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The Role of Clusterin in Alzheimer's Disease: Pathways, Pathogenesis, and Therapy

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Abstract Genetic variation in clusterin gene, also known as apolipoprotein J, has been associated with Alzheimer's disease (AD) through replicated genome-wide studies, and plasma clusterin levels are associated with brain atrophy, baseline prevalence and severity, and rapid clinical progression in patients with AD, highlighting the importance of clusterin in AD pathogenesis. Emerging data suggest that clusterin contributes to AD through various pathways, including amyloid-β aggregation and clearance, lipid metabolism, neuroinflammation, and neuronal cell cycle control and apoptosis. Moreover, epigenetic regulation of the clusterin expression also seems to play an important role in the pathogenesis of AD. Emerging knowledge of the contribution of clusterin to the pathogenesis of AD presents new opportunities for AD therapy.

Keywords Alzheimer's disease · Clusterin · Genetics · Amyloid-β · Pathogenesis · Therapy

Introduction

Clusterin gene (CLU), also known as apolipoprotein J (ApoJ), is currently the third most associated LOAD risk gene according to Alzgene database (http://www.alzgene.org/), and it may explain around 9% of the AD attributable risk [1]. Clusterin levels have been found to be increased in brain and cerebrospinal fluid of patients with AD, and plasma clusterin was recently reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in patients with AD [2, 3]. Similar to its predecessor apolipoprotein E (ApoE), several studies have presented compelling evidence implicating clusterin in the pathogenesis of AD. Although the mechanisms that underlie the pathogenic nature of clusterin in AD are still not completely understood, several pathways have been identified in vitro and in vivo. In this review, we aim to assess evidence for associations of clusterin with risk for AD, with particular attention to amyloid-β (Aβ)-dependent and Aβ-independent pathways through which clusterin contributes to AD pathogenesis. Here, we also present the recent advances and challenges in targeting clusterin for AD therapy.

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Biochemical Properties of Clusterin

In humans, CLU gene maps on chromosome 8p21–p12 proximal to the lipoprotein lipase gene locus. It is organized in nine exons of variable size, ranging from 126 to 412 bp and spanning a region of 17,877 bp (Fig. 1) [4]. The recent GenBank update has earmarked three transcriptional isoforms of human CLU given as RefSeq, named Isoform 1, Isoform 2, and Isoform 3 (also called 11036) (GenBank accession numbers NM_001831.2 NM_203339.1 and NM_001171138.1, respectively). These three transcripts are probably originated from three alternative transcriptional initiation start sites and only produced in humans and chimpanzees [4]. The three primary transcript isoforms produced by ribonucleic acid (RNA) polymerase contain 9 exons, 8 introns, and a terminal 3′-untranslated region.

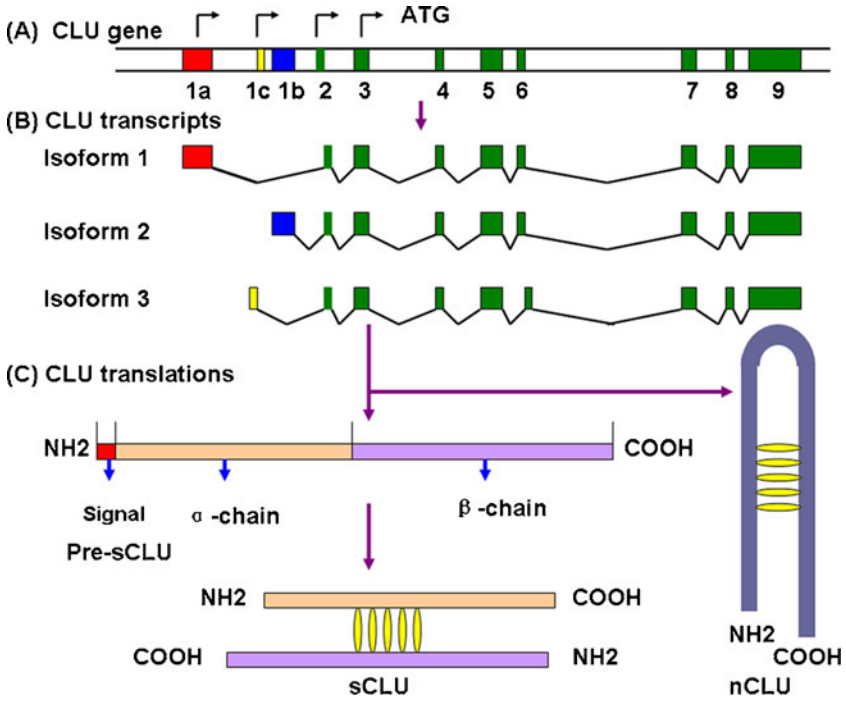

Fig. 1 Structure of clusterin: gene, transcription and translation products. **a** Schematic overview of clusterin gene organization on human chromosome 8. *Green blocks* represent exons from 2 to 9, common to all transcripts, while *red*, *blue*, and *yellow blocks* represent unique exon 1 of different transcriptional variants of clusterin. Isoform 1 exon 1 (*1a*) is indicated in *red*, isoform 2 exon 1 (*1b*) in *blue*, and isoform 3 exon 1 (*1c*) in *yellow*. Transcriptional start sites which are in-frame in the clusterin sequence are represented with *black arrows*. **b** Schematic representation of known exonic structure of clusterin mRNA variants. **c** Schematic representation of secreted (sCLU) and nuclear clusterin (nCLU). Translation of sCLU produces a 427-amino acid sequence that is then targeted to the ER by an initial leader peptide. The intracytoplasmic precursor called pre-sCLU (pre-secretory form) is an uncleaved, unglycosylated protein before ER processing. This precursor protein is processed by first removing the N-terminal signal peptide and subsequently the peptide is further glycosylated and proteolytically cleaved to α and β peptides at an internal site between Arg^{205} and Ser^{206}. The two subunits are linked to each other by five disulfide bonds (*yellow ellipses*). However, nCLU, which also contains 5 disulfide bonds (*yellow ellipses*), lacks the leader peptide and does not undergo glycosylation and α/β cleavage

