Chapter 13

Clusterin (APOJ) in Alzheimer’s Disease: An Old Molecule with a New Role

Sarah K. Woody and Liqin Zhao

Additional information is available at the end of the chapter

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Abstract

Clusterin (CLU), initially identified in 1983 as a “clustering factor” in ram rete testis fluid, is a multifaceted protein that was re-discovered and subsequently renamed eight times from 1983 to 1992. CLU exists as multiple protein isoforms including the 80 kDa glycosylated mature/secreted form of CLU (mCLU) and the smaller non-modified nuclear and intracellular forms of CLU (nCLU and icCLU, respectively). These isoforms, which are expressed at the highest levels in the brain, are suggested to play distinct roles in various disease processes such as those involving inflammation and apoptosis. Currently, CLU, also known as apolipoprotein J (APOJ) which belongs to the same protein family as apolipoprotein E (APOE), is the third most significant genetic risk factor for the development of late-onset Alzheimer’s disease (LOAD); however, an extensive gap exists in the literature in understanding the physiological roles of CLU in normal brain and the pathogenic mechanisms conferred by CLU polymorphisms in the onset of LOAD. In this chapter, we discuss the status of the current knowledge regarding the generation and regulation of CLU protein isoforms, the clinical evidence and possible mechanisms involved in LOAD, and provide our perspectives for future studies.

Keywords: late-onset Alzheimer’s disease (LOAD), genetic risk factors, clusterin (CLU), apolipoprotein J (APOJ), apolipoprotein E (APOE)

1. Introduction

1.1. Alzheimer’s disease: current status and challenges

Alzheimer’s disease (AD) currently affects 35 million people worldwide, including 5.4 million Americans; a number that is estimated to triple by the year 2050 [1]. As the prevalence of AD
increases, the AD-associated economic burden will also increase. In 2015, the direct costs associated with the care of AD patients in the United States reached $226 billion. This number is predicted to reach $1.1 trillion by the year 2050 making AD one of the most costly chronic illnesses in the world [1]. At present, AD is the sixth leading cause of death in the United States and is the only leading cause of death that cannot be prevented or cured. There are currently five FDA-approved drugs available to treat AD; however, these drugs do not address the underlying cause of AD and provide only temporary therapeutic relief in a fraction of the patients to whom they are administered. An extensive amount of clinical trials aimed at treating AD have been performed in the last 15 years; all of which have failed [2, 3]. These unanticipated challenges combined with the estimated rapid increases in AD prevalence stress the importance of identifying the underlying AD risk mechanisms that would allow prevention, risk reduction, and early intervention in the preclinical stage of AD.

1.2. Late-onset AD: complex etiology and risk factors

There are two types of AD: early-onset familial AD (FAD) and late-onset sporadic AD (LOAD). FAD is rare and mostly caused by inherited genetic mutations that result in abnormal overproduction of neurotoxic β-amyloid (Aβ) peptides. LOAD, the most common form of AD representing 95% of human cases, develops after age 60 and involves a heterogeneous and multifactorial etiology. It is now widely accepted that a person’s risk for developing LOAD is primarily influenced by a combination of complex interactions between genetic and environmental risk factors. At present, age remains the most predominant risk factor for LOAD. It is estimated that one in nine (11.1%) senior citizens aged 65 or older have been diagnosed with LOAD; a ratio that increases to one in three (33.3%) by age 85 [1]. The National Institute on Aging (NIA) indicates that the risk of developing LOAD doubles every 5 years past the age of 65 [4]. Additionally, epidemiologic studies from the NIA estimate that the total percentage of senior citizens in the United States will increase by 7% by 2030 making senior citizens the fastest-growing age group in the United States and consequently the most at-risk population [4].

Sex also plays a significant role in the development of LOAD. Of the 5.4 million Americans currently living with AD, approximately 65% are women [1]. It was originally postulated that the higher percentage of women living with AD was due to the increased life span of the female population; however, as the average worldwide life expectancy of men and women differs by only 4 years, this presumption is invalid. A meta-analysis of seven sex-specific clinical studies revealed that women are 1.5 times more likely to develop AD than age-matched men, indicating that the female sex confers AD risk independent of age [5]. In addition to a higher incidence of AD, it is now well established that sex influences both the development and progression of LOAD. For example, female AD patients have been shown to exhibit more severe cognitive decline than men during the progression of AD pathology [6–8]. While the exact mechanisms underlying this sex bias are currently unknown, mounting evidence suggests that female vulnerability to AD is largely associated with the irreversible decline of female sex hormones during the onset of menopause [9–11]. However, despite these findings, the precise molecular mechanisms underlying female vulnerability remain uncharacterized.
Genetic predisposition is another prominent risk factor associated with the development of AD. A long-standing observation in the field of LOAD research is the significantly increased AD risk associated with possession of the human apolipoprotein E ε4 allele (APOE ε4) [12], the most predominant genetic risk factor for LOAD. Possession of the ε4 allele is clinically associated with an increased rate and severity of cognitive decline, a younger age of onset, and altered response to AD treatments [13–16]. Moreover, the ε4 allele has been shown to reduce brain glucose utilization [17], increase neuronal inflammation [18], and is associated with increased Aβ dyshomeostasis [19, 20]. In addition to these data, studies have demonstrated that the APOE ε4-associated AD risk is significantly more pronounced in the female population. For example, a recent clinical study conducted in a cohort of 8084 elderly individuals (healthy controls: n = 5496; MCI cases: n = 2588) demonstrated that the risk of clinical conversion from healthy aging to MCI or from MCI to AD conferred by the ε4 allele was significantly greater in women than in men, a finding that corresponds with several earlier reports [21–25].

In addition to APOE ε4, two of the largest genome-wide association (GWA) studies ever conducted to date have recently identified several other genetic risk factors that confer a significantly increased risk of developing LOAD [26, 27]. Of the genetic risk factors identified, clusterin (CLU), also known as apolipoprotein J (APOJ), was established as the third most predominant genetic risk factor for LOAD. CLU, which belongs to the same protein family as APOE, has been shown to regulate inflammation, oxidative stress, and amyloid homeostasis in the brain. Moreover, a recent study conducted by our laboratory indicated that CLU mRNA and protein expression levels are significantly reduced specifically in female brain during a time period that likely corresponds to the onset of reproductive senescence. These data suggest that, similar to APOE ε4, CLU is also influenced by sex in the brain aging process and the pathogenesis of LOAD [28]. In the following sections, we summarize the current understanding of CLU protein isoforms and their biological functions with specific emphasis on the neuroprotective potential of CLU protein isoforms in the brain.

