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HIGHLIGHT

Microglial APOE4 promotes neuron degeneration in Alzheimer's disease through inhibition of lipid droplet autophagy

KEY WORDS

APOE4;
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The presence of glial lipid droplet (LD) and the apolipoprotein E4 (APOE4) genotype have been documented as two major risk factors for the development of Alzheimer's disease (AD)[1,2]. However, the intricate molecular interplay between these two factors remains elusive and warrants further investigation. A recent study published in Nature has shed light on this issue by offering valuable insights into APOE4 activity in control of LD formation in the human brain[3].

The brain is rich in lipid content with cholesterol, fatty acids and triglycerides. The brain lipid disorder is a pathological marker of AD, a progressive neurodegenerative disorder from aging characterized by cognitive decline and memory impairment, posing a significant public health challenge for aging populations globally[1]. Despite decades of intensive research in both clinical and laboratory settings, the search for an effective and safe therapeutic intervention to reverse or delay the progression of AD remains unsuccessful, primarily due to a lack of comprehensive understanding of AD etiology[1]. Intriguingly, Alois Alzheimer's initial report on AD described the presence of adipocyte-like morphology in brain cells of AD patients, a pathological feature that was observed together with the accumulation of Aβ plaques and Tau tangles in the AD brain[1]. However, this observation gained limited attention within the AD research field until recently. A groundbreaking study on genome-wide association in AD brain tissues has directed the focus towards lipid metabolism in glial cells, identifying several genes that play pivotal roles in this process[2]. Consequently, brain lipid metabolism has emerged as a vibrant area of research in elucidating the etiology of AD.

Recent research consistently underscores the role of APOE4 in AD through its regulation of lipid metabolism in the brain[2]. APOE is a class of apolipoproteins that mediate lipid metabolism in mammals, with APOE being the most abundant in the brain[1]. This protein exhibits three distinct variants—APOE2, APOE3, and APOE4—each playing a role in the metabolism of triglycerides and cholesterol. Notably, APOE4 has been identified as a genetic risk factor for AD, while APOE2 has been found to reduce this risk[1]. In the microenvironment of human neurons, APOE4 expression is significantly upregulated in microglia of AD patients, and this upregulation is closely associated with an increased glial neuroinflammatory status, as evidenced by single-cell RNA sequencing studies[4]. Furthermore, in cellular models, APOE4 expression leads to a marked accumulation of LDs in microglia derived from human pluripotent stem (iPS) cells[5]. This LD accumulation induces a dysfunctional state in microglia, termed LD-accumulating microglia (LDAM), which has been observed in aged mice and chimeric human-mouse AD models[6]. Notably, LDAM formation is triggered in myeloid cells upon stimulation by Toll-like receptor signals, such as those emanating from bacteria[7].

LDs are subcellular organelles with a single layer of membrane, which has been evolutionarily conserved in macrophages for immune defense by exhibiting anti-microbial properties[6]. In the demyelination mouse models and human iPS cell models, cholesterol-rich lysosomes and LDs have been observed in the dysfunctional microglia[6,8]. Similarly, in the brains of ageing *Drosophila*, senescent glia promotes lipid accumulation in non-senescent glia in response to mitochondrial dysfunction in neurons[9]. Collectively, these studies suggest that lipid accumulation in microglia is intricately linked to neurodegeneration and is

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associated with APOE4 expression. The cumulative evidence demonstrates that APOE4 disrupts lipid metabolism to increase accumulation of LDs in microglia, thereby exacerbating the pathological changes observed in AD patients.

The recently published study in Nature digs into the intricate interplay between APOE4 and LDs within microglia of the brain tissue of AD patients[3]. Leveraging single-nucleus RNA sequencing (snRNA-seq) technology, the research team analyzed fresh-frozen frontal cortex tissue samples, comparing three distinct cohorts: AD individuals with the APOE4/4 genotype, AD individuals with the APOE3/3 genotype, and non-AD APOE3/3 individuals. Their findings highlight a profound discrepancy in the expression of acyl-CoA synthetase long-chain family member 1 (ACSL1), a lipid-associated enzyme, among these groups. Specifically, ACSL1 expression was markedly upregulated in microglia from AD patients, with an even more pronounced increase observed in those harboring the APOE4/4 genotype compared to APOE3/3 carriers. The ACSL1 expression positively correlated with LD accumulation in microglia, suggesting a functional link between these two factors. Furthermore, ACSL1 expression was found to be associated with metabolic regulators such as nicotinamide phosphoribosyltransferase (NAMPT) and dihydropyrimidine dehydrogenase (DPYD) in microglia, pointing to a broader network of interconnected pathways. Given the strong relationship between LDs and ACSL1, the researchers coined the term "LDAM" (lipid droplet-associated microglia) to describe $ACSL1^{+}$ microglia, which constituted the largest subpopulation of microglia in APOE4/4 AD patients. These findings underscore the positive association of APOE4 with LDAM numbers in AD patients, mediated through the *ACSL1* gene.

