



cells



Perspective

Detrimental Effects of ApoE ϵ 4 on Blood–Brain Barrier Integrity and Their Potential Implications on the Pathogenesis of Alzheimer’s Disease

Kevin Kirchner, Linda Garvert, Luise Kühn, Sarah Bonk, Hans Jörgen Grabe and Sandra Van der Auwera



<https://doi.org/10.3390/cells12212512>

Perspective

Detrimental Effects of ApoE ϵ 4 on Blood–Brain Barrier Integrity and Their Potential Implications on the Pathogenesis of Alzheimer’s Disease

Kevin Kirchner ¹, Linda Garvert ¹ , Luise Kühn ¹ , Sarah Bonk ¹, Hans Jörgen Grabe ^{1,2} and Sandra Van der Auwera ^{1,2,*}

¹ Department of Psychiatry and Psychotherapy, University Medicine Greifswald, 17475 Greifswald, Germany

² German Centre for Neurodegenerative Diseases (DZNE), Partner Site Rostock/Greifswald, 17475 Greifswald, Germany

* Correspondence: sandra.auwera@uni-greifswald.de

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disease representing the most common type of dementia in older adults. The major risk factors include increased age, genetic predisposition and socioeconomic factors. Among the genetic factors, the apolipoprotein E (ApoE) ϵ 4 allele poses the greatest risk. Growing evidence suggests that cerebrovascular dysfunctions, including blood–brain barrier (BBB) leakage, are also linked to AD pathology. Within the scope of this paper, we, therefore, look upon the relationship between ApoE, BBB integrity and AD. In doing so, both brain-derived and peripheral ApoE will be considered. Despite the considerable evidence for the involvement of brain-derived ApoE ϵ 4 in AD, information about the effect of peripheral ApoE ϵ 4 on the central nervous system is scarce. However, a recent study demonstrated that peripheral ApoE ϵ 4 might be sufficient to impair brain functions and aggravate amyloid-beta pathogenesis independent from brain-based ApoE ϵ 4 expression. Building upon recent literature, we provide an insight into the latest research that has enhanced the understanding of how ApoE ϵ 4, secreted either in the brain or the periphery, influences BBB integrity and consequently affects AD pathogenesis. Subsequently, we propose a pathway model based on current literature and discuss future research perspectives.

Keywords: apolipoprotein E; amyloid beta; blood–brain barrier; cyclophilin A; low-density lipoprotein receptor-related protein1; matrix metalloproteinase 9; tissue inhibitor of metalloproteinase 3



Citation: Kirchner, K.; Garvert, L.; Kühn, L.; Bonk, S.; Grabe, H.J.; Van der Auwera, S. Detrimental Effects of ApoE ϵ 4 on Blood–Brain Barrier Integrity and Their Potential Implications on the Pathogenesis of Alzheimer’s Disease. *Cells* **2023**, *12*, 2512. <https://doi.org/10.3390/cells12212512>

Academic Editor: Christoph Englert

Received: 14 September 2023

Revised: 12 October 2023

Accepted: 20 October 2023

Published: 24 October 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease representing the most common type of neurodegenerative disorders worldwide [1]. The healthcare expenses for treating Alzheimer’s disease in 2020 are estimated at USD 321 billion for the United States alone, and it is anticipated that these costs will surpass USD 1 trillion by 2050 as the population ages [2]. AD accounts for 60% to 80% of dementia cases [3], and with increasing life expectancy [4], a further rise in prevalence can be expected [1]. Hence, the development of effective preventive or therapeutic solutions is a high priority for healthcare systems worldwide [1]. Over the last decades, researchers sought to enhance our understanding of the pathological mechanisms behind AD, which has led to the development of diverse hypotheses and research approaches. Major risk factors that have been identified are increased age, genetic predisposition and socioeconomic factors [5]. Genetic analyses, including large genome-wide association studies [6], have identified the apolipoprotein E (ApoE) ϵ 4 allele as the strongest known genetic risk factor for AD, making it a main focus in AD research. Notably, ApoE is synthesised and secreted not only in the brain but also in peripheral tissues, especially the liver [7]. The question of how both brain-derived and peripheral ApoE (bApoE and pApoE, respectively) might affect the central nervous system and the pathogenesis of AD remains a topic of debate. Briefly, established

mechanisms include the accumulation of extracellular amyloid beta (A β) peptides or hyperphosphorylated tau protein fibrillary aggregation (tangles) in the brain [8,9]. In addition, other mechanisms via vascular processes have been proposed [7,10–14].