2. Clusterin: from form to function

2.1. CLU: discovery and nomenclature

In 1983, Blaschuk et al. identified a high-molecular weight protein in ram rete testis fluid [29]. Further analyses indicated that this unknown protein was capable of eliciting the “clustering” of Sertoli cells, mouse testis TM-4 cells, and erythrocytes resulting in the name clusterin. In 1984, Griswold and colleagues purified a dimeric acidic glycoprotein (DAG) from the Sertoli cells of rat testes [30]. This abundantly expressed but uncharacterized protein was detected at several molecular weights via reducing chromatography (41 and 29 kDa), western blot (27 and 21 kDa), and immunoprecipitation (70 kDa) [30]. In 1988, another study identified a “novel” protein in human serum. This heterodimeric protein had a molecular mass of 80 kDa, was composed of two 40-kDa chains, and was sequentially unique to all other proteins. Furthermore, it was concluded that this protein, which was deposited in the renal glomeruli of patients with glomerulonephritis, was integrally involved in kidney health [31]. As a result of these
observations, Murphy and colleagues named this protein serum protein 40 kDa (SP-40,40) [31]. In 1990 and the years following, Harmony and colleagues identified and extensively characterized a component of high-density lipoproteins in human plasma which was referred to as apolipoprotein J (APOJ) [32]. However, upon the advent of DNA sequencing technology, it was determined that clusterin, DAG, SP-40,40, and APOJ were in fact the same protein. In the following decade, clusterin was “re-discovered” and subsequently re-labeled with other alternative names including testosterone-repressed prostrate message-2 (TRPM-2) [33], KU70-binding protein 1 (KUB1) [34], complement lysis inhibitor (CLI) [35], and sulphated glycoprotein-2 (SGP2) [36]. In 1992, a forum conducted at Cambridge University officially deemed this diverse protein clusterin (CLU).

2.2. CLU: from gene to protein

CLU is a single-copy gene located on the short arm of chromosome 8 (8p21-12) [37, 38] where it spans approximately 18,115 base pairs (bp). Upon the splicing of eight introns, this nine-exon product spans approximately 2877 bp, and is transcribed into at least two distinct mRNA transcripts. CLU mRNA transcript 1 (NM_001831.3), the most extensively characterized transcript, is translated into the mature/secreted isoform of CLU (mCLU) that has been predominantly identified and studied in the field of CLU research. CLU mRNA transcript 1 is initially translated into a 449-amino-acid precursor protein (pCLU, 60 kDa) beginning at a canonical translational start site located at base pair 187 in exon 2. This pCLU protein contains an N-terminal 22-amino-acid endoplasmic reticulum (ER)-targeted signaling peptide (amino acids 1–22 or bp 187–252) and two nuclear localization sequences in exon 3 (amino acids 78–81 or bp 418–429) and exons 8–9 (amino acids 443–447 or bp 1513–1528). The translated pCLU protein is then targeted to the ER where the 22-amino-acid leader sequence (LS) is cleaved. Following LS cleavage, the peptide bond between R227 and S228 is cleaved resulting in the formation of two individual CLU subunits: the alpha subunit (CLUα, 34–37 kDa) and the beta subunit (CLUβ, 36–39 kDa). These two subunits are subsequently linked by five disulphide bonds to form an anti-parallel heterodimer [39]. N-glycosylation at six glycosylation sites is the final step in the generation of mCLU which, under nonreducing conditions, has a molecular weight of 75–80 kDa (Figure 1A) [40]. Alternatively, complete removal of exon 2 via alternative splicing results in the fusion of exons 1 and 3, thereby creating CLU mRNA transcript 2. In this secondary transcript, translation is initiated at a canonical translational start site located in exon 3. This results in the production of a CLU protein isoform that lacks the ER LS but retains the nuclear localization sequences. This alternative CLU isoform, which is non-ER targeted and unglycosylated, is shuttled between the cytoplasm and the nucleus and is referred to as “nuclear” CLU (nCLU, 49 kDa, Figure 1B). In addition to mCLU and nCLU, emerging evidence indicates that several different splicing variants of CLU also exist. These splicing variants, which are relatively uncharacterized, are suggested to lack portions of exon 2 and/or exon 5 and are generally referred to as “intracellular” isoforms (icCLU, 45, 50, and 53–55 kDa) [41–43].

Initial characterization by Harmony et al. indicated the expression of CLU mRNA in liver, lung, spleen, heart, reproductive tissues, and brain with predominant expression in brain and reproductive tissues [44]. Since this initial characterization, several other research groups,
including our own, have detected CLU mRNA and protein expression in many cell lines and tissue types tested. Moreover, CLU appears to be ubiquitously expressed on the subcellular level with multiple studies demonstrating CLU expression in the cytosol [45], nucleus [41], ER, and Golgi apparatus. Within the brain, CLU expression has been detected within neurons [46], astrocytes [46–48], microglia [49], and within the extracellular space [50]. While initial reports indicated that CLU was solely synthesized and secreted from the astrocytes in a manner similar to APOE [51], our recent findings demonstrate that pure cultures of primary neurons express

**Figure 1.** CLU transcription and translation. In humans, CLU is a single-copy gene located on the short arm of chromosome 8 that is comprised of nine exons spanning approximately 2.8 kb. (A) The mCLU isoform is generated from mRNA transcript 1 from a canonical translational start site in exon 2. The resulting precursor protein (pCLU), which contains an N-terminal ER-targeting leader sequence (LS), is transported to the ER where the 22-amino-acid LS is removed. CLU is then cleaved into the alpha and beta subunits and rapidly disulfide bonded and glycosylated to form an anti-parallel, heterodimeric glycoprotein: mCLU. (B) Alternatively, the nCLU isoform is generated from mRNA transcript 2. In this transcript, a splicing event removes exon 2 resulting in a truncated CLU isoform that lacks the ER-targeting LS. Therefore, the nCLU isoform, which retains the nuclear localization sequence, bypasses the ER/Golgi apparatus and is shuttled between the cytosol and the nuclear compartment.
mCLU, nCLU, and to a lesser extent icCLU isoforms indicating that neurons are also capable of generating de novo CLU. Though the exact physiological functions of CLU remain a mystery, the nearly ubiquitous nature of CLU indicates the significance of this protein in cellular homeostasis.

### 2.3. CLU: transcriptional regulation

Though the gene promoter of CLU is highly conserved across species, the transcriptional regulation of CLU is complex as the predominant CLU transcriptional regulators appear to differ between tissue and cell type. However, despite the controversy in the literature, it is generally agreed that CLU is primarily upregulated by cellular injury, cytotoxic insult, and various stress stimuli [52–54]. For instance, Loisen and colleagues demonstrated that the CLU gene promoter contains an MG132 responsive region and a heat-shock element (HSE) indicating that proteasomal stress directly influences CLU transcription [52]. Another study demonstrated that the CLU gene promoter contains both HSEs and an activator protein-1 (AP-1) response element indicating direct transcriptional regulation by stimuli derived from cellular proliferation and differentiation [54]. In addition to these data, alternative stress-related transcription factor response elements have been identified in the CLU gene promoter including a cAMP response element (CRE), an AP-2 response element, a specificity protein-1 (SP1) response element, and a glucocorticoid response element (GRE) [33, 53]. It has also been demonstrated that apoptotic stimuli modulates CLU transcription, specifically in cancer. An early study from Cervellera et al. identified a MYB binding site in the 5’ flanking region of CLU and that B-MYB, a MYB family member that regulates cellular proliferation and apoptosis, directly bound to and transactivated the CLU gene [55]. CLU transcription is also regulated by several different growth factors including nerve growth factor (NGF) and transforming growth factor beta (TGFβ) [56–58]. For instance, it has been demonstrated that TGFβ induces the upregulation of CLU gene expression by stimulating the interaction between the CLU gene promoter and AP-1 [57]. An extension of these studies demonstrated that TGFβ deficiency resulted in the repression of CLU gene expression via interaction between c-Fos and the CLU gene promoter; an interaction that was abrogated upon cellular stimulation with TGFβ [58].

