Mini review

Apolipoprotein E and matrix remodeling – a link to neurodegeneration in Alzheimer's disease

Jelena BAŠIĆ[1*], Vuk MILOŠEVIĆ[2,3], Branka ĐORĐEVIĆ[1], Vladana STOJILJKOVIĆ[1], Milica ŽIVANOVIĆ[4], Tatjana JEVTOVIĆ STOIMENOV[1], Ivana STOJANOVIĆ[1]

[1] *Department of Biochemistry, Faculty of Medicine, University of Niš, Bulevar dr Zorana Đinđića 81, 18000 Niš, Serbia*
[2] *Clinic of Neurology, University Clinical Center Niš, Bulevar dr Zorana Đinđića 48, 18000 Niš, Serbia*
[3] *Faculty of Medicine, University of Niš, 18000 Niš, Serbia*
[4] *Radiology Center, University Clinical Center Niš, 18000 Niš, Serbia*

Summary. Apolipoprotein E (APOE) is a glycoprotein primarily produced by astrocytes and microglia. It plays a crucial role in complexing with amyloid β (Aβ) to accelerate its clearance. APOE genotyping holds great importance in determining whether an individual carries the APOE ε2/ε3/ε4 allele and the corresponding APOE2/E3/E4 protein isoform. Carrying the APOE ε4 allele has been associated with an increased risk of Aβ accumulation, amyloid plaque formation, and late-onset Alzheimer's disease (LOAD). The identification of novel biomarkers that indicate the earliest pathophysiological processes involved in Alzheimer's disease (AD) and the analysis of their diagnostic value in patients, especially through less invasive and cost-effective procedures that can visualize AD in a minimally invasive manner, are the focuses of numerous researchers. Matrix metalloproteinases (MMPs), their tissue inhibitors (TIMPs), and activators play a significant role in extracellular matrix remodeling, disruption of blood-brain barrier integrity, prolonged neuroinflammation, and Aβ clearance. These biomarkers are showing promise as potential blood-based diagnostic markers for patients with AD. In this context, we will discuss the possible mechanisms underlying the interrelation between APOE ε4 carrier status, matrix remodeling enzymes, and neurodegeneration in AD. Additionally, we will explore the diagnostic accuracy of these biomarkers in AD dementia patients based on the results obtained by our research group.

Keywords: apolipoprotein E, Alzheimer's disease, matrix metalloproteinase, tissue inhibitor of metalloproteinase.

INTRODUCTION

Dementia is a syndrome characterized by impaired cognitive functions, and changes in personality and behavior, leading to disturbances in activities of daily living (ADL). Alzheimer's disease (AD), the most frequent cause of dementia is responsible for 60-70% of all dementia cases. Dementia is the 5th leading contributor to Disability Adjusted Life Years burden among people aged 60 years and over, according to the WHO GBD Study (2016) (Nichols et al. 2019). Deaths due to dementias more than doubled between 2000 and 2016, making it the 5th leading cause of global deaths in 2016 and the 3rd in the European WHO region. The World Alzheimer Report showed that there were almost 900 million people aged 60 years and over living worldwide (Prince et al. 2015). It is estimated that there are more than 10 million people with dementia in Europe and more than 50 million in the world. According to the WHO GBD Study (2019), the prevalence of dementia cases in Serbia for the year 2019 was approximately 130,000, with projections indicating an anticipated increase to around 180,000 cases by the year 2050 (Nichols et al. 2022).

There are two forms of AD, the familial or autosomal dominant form with early-onset, and the more common spo-

E-mail: jelena.basic@medfak.ni.ac.rs

radic or late-onset form (LOAD). The autosomal dominant form is characterized by mutations in the genes for amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) and occurs in less than 1% of patients, while the most common form is LOAD, associated with variations in the gene for apolipoprotein E (APOE) (Frisoni et al. 2022).

Three key factors in the pathogenesis of AD are proteinopathy, neurodegeneration, and neuroinflammation. According to the amyloid cascade hypothesis, the most significant pathohistological characteristics of the disease are the extracellular deposition of β amyloid (Aβ), predominantly composed of $Aβ_{1-42}$ peptide in brain tissue, and the formation of neurofibrillary tangles constructed of hyperphosphorylated and inadequately folded tau protein in nerve cell axons, leading to progressive neuronal loss, brain atrophy, and consequent development of cognitive impairment, which is a key feature of dementia syndrome.

The clinical presentation of the disease can be divided into three phases. The first phase is the asymptomatic phase, during which individuals do not exhibit any noticeable symptoms of the disease. Following this phase, individuals may enter the stage of mild cognitive impairment (MCI), where they experience mild cognitive deficits that are noticeable but do not significantly impair their ability to perform daily activities. Finally, the disease progresses to dementia, which is characterized by a decline in ADL due to cognitive impairment (Jack Jr et al. 2011).