Each of these transcripts has a unique fragment of exon 1 and shares the remaining sequence from exon 2 to exon 9 (Fig. 1). Two different sets of CLU protein isoforms have been described in mammalian cells, a conventional secretory isoform set (pre-secretory and mature secretory clusterin proteins, psCLU and sCLU, respectively) that has pro-survival functions and another isoform set of intracellular isoforms, appearing in the cytoplasm (pre-nuclear CLU, pnCLU) in basal cells and in the nucleus as an ~55-kDa mature nuclear clusterin (nCLU) form that has pro-apoptotic cell death functions (Fig. 1) [4]. Clusterin is unique among other characterized human apolipoproteins in its structure and tissue distribution. The sCLU is a 75–80-kDa glycosylated α–β-heterodimer, consisting of two chains of about 40 kDa each, linked by five disulphide bonds (Fig. 1) [5]. Translation of sCLU starts from the ATG in exon 2 and produces a 427-amino acid sequence that is then targeted to the ER by an initial leader peptide. The intracytoplasmic precursor psCLU is an uncleaved, unglycosylated protein with an apparent size of 60–64 kDa before ER processing. This precursor protein is processed by first removing the N-terminal signal peptide, and subsequently, the peptide is further glycosylated and proteolytically cleaved to α and β peptides at an internal site between Arg^{205} and Ser^{206}, both of which are 40 kDa in size. The two subunits are linked to each other by five disulfide bonds [6]. A shorter form of about 50 kDa targeting the cell nucleus has also been identified (nCLU) [6]. nCLU lacks the leader peptide and does not undergo glycosylation and α/β cleavage (Fig. 1). The clusterin mRNA is present in relatively high levels in the brain, ovary, testis, and liver; is less abundant in the heart, spleen, lung, and breast; and is absent in the T lymphocytes [5]. Clusterin is a stress-induced protein implicated in a variety of physiological functions, including roles in apoptosis, complement regulation, lipid transport, sperm maturation, endocrine secretion, membrane protection, promotion of cell interactions, and as a chaperone [6, 7]. It is differentially regulated in many severe physiological disturbance states including central nervous system disorders, kidney degenerative diseases, tumorigenesis, atherosclerosis, inflammation, and cell death [4–7]. Clusterin and apolipoprotein A-I (ApoA-I) are two major lipid binding proteins and constituents of high density lipoproteins (HDL) [8]. Computer sequence analysis indicates a significant relationship between

clusterin and ApoA-I [9]. However, the amino acid compositions, amino-terminal sequences, and the immunochemical reactivities of the clusterin subunits provide strong evidence that they are distinct from ApoA-I [8]. Clusterin-HDL has α2 electrophoretic mobility and is unstable while ApoA-I-HDL electrophorese in the pre-beta region. Both clusterin and ApoA-I are secreted by the epithelial epididymal principal cells [8] into the lumen of the duct where they interact with sperm plasma membranes [8]. After a transient interaction with the sperm surface, both clusterin and ApoA-I dissociate and are internalized by the epididymal epithelial cells via the endocytotic receptors, megalin and cubilin [8].

Genetics of Clusterin Gene in AD

CLU locates in a chromosomal region of interest in LOAD defined by the genome-wide linkage study [10]. Four large genome-wide association studies (GWAS) published in the last 2 years have identified the CLU gene locus as a strong genetic locus involved in AD [11–14]. The main associated single nucleotide polymorphisms (SNPs) include rs11136000, rs9331888, rs2279590, rs7982, and rs7012010 (Table 1). Several independent candidate gene studies have replicated and confirmed these results in various Caucasian populations, although the strongest associated variant sometimes differed (Table 1) [15–19]. We also recently reported that variation in the CLU gene plays an important role in sporadic LOAD in the Han Chinese population [20]. Using a large multicenter data set for 15,239 subjects, a meta-analysis has also confirmed that CLU is AD susceptibility locus in European ancestry populations [21]. However, the association of CLU polymorphisms with AD was not replicated in the African-American, Arab, and Hispanic populations. As the negative results are possibly because of small sizes of these groups, further analysis is merited in these racial/ethnic groups using larger cohorts [21]. Interestingly, the CLU genetic association was also replicated and confirmed in the largest family-based GWAS of LOAD [22].

Efforts to identify functional variations through exon sequencing and examining effects of SNPs on CLU expression in brain tissue have not yet provided a functional link between the associated polymorphisms and AD [23], such as is seen in ApoE. However, the risk allele of the AD-associated SNP rs9331888 was found to be associated with the alternative splicing of CLU gene [24]. It increases the relative abundance of transcript NM_203339. Schürmann B et al. identified that the risk allele of the AD-associated SNP rs11136000 was significantly associated with lower clusterin plasma levels in an allele-dose-dependent manner [25]. Coincidently, the results of our recent study also revealed that the AD risk rs9331888 allele was associated with a decrease in CLU plasma levels [26]. However, clusterin levels have been found to be increased in brain and cerebrospinal fluid of patients with AD, and plasma clusterin was recently reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in patients with AD [2, 3]. Several studies have provided evidences for a protective role of clusterin in AD pathogenesis, such as prevention of Aβ fibrillization, clearance of Aβ, inhibition of the complement system and neuronal apoptosis, and promotion of neurite outgrowth [3, 7]. Moreover, these data from mouse and man suggest that clusterin is acting as disease response agent, specifically amyloid response agent [27, 28]. The mouse data suggest that clusterin is beneficial agent, perhaps acting to remove amyloid [27]. Therefore, interestingly, the fact that the AD risk alleles are associated with less accumulation or production of clusterin while in the disease there appeared to be excessive accumulation or production perfectly fits the hypothesis that carriers of the clusterin gene AD-risk variants may increase vulnerability to developing AD later in life by attenuating the neuroprotective response increase of clusterin. Additionally, as the two risk alleles (rs11136000 and rs9331888) are in near complete linkage disequilibrium (LD) with each other [20, 24], it is also likely that neither rs11136000 nor rs9331888 are causative variants that actually cause expression changes in the clusterin protein, but they both point to the true causative variant in that LD block.

A more accurate prediction of disease progression based upon CLU genetic variants may allow for better clinical trial design. If the CLU genetic variants do differentially affect the progression and response to therapies, it will be critical to subdivide subject groups and analyze the rate of cognitive decline separately according to CLU genotype. Hence, genetic analysis of CLU in AD requires more in-depth investigation in the future.