### 2.4. CLU: posttranslational modification

CLU is regulated by several types of posttranslational modification (PTM), the most predominant type being N-linked glycosylation. As previously indicated, mCLU is N-glycosylated at six different asparagine residues (N86, N103, N145, N291, N354, and N374) during ER-Golgi processing: a modification that comprises approximately 20–25% of the total mass of mCLU [59]. While glycosylation status was originally thought to have little to no impact on CLU function [40, 60], a recent study demonstrated that the chaperone activity of mCLU is dependent upon mCLU glycosylation [61]. This study also demonstrated that the glycosylation of nCLU did not result in chaperone activity indicating that glycosylation-mediated effects are specific to the mCLU isoform. It has also been established that complete deglycosylation of mCLU results in a 70–90% decrease in mCLU chaperone activity and a significant decrease in
the number of α-helices in the secondary structure of CLU. These data suggest that the lack of chaperone activity in deglycosylated mCLU could be, in part, due to the significant changes in secondary structure. Additionally, this study indicates that partially glycosylated mCLU retains chaperone activity suggesting that “core” glycosylation sites are crucial for mCLU function, while peripheral glycosylation may be dispensable [61]. Parallel to these findings, a study by Kang et al. indicated that ER stress, which inhibits protein glycosylation, resulted in rapid retro-translocation of mCLU from the ER yielding several hypo-glycosylated CLU isoforms. These hypo-glycosylated isoforms, which are misfolded and generally nonfunctional, are rapidly poly-ubiquitinated under normal conditions and cleared through proteasomal degradation. However, if the proteasome is chemically inhibited following ER stress, hypo-glycosylated CLU accumulates in the cytosolic compartment resulting in cytotoxicity [62]. Collectively, these studies indicate that, contrary to what was originally postulated, glycosylation is crucial for mCLU chaperone activity.

In addition to N-linked glycosylation, CLU is also a primary target for ubiquitination and phosphorylation. It has been demonstrated that nCLU is a target for K63 ubiquitination through the ubiquitin E3 ligase, a product of von Hippel-Lindau (pVHL). However, contrary to the canonical function of protein ubiquitination, K63-linked ubiquitination of nCLU does not target nCLU for destruction, rather it promotes nCLU nuclear translocation for reasons that are currently unknown [63]. Pertaining to CLU phosphorylation, a recent proteomics study which focused on the identification of the serum phospho-proteome has identified three different phosphorylation sites at residues Thr393, Ser394, and Ser39 within the CLU protein. Additionally, a more recent study indicated that treatment of hepatocytes with 10-mM glucose and fructose significantly increased the levels of mCLU serine phosphorylation. This same study demonstrated increased mCLU serine phosphorylation in both the skeletal muscle and the liver of rats that were orally administered high doses of glucose and/or fructose indicating that phosphorylated CLU may interact with or respond to the activation of glucose-sensitive cellular bioenergetic pathways. In addition to ubiquitination and phosphorylation, an early report indicated that CLU is iodinated at 1 of the 12 tyrosine residues within the CLU protein. This iodination occurs within the apical plasma membrane of thyrocytes and is suggested to serve as a mechanism by which the thyroid gland can conserve iodine, which is relatively rare in the body [64]. It is also suggested that CLU activity is regulated by both sialylation [65] and acetylation [66]; however, definitive acetylation or sialylation sites have not been identified.

3. CLU in Alzheimer’s disease: clinical findings

3.1. CLU polymorphisms in LOAD

Since the initial determination of CLU SNP-associated AD risk by Harold et al. and Lambert et al. [26, 27], there have been approximately 40 independent follow-up meta-analyses and case-control studies that have examined the association between CLU SNPs and AD risk (Table 1). These reports were located through a PubMed search focused on topics pertaining to CLU SNPs in AD. Resulting articles were reviewed and those studies which provided a
listing of the CLU SNP(s) studied, population demographics, and a thorough description of cognitive assessment and statistical analysis were included in Table 1. Though conflicting evidence exists, the majority of the studies indicate that genetic variation in CLU increases the risk of developing AD and that this association is independent of APOE ε4 status. There are approximately 355 identified SNPs in the CLU gene [67]; however, it appears that the primary risk-conferring CLU SNP is rs11136000. Of the 33 studies summarized in Table 1, 25 studies either include or exclusively focus on the impact of the rs11136000 SNP on AD risk; however, the results are inconsistent. Thirteen studies conclude that possession of rs11136000 does confer increased AD risk [26, 27, 68–77], while ten studies conclude no significant association between rs11136000 and AD [78–85]. Moreover, two studies conclude that possession of the rs11136000 SNP reduces risk of AD development [86, 87]. A possible explanation for these discrepancies may be found by examining the population ethnicities. Of the 13 studies that conclude rs11136000 confers AD risk, 11 studies are performed in a predominantly or exclusively western European or American Caucasian population. Alternatively, nine of the 10 studies that conclude no significant association (NSA) between rs11136000 and AD were performed in Asian, eastern European and Russian, Middle Eastern, or Hispanic populations indicating that the risk associated with the rs11136000 SNP may vary based on population ethnicity. Contrary to these data, two separate studies performed in exclusively German and American Caucasian populations found NSA between rs11136000 and AD risk. Moreover, the notion that rs11136000 does not confer AD risk in Asian populations is contradicted by two independent studies that indicate rs11136000-mediated AD risk in exclusively Chinese populations. As all the presented studies performed in Asian populations are adjusted for age, gender, and APOE status, and are comprised of numerically similar sample sizes, it is difficult to identify the exact reason underlying these discrepancies. One observation is that some studies have divided study populations into much smaller groups based upon the specific nucleotide substitution located at the rs11136000 SNP site (i.e. C,T,A substitution), while others have examined only rs11136000 carriers vs. non-carriers. The failure to stratify study populations based on the rs11136000 allele/genotype would have a significant impact on study outcome as the C allele of rs11136000 is considered the risk-conferring allele, while the A allele and T allele are considered normal and neuroprotective, respectively (i.e. C = risk allele, A = normal, and T = protective). Specifically, studies have indicated that the C allele confers a 1.16-fold increased chance of developing LOAD and that 36% of Caucasians carry two copies of this AD-risk variant [26, 27]. Moreover, the C allele is associated with faster cognitive decline in preclinical AD [66] and lower memory scores in healthy elderly controls and elderly AD patients [67]. Young healthy carriers of the C allele exhibit neural hyperactivation in memory-associated brain regions during working memory tasks [73], neural inefficiency in memory-related prefrontal and limbic areas during working memory [88], and reduced coupling between hippocampus and prefrontal cortex during memory processing [89]. Structurally, possession of the C allele is associated with diminished white matter integrity in several brain regions [90] and increased longitudinal ventricular expansion in elderly patients independent of APOE ε4 and dementia status [91]. Taken together, these data indicate that the rs11136000 SNP is significantly associated with the development of AD in predominantly Caucasian
populations and that the rs11136000 AD-associated risk may be initiated several decades prior to the onset of AD.