Diagnosis of AD includes the use of neuroradiological and biochemical biomarkers. For the assessment of morphological changes, magnetic resonance imaging (MRI) has an inevitable role, as a diagnostic and follow-up tool. Brain MRI shows different patterns of brain structure atrophy using visual rating scales and volumetric techniques as well as characteristic patterns of brain perfusion (Patel et al. 2020). Following the current National Institute on Aging-Alzheimer's Association diagnostic guidelines (NIA-AA (2011)) criteria for diagnosing AD, the concentrations of biochemical biomarkers in the cerebrospinal fluid (CSF) are analyzed (Aβ42, Aβ40, total Tau (t-tau), and phosphorylated Tau (p-tau,181P)) (McKhann et al. 2011). The positivity of selected CSF AD biomarkers is determined using the A/T/N classification (2016) (Jack et al. 2016). The Aβ pathology (biomarker "A") is assessed with CSF Aβ42, and/or Aβ42/Aβ40 ratio, the tau pathology (biomarker "T") with CSF p-tau, and neurodegeneration (biomarker "N") with CSF t-tau. Biomarker changes may present themselves as early as 20 years before disease onset (Prins et al. 2022).

Identifying novel biomarkers indicative of the earliest pathophysiological processes involved in AD, and using preferably minimally invasive methods to identify AD pathology is a focus of researchers in preclinical and clinical studies.

NEUROINFLAMMATION AND MATRIX REMODELING ENZYMES IN AD

It has been shown that the inflammatory response plays an important role in this disease and is controlled by microglia and astrocytes. By sensing signals of damage or injury, astrocytes, and microglia suffer a gradual activation process, followed by the release of proinflammatory mediators leading to chronic inflammation, further neuronal damage, and disease progression. It has been demonstrated that Aβ protofibrils activate microglia, leading to the subsequent release of proinflammatory cytokines such as tumor necrosis factor (TNF-α), interleukins (IL-1β, IL-6, IL-12), interferon-gamma (INF-γ), as well as chemokines like monocyte chemoattractant protein-1 (MCP-1) and eotaxin-1. This activation also results in the generation of reactive oxygen species and nitric oxide (NO). In response to this inflammatory process, astrocytes express various receptors for inflammatory cytokines (e.g., IL-1β and TNFα), chemokines, and damage signals (including TLR ligands). Furthermore, other receptors and mediators of inflammation may be induced after appropriate activation signals from other brain cells (Prins et al. 2022). Elevated levels of specific inflammation-related biomarkers have been detected in the sera or CSF of patients with AD. These biomarkers include TNFα, IL-1β, YKL-40 (also known as chitinase-3-like protein-1 (CHI3L1)), and glial fibrillary acidic protein (GFAP). Additionally, two chemokines, MCP-1, and eotaxin-1, have previously been associated with greater memory impairment in individuals with MCI and AD (Angiulli et al. 2021; Prins et al. 2022).

Furthermore, a great number of inflammation-related biomarkers are attracting attention as novel AD-related biomarkers. Matrix metalloproteinases (MMPs), and their tissue inhibitors (TIMPs), responsible for disrupting the blood-brain barrier (BBB) integrity, prolonged neuroinflammation, as well as Aβ clearance (Abe et al. 2020), are finding their place as potential promising biomarkers in both preclinical and clinical studies.

MMPs are zinc-dependent enzymes, which belong to the endopeptidases and play a central role in the homeostasis of the extracellular matrix (ECM) (Galis and Khatri 2002). There are 28 types of MMPs in humans. MMPs are recognized for their role in the degradation of extracellular proteins and have been implicated in the direct breakdown of Aβ, consequently reducing Aβ deposits. However, it is noteworthy that activated microglia release proinflammatory cytokines and MMPs. The secreted MMPs can degrade Aβ, but they can also exacerbate inflammation within the brain, potentially contributing to neuronal death (Tuna et al. 2018).

Emerging evidence suggests that MMPs play additional roles in AD encompassing the regulation of inflammatory processes, BBB permeability, and involvement in APP

processing along the amyloidogenic pathway. Specifically, MMP13 has been demonstrated to exert proamyloidogenic effects by regulating the translation of β-site APP cleaving enzyme 1 (BACE1). The involvement of the phosphoinositide 3-kinases signaling pathway has been proposed in cultured neuronal cells and APPswe/PS1E9 mice (Zhu et al. 2019). Furthermore, pro-amyloidogenic effects have been reported for MT1-MMP and MT5-MMP in Swedish APP-expressing human embryonic kidney 293 (HEK293) cells and transgenic mouse model of AD (Baranger et al. 2016). In addition, MT1-MMP has been found to promote the localization of APP/Aβ in endosomes and exhibit properties that mimic those of β-secretase. This suggests that MT1-MMP may contribute to the amyloidogenic processing of APP and the generation of Aβ. The ability of MT1-MMP to facilitate the accumulation of APP/Aβ in endosomal compartments highlights its potential involvement in the pathological mechanisms underlying AD (Paumier et al. 2019). Furthermore, elevated expression of MMP-1 and MMP-3 is found to be associated with the clinical diagnosis of probable AD (Iulita et al. 2019; Hoogmartens et al. 2021).

In recent years, significant research attention has been devoted to MMP-9, a member of the gelatinase group, due to its perceived importance in the pathogenesis and progression of AD (Tuna et al. 2018). MMP-9 is primarily produced by microglia, astrocytes, and endothelial cells in response to proinflammatory cytokines, particularly TNFα and IL-1β. Initially secreted in the form of pro-MMP-9, it is activated extracellularly by other proteases and by the transmembrane glycoprotein Extracellular matrix metalloproteinase inducer, also known as CD147 or basigin. The inhibitor of MMP-9, TIMP-1, is secreted alongside MMP-9 and forms complexes with it in the extracellular space. The balance between the activity of MMP-9, CD147, and TIMP-1 is crucial for maintaining ECM homeostasis and promoting Aβ clearance, a hallmark of AD (Du et al. 2019).