Epigenetics of Clusterin Gene in AD

Recent studies have suggested that epigenetic mechanisms may play a pivotal role in the course and development of AD [29]. Indirect evidence demonstrating epigenetic alterations associated with various risk factors for AD, such as nutritional factors, stress, depression, and brain trauma, implies that epigenetic processes may be the key mechanism mediating gene × environment interactions in AD. Therefore, both genome-wide and more targeted, i.e. gene specific, techniques to establish DNA methylation profiles and histone modification maps could add significantly to information on genetic variations and gene expression profiles for genes implicated in AD.

The expression of CLU is clearly increased after neuronal injuries and degeneration as well as during aging and neurodegenerative diseases [6]. The promoter region of CLU contains several binding sites for stress-related transcription factors, e.g., AP1, HSF, and CREB [30] and a CpG-rich

Table 1 Reported association studies of CLU variants with AD

No. and type of subjects	SNPs	OR (95% CI)	*P* value	Population type	Refs
GWAS, 3,941 cases, 7,848 controls	rs11136000	0.84 (0.79–0.89)	1.4×10^{-9}	GWAS: GERAD1 includes UK/ Ireland, Germany, and USA	[11]
	rs7982	NA	1.4×10^{-9}		
	rs3087554	NA	NA		
	rs9331888	NA	1.4×10^{-9}		
	rs7012010	1.11 (1.04–1.18)	7.6×10^{-4}		
Follow-up, 2,023 cases, 2,340 controls	rs11136000	0.91 (0.79–0.89)	0.017	Follow-up sample: Belgium, Germany, Greece, UK, and UK/Ireland	
	rs7982	NA	0.032		
	rs3087554	NA	0.146		
	rs9331888	NA	0.304		
	rs7012010	NA	0.309		
Combined, 5,964 cases, 10,188 controls	rs11136000	0.86 (0.82–0.90)	8.5×10^{-10}		
	rs7982	0.8	8.0×10^{-10}		
	rs3087554	1.09	0.146		
	rs9331888	1.05	0.304		
	rs7012010	1.10	1.0×10^{-4}		
GWAS, ≤2,025 cases, ≤5,328 controls	rs2279590	0.83 (0.77–0.90)	1.0×10^{-6}	GWAS	[12]
	rs11136000	0.83 (0.77–0.90)	1.5×10^{-6}	EADI1 includes French Caucasian	
	rs9331888	1.19 (1.11–1.30)	1.8×10^{-5}		
Follow-up, ≤3,862 cases, ≤3,180 controls	rs2279590	0.88 (0.81–0.95)	8.2×10^{-4}	Follow-up sample: Belgium, Finland, Italy, and Spain	
	rs11136000	0.88 (0.81–0.95)	8.8×10^{-4}		
	rs9331888	1.12 (1.04–1.21)	2.9×10^{-3}		
Combined, ≤5,887 cases, ≤8,508 controls	rs2279590	0.86 (0.82–0.91)	8.9×10^{-9}		
	rs11136000	0.88 (0.81–0.90)	7.5×10^{-9}		
	rs9331888	1.16 (1.10–1.23)	9.4×10^{-8}		
GWAS, 3,006 cases, 14,642 controls	rs11136000	0.89 (0.83–0.94)	4.98×10^{-4}	Stage 1: White from CHARGE, TGEN, Mayo AD GWAS	[13]
Pooling 1, 5,038 cases, 19,974 controls	rs11136000	0.85 (0.81–0.90)	1.49×10^{-9}	Stage 2: White from stage 1 and EADI1	
Pooling 2, 8,371 cases, 26,969 controls	rs11136000	0.85 (0.82–0.88)	1.6×10^{-16}	Stage 3: White from stage 1 and GERAD1 (excluding the Mayo AD GWAS)	
1,140 cases, 1,209 controls	rs11136000	0.82 (0.77–0.99)	0.03	Replication stage: Spanish	
1,367 cases (973 incident), 7,962 controls	rs11136000	0.90 (0.82–0.98)	0.02	Replication stage: White from CHARGE	
8,309 cases, 7,366 controls	rs1532278	0.90 (0.85–0.95)	5.6×10^{-5}	Stage 1: ADGC GWAS includes ACT/eMERGE, ADC, ADNI, GenADA, UM/VU/MSSM, OHSU, NIA-LOAD, and TGEN2.	[14]
3,531 cases, 3,565 controls	rs1532278	0.87 (0.81–0.94)	2.6×10^{-4}	Stage 2: Follow-up replication includes Mayo Clinic, ROSMAP, UP, and WU.	
Combined, 11,840 cases, 10,931 controls	rs1532278	0.89 (0.85–0.93)	8.3×10^{-8}		
2,654 cases, 1,175 controls	rs2279590	0.83 (0.73–0.95)	0.03	Family based USA	[15]
	rs11136000	0.84 (0.73–0.96)	0.06		
	rs9331888	1.06 (0.92–1.23)	0.19		
214 cases, 211 controls	rs2279590	1.05 (0.83–1.33)	0.63	Germany	
	rs11136000	0.99 (0.83–1.27)	0.49		
	rs9331888	1.20 (0.93–1.55)	0.12		
1,019 cases with clinically characterized and neuropathologically verified AD, 591 controls without neuropathologic AD	rs11136000	0.86 (0.74–0.99)	0.04	White, United States, United Kingdom, and the Netherlands	[16]
549 cases, 544 controls	rs881146	?	0.037	Caribbean Hispanic individuals	[17]

Table 1 (continued)