The *APOE4* gene was found to enhance the formation of LDs, a process that was further potentiated by the presence of the amyloid-β (Aβ) protein. Utilizing oil red O staining, the researchers quantified intracellular lipids within brain samples[3], revealing an abundance of liposomes resembling LDs in AD-APOE4/4 brain tissue, which exhibited a perinuclear distribution pattern within microglia. Notably, these lipid droplet-associated microglia (LDAMs or oil red O-positive cells) were observed to be proximal to or situated at the core of Aβ plaques, mirroring the distribution pattern observed for $ACSL^{+}$ cells. To gain insight into the role of APOE in LD accumulation within microglia, the researchers generated APOE4/4 and genetically matched APOE3/3 induced pluripotent stem cell (iPSC)-derived microglia (iMGs) as reported[2]. Live-cell microscopy employing a fluorescent probe specific for neutral lipids (LipidSpot) revealed a significant increase in LD accumulation within APOE4/4 iMGs compared to their APOE3/3 counterparts. Furthermore, exposure of iMGs to fibrillar Aβ (fAβ) resulted in a robust enhancement of LD formation, which was notably exacerbated in the presence of the APOE4 allele. Conversely, the effect of fAβ on LD accumulation was absent in APOE-knockout (KO) microglia, underscoring the specificity of APOE4 in this process. Consistent with these findings, the expression pattern of the lipid droplet-associated gene PLIN2 mirrored that of ACSL1 in APOE4 microglia, providing further evidence of a mechanistic link between APOE4, Aβ, and LD formation. Collectively, these observations suggest that Aβ promotes LD formation in microglia through APOE4, highlighting a potential therapeutic target for the mitigation of neurodegenerative processes associated with Alzheimer's disease.

LD accumulation promotes inflammatory response in microglia[3]. The relationship APOE4 and Aβ was observed in the primary rat microglia, primary human macrophages, and mouse BV2 microglia cell lines treated with fAβ[3]. Using coherent anti-Stokes Raman scattering (CARS) imaging, the researchers found that the LD spectra of fAβ-treated iMG overlapped with the unsaturated (triglyceride) spectra. Lipidomics analysis showed that these lipids were synthesized de novo in microglia in response to fAβ. Genome-wide CRISPR-KO screen in the monocyte cell lines U937 revealed the regulators of triglyceride metabolism as top category of genes and ACSL1 as one of the most significant genes in the LD formation. To assess the transcriptomic and epigenetic states of LD microglia, the researchers compared ATAC-seq and RNA-seq in the isolated LD-high and LD-low microglia. The results showed that LD-high microglia displayed more motifs related to the NF-κB family of transcription factors with higher expression of NF-κB-related pro-inflammatory cytokines, lower expression of microglial homeostasis markers over the LD-low microglia. Phenotypic measurements suggest that LD-containing APOE4/4 microglia had phagocytosis defect, lysosome accumulation and high secretion of inflammation-associated chemokines in the cell culture media. This group data suggests that APOE4 may promote microglial inflammatory response through induction of LD accumulation.

Finally, to explore into the impact of LDAM on neuronal activity, the research team generated conditioned media from APOE4 microglia with high and low levels of lipid deposition, accomplished by nurturing them in neurobasal media for a period of 12 h. These media were then employed to treat APOE4/4 iPS cell-derived human neurons, with the objective of assessing their biological effects. The outcomes unveiled that the medium from microglia with high levels of LDs triggered the neurons to produce significantly higher concentrations of phosphorylated tau protein at serine 181 residue (pTau-181), as compared to untreated neurons. Furthermore, the presence of pTau was notably increased in human neurons cultured in conditioned media from both APOE3/3 and APOE4/4 microglia, albeit this elevation was absent in media of APOE-KO microglia. The activity of caspase, an indicator of apoptotic processes, was elevated in neurons exposed to microglia of high lipid deposition of APOE3/3 and APOE4/4 iPS cells. Conversely, media conditioned by APOE-KO iMGs failed to elicit such a response. Intriguingly, the human neurons treated with media conditioned by high-LD microglia manifested an augmentation in lipid droplet (LD) count, a phenomenon attributed to the high concentrations of triglycerides present in the conditioned media, which were in turn stimulated by Aβ in APOE4 microglia.