MMP-9 plays a critical role in the degradation and clearance of Aβ and its increased expression has been observed in the brains of individuals with AD, particularly in astrocytes surrounding amyloid plaques. This MMP also influences endothelial tight junctions, alters pericyte phenotypes, and contributes to increased BBB permeability. Furthermore, previous studies have revealed that MMP-9 can target tau protein, a key protein involved in the formation of neurofibrillary tangles in AD. MMP-9 has been shown to induce tau aggregation, suggesting its potential role as a potent inducer of tau pathology. Collectively, the involvement of MMP-9 in Aβ degradation, BBB integrity, and tau pathology highlights its multifaceted contribution to the pathogenesis and progression of AD (Tuna et al. 2018). However, the changes in circulating levels of MMP-9 and its inhibitor

in patients with AD have shown inconsistent results. In the studies conducted by Lorenzl et al. (2003, 2008), the plasma level of MMP-9 was found to be significantly higher in AD patients compared to both the control group and patients with MCI-AD. However, these studies did not observe any difference in the level of TIMP-1 between patients and controls. Conversely, other studies have indicated that the plasma level of MMP-9 in AD patients was significantly lower compared to controls (Horstmann et al. 2010). Some studies failed to detect a significant difference in the plasma level of MMP-9 and the MMP-9/TIMP-1 ratio between patients and controls (Lim et al. 2011; Abe et al. 2020).

Previous studies have indicated the involvement of CD147 in the production and degradation of Aβ in AD, but the specific molecular mechanism remains unclear. One study suggested that CD147 acts as another subunit of γ-secretase complexes, which play a role in the negative regulation of β-amyloid precursor proteins, ultimately leading to Aβ production (Zhou et al. 2005). Another study proposed that CD147 modulates Aβ levels through the extracellular degradation of secreted Aβ (Vetrivel et al. 2008). Despite its involvement in Aβ metabolism, there is limited knowledge regarding the factors that regulate CD147 expression in AD.

In our study, we enrolled participants with dementia due to AD as well as cognitively unimpaired individuals (Milošević et al. 2022). The findings revealed that the plasma levels of MMP-9 and TIMP-1 were significantly higher in patients with AD compared to cognitively unimpaired individuals. However, there was no significant difference observed in the level of CD147 or the ratio of MMP-9/TIMP-1 between the two groups. Receiver Operating Characteristic (ROC) curve analysis was performed, and the results indicated that only the areas under the curve (AUC) for MMP-9 and TIMP-1 differed significantly from the reference area. Based on the Swets criterion (Swets 1988), the calculated AUC values for MMP-9 and TIMP-1 demonstrated moderate diagnostic accuracy in distinguishing individuals with AD dementia from cognitively unimpaired individuals. This suggests that increased MMP-9 and TIMP-1 expression might serve as potential biomarkers for AD diagnosis. The dysregulation of matrix remodeling enzymes in plasma could reflect the underlying pathological processes occurring in the brain and may provide valuable insights into the diagnosis and progression of AD.

APOLIPOPROTEIN E AND MATRIX REMODELING ENZYMES IN AD

APOE is a 299 amino acid glycoprotein synthesized in the liver and brain, characterized by a high arginine content. The APOE gene, located on chromosome 19q13.32, encodes

a 22 kDa protein and consists of four exons. Two Single Nucleotide Polymorphisms, rs429358 and rs7412, play a crucial role in determining an individual's APOE ε2, ε3, or ε4 allele status, and consequently, the corresponding APOE2, E3, or E4 protein isoforms. The APOE2 isoform features cysteine at positions 112 and 158, the APOE3 isoform has cysteine at position 112 and arginine at position 158, while the APOE4 isoform contains arginine at both positions.

APOE plays a crucial role in the clearance of Aβ, which is eliminated from the brain through the BBB or via cellular and enzymatic degradation. It is synthesized in microglia, astrocytes, and neurons under stressful conditions (Troutwine et al. 2022). Once lipidated, APOE binds soluble Aβ and enhances its clearance, including uptake through the LDL receptor (LDLR) and LDL Receptor-Related Protein 1 (LRP1) (cellular clearance), as well as transport across the BBB (perivascular clearance). The APO E4 isoform exhibits a lower affinity for Aβ and binds to it less strongly compared to APO E3. This impairs cellular and perivascular clearance, resulting in Aβ accumulation in the brain and further disease progression (Bachmeier et al. 2014; Frisoni et al. 2022). Studies have also indicated that microglia in APOE ε4 carriers display reduced phagocytic activity and decreased expression of enzymes (neprilysin and insulin-degrading enzyme) involved in Aβ removal. Additionally, APOE ε4 carrier state is associated with the deposition of tau, α-synuclein, and other proteins linked to neurodegeneration, independent of Aβ (Wang et al. 2018; Iannucci et al. 2021; Frisoni et al. 2022; Troutwine et al. 2022). Furthermore, research has shown that patients carrying the APOE ε4 allele exhibit distinct patterns of brain region involvement in the pathohistological process compared to APOE ε4-negative patients, leading to different clinical presentations. Specifically, APOE ε4-negative patients experience cognitive decline in other domains (language, behavior, attention) before memory decline (amnestic AD), which is the initial symptom in APOE ε4-positive patients (Frisoni et al. 2022).

