 years) (*n*=76)**	***P***
Age, years	58.9±7.5	52.7±10	<0.001	76.5±4.9	75.4±4.6	0.095
Age of onset, years	54.7±7			72.1±4.9		
Gender, *n* (%)						
Male	62 (35.8)	125 (46.6)	0.03	70 (45.5)	33 (43.4)	0.771
Female	111 (64.2)	143 (53.4)		84 (54.5)	43 (56.6)	
MMSE	16.78±8.4 (*n*=109)	29.3±1.1 (*n*=32)	<0.001	19.3±6.9 (*n*=131)	28.6±1.5 (*n*=18)	<0.001
GDR	10.7±6.2 (*n*=46)	6.7±5.6 (*n*=17)	0.022	11.2±6.7 (*n*=51)	7.4±5.1 (*n*=9)	0.120
APOE ε4 status, *n* (%)			<0.001			<0.001
ε4 carrier	65 (37.8)	37 (14)		60 (39.7)	10 (13.3)	
ε4 noncarrier	107 (62.2)	228 (86)		91 (60.3)	65 (86.7)	

GDS: Geriatric depression scale, MMSE: Mini-Mental State Examination, APOE: Apolipoprotein E, EOAD: Early-onset AD, LOAD: Late-onset AD, AAO: Age at onset

Supplementary Table S2: Demographic and clinical characteristics of patients and controls for which serum samples were available

	Patients (*n*=25)	**Controls (*n*=10)**	***P***
Age at recruitment, years	65.2±12.1	67.8±11.7	0.573
Age of onset, years	60±11.4		
Gender, *n* (%)			0.215
Male	7 (28)	5 (50)	
Female	18 (72)	5 (50)	
MMSE score	15.5±5.9 (*n*=20)	27.8±1.8 (*n*=6)	<0.001
APOE ε4 status, *n* (%)			0.018
ε4 carrier	10 (40)	0 (0)	
ε4 noncarrier	15 (60)	10 (100)	

Continuous variables are presented as mean±SD and dichotomous variables as percentage. A *t*-test was used for comparison of means and Chi-square test for percentages. MMSE: Mini-Mental State Examination, *n*: Number of individuals, APOE: Apolipoprotein E, SD: Standard deviation