No. and type of subjects	SNPs	OR (95% CI)	*P* value	Population type	Refs
	rs70120100		0.159		
	rs17057441		0.045		
	rs11136000		0.582		
1,819 cases, 2,565 controls	rs11136000	0.82 (0.75–0.91)	8.6×10^{-5}	White, United States	[18]
1,348 cases, 1,359 controls	rs2279590	0.91 (0.85–0.97)	0.148	White, United States	[19]
	rs11136000	0.93 (0.87–0.99)	0.258		
	rs9331888	1.02 (0.95–1.09)	0.826		
6,925 cases, 9,748 controls	rs2279590	0.87 (0.84–0.91)	3.07×10^{-9}	White from EADI1, GERAD1 and the present study.	
11,154 cases, 17,786 controls	rs11136000	0.86 (0.83–0.89)	4.4×10^{-16}		
7,209 cases, 9,831 controls	rs9331888	1.11 (1.06–1.17)	6.76×10^{-6}		
324 cases, 388 controls	rs2279590	0.82 (0.62–1.08)	0.157	Northern Han Chinese	[20]
	rs11136000	0.84 (0.64–1.10)	0.194		
	rs9331888	1.39 (1.13–1.72)	0.002		
5,935 cases, 7,034 controls	rs7012010	1.10 (1.03–1.17)	0.0025	White subjects from ADC, ADNI, CAMP	[21]
	rs3087554	1.00 (0.92–1.09)	0.92		
	rs11136000	0.91 (0.85–0.96)	0.0007	FHS, UM/VU/MSSM, MIRAGE, NIA-LOAD, OHSU, and TGEN	
	rs9331888	0.99 (0.92–1.06)	0.76		
	rs7982	0.87 (0.81–0.94)	0.0002		
1,135 cases, 1,135 controls	rs7012010	1.08 (0.95–1.2)	0.24	Non-Caucasian from African American, Arab, and Caribbean Hispanic subjects	
	rs3087554	0.77 (0.53–1.1)	0.14		
	rs11136000	1.05 (0.94–1.2)	0.39		
	rs9331888	0.90 (0.59–1.4)	0.64		
	rs7982	0.97 (0.84–1.1)	0.68		
1,848 cases, 1,991 controls	rs11136000	?	0.0083	NIA-LOAD and NCRAD Family Studies	[22]
	rs7012010		0.2786		
993 cases, 884 controls	rs11136000	?	0.0019	A single case was selected from each family	
	rs7012010		0.0614		
403 cases, 235 controls	c.48C>A	0.752	0.546	White, Portuguese series	[23]
	rs9331892	0.674	0.607		
	rs7982	0.943	0.630		
	rs3216167	1.233	0.145		
	rs9331936	3.507	0.247		
	rs9331937	0.577	0.698		
	rs9331938	1.156	0.906		
	rs9331939	0.287	0.309		
	rs3087554	1.112	0.565		
	g.27511354C/T	1.723	0.500		
489 cases, 632 controls	c.−229G>C	1.50	0.11	White, UK series	
	c.701G.A	0.47	0.51		
	rs41276297	0.53	0.35		
	rs7982	0.93	0.41		
	c.965T>C	1.42	0.73		
	rs3216167	1.68	0.04		
	rs9331939	0.68	0.68		

ACT/eMERGE the Adult Changes in Thought/Electronic Medical Records and Genetics study, *ADC* Alzheimer's Disease Centers cohort, *ADGC* Alzheimer Disease Genetics Consortium, *ADNI* Alzheimer's Disease Neuroimaging Initiative cohort, *CAMP* Collaborative Aging and Memory Project cohort, *CHARGE* Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium, *CI* confidence intervals, *EADI1* European AD Initiative 1 Consortium, *FHS* Framingham Heart Study cohort, *GenADA* Genotype–Phenotype Associations in Alzheimers Disease Study, *GERAD1* Genetic and Environmental Risk in AD 1 Consortium, *GWAS* Genome-Wide Association Studies, *JHU* Johns Hopkins University cohort, *MIRAGE* Multi-Institutional Research on Alzheimer's Genetic Epidemiology cohort, *NCRAD* National Cell Repository for Alzheimer Disease, *NIA-LOAD* National Institute on Aging Late-Onset Alzheimer's Disease cohort, *OHSU* Oregon Health and Science University cohort, *OR* odds ratios, *ROSMAP* the Rush University Religious Orders Study/Memory and Aging Project, *SNP*, single nucleotide polymorphism, *TGEN* Translational Genomics Research Institute cohort, *TGEN2* Translational Genomics Research Institute series 2, *UM/VU/MSSM* University of Miami/Vanderbilt University/Mount Sinai School of Medicine cohort, *UP* University of Pittsburgh, *WU* Washington University, *?* no data

methylation domain [31–33]. Since aging affects both DNA methylation and histone acetylation status, epigenetic regulation might have an important role in clusterin/Apo J expression [31]. CLU has been epigenetically regulated in prostate cancer cell lines, neural cells, and retinal pigment epithelial cells [31–33]. The demethylation with 5-aza-2′-deoxycytidine in prostate cancer cell lines significantly increases the expression of CLU [31], and the treatment with histone deacetylase inhibitors and 5-aza-2-deoxycytidine also strongly increase the expression of CLU and secretion of CLU protein in human neural cells [32] and retinal pigment epithelial cells [33]. A similar mechanism of epigenetic regulation has also been described in tumor-conditioned endothelial cells [34]. Meanwhile, epigenetic regulation is also generally believed to be linked to aging process and age-related diseases [35]. Aberrant epigenetic regulation of the CpG islands could affect the risk of developing LOAD [36]. Recent evidence has also shown that epigenetic factors could affect the amyloidogenesis in AD [37]. Moreover, histone deacetylase (HDAC) inhibitors have several neuroprotective and neurotrophic effects and have demonstrated therapeutic potential in several neurodegenerative diseases [38]. They can even prevent the pathogenesis in transgenic AD mice [39]. HDAC inhibitors are also promising therapeutic agents in clinical trials to combat different cancer diseases [40].

Clusterin Levels in AD

Brain Clusterin in AD

In 1990, mRNA for clusterin was initially found to be significantly elevated in AD affected brain regions when compared to control brains [41]. The clusterin protein levels are increased in the frontal cortex and hippocampus of postmortem AD brains [42]. Moreover, clusterin immunoreactivity is present in amyloid deposits, neuropil threads, dystrophic neurites in senile plaques, and neurofibrillary tangle (NFT)-free neurons but is rarely observed in NFT-containing neurons [43]. Using PET imaging, it was also demonstrated that increased plasma clusterin concentrations were positively associated with fibrillar Aβ burden in the entorhinal cortex in AD patients [2]. Moreover, reduced levels of ApoE and increased levels of clusterin were correlated with the number of ε4 alleles [44], while the opposite was found in another study, which demonstrated that the presence of the ApoE ε4/4 allele significantly decreased the amount of clusterin in the frontal lobe in AD patients [45].