The APOE ε4 allele is widely recognized as the primary risk factor for sporadic AD and is associated with an earlier onset of the disease, depending on an individual's carrier status. Individuals carrying the APOE ε4 allele (APOE ε4+) are estimated to experience disease onset on average 12 years earlier than those without the allele (APOE ε4-) (Belloy et al. 2019). Moreover, research indicates that individuals with one APOE ε4 allele have a 2-3 times higher risk of developing AD, while those with two APOE ε4 alleles face an even higher risk, estimated at 10-15 times greater than non-carriers, for AD development (Troutwine et al. 2022).

Our study revealed significant differences in the distribution of six possible APOE genotypes (ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4, ε4/ε4) between patients with dementia due to AD and cognitively unimpaired individuals. We observed higher frequencies of genotypes ε3/ε4 and the ε4 allele in patients compared to the control group. Specifically, the presence of the APOE ε4 allele was associated with a 3-fold higher risk of developing dementia due to AD compared to the reference allele APOE ε3 in the studied population (Bašić et al. 2023).

Although APOE ε4 represents the principal genetic risk factor for LOAD, its presence does not assure the development of AD. This suggests that other factors need to interact with APOE4 to fully explain AD's origins, including metal ions. Certain factors that accelerate protein conformational changes, like trace elements such as zinc, copper, and iron, can facilitate the formation of Aβ oligomers (Kawahara et al. 2017). Moreover, emerging evidence indicates that interactions between brain metal ions and APOE might constitute a mechanism for neurodegeneration. Research has revealed that APOE binds to zinc with isoform-dependent affinities (APOE2 > APOE3 > APOE4). APOE2, characterized by cysteine residues at positions 112 and 158, is predicted to exhibit a stronger zinc-binding affinity, while APOE4, with arginine residues at those positions, demonstrates reduced zinc binding. APOE2 effectively shields Aβ from zinc-induced precipitation compared to APOE4. Additionally, zinc binding to or surrounding APOE/Aβ complexes or Aβ aggregates may confer protection against proteolytic degradation. APOE4 carriers harbor more neurotoxic APOE4 fragments in their brain and plasma, contributing to AD progression (Xu et al. 2015). APOE binds to copper with a lower affinity than zinc. In the presence of APOE, copper-induced Aβ aggregation is heightened, with APOE4 showing the most pronounced copper-Aβ precipitation. Individuals carrying APOE4 are more susceptible to copper toxicity, as serum/plasma copper levels are elevated in APOE4 carriers compared to APOE2 and APOE3 carriers (Zhang et al. 2022). Although the role of APOE4 in causing iron dysregulation in AD remains disputed, it is considered a modulator of iron-associated brain damage in AD pathogenesis. APOE4 promotes brain iron accumulation, leading to elevated oxidative stress and cell death (ferroptosis), thus contributing to the elevated risk of AD. As both iron and APOE4 are linked to cognitive decline (Ayton et al. 2020), their synergistic interaction could exacerbate cognitive impairment during AD progression.

Based on extensive research, the latest probabilistic model of Alzheimer's disease (AD) suggests the existence of two sporadic forms: APOE ε4-related AD and APOE ε4-unrelated AD. The determination of APOE ε4 status is considered crucial not only for assessing the individual's risk but also for patient stratification. Consequently, it is recommended to include APOE ε4 status determination in the guidelines for the diagnosis and treatment of AD (Frisoni et al. 2022).

Studies have demonstrated that microglia expressing the human APOE ε4 allele exhibit a significant increase in the production of pro-inflammatory mediators, including TNFα, IL-6, and NO. Furthermore, such microglia also display impaired migration and reduced phagocytic function. This evidence highlights the role of APOE in regulating neuroinflammatory processes. Additionally, the involvement of APOE in neuroinflammation is further supported by the finding that APOE signaling through the triggering receptor expressed on myeloid cells 2 (TREM2) triggers a switch in microglia towards a neurodegenerative phenotype (Iannucci et al. 2021; Troutwine et al. 2022). Furthermore, it has been observed that all APOE isoforms can induce increased expression of IL-1β, TNFα, and IL-6 in human astrocytes, with APOE4 leading to the highest level of cytokine expression (Iannucci et al. 2021).

While a previous in vitro study indicated that MMP-7 could potentially degrade Aβ1-42, leading to the prevention of Aβ aggregation (Taniguchi et al. 2017), biochemical investigations have revealed elevated levels of pro-inflammatory mediators and MMP-7 in the brains of individuals with AD (Sorrentino et al. 2021). Additionally, CSF levels of MMP-7 have shown a positive correlation with the severity of white matter lesions in individuals with amnestic MCI (Sasaki et al. 2021). It has been demonstrated that elevated APOE protein levels lead to increased AP-1 activity, subsequently augmenting MMP-7 expression in certain cell types. However, the precise mechanism by which APOE influences MMP-7 in AD remains not fully understood (Jayakar et al. 2017).