This study has uncovered a pivotal role of APOE4 in fostering the development of LDs within microglia, a process that is mediated through the inhibition of lipid degradation by suppressing autophagy. Notably, the activity of APOE4 is further augmented by inflammatory cues and the stimulation of Aβ, thereby exacerbating the accumulation of LDs. In turn, this accumulation of LDs within microglia triggers an inflammatory cascade, characterized by the secretion of inflammatory cytokines and the activation of the NF-κB signaling pathway. The findings reveal that the APOE4 genotype predisposes microglia to transition into an evolutionarily conserved yet maladaptive and destructive state known as LDAM, in response to innate immune triggers such as Aβ and lipopolysaccharide (LPS). Moreover, APOE4 and Aβ proteins collaborate to induce the expression of ACSL1, a key enzyme involved in triglyceride synthesis, thereby facilitating LD accumulation within microglia. Ultimately, microglia laden with LDs secrete neurotoxic factors that contribute to neuronal apoptosis, lipid accumulation, and increased levels of pTau-181, thereby exacerbating the neurodegenerative hallmarks of AD.

The study enforces the value of APOE4 as a therapeutic target along other studies in literature[5] (Fig. 1). Notably, single

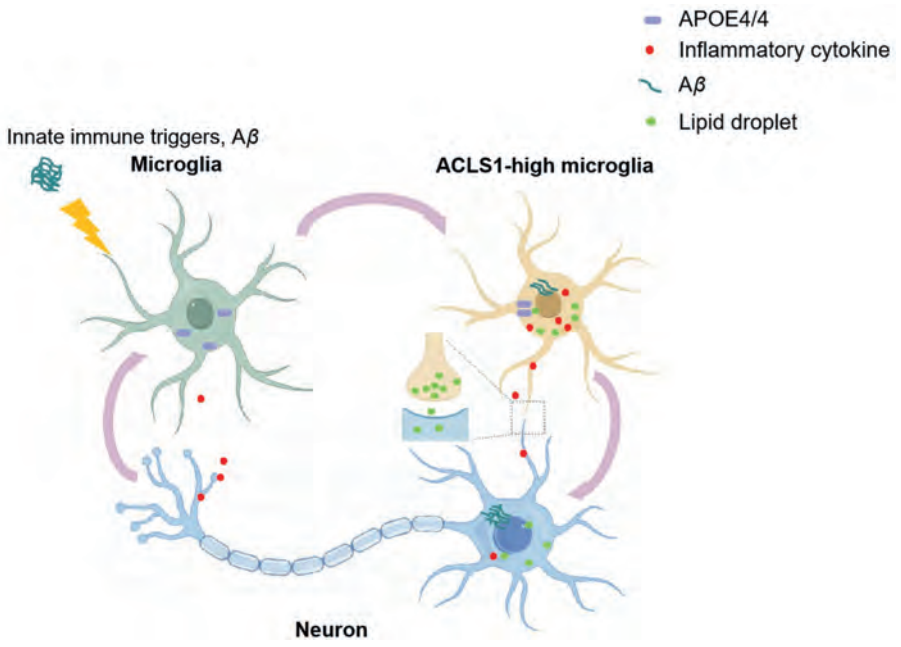

Figure 1 APOE4 in regulation of microglial lipid droplets.

cell transcriptomic study of diverse brain cell types has elucidated how human APOE4 prompts lipid disorders in astrocytes and microglia by enhancing *de novo* cholesterol synthesis even under conditions of high intracellular cholesterol levels[8]. In astrocytes derived from iPS cells, APOE4 could disrupt intracellular lipid equilibrium by enhancing triglyceride production for accumulation of intracellular LDs[2]. Remarkably, the pathological alterations could be mitigated by choline supplementation[2]. APOE4 has been shown to diminish fatty acid oxidation, contributing to lipid accumulation in astrocytes in the hippocampus, ultimately impairing neuronal function[5]. These studies consistently suggest that APOE4 or the signaling pathways is an ideal drug target for effective intervention of AD. Currently, various strategies have been employed to modify APOE4 activities, which include genetic therapies mediated by adeno-associated virus (AAV) vectors, antisense oligonucleotides (ASOs), and RNA interference (RNAi); antibody-based approaches; and small molecule drugs[10]. APOE4 provides an alternative pathway to the existing targets including neurotransmitter receptors, inflammation, Aβ protein, pTau protein and synaptic plasticity in the current drug pipeline for AD[5].

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Author contributions

Meng Mao: Writing − original draft. Xiwen Ma: Investigation. Xiaochuan Wang: Investigation. Jianping Ye: Writing − review & editing, Supervision.

Conflicts of interest

The authors declare no conflict of interest.

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