CSF Clusterin in AD

Clusterin has been demonstrated to be present in lipoprotein particles in CSF [46]. The analysis of the clusterin level in CSF has proved difficult since its glycosylation level can change [47] and clusterin can also form complexes with Aβ peptides and fibrils [48]. Initial studies did not find any difference between control and AD patients [45], but more recent studies using modern techniques have revealed that the level of clusterin protein in CSF is significantly increased in AD patients [48, 49].

Plasma Clusterin in AD

Interestingly, using a combined proteomic and neuroimaging approach, plasma clusterin was recently reported to be positively associated with brain atrophy in the hippocampus and entorhinal cortex, baseline disease severity, and rapid clinical progression in AD, suggesting its possible use as a plasma biomarker of AD [2]. This result was confirmed in transgenic mice that had marked cerebral Aβ deposition and cognitive defects [2]. In an independent follow-up study, plasma clusterin levels were replicated to be significantly associated with baseline prevalence and severity of AD, but not with incidence of AD [3]. Moreover, plasma clusterin concentration is also associated with longitudinal brain atrophy in mild cognitive impairment [50]. Furthermore, plasma concentration of clusterin also appears to reflect its concentration within brain regions vulnerable to AD pathology. However, plasma clusterin levels were not found to be increased in presymptomatic AD in an independent small case–control study [51]. The negative results are possibly because of the small size of the group. More studies are required to further elucidate whether the clinical utility of plasma CLU concentration as a stand-alone biomarker for AD is feasible.

Aβ-Dependent Roles for Clusterin in AD

Effects of Clusterin on Aβ Aggregation

Although several reasons may underlie the clusterin specific effect on the risk of developing AD (Fig. 2), convincing evidence suggests that the physical interaction of clusterin with Aβ plays an important role in AD pathogenesis. Clusterin, as a chaperone, can bind via its flexible structures to a wide array of physiological ligands putatively involved in Alzheimer's pathology, such as Aβ peptides and fibrils, complement components and important lipids, e.g., cholesterol and phospholipids [52].

Both in vitro and in vivo studies have shown that clusterin/Aβ interactions play an important role in amyloid formation and toxicity [27, 53–58]. Clusterin has been demonstrated to be fully active to interact with soluble Aβ ($Aβ_{1–40}$ and $Aβ_{1–42}$) in vitro [53]. Furthermore, the complex formation significantly prevents aggregation and

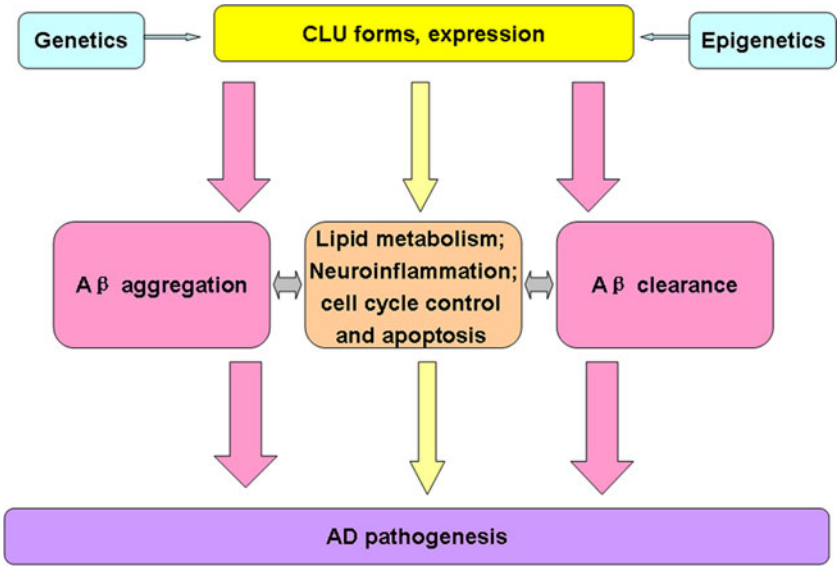

Fig. 2 Pathogenic mechanisms of clusterin in AD. Several mechanisms have been proposed to understand the important roles of clusterin in AD pathogenesis. Evidence suggests that the major effect of clusterin on the risk of developing AD is via its effect on Aβ aggregation and clearance, influencing the onset of Aβ deposition. Other mechanisms, such as the involvement in neuroinflammation, the modulation of brain cholesterol and lipid metabolism, as well as the inhibition of neuronal apoptosis/potentiation of neuroprotection, may also contribute to the disease process. Clusterin forms or expression changes produced by genetic polymorphisms or epigenetic modifications may also mediate processes involved in AD pathogenesis

polymerization of soluble Aβ. In addition, the binding to clusterin protects soluble Aβ from proteolytic degradation [53]. It was also observed that clusterin regulated amyloid formation in a biphasic manner with low clusterin to Aβ peptides ratios enhancing and higher ratios inhibiting amyloid formation, respectively [54]. Despite potential neuroprotective effects of clusterin, it was also observed to promote the formation of toxic soluble oligomeric forms of Aβ in vitro [55, 56]. Clusterin decreased aggregation of Aβ42 and that subsequent incubation of the less aggregated material to PC12 cells significantly increased amyloid induced toxicity and oxidation [55]. Small diffusible oligomers of Aβ42 induced by the presence of clusterin were associated with increased neuronal toxicity in organotypic central nervous system cultures at nanomolar concentrations [56]. Interestingly, using highly sensitive single-molecule fluorescence methods, $A\beta_{1-40}$ is recently found to form a heterogeneous distribution of small oligomers, all of which interact with clusterin to form long-lived, stable complexes [57]. Consequently, clusterin is able to influence both the aggregation and disaggregation of Aβ(1–40) by sequestration of the Aβ oligomers, indicating the protective role of clusterin [57]. A preliminary in vivo study showed that that expression of murine clusterin in some way facilitates the conversion of a larger percentage of aggregated Aβ into thioflavine-S-positive amyloid as compared with clusterin-deficient mice [58]. Although these results may seem contradictory to some in vitro findings which have demonstrated that at certain concentrations, purified clusterin can interact with Aβ and result in an inhibition of fibril formation [53, 55], it is possible that the absence of clusterin results in a compensatory change in other molecules that results in the phenotype observed. Moreover, they also observed that the thioflavine-S-positive amyloid that deposits in the absence of clusterin was associated with far less neuritic dystrophy than amyloid present in clusterin-expressing PDAPP mice [58]. However, in a background of ApoE-negative ($ApoE^{-/-}$) PDAPP mice, the ablation of clusterin expression had both earlier onset and markedly increased Aβ and amyloid deposition [27]. The study strongly demonstrates that ApoE and clusterin cooperatively suppress Aβ deposition and that ApoE is contributing to this effect via directly influencing the metabolic fate of soluble, extracellular Aβ [27]. Such evidence shows that the in vivo effects of clusterin on amyloid formation are likely to involve multiple interactions and processes. Moreover, in these PDAPP transgenic mice, cortical Aβ plaques were shown to contain clusterin, and both Aβ burden and clusterin deposition increased with age [2]. Using PET imaging, it was also demonstrated that increased plasma clusterin concentrations were positively associated with fibrillar Aβ burden in the entorhinal cortex [2].