Stomrud et al. (2010) reported higher CSF MMP-9 levels in subjects with positive biomarkers of AD (low Aβ, high tau, and APOE4 ε4+) in comparison to biomarker negative subjects, suggesting a role of MMP-9 in the pathophysiology of AD at an early stage, even before the development of cognitive decline. Furthermore, high MMP-9 activity, as well as an inverse correlation between Mini-Mental State Examination (MMSE) score and MMP-9 activity in the brains of AD and MCI patients were observed (Bruno et al. 2009).

The mechanisms underlying the impact of MMP-9 on neurodegeneration and cognitive decline in APOE ε4+ individuals are currently not fully understood. However, some studies suggest that APOE influences the disposition of MMP-9 in the brain in an isoform-dependent manner. For instance, Ringland et al. (2020) demonstrated that APOE affects the levels of MMP-9 in conditioned media derived from brain endothelial cells, facilitates the conversion to active MMP-9, and inhibits MMP-9 activity in a dose-dependent manner. Importantly, APOE4 was found to be the least effective isoform in modulating these processes compared to other APOE isoforms. As a result, the presence of APOE4 leads to elevated levels of MMP-9 in the cerebrovasculature, as observed in both human and animal specimens of AD brains (Bachmeier et al. 2014; Shackleton et al. 2019).

Targeting MMP-9 may offer a promising strategy to mitigate the pathophysiology of AD, particularly in individuals with the APOE ε4 allele. In a study by SB-3CT, an MMP-9 inhibitor, treatment was found to reduce Aβ-induced shedding of lipoprotein receptors in brain endothelial cells and the brains of APOE targeted replacement (APOE-TR) mice carrying the APOE ε4 allele (Shackleton et al. 2019). Furthermore, SB-3CT treatment led to significantly enhanced clearance of Aβ from the brain to the periphery following intracranial administration of Aβ in APOE4-TR mice. These findings suggest that APOE influences MMP-9 function, and the impact of APOE isoforms on lipoprotein receptor shedding and Aβ clearance across the BBB (Bachmeier et al. 2012, 2014; Ringland et al. 2020).

Moreover, the APOE ε4 allele is associated with increased levels of IL-1b (Dafnis et al. 2012) decreased BBB integrity (Kloske and Wilcock 2020), and compromised tight junctions responsible for maintaining BBB integrity (Nishitsuji et al. 2011). It has been suggested that pericytes and MMP-9 may mediate these effects (Troutwine et al. 2022). In addition, Stomrud et al. (2010) reported that a decrease in the TIMP-1/MMP-9 ratio in AD patients was associated with high levels of CSF t-tau, which is a marker of neurodegeneration. Taken together, these findings highlight the potential involvement of MMP-9 in AD pathophysiology, particularly in APOE ε4+ individuals, and suggest that targeting MMP-9 could be a valuable approach for therapeutic intervention.

Abe et al. (2020) conducted a study that demonstrated declines in hippocampal volumes as observed through brain MRI scans and cognitive function assessed by the MMSE after a four-year follow-up period. The study found that these declines were significantly more rapid in individuals with MCI-AD who had high levels of MMP-9 at baseline compared to those with middle and low levels of MMP-9. Additionally, MCI-AD patients with high levels of TIMP-1 at baseline exhibited a significantly faster decline in hippocampal volume compared to those with low levels of TIMP-1 at baseline. Importantly, these changes were particularly significant in APOE4 ε4+ MCI-AD patients during a 15-year follow-up period (Abe et al. 2022).

In our study, we did not observe a significant difference in MMP-9, TIMP-1, CD147 levels, or the MMP-9/TIMP-1 ratio between APOE ε4+ and APOE ε4- patients with AD dementia. However, when we conducted a volumetric analysis based on brain MRI scans (Živanović et al. 2023), we found that APOE ε4+ patients exhibited decreased hippocampal volume in various measures, including total volume, total/intracranial volume (ICV), right hippocampus, right/ICV, and left hippocampus, when compared to APOE ε4- patients.

Additionally, we observed a positive correlation between the MMP-9/TIMP-1 ratio and the Asymmetry Index (AI). The AI is considered a neuroradiological marker of disease progression, and our findings suggest that the MMP-9/TIMP-1 ratio might serve as a promising plasma biomarker for monitoring disease progression in AD. These results highlight the potential role of MMP-9 and TIMP-1 as mediators in the neurodegenerative processes associated with AD. The results of our research can be followed up on studies that showed increased production and concentration of inflammatory mediators in the CSF of patients with AD and increased expression of MMP-9 (Morales et al. 2014; Abe et al. 2020; Prins et al. 2022), mediation of MMP-9 in the accelerated proteolysis of receptors that participate in the removal of lipoprotein complexes and Aβ (LDLR and LRP1) and damage to tight junctions responsible for maintaining the integrity of the BBB (Ringland et al. 2020; Troutwine et al. 2022), as well as studies that showed an increase in the concentration of MMP-9 in the plasma of patients with AD (Lorenzl et al. 2003, 2008).