Recent evidence from animal and human studies suggests that cerebrovascular dysfunctions, leading to blood–brain barrier (BBB) leakage, might also be associated with ApoE ϵ 4 and AD pathology [13–15]. For instance, cerebral microinfarcts and microbleeds, which are associated with the disruption of structural connections in the brain [16], represent a common pathological feature in patients with dementia and cerebrovascular diseases [17]. The association between cerebrovascular dysfunctions and AD pathology is further supported by the joint risk factor profile of AD and cardiovascular diseases consisting of the ApoE ϵ 4 genotype, diabetes, hypoperfusion, hypertension and hypercholesterolemia [15].

In this work, we intend to illuminate the mechanisms underlying the interplay between ApoE ϵ 4, blood–brain barrier leakage and AD. To achieve this, we provide an overview of the hypothesised pathways and mechanisms illustrating how ApoE ϵ 4, secreted either in the brain or peripheral tissues, might impact BBB integrity and, thus, affect AD pathogenesis. Subsequently, we propose a combined pathway model based on current literature and discuss future research perspectives.

2. Blood–Brain Barrier and ApoE—Structure and Function

2.1. Blood–Brain Barrier

The blood–brain barrier is a highly selective semipermeable barrier primarily constituted within the microvasculature (capillaries), thereby separating the bloodstream from the brain and its extracellular fluid. It enables the controlled entry of nutrients into the brain and the removal of waste substances out of the brain, such as excessive glutamate and A β [7,18]. Unless a specific transport system is present, larger molecules, such as peptides and proteins, are unable to cross an intact blood–brain barrier [7]. On the cellular level, the BBB comprises predominantly a single layer of endothelial cells, surrounded by a basement membrane (BM) as well as pericytes and astrocytes [7,16,18] forming the neurovascular unit [19] (Figure 1). The endothelial cells are connected by tight junctions (TJs), specialised structures of various transmembrane proteins that form the semipermeable barrier crucial for preventing the free passage of substances [7,16]. Within the BBB, there are two distinct types of basement membranes (endothelial and parenchymal) that enclose the pericytes. Both types represent a specialised extracellular matrix that supports different functions such as structural support, cell anchoring and signalling transduction [20]. Pericytes are embedded in the BMs, where they regulate BBB permeability and the maintenance of TJs. Thus, they significantly impact cerebrovascular integrity and stability [7,16]. Astrocytes completely surround the capillaries with their endfeet, serve as the outer surface of the BBB and significantly contribute to the maintenance of the BBB [16]. They play a crucial role in neurotransmitter recycling, maintenance of tissue ion homeostasis and the regulation of synaptic transmission via the release of gliotransmitters [21].

2.2. Apolipoprotein E (ApoE)

ApoE was discovered in the early 1970s as a protein associated with cholesterol- and triglyceride-rich plasma proteins [7]. It is synthesised and secreted in the brain and peripheral tissues, predominantly the liver [7,22], and regulates lipid-related events [23]. The protein comprises 299 amino acids and exists in three major isoforms that differ only in one or two amino acids at positions 112 and 158: ApoE ϵ 2 (cys112, cys158), ApoE ϵ 3 (cys112, arg158) and ApoE ϵ 4 (arg112, arg158). These variations modify both the structure and function of ApoE [24]. The ApoE ϵ 4 allele has been identified as the strongest genetic risk factor for AD [9,25]. Carriers of the ApoE ϵ 4 allele show an increased risk and decreased age of onset for AD as well as an earlier and ampler amyloid beta (A β) pathology compared with ApoE ϵ 2 and ApoE ϵ 3 allele carriers [23]. Amyloid beta denotes peptides derived from the amyloid-precursor protein (APP) and form the amyloid oligomers and plaques commonly seen in AD patients [3]. Although the function of A β is not well understood, the

idea that A β plaque depositions are the main drivers for the development of AD already emerged in the 1980s with the amyloid cascade hypothesis by Hardy and Higgins, which subsequently dominated the research field during the past decades [26]. A β oligomers, in particular, were found to be associated with cognitive deficits [3], accelerated memory impairments and early cognitive decline in ApoE ϵ 4 carriers [27] and were identified as primary drivers for neurotoxic processes in AD [28]. Of the three ApoE isoforms, ApoE ϵ 4 shows the lowest A β clearance rate [7,29], which might be due to its weakened interaction with the low-density lipoprotein receptor (LDLR) and the LDLR-related protein 1 (LRP1), the two main contributors of ApoE-driven A β removal at the BBB [29]. Apart from its involvement in A β accumulation and clearance, studies have shown that bApoE ϵ 4 is also associated with reduced BBB repair after traumatic brain injury, reduced TJ proteins in vitro, reduced pericyte coverage of blood vessels in human post-mortem tissue and increased BBB leakage [18].

