# Clusterin: a multifaceted protein in the brain

**Hee-Jung Moon, Sarah K. Herring, Liqin Zhao***

Late-onset Alzheimer's disease (LOAD), the most common cause of dementia, currently affects 5.6 million Americans ages 65 and older. LOAD is a neurodegenerative disorder characterized by progressive loss in synaptic function, notable bioenergetic decline, increased neuronal death and brain atrophy, and significant cognitive impairment. Because the etiology of LOAD remains unknown, a treatment for LOAD has not yet been formulated, a fact that is clearly demonstrated by the more than 200 failed clinical trials. These failures underscore the significance of identifying the LOAD risk mechanisms that would allow early intervention in the preclinical stage of LOAD. Genome-wide association studies have identified more than a dozen genetic risk variants that are associated with the development of LOAD. Clusterin (*CLU*), also known as apolipoprotein J (*APOJ*), has been established as the third most prominent genetic risk factor for LOAD after apolipoprotein E (*APOE*) and bridging integrator 1 (*BIN1*) (Harold et al., 2009; Lambert et al., 2009). A number of single nucleotide polymorphisms (SNPs) within the *CLU* locus, with the majority being intronic, have been linked to significantly altered LOAD risk, independent of *APOE* status (**Figure 1A**; (Medical Genetics and Human Variation, 2019)); however, it is unclear how these SNPs affect CLU mRNA, protein isoform expression and function.

CLU was first discovered in 1983 as a highly abundant glycoprotein in ram rete testis fluid that elicited the "clustering" of cells (Fritz et al., 1983). CLU was subsequently identified in a variety of additional tissues and, because of its diverse distribution and function, acquired several names from independent research groups. These included sulphated glycoprotein-2, testosterone-repressed prostate message 2, complement-associated protein SP-40, complement lysis inhibitor, ionizing radiation-induced protein-8, Ku70-binding protein 1, aging-associated gene 4 protein, and APOJ. In 1992, a forum conducted at Cambridge University officially agreed on the name clusterin (CLU).

*CLU*, a single-copy gene containing 9 exons, is located on chromosome 8 in humans (8p21-p12). The primary mRNA transcript (NM_001831.3) transcribed from *CLU* encodes a widely distributed ~75–80 kDa secreted, chaperone-like protein traditionally referred to as mature CLU (mCLU). Translation of NM_001831.3 results in the production of the 449 amino acid (aa) mCLU preprotein, which contains an N-terminal 22 aa endoplasmic reticulum (ER)-targeting sequence and 2 nuclear localization sequences. This preprotein is immediately targeted to the ER, where the ER-targeting sequence is cleaved followed by substantial processing, including proteolytic cleavage at an internal site between Arg227 and Ser228 to form the α and β chains, *N*-glycosylation at 6 discrete sites (i.e., Asn86, Asn103, Asn145, Asn291, Asn354, and Asn374), and disulfide bonding at 5 sites. Collectively, this processing results in the production of the heterodimeric, antiparallel, secreted mCLU protein isoform (**Figure 1B**), which has been the primary focus of the bulk of the CLU-related literature. However, recent studies, including our own, have uncovered several minor or alternative CLU protein isoforms that localize and function differently from mCLU (Foster et al., 2019; Herring et al., 2019). Complete removal of Exon 2 via alternative splicing results in the absence of the ER-targeting sequence and produces a non-glycosylated, non-secreted CLU protein described in the literature as the pro-apoptotic nuclear CLU (nCLU, 45–50 kDa) protein isoform. In addition, some studies indicate the presence of intracellular CLU protein isoforms that lack Exon 2 and/or Exon 5 (icCLU, 45–50 kDa, and 53–55 kDa) (Rohne et al., 2016).

Within the brain, CLU is the second most abundantly expressed apolipoprotein putatively involved in LOAD pathology (Woody and Zhao, 2016). Thus, a large portion of brain-based CLU research focuses on the role of mCLU in the regulation of amyloid beta (Aβ) deposition, aggregation, and clearance and describes mCLU as both pro- and anti-amyloidogenic. mCLU expression is induced by stress or inflammation and functions as a chaperone-like protein to clear misfolded proteins and protein aggregates from the cytosol and extracellular space. Moreover, expression of mCLU has been indicated to increase the solubility of Aβ to prevent Aβ aggregation and modulate Aβ clearance through the blood-brain barrier via LDL receptor-related protein 2-mediated transport. In contrast, it has also been demonstrated that increased mCLU expression may exacerbate Aβ-induced neurotoxicity and that Aβ plaque formation may be facilitated by mCLU expression. Most recently, mCLU has been hypothesized to both attenuate and exacerbate Aβ-induced toxicity depending on the molar ratio of mCLU to Aβ within its respective environment (Yerbury et al., 2007).