We propose that the elevation of plasma MMP-9 levels in patients with dementia due to AD may serve as an indicator of neuroinflammation. In this scenario, increased production of MMP-9 occurs in response to pro-inflammatory cytokines, particularly TNF-α and IL-1β. This heightened MMP-9 activity leads to accelerated proteolysis of LDLR and LRP1, resulting in prolonged half-life and accumulation of the extracellular receptor-Aβ complex. Consequently, this accumulation contributes to the induction of tau protein hyperphosphorylation, neurodegeneration, brain atrophy, and a reduction in hippocampal volume, ultimately leading to cognitive decline. Notably, the presence of the APOE ε4 allele is implicated in mediating and accelerating these processes. Furthermore, the increase in TIMP-1 levels may be considered a compensatory response to counterbalance the elevated MMP-9 activity. These hypotheses provide insights into the potential mechanisms underlying the interplay between inflammation, MMP-9, Aβ accumulation, and neurodegeneration in AD.

However, more evidence is required to elucidate the exact roles of MMPs and TIMPs in the pathogenesis of AD and evaluate the predictive value of their plasma levels to estimate future neurodegeneration.

CONCLUSIONS AND FUTURE DIRECTIONS

Previous results demonstrate that patients with dementia due to AD exhibit a significantly higher frequency of the APOE ε4 allele compared to the group of cognitively unimpaired individuals, indicating that carriers of the APOE ε4 allele are at a greater risk of developing dementia due to AD when compared to those with the APOE ε3 allele. MMP-9

plays an important role in the degradation and clearance of Aβ, its expression is increased in the brains of patients with AD, especially in astrocytes surrounding the amyloid plaque. This proteinase also affects endothelial tight junction proteins, changes pericytes' phenotype, and increases the permeability of the BBB, but can also be a strong inducer of tau protein aggregation, further leading to brain atrophy and a cognitive decline. These changes could be enhanced by the presence of the APOE ε4 genotype. Indeed, the dysregulation of matrix remodeling enzymes, such as MMP-9, and tissue inhibitors, like TIMP-1, in plasma holds promise as potential biomarkers for AD diagnosis. Increased expression of these proteins in the bloodstream may reflect the pathological processes occurring in the brain and provide valuable insights into the diagnosis and progression of AD. However, it is essential to emphasize that further evidence is needed to fully understand the precise roles of MMPs and TIMPs in the pathogenesis of AD. Future research focused on neuroimaging, particularly amyloid and tau PET scans, will be necessary to confirm the role of these biomarkers in both the diagnosis and stratification of patients for further examination and assessment of their risk for disease progression.

REFERENCES

Abe K, Chiba Y, Hattori S, Yoshimi A, Asami T, Katsuse O, Suda A, Hishimoto A, Alzheimer's Disease Neuroimaging Initiative. 2020. Influence of plasma matrix metalloproteinase levels on longitudinal changes in Alzheimer's disease (AD) biomarkers and cognitive function in patients with mild cognitive impairment due to AD registered in the Alzheimer's Disease Neuroimaging Initiative database. Journal of the Neurological Sciences. 416:116989.

Abe K, Chiba Y, Ide K, Yoshimi A, Asami T, Suda A, Odawara T, Hishimoto A, Alzheimer's Disease Neuroimaging Initiative. 2022. Plasma MMP-9 levels as the future risk of conversion to dementia in ApoE4-positive MCI patients: Investigation based on the Alzheimer's disease neuroimaging initiative database. The Journal of Prevention of Alzheimer's Disease. 9:331–337.

Angiulli F, Conti E, Zoia CP, Da Re F, Appollonio I, Ferrarese C, Tremolizzo L. 2021. Blood-based biomarkers of neuroinflammation in Alzheimer's disease: A central role for periphery? Diagnostics. 11:1525.

Ayton S, Wang Y, Diouf I, Schneider JA, Brockman J, Morris MC, Bush AI. 2020. Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. Molecular Psychiatry. 25:2932–2941.

Bachmeier C, Paris D, Beaulieu-Abdelahad D, Mouzon B, Mullan M, Crawford F. 2012. A multifaceted role for apoE in the clearance of beta-amyloid across the blood-brain barrier. Neurodegenerative Diseases. 11:13–21.

Bachmeier C, Shackleton B, Ojo J, Paris D, Mullan M, Crawford F. 2014. Apolipoprotein E isoform-specific effects on lipoprotein receptor processing. Neuromolecular Medicine. 16:686–696.

Baranger K, Marchalant Y, Bonnet AE, Crouzin N, Carrete A, Paumier JM, Py NA, Bernard A, Bauer C, Charrat E. 2016. MT5-MMP is a new pro-amyloidogenic proteinase that promotes amyloid pathology and cognitive decline in a transgenic mouse model of Alzheimer's disease. Cellular and Molecular Life Sciences. 73:217–236.

Bašić J, Milošević V, Đorđević B, Stefanović N, Cvetković M, Jevtović-Stoimenov T, Stojanović I. 2023. Association of the apolipoprotein E ε4 allele in a Serbian population with Alzheimer's dementia. Archives of Biological Sciences. https://doi.org/10.2298/ABS230614026B.

Belloy ME, Napolioni V, Greicius MD. 2019. A quarter century of APOE and Alzheimer's disease: progress to date and the path forward. Neuron. 101:820–838.

Bruno MA, Mufson EJ, Wuu J, Cuello AC. 2009. Increased matrix metalloproteinase 9 activity in mild cognitive impairment. Journal of Neuropathology and Experimental Neurology. 68:1309–1318.