In addition to rs11136000, another CLU SNP, rs9331888, which was also identified by Lambert and colleagues in the original GWA studies, has also been repeatedly investigated as an AD risk SNP. Of the 33 studies presented in Table 1, seven clinical studies and two meta-analyses examined the association of rs9331888 with AD risk [27, 69, 81, 83, 84, 92–95]. However, similar to that of rs11136000, the results vary and appear to be dependent upon population ethnicity. For instance, two separate meta-analyses conclude that rs9331888 confers AD risk in Caucasian but not Asian populations [92, 95]. However, two separate case-control studies performed in exclusively Chinese populations both indicate that rs9331888 is significantly associated with AD risk [84, 94]. In addition to differing and/or small sample sizes, one possible confounding factor could be sex of the study population. As sex modulates an individual’s risk for LOAD, it is likely that stratification of study populations by sex will have a significant impact on the study results.

| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|------------------|-------------------------------|---------------------------|--------------------|---------------|
| rs11136000       | Harold et al. (2009)          | GWA study in European and US population: Stage 1 population: AD cases: n = 3941, control cases: n = 7848. Stage 2 population: AD cases: n = 2023, control cases: n = 2340. | AD diagnoses: DSM-IV and NINCDS criteria for probable AD or CERAD criteria for definite AD. | rs11136000 SNP was significantly associated with the development of LOAD but not the age of onset. |
| rs2279590        | Lambert et al. (2009)         | GWA study in French, Finnish, Italian, Spanish, and Belgian population: Stage 1 population: AD cases: n = 2032, control cases: n = 5328. Stage 2 population: AD cases: n = 3978, control cases: n = 3297. | AD diagnoses: DSM-III-R and NINCDS-ADRDA criteria for probable AD dementia criteria and with integrity of their cognitive functions (MMSE >25). | All CLU polymorphisms examined showed a significant association with AD development. |
| rs9331888        | Yu et al. (2010)              | Case-control study in Han Chinese population: AD cases: n = 324, AOO >65 years, 181 history of dementia. Control cases: n = 388, 211 females: age = 75.93 ± 4.69. | AD diagnoses: NINCDS-ADRDA criteria for probable AD; no family history of dementia. Control criteria: Healthy and neurologically normal individuals as determined by medical records. | - rs9331888 SNP was significantly associated with increased AD risk. - rs2279590 showed |
| rs11136000       |                               |                           |                    | - rs11136000 was not significantly associated with increased AD risk in |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|------------------|-------------------------------|--------------------------|-------------------|---------------|
| rs11136000       | Seshadri et al. (2010)        | Three-stage GWA study in a white population: | Dementia diagnoses: DSM-IV criteria | rs11136000 was significantly associated with increased risk for LOAD in all study populations analyzed. |
|                  |                               | Stage 1 population: DementiaAD diagnoses: NINCDS-ADRDA criteria for definite, LOAD in all study populations analyzed. | AD diagnoses: NINCDS-ADRDA criteria for definite, probable, or possible AD; AD pathology confirmed at autopsy | Presence of the rs11136000 risk allele did not improve ability to predict AD onset. |
|                  |                               | n = 8935, AD cases: 2033, dementia-free control cases: 14,642 |                                |               |
|                  |                               | Stage 2 population: AD cases: n = 2032, control cases: 5328 |                                |               |
|                  |                               | Stage 3 population: AD cases: n = 3333, control cases: 6995 |                                |               |
|                  |                               | Independent case-control replication population: Ethnicity—Spanish, AD cases: n = 1140, age = 78.8 ± 7.9, 69.9% female; control cases: n = 1209, age = 49.9 ± 9.2, 58.2% female |                          |               |
| rs7982           | Jun et al. (2010)             | Meta-analysis in nine European white cohorts and five non-European cohorts (African American, Israeli-Arab, and Caribbean Hispanic): AD cases: n = 7070 | Not provided | - All CLU polymorphisms examined demonstrated a significant association with AD in only white cohorts. |
| rs7012010        |                               | Control cases: n = 8169 |                                |               |
| rs11136000       | Corneveaux et al. (2010)      | GWA study of a European population AD cases: n = 1019, 652 females, 367 males | AD diagnoses: Clinically diagnosable dementia at time of death and neuropathological confirmation of AD (Braak stage V or VI) upon autopsy | rs11136000 SNP was significantly associated with LOAD. |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|-----------------|-----------------------------|---------------------------|-------------------|---------------|
| rs11136000      | Jessen et al. (2010)        | Longitudinal cohort study in German population: No memory impairment: 685 females, 342 males, age = 79.4 ± 3.4 Memory impairment without worry: 591 females, 415 males, age = 79.8 ± 3.6 Memory impairment with worry: 273 females, 109 males, age = 79.8 ± 3.5 | AD diagnoses: NINCDS-ADRDA criteria for probable AD | - The rs1113600 AD-risk variant is associated with low plasma CLU levels in cognitively intact healthy controls and numerically (but non-significantly) associated with lowered plasma CLU in AD cases. |
| rs11136000      | Lancaster et al. (2011)    | fMRI study in young Caucasian cohort: Subjects: n = 43, 22 males, 21 females, age 18–51 Subjects were genotyped for rs11136000 SNP and pooled according to genotype: CC = risk group (n = 13) and CT/TT = non-risk group (n = 24/6) | Inclusion criteria: No personal or family history of neuropsychiatric, neurological, or neurodegenerative disease; no chronic somatic illnesses or history of substance abuse | - Carriers of the CLU risk genotype (CC) exhibited neural hyperactivity during working memory tasks in the frontal and posterior cingulate cortex and the hippocampus compared to participants in the non-risk group. |
| rs11136000      | Schurmann et al. (2011)    | GWA study on a subset of participants from the German Study on Aging, Cognition and Dementia: AD cases: n = 67, 47 females, 20 males, age = 85.3 ± 3.7 Control cases: n = 191, 134 females, 57 males, age = 83.7 ± 3.2 | Not provided | - rs11136000 AD-risk variant was associated with low plasma CLU levels. |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|------------------|-------------------------------|---------------------------|--------------------|---------------|
| Rs7982           | Komatsu et al. (2011)         | Case-control study in Japanese population: AD cases: n = 180, 101 females, 79 males, age = 67.4±6.7 | AD diagnoses: NINCDS-ADRDA criteria; subjects had no family history of AD | - No association was detected between CLU SNPs and AD in a Japanese population. |
| Rs572844         |                               |                           |                    |               |
| rs1532277        |                               |                           |                    |               |
| rs2279590        |                               |                           |                    |               |
| Rs9331888        |                               |                           |                    |               |
| Rs10503814       |                               |                           |                    |               |
| Rs11136000       | Golenkina et al. (2010)       | Cohort study in a Russian population: AD cases: Early-onset—n = 214, AOO = 56.9 ± 5.38 Late-onset—n = 320, AOO 72.2 ± 5.04 Control cases: Moscow region: n = 343, age range = 35–85, age = 60.96 ± 7.94 Ural region: n = 160, age range = 69–89, age = 73.87 ± 3.87 Siberian region: n = 199, age range = 41–96, age = 61 ± 15.34 | AD diagnoses: NINCDS-ADRDA criteria, ICD-10 criteria, and DSM-IV criteria | - No significant association was detected between rs11136000 SNP and the development of AD in a Russian population. |
| rs881146         | Lee et al. (2011)             | Nested case-control GWAS in a cohort of Caribbean Hispanic subjects: AD cases: n = 549, age of onset = 79.98 ± 8.0 | Dementia diagnoses: Diagnoses established on the basis of all available information gathered from initial and follow-up studies | - rs881146 SNP was significantly associated with LOAD - rs11136000 and other SNPs were not significantly associated with LOAD in a Caribbean Hispanic population. |
| rs11136000       |                               |                           |                    |               |
| rs17057441       |                               |                           |                    |               |
| rs70120100       |                               |                           |                    |               |
| Rs11136000       | Ma et al. (2011)              | Case-control study in Chinese Han population: AD cases: n = 127, 73 females, 54 males, age = 73.12 ± 8.58 Control cases: n = 143, 79 females, 64 males, age = 73.80 ± 6.30 | AD diagnoses: 2007 revised AD diagnoses criteria | - rs11136000 was significantly associated with LOAD in Chinese Han population. |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|-----------------|-------------------------------|---------------------------|-------------------|---------------|
| rs11136000      | Braskie et al. (2011)         | Brain imaging study of Australian Caucasian population: Subjects: n = 398, age range = 20–29, mean age = 23.6 ± 2.2 | Subject criteria: Healthy, young, right-handed Australian Caucasian twins exhibit lower white matter integrity in the genotype and ventricle size corpus callosum, fornix, cingulum, and superior and inferior longitudinal fasciculi. | - Young healthy carriers of the CLU AD-risk SNP |