The somewhat rudimentary but important question still exists as to whether it is better to increase or decrease human clusterin levels in order to reduce Aβ levels. Analyzing whether and to what extent altering human clusterin level affects Aβ pathology will help determine whether targeting clusterin levels may be a viable therapeutic option for influencing Aβ levels and toxicity and ultimately treating AD.

Effects of Clusterin on Aβ Clearance

Currently, it is widely believed that impaired Aβ clearance is a major pathogenic event for LOAD [59]. Aβ has a relatively short half-life in the brain [60]. In human brains, the Aβ clearance rate is 8.3% per hour [61], indicating that Aβ is actively and efficiently cleared from the brain. Clusterin and ApoE are the main escorting proteins of Aβ in brain. Transgenic mouse models of AD have revealed that ApoE and clusterin regulate in a cooperative manner the clearance and deposition of Aβ in brain [27]. Both $ApoE^{-/-}$ and $ApoE^{-/-}$/$clusterin^{-/-}$ mice had elevated CSF and brain interstitial fluid Aβ, as well as significant differences in the elimination half-life of interstitial fluid Aβ [27].

In brief, there are two major pathways by which Aβ is cleared from the brain: receptor-mediated clearance by cells in the brain parenchyma (microglia, astrocytes, and neurons), along the interstitial fluid (ISF) drainage pathway or through the blood–brain barrier (BBB) and through endopeptidase-mediated proteolytic degradation [62]. Low-density lipoprotein receptor-related protein-2 (LRP-2)/megalin, which is expressed in vascular CNS tissues including the choroid plexus, the BBB endothelium, and the ependyma, is the major receptor for the uptake of Aβ complexed with clusterin (Fig. 3) [62, 63]. Using an established in vivo technique, clusterin is rapidly eliminated from mouse brain across the BBB by LRP-2/megalin receptor, and the binding of clusterin to $Aβ_{1–42}$ clearly facilitates its clearance across the BBB (Fig. 3) [63]. This suggests that at physiological concentrations, the net transport of soluble Aβ via clusterin/megalin at the BBB favors its efflux from the brain. Furthermore, chronic ischemic BBB injuries and hemorrhages, such as those occurring in cerebral amyloid angiopathy, can increase the extravasation of serum clusterin/Aβ complexes from blood to brain and in that way increase the amyloid deposition and amyloid binding to neurons, e.g., as observed in ischemic conditions (Fig. 3) [64]. Although perfusion studies in guinea pig demonstrated that this receptor is utilized for the efficient transport of clusterin–$Aβ_{1–40}$ complexes from the blood across the BBB into the brain [65], the fact that at physiological concentrations this transport mechanism is saturated by free clusterin suggests that the clusterin-dependent transport of Aβ via megalin-

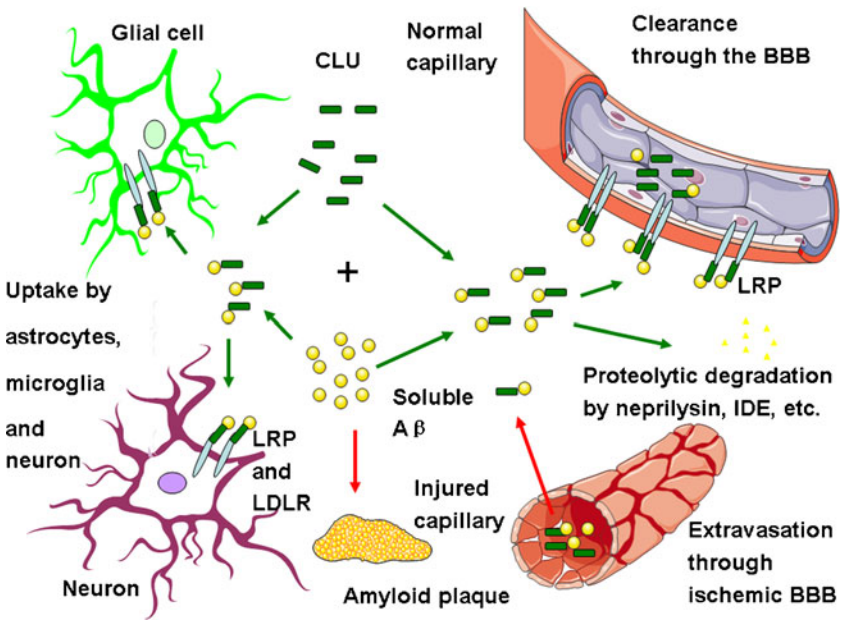

Fig. 3 Effects of clusterin on Aβ clearance. Major Aβ clearance pathways include receptor-mediated clearance by cells in the brain parenchyma (neurons and glia), along the interstitial fluid drainage pathway and through the blood–brain barrier (*BBB*) and proteolytic degradation by endopeptidases. Receptor-mediated clearance of Aβ in the brain is mediated mostly via LRP (low-density lipoprotein receptor-related protein 1), which is widely expressed in neurons, astrocytes, and microglia of the brain parenchyma, as well as in endothelial cells, astrocytes, and smooth muscle cells at the BBB and cerebral arteries, but several other transporters have been described, e.g., MDR1-P glycoprotein (multidrug receptor), scavenger receptors, and receptor for advanced glycation end products. Remarkably, these lipoprotein receptors do not bind Aβ directly, but only as a complex ligand. LRP-2/megalin is the major receptor for the uptake of Aβ complexed with clusterin. Clusterin is rapidly eliminated from brain across the BBB by LRP-2/megalin receptor and the binding of clusterin to Aβ clearly facilitates its clearance across the BBB. In addition to modulating Aβ transport, clusterin could also be involved in the local removal of soluble Aβ form the ISF by receptor-mediated endocytosis of clusterin-bound Aβ into brain cells followed by the intracellular degradation of the complexes. Furthermore, chronic ischemic BBB injuries and hemorrhages can increase the extravasation of serum clusterin/Aβ complexes from blood to brain and in that way increase the amyloid deposition and amyloid binding to neurons. *IDE* insulin-degrading enzyme