Dafnis I, Tzinia A, Tsilibary E, Zannis V, Chroni A. 2012. An apolipoprotein E4 fragment affects matrix metalloproteinase 9, tissue inhibitor of metalloproteinase 1 and cytokine levels in brain cell lines. Neuroscience. 210:21–32.

Du J, Li S, Su Y, Wang H, Liu H, Cui H. 2019. The role of extracellular matrix metalloproteinase inducer on the action of dihydrotestosterone against the cellular damage induced by Aβ42. Molecular and Cellular Endocrinology. 498:110536.

Frisoni GB, Altomare D, Thal DR, Ribaldi F, van der Kant R, Ossenkoppele R, Blennow K, Cummings J, van Duijn C, Nilsson PM. 2022. The probabilistic model of Alzheimer disease: the amyloid hypothesis revised. Nature Reviews Neuroscience. 23:53–66.

Galis ZS, Khatri JJ. 2002. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circulation Research. 90:251–262.

Hoogmartens J, Hens E, Engelborghs S, De Deyn PP, van der Zee J, Van Broeckhoven C, Cacace R. 2021. Investigation of the role of matrix metalloproteinases in the genetic etiology of Alzheimer's disease. Neurobiology of Aging. 104:105-e1.

Horstmann S, Budig L, Gardner H, Koziol J, Deuschle M, Schilling C, Wagner S. 2010. Matrix metalloproteinases in peripheral blood and cerebrospinal fluid in patients with Alzheimer's disease. International Psychogeriatrics. 22:966–972.

Iannucci J, Sen A, Grammas P. 2021. Isoform-specific effects of apolipoprotein E on markers of inflammation and toxicity in brain glia and neuronal cells in vitro. Current Issues in Molecular Biology. 43:215–225.

Iulita MF, Ganesh A, Pentz R, Flores Aguilar L, Gubert P, Ducatenzeiler A, Christie S, Wilcock GK, Cuello AC. 2019. Identification and preliminary validation of a plasma profile associated with cognitive decline in dementia and at-risk individuals: a retrospective cohort analysis. Journal of Alzheimer's Disease. 67:327–341.

Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS. 2016. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 87:539–547.

Jack Jr CR, Albert M, Knopman DS, McKhann GM, Sperling RA, Carillo M, Thies W, Phelps CH. 2011. Introduction to revised criteria for the diagnosis of Alzheimer's disease: National Institute on Aging and the Alzheimer Association Workgroups. Alzheimer's and Dementia: The Journal of the Alzheimer's Association. 7:257.

Jayakar SK, Loudig O, Brandwein-Gensler M, Kim RS, Ow TJ, Ustun B, Harris TM, Prystowsky MB, Childs G, Segall JE. 2017. Apolipoprotein E promotes invasion in oral squamous cell carcinoma. The American Journal of Pathology. 187:2259–2272.

Kawahara M, Kato-Negishi M, Tanaka K. 2017. Cross talk between neurometals and amyloidogenic proteins at the synapse and the pathogenesis of neurodegenerative diseases. Metallomics. 9:619–633.

Kloske CM, Wilcock DM. 2020. The important interface between apolipoprotein E and neuroinflammation in Alzheimer's disease. Frontiers in Immunology. 11:754.

Lim NKH, Villemagne VL, Soon CP, Laughton KM, Rowe CC, McLean CA, Masters CL, Evin G, Li QX. 2011. Investigation of matrix metalloproteinases, MMP-2 and MMP-9, in plasma reveals a decrease of MMP-2 in Alzheimer's disease. Journal of Alzheimer's Disease. 26:779–786.

Lorenzl S, Albers DS, Relkin N, Ngyuen T, Hilgenberg SL, Chirichigno J, Cudkowicz ME, Beal MF. 2003. Increased plasma levels of matrix metalloproteinase-9 in patients with Alzheimer's disease. Neurochemistry International. 43:191–196.

Lorenzl S, Büerger K, Hampel H, Beal MF. 2008. Profiles of matrix metalloproteinases and their inhibitors in plasma of patients with dementia. International Psychogeriatrics. 20: 67–76.

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R. 2011. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's and Dementia. 7:263–269.

Milošević V, Malobabić M, Stojanović I, Bašić J. 2022. Importance of a functional measure in the evaluation of patients in a memory clinic: Validation of the Serbian version of the Amsterdam instrumental activities of daily living questionnaire. Clinical Neurology and Neurosurgery. 214:107165.

Morales I, Guzmán-Martínez L, Cerda-Troncoso C, Farías GA, Maccioni RB. 2014. Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. Frontiers in Cellular Neuroscience. 8:112.

Nichols E, Szoeke CE, Vollset SE, Abbasi N, Abd-Allah F, Abdela J, Aichour MTE, Akinyemi RO, Alahdab F, Asgedom SW. 2019. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology. 18:88–106.

Nichols E, Steinmetz JD, Vollset SE, Fukutaki K, Chalek J, Abd-Allah F, Abdoli A, Abualhasan A, Abu-Gharbieh E, Akram TT. 2022. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. The Lancet Public Health. 7:e105–e125.