| rs11136000      | Ferrari et al. (2012)         | Case-control study in a Caucasian-American population: AD cases: n = 342, age = 76.78 ± 8.6 Control cases: n = 277, age = 70.21 ± 8.6 | AD diagnoses: NINCDS-ADRDA criteria Control criteria: Subject within cognitively normal limits on a standard psychometric test | - rs11136000 was significantly associated with LOAD. |
| rs2279590rs11136000 | Kamboh et al. (2012)          | Case-control study in Caucasian-American population: AD cases: n = 1348, AOO = 72.6 ± 6.4, 65.6% female Control cases: n = 1359, age = 74.7 ± 6.5, 60.8% female | AD diagnoses: NINCDS-ADRDA criteria for probable or definite AD Control criteria: Non-demented Caucasian-American over 60 years of age | - No significant association was observed between CLU SNPs and AD in case-control study. |
| rs7982          | Karch et al. (2012)           | GWA study in Euro-American population: confirmed AD AD cases: n = 73, age = 87 ± 7, 42% male-matched cognitively normal associated with AD controls Control cases: n = 39, age = 86 ± 9, 44% male | AD diagnoses: Autopsy criteria | - rs7982 was associated with disease status. - Elevated CLU levels are associated with AD brains. - CLU is altered at the mRNA level in AD brain. |
| rs3087554       | Lin et al. (2012)             | Case-control study in Taiwanese population: AD cases: n = 268, Control cases: n = 389 | AD diagnoses: DSM-IV criteria and NINCDS-ADRDA criteria Control cases: Assessed via Short Portable Mental Status Questionnaire | - rs11136000 was associated with significantly reduced risk for AD. |
| rs9331942       |                               |                           |                   |               |
| rs9331949       |                               |                           |                   |               |
| rs11136000      |                               |                           |                   |               |
| rs2279590rs9331888 | Chen et al. (2012)            | Case-control study in southern Chinese population: AD diagnoses: NINCDS-ADRDA criteria and no family history of AD | Control cases: n = 389 | - rs2279590 and rs11136000 SNPs confer susceptibility to AD in |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|-----------------|--------------------------------|--------------------------|--------------------|---------------|
| rs11136000      |                                | AD cases: n = 462, Control cases: n = 350 | Control criteria: Cognitively normal individuals as indicated by CDR scale | southern Chinese population. |
|                 | Xing et al. (2012)             | Case-control study: AD cases: n = 104, AOO = ≥65, age = 80.20 ± 5.57, 63 females, 41 males, Control cases: n = 104, age = 79.32 ± 5.37, 58 females, 46 males | AD diagnoses: NINCDS-ADRDA criteria for probable AD, Control criteria: Confirmed healthy by medical history, medical examination, and MMSE score > 28 | - Subjects who were homozygous for the AD-risk allele exhibited a significant decrease in CLU protein and mRNA levels. |
|                 | Klimkowicz-Mrowiec et al. (2012) | Case-control study in a Polish population: AD cases: n = 253, age = 73.9 ± 5.8, 173 females, Control cases: n = 240, age = 73.8 ± 6.9, 138 females | AD diagnoses: NINCDS-ADRDA criteria for probable AD—no family history of AD, Control criteria: MMSE > 26, no family history of dementia, no apparent neurological, psychiatric, or cerebrovascular disease | - No significant association between rs11136000 and the AD in a Polish population. |
|                 | Yu et al. (2013)               | Case-control study in Han Chinese population: AD cases: n = 796, AOO = ≥65, age = 74.3 ± 7.0, 396 females, Control cases: n = 796, age = 73.9 ± 6.5, 388 females | AD diagnoses: NINCDS-ADRDA criteria for probable AD, No family history of neurodegenerative disorders or dementia, Control criteria: Free of cognitive impairment as indicated by neurophysiological and medical exams | - Of the 18 SNPs tested, only the C allele (major allele) of rs9331949 was significantly associated with AD in the Han Chinese population. |
| rs11136000      | Thambisetty et al. (2013)     | Two-part longitudinal study from Baltimore Longitudinal Aging Study: Study 1 population: n = 88, age = 69, age range = 56–86, Study 2 population: n = 599, age = 67.5, age range 60–93 | Inclusion criteria: No history of clinical stroke, head trauma, or CNS inflammation; Subject without cognitive impairment as indicated by memory processes. | - Cognitively normal subjects carrying the CLU risk allele exhibit increased rCBF in brain regions intrinsic to memory processes. - Risk allele carriers who converted to MCI exhibit increased rates of... |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|------------------|------------------------------|---------------------------|-------------------|----------------|
| rs11136000       | Pedroza et al. (2014)        | Association study in a white and black population: AD cases: n = 44 black, n = 432 white, age = 78.9, age of 0 range = 52.2–91.2 Control cases: n = 224 black, n = 2219 white, age = 78.7, age range = 60.5–96.4 | AD diagnoses: NINCDS-ADRDA criteria for probable AD Control criteria: CDR score | Memory decline over non-carriers. - The minor allele of rs11136000 may confer enhanced memory in whites. |
| rs1532278        | Lu et al. (2014)             | Case-control study in southern Han Chinese population: AD cases: n = 499, age = 69.990 ± 9.961 Control cases: n = 592, age = 68.930 ± 9.390 | Not provided | - No significant association was detected between CLU SNPs and LOAD in southern Han Chinese population. |
| rs2279590        | Patel et al. (2014)          | Prospective cohort study in a British Caucasian cohort with Down syndrome: Subjects: n = 304 Down syndrome patients, age > 16 | Dementia diagnoses: ICD-10 research criteria | - No significant association between rs1532278 and the development of dementia in a cohort of Caucasian Down syndrome patients. |
| rs11136000       | Lancaster et al. (2015)      | fMRI study in young Caucasian population: Subjects: n = 85, age range = 19–47 | Inclusion criteria: Healthy, right-handed, young Caucasians with no history of mental illness or drug abuse | - Carriers of the rs11136000 risk variant exhibit higher activation levels in memory-related pre-frontal and limbic areas during working memory tasks. |
| rs11136000       | Sen et al. (2015)            | Case-control study in a Turkish population: AD cases: n = 112, age range = 65–98, age = 73.59 ± 7.59 Control cases: n = 106, age = 74.04 ± 5.29 | AD diagnoses: NINCDS-ADRDA criteria for probable AD—no family history of dementia Control criteria: Cognitively intact | - No significant association was observed between rs11136000 and AD in the entire Turkish population. - Turkish females carrying the rs11136000 TT genotype exhibited increased BEHAVE-AD scores |
| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|-----------------|-----------------------------|--------------------------|-------------------|---------------|
| rs11136000      | Sohrabifar et al. (2015)    | Case-control study in an Iranian population: | Not provided | suggesting a possible association between the TT genotype and female Turkish subjects. |
|                 |                             | *AD cases:* n = 160       |                   | - No significant association between rs11136000 and AD in an Iranian population. |
|                 |                             | *Control cases:* n = 163  |                   |               |
| rs9331888       | Toral-Rios et al. (2015)    | Case-control study in a Mexican population: | *AD diagnoses:* NINCDS-ADRDA criteria, *Control criteria:* MMSE ≥ 24, no memory complaints, no acute or severe chronic illness | - No significant association between rs9331888 and AD in a Mexican population. |
|                 |                             | *AD cases:* n = 94, age > 60 |                   |               |
|                 |                             | *Control cases:* n = 100, age > 60 |                   |               |
| rs9331888       | Shuai et al. (2015)         | Meta-analysis of 11 case-control studies: | *Study inclusion criteria:* (1) Study evaluated rs9331888 SNP and AD risk, (2) Case-control design, (3) Sufficient study population was provided | - Significant association between rs9331888 and AD in Caucasian population among allelic, additive, and recessive models. - No association in combined population or only Asian population. |
|                 |                             | *Ethnicities:* Caucasian and Asian populations |                   |               |
|                 |                             | *AD cases:* n = 8766       |                   |               |
|                 |                             | *Control cases:* n = 11,366 |                   |               |
| rs2279590       | Zhang et al. (2015)         | Meta-analysis of 11 case-control studies: | *Study inclusion criteria:* (1) Study evaluated rs2279590 SNP and AD risk, (2) Case-control design, (3) Study provided the number of rs2279590 genotypes, (4) Study provided OR with a 95% CI | - Significant association detected between rs2279590 and AD in Asian population among additive and recessive models. |
|                 |                             | *Ethnicities:* Caucasian and Asian populations |                   |               |
|                 |                             | *AD cases:* n = 8605       |                   |               |
|                 |                             | *Control cases:* n = 12,050 |                   |               |
| rs9331888       | Zhang et al. (2015)         | Meta-analysis of 12 case-control studies: | *Study inclusion criteria:* (1) Study evaluated rs9331888 SNP and AD risk, (2) Case-control design, (3) Study provided the number of SNP genotypes, (4) Study provided OR with a 95% CI | - Significant association detected in pooled population. - Subgroup analysis demonstrates a significant association between rs9331888 and AD in Caucasian population. |
|                 |                             | *Ethnicities:* Caucasian and Asian populations |                   |               |
|                 |                             | *AD cases:* n = 16,876     |                   |               |
|                 |                             | *Control cases:* n = 19,295 |                   |               |
### 3.2. CLU as an AD biomarker