CLU is also the second major apolipoprotein in the brain involved in the transport of brain cholesterol and phospholipids. The impact of membrane-concentrated cholesterol on the activity of many transmembrane receptors and enzymes, including those that cleave amyloid precursor protein to produce Aβ (i.e. β-secretase and γ-secretase), suggests that CLU may modulate LOAD risk through its regulation of cholesterol metabolism. Furthermore, several studies demonstrated that genetic variants of CLU might incidentally alter susceptibility to LOAD through increased inflammation and cell death. mCLU has been shown to protect cells from oxidative stress and inhibit intrinsic apoptosis by stabilizing the union between Ku70 and the apoptotic protein Bax, while reduced mCLU activity diminishes the binding of Ku70-Bax with the resulting accumulation of pro-apoptotic proteins such as cytochrome C and caspase 9. In contrast, nCLU protein expression may be associated with increased cell death following traumatic brain injury or in response to cytotoxic stimuli. In general, the bulk of brain-based CLU research definitively demonstrates the involvement of mCLU in key LOAD-related processes such as Aβ regulation, lipid transport and metabolism, inflammation, and cellular apoptosis. However, the molecular mechanisms underlying CLU protein isoform involvement in brain function are still unclear, largely due to a lack of foundational information concerning CLU mRNA transcripts, corresponding protein isoforms, and the localization and function of each protein isoform.

To address this research gap, our laboratory has recently conducted a wide-ranging evaluation of brain CLU mRNA and protein isoforms using various rodent and human models. The data provide a comprehensive listing of CLU mRNA transcripts and corresponding protein isoforms as well as the cellular and subcellular localization of each identified isoform in brain tissue, neurons, and astrocytes (Herring et al., 2019). Specifically, the data indicate that both astrocytes and neurons possess the capability of generating *de novo* mCLU as both express CLU mRNA containing Exon 2, the mCLU precursor protein (CLU_49 kDa), immature mCLU (CLU_60 kDa) and some variants of the fully processed mCLU (i.e. deglycosylated mCLU subunits). The data also indicate subtle differences between astrocytes and neurons such as the presence of alternative non-secreted CLU isoforms primarily expressed in neurons (i.e. CLU_53 kDa and CLU_68 kDa) and an additional mRNA transcript capable of producing mCLU in astrocytes (i.e. Exon 1A-3-containing mRNA). However, chief among the brain-based CLU findings is the discovery of the unglycosylated, non-secreted mitochondrial CLU protein isoform (CLU_45 kDa, or mitoCLU), which is localized to the mitochondrial matrix of healthy rodent brain tissue and is expressed in both rodent and human neurons and astrocytes. Lack of *N*- or *O*-linked glycosylation on the mitoCLU protein isoform indicates translation from an Exon 2-deficient CLU mRNA transcript. Sequential assessment of human and murine CLU indicated a canonical translational start site (AUG) in Exon 3 at aa 34 in human CLU and a non-canonical translational start site [CUG; (Leucine capable of initiating translation)] in Exon 3 at aa 33 in murine CLU (**Figure 1B**). Generation and transfection of three human CLU aa 34 variants [34_AUG (Met; representing human CLU), 34_CUG (Leu; representing murine CLU), and 34_CUA (Leu; incapable of initiating translation) confirmed that the translation of mitoCLU is initiated in Exon 3, specifically from an in-frame AUG [aa 34 (Met), human] or a non-canonical CUG [aa 33 (Leu), murine] (Herring et al., 2019).

Although the data clearly indicate the presence of a mitochondrial CLU protein isoform, the mechanism of mitoCLU protein import is yet to be determined. As over 98% of mitochondrial proteins are synthesized in the cytosol as preproteins with a mitochondrial targeting sequence (MTS), mitoCLU should be examined for a possible MTS. The most common type of MTS is an amino-terminal extension of the preprotein termed the presequence. This MTS directs non-mitochondrial passenger proteins across both outer and inner membranes into the mitochondrial matrix. The presequences of different mitochondrial proteins are not necessarily sequentially consistent but have similar physiochemical properties. The signal is typically described as an N-terminal motif that is ~15–70 residues in length and enriched in positively charged amino acids