mediated transcytosis is considered to have no significant influence on the influx of plasma Aβ into the brain [66]. Despite these findings, more work is needed to determine the exact role that the BBB plays in mediating Aβ clearance, how clusterin plays a role in this process.

In addition to modulating Aβ transport, clusterin could also be involved in the local removal of soluble Aβ from the ISF by receptor-mediated endocytosis of clusterin–Aβ complexes into brain cells followed by the intracellular degradation of the complexes (Fig. 3). Uptake of Aβ into differentiated mouse teratocarcinoma F9 cells upon co-incubation with clusterin is enhanced and preformed clusterin–Aβ complexes are internalized and degraded [67]. Both processes could be blocked by receptor-associated protein and by anti-megalin antibodies, demonstrating that clusterin–Aβ complexes are endocytosed via megalin. Exposition to extracellular Aβ leads to an activation of astrocytes and to a rise in cell-associated clusterin with a concomitant decline in the amount of clusterin in the culture medium pointing to an enhanced endocytosis of clusterin in the presence of Aβ peptide [68]. Interestingly, induction of cytoplasmic vacuoles containing fibrillar amyloid material in human and rat astrocytes upon exposure to $A\beta_{1-42}$ peptides and fibrils is paralleled by an enhanced expression of clusterin [69]. However, more studies are necessary to solidify whether clusterin facilitates the uptake of Aβ into the various cell types found in the brain and by what mechanism this may occur.

Aβ-Independent Roles for Clusterin in AD

The Effect of Clusterin on Lipid Metabolism

Strikingly, clusterin encodes the second major apolipoprotein of the brain, ApoJ. It shares many of ApoE's properties, not only in relation to Aβ but also to lipid transport. It is involved in the transport of cholesterol and phospholipids [70], and increased clusterin levels have been observed in atherosclerosis [71]. Clusterin promotes the efflux of cholesterol from lipid-loaded mouse macrophages [72]. Polymorphisms in clusterin have been associated with lipid levels and carotid intima media thickness [73]. This suggests that genetic variation in clusterin might also indirectly modify susceptibility to AD by increasing the risk of cerebrovascular disease, which in turn could accelerate the primary neurodegenerative process.

The brain is a lipid-rich organ with lipids in cellular membranes and in the myelin sheathes of axons. The insoluble nature of lipids means they cannot be transported between cells that are not contiguous unless they are solubilized so that the lipids are transported in soluble lipoprotein particles. Clusterin is one of the main brain cholesterol transport lipoproteins [70, 74]. The effects of cholesterol on AD susceptibility indicate that it is likely that the roles of these brain cholesterol transport lipoproteins in lipoprotein particles and lipid metabolism modulate their role in Aβ-related pathways [74]. Further studies will be important to clarify the role of clusterin-related brain lipid metabolism and whether the presence of CLU polymorphism directly promotes the metabolic changes that occur during the disease process or it indirectly affects brain lipid metabolism via effects on amyloid or possibly effects on the cerebrovasculature.

The Role of Clusterin in Neuroinflammation

Inflammation of the brain is a prominent pathological feature of AD [75]. Currently, there have been increasing evidence suggesting that inflammatory mechanisms are not merely bystanders in neurodegeneration but powerful pathogenetic forces in the disease process [75, 76], as long-term treatment with non-steroidal anti-inflammatory drugs reduces AD risk and may delay disease progression [77, 78]. The innate immune response and resulting neuroinflammation appears to be responsible for local activation of microglia, astrocytes, and the complement system, the subsequent local initiating a pro-inflammatory cascade that results in the release of potentially cytotoxic molecules, cytokines, and other related compounds, causing neurodegeneration [75, 79]. Several studies have suggested that clusterin is involved directly and indirectly in numerous ways related to inflammation and immunities, also in brain [80, 81]: (a) regulation of complement activation [82, 83], (b) negative regulator of NF-κB [84, 85], and (c) activation of microglia and bidirectional regulation of and by major proinflammatory cytokines such as TNF-α, TGF-1β, and IL-6 [86–90]. However, the role of clusterin in the inflammation associated with AD pathogenesis needs to be further investigated with genetic and pharmacological manipulations of specific inflammatory pathways.

The Effect of Clusterin on Cell Cycle Control and Apoptosis

nCLU is pro-apoptotic, while sCLU is pro-survival [91]. Both the nCLU and sCLU forms of clusterin have been implicated in various cellular functions, including DNA repair apoptotic cell death and cell cycle regulation. Various studies have shown that forced over-expression of full-length clusterin mRNA can non-physiologically force expression of nCLU, which acts as a pro-cell death signal that leads to cell growth inhibition and lethality [4, 92]. Moreover, a recent proposal suggests that tumor cell survival is connected with over-expression of sCLU and loss of nCLU [93]. This theory has been supported by recent data proposing that cells must suppress sCLU to stimulate cell death. Moreover, recent studies have shown that clusterin is involved in DNA repair signaling pathways, especially in the non-homologous end-joining DNA double-strand break repair pathway. nCLU protein

binds to Ku70, forming a trimeric protein complex with Ku80. Over-expression of nCLU reduces the binding activity of Ku70/Ku80 to DNA in whole-cell extracts [4, 90]. In addition, clusterin represents a key player in the regulation of cell cycle progression. Forced overexpression of full-length CLU mRNA, which probably resulted in expression of nCLU, in immortalized human prostate epithelial cells resulted in an increased accumulation of cells at the G0/G1 phases of the cell cycle, accompanied by slow down of cell cycle progression and a reduction of DNA synthesis [94]. High levels of sCLU were reported to cause G1 cell cycle arrest in distinct cell types [95]. The question of clusterin roles in DNA repair, cell cycle regulation, and apoptotic cell death is important in AD pathogenesis since dysfunctions in these regulatory processes have been linked to AD pathology [96].