Nishitsuji K, Hosono T, Nakamura T, Bu G, Michikawa M. 2011. Apolipoprotein E regulates the integrity of tight junctions in an isoform-dependent manner in an in vitro blood-brain barrier model. Journal of Biological Chemistry. 286:17536–17542.

Patel KP, Wymer DT, Bhatia VK, Duara R, Rajadhyaksha CD. 2020. Multimodality imaging of dementia: clinical importance and role of integrated anatomic and molecular imaging. Radiographics. 40:200–222.

Paumier JM, Py NA, García-González L, Bernard A, Stephan D, Louis L, Checler F, Khrestchatisky M, Baranger K, Rivera S. 2019. Proamyloidogenic effects of membrane type 1 matrix metalloproteinase involve MMP-2 and BACE-1 activities, and the modulation of APP trafficking. The FASEB Journal. 33:2910–2927.

Prince MJ, Wimo A, Guerchet MM, Ali GC, Wu YT, Prina M. 2015. World Alzheimer Report 2015-The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends.

Prins S, de Kam ML, Teunissen CE, Groeneveld GJ. 2022. Inflammatory plasma biomarkers in subjects with preclinical Alzheimer's disease. Alzheimer's Research and Therapy. 14:1–9.

Ringland C, Schweig JE, Paris D, Shackleton B, Lynch CE, Eisenbaum M, Mullan M, Crawford F, Abdullah L, Bachmeier C. 2020. Apolipoprotein E isoforms differentially regulate matrix metallopeptidase 9 function in Alzheimer's disease. Neurobiology of Aging. 95:56–68.

Sasaki Y, Kimura N, Aso Y, Yabuuchi K, Aikawa M, Matsubara E. 2021. Relationship between cerebrospinal fluid matrix metalloproteinases levels and brain amyloid deposition in mild cognitive impairment. Biomolecules. 11:1496.

Shackleton B, Ringland C, Abdullah L, Mullan M, Crawford F, Bachmeier C. 2019. Influence of matrix metallopeptidase 9 on beta-amyloid elimination across the blood-brain barrier. Molecular neurobiology. 56:8296–8305.

Sorrentino S, Ascari R, Maderna E, Catania M, Ghetti B, Tagliavini F, Giaccone G, Di Fede G. 2021. Microglial heterogeneity and its potential role in driving phenotypic diversity of Alzheimer's disease. International Journal of Molecular Sciences. 22:2780.

Stomrud E, Björkqvist M, Janciauskiene S, Minthon L, Hansson O. 2010. Alterations of matrix metalloproteinases in the healthy elderly with increased risk of prodromal Alzheimer's disease. Alzheimer's Research and Therapy. 2:1–8.

Swets JA. 1988. Measuring the accuracy of diagnostic systems. Science. 240:1285–1293.

Taniguchi M, Matsuura K, Nakamura R, Kojima A, Konishi M, Akizawa T. 2017. MMP-7 cleaves amyloid β fragment peptides and copper ion inhibits the degradation. Biometals. 30:797–807.

Troutwine BR, Hamid L, Lysaker CR, Strope TA, Wilkins HM. 2022. Apolipoprotein E and Alzheimer's disease. Acta Pharmaceutica Sinica B. 12:496–510.

Tuna G, Yener GG, Oktay G, İşlekel GH, Kİrkalİ FG. 2018. Evaluation of matrix metalloproteinase-2 (MMP-2) and-9 (MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) in plasma from patients with neurodegenerative dementia. Journal of Alzheimer's Disease. 66:1265–1273.

Vetrivel KS, Zhang X, Meckler X, Cheng H, Lee S, Gong P, Lopes KO, Chen Y, Iwata N, Yin KJ. 2008. Evidence that CD147 modulation of β-Amyloid (Aβ) levels is mediated by extracellular degradation of secreted Aβ. Journal of Biological Chemistry. 283:19489–19498.

Wang C, Najm R, Xu Q, Jeong D eun, Walker D, Balestra ME, Yoon SY, Yuan H, Li G, Miller ZA. 2018. Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. Nature Medicine. 24:647–657.

Xu H, Gupta VB, Martins IJ, Martins RN, Fowler CJ, Bush AI, Finkelstein DI, Adlard PA. 2015. Zinc affects the proteolytic stability of Apolipoprotein E in an isoform-dependent way. Neurobiology of Disease. 81:38–48.

Zhang Y, Gao H, Zheng W, Xu H. 2022. Current understanding of the interactions between metal ions and Apolipoprotein E in Alzheimer's disease. Neurobiology of Disease. 172(1):105824.

Zhou S, Zhou H, Walian PJ, Jap BK. 2005. CD147 is a regulatory subunit of the γ-secretase complex in Alzheimer's disease amyloid β-peptide production. Proceedings of the National Academy of Sciences. 102:7499–7504.

Zhu BL, Long Y, Luo W, Yan Z, Lai YJ, Zhao LG, Zhou WH, Wang YJ, Shen LL, Liu L. 2019. MMP13 inhibition rescues cognitive decline in Alzheimer transgenic mice via BACE1 regulation. Brain. 142:176–192.

Živanović M, Trenkić AA, Milošević V, Stojanov D, Mišić M, Radovanović M, Radovanović V. 2023. The role of magnetic resonance imaging in the diagnosis and prognosis of dementia. Biomolecules and Biomedicine. 23:209.