In 1992, it was suggested that peripheral CLU (then referred to as SGP-2) expression may serve as a potential biomarker for predicting the onset and/or severity of neurodegenerative disorders such as LOAD [96]. Though this concept was proposed over 20 years ago, the possibility of CLU as an AD biomarker is only recently being examined. Since 2010, 10 different studies have been performed with the aim of determining the validity of CLU as an AD biomarker (Table 2). However, the conclusions of these studies are contradictory at best. Of the 10 studies presented in Table 2, six studies conclude that increased plasma CLU levels are associated with increased rate of cognitive decline [97], increased white matter atrophy [98], increased risk for AD [99], and were indicative of greater fibrillar Aβ burden [100]. However, contrary to these findings, four studies conclude that CLU levels are not significantly different between control subjects and subjects with MCI, AD, or dementia, suggesting that peripheral CLU is unreliable as an AD biomarker [101–105]. One primary difference between these studies is the fluid that was analyzed for CLU concentration. The six studies concluding that CLU would be a reliable biomarker utilize plasma samples for analysis, whereas the three of the four studies indicating no difference between control and AD subjects measure serum or platelets. Another key difference between these conflicting reports is the sample size. In three of the four studies concluding that CLU would not be a reliable AD biomarker, the sample size per group is less than 70 subjects, whereas most of the studies indicating the possibility of CLU as a peripheral biomarker contain several hundred subjects per group. Therefore, it is also possible that these differences are the result of inadequate sample size. Despite these discrepancies, these studies collectively suggest that at least plasma CLU could provide a predictive biomarker for determining the risk for AD.

### Table 1. CLU polymorphisms in AD (2009–2016).

| CLU gene variant | Study and year of publication | Study design and subjects | Diagnoses criteria | Major findings |
|------------------|------------------------------|---------------------------|-------------------|---------------|
|                  | Thambisetty et al. (2010)    | Prospective cohort study: Subjects: n = 844 | AD diagnoses: NINCDS-ADRDA criteria for probable AD | -Increased plasma concentration of CLU was population but not Asian population. |