Roles of Clusterin in Cognitive and Behavioral Impairments

Although clusterin has been implicated in the pathophysiology of AD [97], little is known about how the gene and its protein product contribute to the manifestation of the disease. Clusterin levels have been correlated with symptom severity, entorhinal/hippocampal cortex atrophy, and Aβ burden [2]. These results are confirmed and extended by a later study [3], which confirms that higher clusterin levels are associated with both the occurrence of Alzheimer disease and disease severity and additionally shows that there is little evidence of higher plasma clusterin levels predicting disease occurrence. However, within the mild cognitive impairment (MCI), higher baseline concentration of plasma clusterin was associated with slower rates of brain atrophy in these regions including whole brain, ventricular CSF, temporal gray matter as well as parahippocampal, superior temporal, and cingulate gyri [50]. One plausible explanation might be that in individuals with MCI, elevated plasma clusterin is associated with decreased rate of atrophy as would be expected for a protective mechanism. However, in individuals with established AD, the association of plasma clusterin with pathology may reflect the eventual failure of such protective mechanisms in the setting of genetic risk factors and/or adverse environmental influences [50]. Several findings also suggest an influence of this multi-functional protein on preclinical stages of AD. Firstly, the Alzheimer risk variant of the clusterin gene (CLU) was associated with lower white matter integrity in young healthy adults [98]. Secondly, using functional magnetic resonance imaging during working memory to probe the effect of the risk variant on brain activation in healthy individuals, participants with the AD risk genotype on the CLU gene had higher activity than participants with the protective allele in dorsolateral prefrontal cortex, hippocampus, and cingulate, particularly during high memory demand [99]. Additionally, the risk allele of the AD-associated SNP rs11136000 was significantly associated with worse cognitive functioning, measured both by MMSE and the cognitive composite score [100]. However, the precise mechanism of the cognitive links of clusterin to cognitive and behavioral impairments has not yet been explored. Also, numerous mentions of in vivo rodent model work are made and some involving reducing Aβ load with clusterin changes, yet again not much in terms of how this may change cognition and behavior. Can clusterin directly impact neural function to the level of behavioral or cognitive changes? Or is this an indirect event, mediated by inflammation or amyloid peptides mentioned in the above sections? Further experimental and in vivo studies are warranted to understand the underlying mechanism.

Clusterin as a Therapeutic Target for AD: Future Perspective

Most therapeutic approaches to AD have been designed to reduce Aβ production or aggregation or to promote its clearance. Because clusterin has crucial roles in both Aβ-dependent and Aβ-independent AD pathogenic pathways, it is logical to also consider potential therapeutic attempts to regulate disease states through modulation of its expression and functions. It has been demonstrated that the subcutaneous injection of recombinant human clusterin protein could evoke many beneficial effects in several animal models of peripheral neuropathies [101]. It was also showed that the orally active clusterin peptide (D-[113-122]apoJ) made the HDL particles which incorporated this moiety anti-inflammatory and this dramatically reduced atherosclerosis in ApoE-null mice [102]. The therapeutic approach can affect the cholesterol transport in plasma and protect against atherosclerosis and probably also against cerebral amyloid angiopathy in AD. Whether these strategies are worth pursuing in AD not only depends on the potential of the compounds to cross the BBB but also on the underlying mechanism of action through which the gene is involved in AD. A more in-depth genetic screening is likely to uncover functional variants that shed light on these mechanisms by their nature (gain or loss of function) and/or location in specific functional domains, splice sites (e.g., alternative splicing of CLU can give rise to a nuclear variant that is involved in apoptosis) or regulatory regions [103].

Moreover, HDAC inhibitors, such as valproic acid and Vorinostat, can induce the expression and secretion of clusterin in several neuroblastoma, astrocytes, and glioma cell lines at the therapeutical concentrations [104]. Hence, one could postulate that HDAC inhibitors may be able to prevent Aβ aggregation or increase Aβ clearance in AD by increasing clusterin expression. However, it is well-known that sCLU is

pro-survival and nCLU is pro-apoptotic. Can we simply find a way to suitably stimulate the expression of clusterin so that clusterin can only elicit therapeutic intervention to save neurons? Furthermore, since clusterin can stimulate microglial cells, how can one save neurons without inducing detrimental effects from microglia? These are all important questions that need further investigation. Ultimately, it may be necessary to target or tailor a specific type of clusterin to a particular therapeutic aim.

Concluding Remarks

CLU is currently the third most associated LOAD risk gene, albeit with an effect size much smaller than that of ApoE gene. However, the association signals reported by the GWAS and candidate gene studies do not appear to exert their effects by altering expression or common coding variants, such as is seen in ApoE and rare coding variants are not likely to be responsible for familial disease by now. Hence, genetic analysis of CLU in AD requires more in-depth investigation in the future. Interestingly, epigenetic regulation of the CLU expression also seems to play an important role in the pathogenesis of AD. Furthermore, the epigenetics of CLU in AD also requires more in-depth investigation both in vivo and in vitro.

Understanding whether CLU plays a mechanistic role in the progression of AD is an important question to address in the future and will provide further insights into its pathophysiological role in AD. An emerging body of data has identified multiple pathways that could explain the pathogenic nature of clusterin in AD. In addition to possibly being involved in aggregation and clearance of Aβ, clusterin has also been reported to be involved in regulation of brain cholesterol and lipid metabolism, inflammation of the brain, and the inhibition of neuronal apoptosis/potentiation of neuroprotection. From these, we must identify the pathways that are most relevant to AD pathogenesis and those that present targets with tractable efficacy for AD therapy. Drugs are normally designed against a central pathway for a particular disease. Thus, clusterin is not a typical target, since it has so many functions and isoform-specific effect. However, this may be an advantage for a multi-etiological disorder such as AD. Further studies are warranted to investigate clusterin-based therapeutic strategies for AD.

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Conflicts of Interest We declare that we have no conflicts of interest.

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