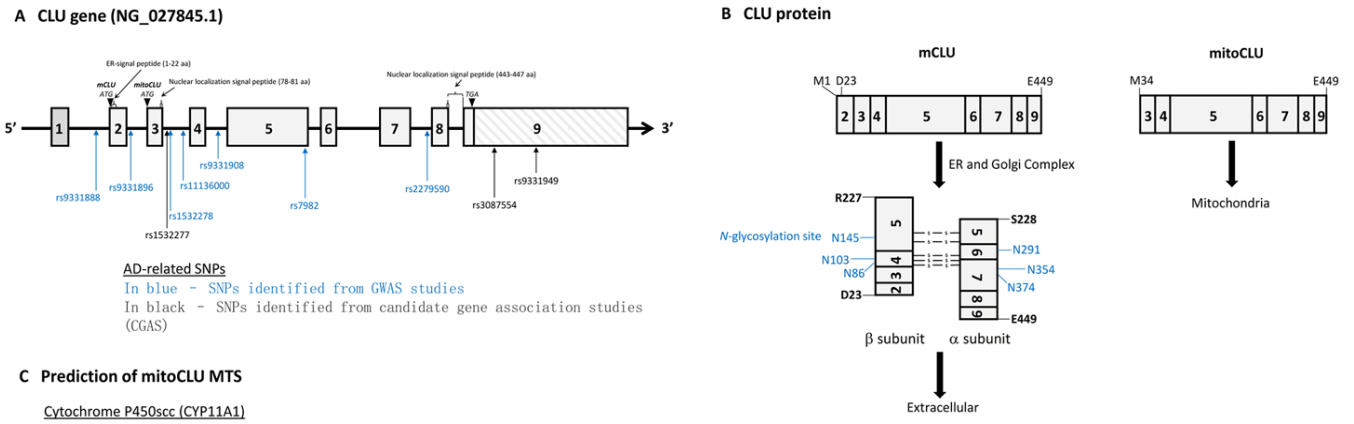


**Figure 1** | **Summary of human *CLU* gene and protein isoforms in the brain.**
(A) CLU is a single-copy gene localized on human chromosome 8 that contains 9 exons. A number of AD-related SNPs in CLU have been identified, mostly intronic. (B) Schematic representation of mature (mCLU) and mitochondrial CLU (mitoCLU) protein isoforms. The mCLU is translated at the translational start site located in Exon 2, and the protein is heavily glycosylated and secreted. The translation of mitoCLU starts at the translational start site located in Exon 3, and the protein is unglycosylated and non-secreted. (C) Physiochemical comparability of the N-terminal amino acid sequence of mitoCLU with mitochondrial targeting signals of human cytochrome P450scc (CYP11A1) and sterol 26-hydroxylase. AD: Alzheimer's disease; CLU: clusterin; GWAS: genome-wide association studies; MTS: mitochondrial targeting sequence; SNP: single nucleotide polymorphism.

(Devi and Anandatheerthavarada, 2010). These sequences have the potential to form amphiphilic α-helices that line one side, whereas the other side is uncharged and hydrophobic. The comparative analyses of the N-terminal sequence of mitoCLU with mitochondrial cytochrome P450scc (CYP11A1) and sterol 26-hydroxylase indicate that the first 50 amino acids of the N-terminus of mitoCLU contain 11 positively charged amino acids (Lys and Arg) which are capable of forming a helical structure consistent with the physiochemical properties of the typical MTS (**Figure 1C**). This suggests mitoCLU may contain a cryptic N-terminal MTS which may target mitoCLU to the mitochondria; however, this prediction must be experimentally investigated.

Just as the mechanism of mitoCLU recognition and import requires further investigation, the molecular function of mitoCLU should be explored in future studies. Recent advances in the study of brain bioenergetics during preclinical LOAD have indicated that bioenergetic dysfunction occurs throughout the preclinical phase of LOAD, with an observable reduction in glucose utilization occurring several decades before clinical onset. Therefore, the discovery of mitoCLU highlights an important and novel avenue of brain-based CLU research in which the identification of mitoCLU protein interacting partners may provide key insight into the understanding of the molecular function of mitoCLU in LOAD development during the preclinical phase.

In summary, CLU is classically described as a heterodimeric, secreted, multi-functional glycoprotein that is involved in cellular stress or neurotoxicity. However, alternative CLU protein isoforms have been recently detected. Our findings (Herring et al., 2019) clarify the subcellular localization of CLU protein isoforms in the brain. Consistent with all *in vivo* and *in vitro* rodent data, mCLU isoforms (CLU_60 kDa, CLU_49 kDa, and CLU_37 kDa) are expressed by human CLU Exon 2–9 and mature mCLU is secreted from the cytosol. Meanwhile, human CLU Exon 3–9 produces a single robust isoform of 45-kDa (CLU_45 kDa), a finding generated in both human and murine cells. MitoCLU is localized to the mitochondrial matrix, although the mechanisms behind protein recognition and import as well as the function of mitoCLU are currently unknown. We believe that this foundational knowledge is critical for our fundamental understanding of the contribution of CLU in the development and intervention of LOAD.

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