**Abbreviations:** Age of onset (AOO), behavioural pathology in Alzheimer’s disease (BEHAVE-AD), the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD), Clinical Dementia Rating (CDR), congestive heart failure (CHF), Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R), Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), functional magnetic resonance imaging (fMRI), genome-wide association (GWA), mini-mental state examination (MMSE), National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA), odds ratio (OR), type 2 diabetes mellitus (T2DM).
| Study and year of publication | Fluid analyzed | Study design and subjects | Diagnoses criteria | Major findings |
|-------------------------------|---------------|---------------------------|-------------------|---------------|
| Schrijvers et al. (2011)      | Plasma        | Case-cohort study from the Rotterdam Study in the Netherlands: Subjects: 60 individuals with prevalent AD at baseline, a sub-cohort of 926 subjects, and an additional 156 subjects diagnosed with AD throughout follow-up time | MCI diagnoses: Subjective memory complaints, CDR scores of less than 1, and evidence of objective memory impairment using the CERAD criteria | Predictive of greater fibrillar Aβ burden. |
| Ijsselstijn et al. (2011) Serum | Case-control study derived from the Rotterdam Scan Study: AD cases: n = 43, age = 78 ± 6.5, 32 females Control cases: n = 43, age = 78 ± 6.8, 32 females | AD diagnoses: DSM-III R criteria Control criteria: MMSE ≥ 28 | Study outcome: Prevalent AD AD diagnoses: Severity of AD measured by the MMSE score, and the risk of developing AD during follow-up examinations | In AD patients, higher plasma CLU was associated with more severe AD. - The likelihood of prevalent AD increased with increasing plasma CLU levels. |
| Thambisetty et al. (2012) Plasma | Longitudinal cohort study: 139 cognitively intact subjects, age = 70.5 | Baseline criteria: Free of clinical diagnosis of dementia at evaluation MCI diagnoses: Petersen criteria Dementia diagnoses: DSM III criteria | - No significant difference in serum CLU levels between pre-symptomatic AD and controls (p-value = 0.54). |
| Mukaetova-Ladinska et al. (2012) Platelets | Case-control study: AD cases: n = 25, age = 78.08 ± 1.0, 10 females Control cases: n = 26, age = 70.81 ± 1.98, 18 females | AD diagnoses: NINCDS-ADRDA criteria for probable AD Control criteria: Subjects with no cognitive and/or neurological problems | - Higher baseline concentration of plasma was associated with slower rates of brain atrophy. - Peripheral concentrations of CLU appear to reflect concentrations in AD-vulnerable brain regions. |
| Silajdzic et al. (2012)Plasma | Quantitative ELISA assessment of plasma | AD diagnoses: DSM-IIIR criteria and NINCDS- | - No significant difference in platelet CLU levels between control and AD patients. | - No significant difference in plasma CLU levels |
| Study and year of publication | Fluid analyzed | Study design and subjects | Diagnoses criteria | Major findings |
|------------------------------|---------------|---------------------------|-------------------|---------------|
| Song et al. (2012)           | Plasma        | Longitudinal cohort study—Sydney Memory and Aging Study: | MCI diagnoses: International consensus criteria and CDR > 0.5 | - CLU plasma levels were negatively correlated with gray matter volume and positively correlated with CSF volume. - Higher plasma CLU levels predict white matter atrophy over 2 years in elderly subjects. |
| Sattlecker et al. (2014)     | Whole blood   | Prospective cohort study—AddNeuroMed Biomarker Study: | Not provided | - Increased plasma CLU is significantly associated with increased rate of cognitive decline. |
| Jongbloed et al. (2015)      | CSF and plasma| Quantitative diagnostic study: | AD diagnoses: NINCDS-ADRDA criteria for probable AD | - Elevated plasma CLU was associated with increased risk for AD and related to cognitive decline in MCI patients. - Plasma CLU is inversely related to cognitive decline in AD patients. |
| Dukic et al. (2016)          | Serum         | Quantitative comparison of serum CLU levels: | AD diagnoses: NINCDS-ADRDA criteria | - Serum concentrations of CLU did not differ between groups. |
4. CLU in the brain: mechanisms of action

Of the known CLU isoforms, mCLU is by far the most studied and has been described as a chaperone-like protein that clears misfolded proteins, cellular debris, and protein aggregates from the cytosol and extracellular space [106–113]. However, the nCLU and icCLU isoforms remain relatively uncharacterized. Several reports have suggested that nCLU and icCLU exhibit solely proapoptotic characteristics; however, results vary across laboratories and are inconsistent [41, 42, 114–116]. This section reviews the available literature pertaining to CLU isoforms in the brain with particular emphasis on the molecular mechanisms by which CLU protein isoforms regulate amyloid homeostasis, inflammation, and apoptosis.

4.1. CLU and Aβ homeostasis

In the early 1990s, CLU mRNA and protein levels were found to be significantly elevated in AD brain, specifically in the frontal cortex and hippocampus of post-mortem AD brain tissue [117, 118]. Shortly after these discoveries, McGeer et al. demonstrated robust CLU immunoreactivity within senile plaques [119]. It was further demonstrated that mCLU-bound soluble Aβ proteins in the cerebral spinal fluid (CSF) [120] and that CLU expression increased the solubility of Aβ and prevented Aβ aggregation [121]. These data strongly suggested that CLU may play an important role in the pathogenesis of AD via regulation of brain amyloid burden. However, contrary to these findings, it has also been demonstrated that increased CLU expression exacerbated Aβ-induced neurotoxicity [122]. Moreover, DeMattos et al. demonstrated that Aβ plaque formation was facilitated by CLU in an animal model of AD suggesting that CLU exerts a negative impact on the brain in the development of AD pathology [123]. These literary contradictions continued to persist until 2007 when a study by Yerbery and colleagues provided a possible explanation for the simultaneously pro- and anti-amyloidogenic effects associated with mCLU [124]. This study indicated that the pro-amyloidogenic effects of mCLU were restricted to conditions in which Aβ was present in a very large molar excess. Under these conditions, mCLU, which functions as a chaperone-like protein to temporarily stabilize misfolded proteins [125], bound to and stabilized Aβ thereby facilitating Aβ aggregation. Alternatively, when mCLU was present at much higher but still substoichiometric levels (i.e. a molar ratio of clusterin:Aβ = 1:10), mCLU provided substantial anti-amyloidogenic effects by inhibiting plaque formation [124]. These data suggest that CLU may exhibit neuroprotective characteristics in preclinical or early stages of AD when brain amyloid burden is significantly
lower. Alternatively, CLU may exert a negative impact during later stages of AD when brain amyloid burden is extensive, though this hypothesis is yet to be tested. Parallel to this notion, a more recent study performed in rat brains indicated that mCLU prevented Aβ42-induced learning and memory impairments, reduced Aβ42-induced glia inflammation, and reduced Aβ42-mediated neuronal degeneration when Aβ42 oligomers were incubated with mCLU prior to brain injection. However, these effects were not observed in rats injected with pre-formed Aβ42 oligomers and mCLU without pre-incubation indicating that mCLU does prevent Aβ42-induced neurotoxicity prior to extensive Aβ42 oligomerization [126]. In addition to these studies, mCLU has been shown to impact the rate of Aβ42 clearance. A study by Bell and colleagues demonstrated that the rate of Aβ clearance was increased by as much as 83% when bound to CLU. This same study further demonstrated that CLU-bound Aβ is transported across the blood-brain barrier specifically through LRP2-mediated transport, while APOE-bound Aβ was transported through LRP-1 [127]. While the regulation of Aβ by mCLU is relatively well characterized, one question that remains unanswered is whether alternative CLU isoforms exert some impact on amyloid homeostasis. It has been demonstrated that Aβ toxicity induces the expression of intracellular CLU (icCLU) in neurons; however, the physiological impact of increased icCLU expression was not determined in this study [128]. At present, no literature specifically implicates a role for nCLU/icCLU isoforms in the regulation of Aβ; however, as nCLU/icCLU isoforms are reportedly induced by cellular stress in multiple peripheral cell lines and nCLU is induced upon treatment with exogenous Aβ, it is likely that nCLU/icCLU isoforms mediate some effect on amyloid homeostasis in the brain; however, more research is needed before a conclusion can be made.

4.2. CLU and inflammation

It is well established that persistent inflammation likely caused by the deposition of neurotoxic protein aggregates in the brain is a key component of LOAD [129]. Early studies suggest that CLU inhibits the activation of the complement system in the brain [31, 130–132]. For instance, several early publications indicated that CLU (then referred to as SP-40,40) prevented the formation of the membrane attack complex (MAC), suggesting that increased CLU would suppress initiation of acute inflammation. However, these data were contradicted by a more recent study that demonstrated CLU-mediated activation of the major histocompatibility complex class II (MHC II) antigen in primary cultures of rat microglia. This same study showed that administration of exogenous CLU resulted in the direct activation of microglia in the brain and the subsequent secretion of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α) indicating that increased CLU expression induces the acute inflammatory response [49]. These findings were corroborated by another study that demonstrated increased CLU staining within reactive microglia in the cortices of rats following cerebral ischemia [133]. Collectively, these studies suggest that increased CLU expression results in the activation of glial cells and the subsequent secretion of pro-inflammatory mediators. Therefore, it is possible that the increased secretion of cytokines such as TNF-α could contribute to chronic inflammation in AD brain; however, this hypothesis requires further testing.
4.3. CLU and apoptosis

Several studies performed in human cancer cell lines have demonstrated that mCLU and nCLU exhibit opposing effects on cell death pathways. mCLU has been shown to protect cells from oxidative stress and inhibit intrinsic apoptosis by interacting with and stabilizing the KU-70-Bax protein complex [134–138]. In contrast, nCLU is suggested to initiate intrinsic apoptotic pathways resulting in rapid cell death [115, 136]. The contrasting functions of mCLU and nCLU appear to also exist in the brain; however, unlike cancer-focused studies, relatively few brain-based investigations have included an examination of the apoptotic characteristics of nCLU. An early study by Schreiber et al. demonstrated that CLU (then referred to as SGP-2) mRNA expression was rapidly and transiently increased in astrocytes, but not CA3 and CA1 neurons, following administration of kainic acid (KA), a neurotoxic seizure-inducing compound [139].

Another study performed in WT, human CLU overexpressing (hCLU-OE) mice and Clu-knockout (Clu-/-) mice subjected to middle cerebral artery occlusion (MCAO) indicated that CLU overexpression resulted in reduced brain injury. Specifically, this study demonstrated a 30–50% increase in CLU mRNA expression 7 days post-ischemia in the ischemic brain hemisphere specifically in the penumbral area (the area that separates necrotic from normal brain tissue). Morphometric analysis of the ischemic hemisphere revealed that the penumbra was significantly thinner in hCLU-OE mice and significantly thicker in Clu-/- mice when compared with WT mice indicating an inverse relationship between CLU mRNA expression and brain injury [140]. Collectively, these two studies strongly support a neuroprotective role for CLU in the brain following significant brain injury. In contrast, ethanol-mediated toxicity has been shown to significantly increase CLU expression in the cortex and amygdala. This upregulated CLU, which was shown to interact with Bcl-XL, was translocated to the nucleus upon exposure to ethanol, and was associated with increased cell death suggesting that these effects were mediated by nCLU [135]. Another study performed in neonatal mice subjected to hypoxic-ischemic brain injury indicated that CLU accumulated in dying neurons following brain injury. Moreover, this study indicated that CLU-deficient mice exhibited 50% less brain injury when compared to wild-type controls indicating that CLU expression exacerbates neuronal cell death following brain injury [141]. Collectively, these studies indicate that nCLU protein expression may be associated with increased cell death following traumatic brain injury or in response to cytotoxic stimuli.

5. Future perspectives

CLU is currently the third most significant genetic risk factor for the development of LOAD; however, an extensive gap exists in the literature in understanding the neurophysiological and neuropathological functions of CLU. Moreover, the bulk of brain-based CLU research refers to CLU as a single protein with few studies including a characterization of its isoforms. As CLU isoforms appear to mediate different physiological processes, the tendency to focus on the effects of CLU as a singular protein could lead to conflicting reports in the literature that are currently unresolved. Therefore, before researchers can fully ascertain the therapeutic
potential of CLU from a clinical perspective, it is vital that these key deficiencies are addressed at the molecular level.

First, it is crucial that current and future studies strive to examine CLU isoforms individually, with particular emphasis on separating the nCLU and mCLU isoforms. Of the studies published pertaining to CLU in the brain, approximately five studies include an examination of nCLU. While it is possible that nCLU does function to regulate apoptosis, recent findings from our laboratory indicate roughly equivalent expression levels of both mCLU and nCLU in healthy primary cortical neurons suggesting that nCLU may be integrally involved in cellular homeostasis. Moreover, our recent data indicate that a nCLU or icCLU isoform is localized to the mitochondria suggesting that these alternative CLU isoforms may play an important role in the regulation of brain mitochondria function. While these studies are still underway, future work should focus on identifying the exact CLU isoforms expressed in other types of brain cells including astrocytes and microglia. Moreover, these studies should examine the cellular distribution, key protein modulators, and the neurophysiological function of each nCLU/icCLU isoform.

An emerging topic in the study of AD is the impact of sex on the development and progression of LOAD. As previously discussed, the female population is more susceptible to developing LOAD and the risk conferred by genetic factors, such as APOE, is greater in females. Moreover, our recent analyses have demonstrated that CLU expression is significantly reduced in the early aging of female but not male brain during a time that corresponds with the onset of reproductive senescence [28]. These data strongly suggest that CLU expression is modulated, in part, by sex hormone signaling pathways in the brain. Parallel to these findings, our recent studies have revealed that brain CLU isoform expression is regulated via estrogen receptor (ER) signaling. Additionally, we find that testosterone (TT) differentially regulates mCLU and nCLU expression; TT increases mCLU expression and decreases nCLU expression. An extension of these studies revealed that TT-mediated upregulation of mCLU expression results from the aromatization of TT to 17β-estradiol (E2). These data are particularly interesting when considered in the context of sex hormone changes between men and women throughout the aging process. It is well established that menopausal onset results in a significant and irreversible decline in ovarian sex hormones, such as E2. However, TT levels in males gradually decline with age at a rate of approximately 2% per year [142]. Therefore, it is possible that TT-mediated upregulation of the neuroprotective mCLU isoform may, in part, contribute to the reduced incidence of AD in men. Likewise, the significant reduction in E2 levels in menopausal and/or postmenopausal women may result in significantly reduced mCLU levels thereby contributing to female vulnerability. While more research is needed to fully elucidate the interactions between sex hormones and neuronal CLU isoforms, these data underscore the importance of including sex as a variable in the study of risk factors that mediate the development of LOAD.

It is particularly interesting that two of the top five genetic risk factors associated with the development of LOAD are members of the apolipoprotein family: APOE and CLU. Therefore, another avenue of research to be considered in the AD field is the possibility of intersecting or overlapping risk pathways mediated by these two genetic factors. Studies have shown that
APOE and CLU share a number of important physiological properties. For instance, they are among the few proteins associated with brain lipoproteins [143, 144]. They interact with a shared set of cell-surface receptors [108] and both APOE and CLU promote neurite outgrowth [145, 146]. Moreover, elimination of either protein in an AD mouse model results in increased accumulation of Aβ [147]. Furthermore, presence of the C allele of the CLU AD-risk SNP has been shown to exacerbate the APOE ε4-mediated decrease in brain activity during executive attention tasks in young healthy dementia-free adults [148]. In addition, the genetic variance that results in increased AD risk from both genes is also associated with compromised or reduced protein expression and/or binding capabilities. Our data indicate that APOE protein expression levels are significantly increased in 6-month-old female Clu-/- mice. However, mCLU expression levels are significantly reduced in 6-month-old female human APOE ε4 gene targeted-replacement mice when compared to APOE ε3 mice indicating that reduced CLU expression may contribute to APOE ε4-mediated AD risk. Collectively, these studies indicate that APOE and CLU could share common risk pathways that contribute to the development of LOAD. Delineation of such pathways will potentially provide valuable insights for an increased understanding of the etiology of LOAD and ultimately help to devise therapeutic strategies to prevent or reduce the risk of developing the disease.

Author details

Sarah K. Woody¹ and Liqin Zhao¹,²*

*Address all correspondence to: lzhao@ku.edu

1 Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS, USA

2 Neuroscience Graduate Program, University of Kansas, Lawrence, KS, USA

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