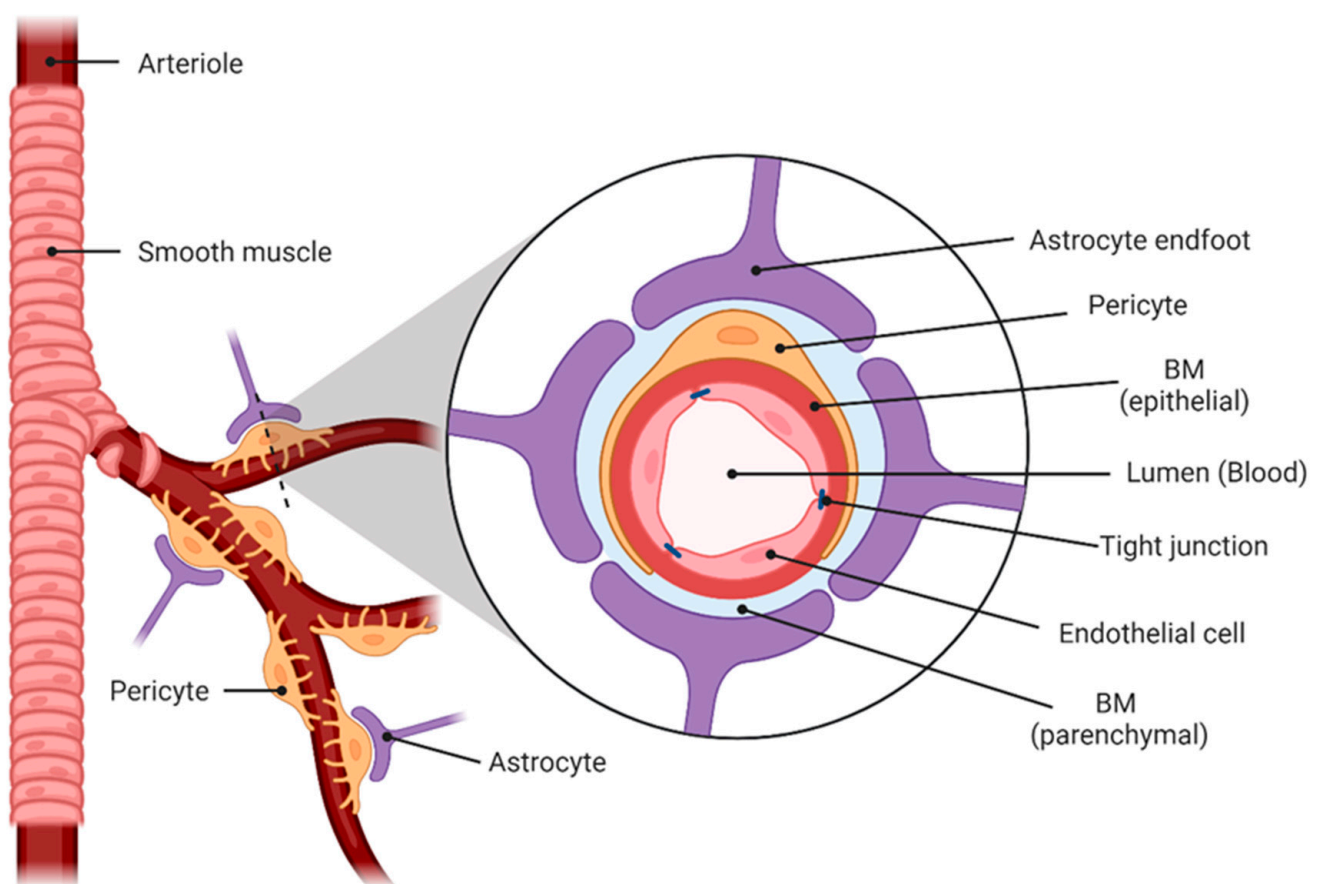


Figure 1. Visualization of the blood–brain barrier constituted within the microvasculature. BM = base-membrane. Created with [BioRender.com](https://www.biorender.com) accessed on 1 September 2023.

As ApoE ϵ 4 is secreted and synthesised not only in the brain but also in peripheral tissues such as the liver [22], it is debated whether pApoE ϵ 4 might also affect the central nervous system and contribute to BBB degradation [23]. Although previous research generally assumed that bApoE ϵ 4 and pApoE ϵ 4 act independently and are separated by the BBB [18,30], a recent study by Liu and colleagues [23] demonstrated that pApoE ϵ 4 might be sufficient to impair brain functions and aggravate A β pathogenesis. However, the exact roles of bApoE ϵ 4 and pApoE ϵ 4 and the BBB in the pathogenesis of AD still lack clear understanding.

3. The Impact of ApoE on the Blood–Brain Barrier

Dysfunctions of the BBB are linked to both the natural ageing process and several neurodegenerative disorders [23]. Studies indicate that both brain as well as peripheral ApoE ϵ 4 might be able to induce neurodegenerative processes by affecting BBB integrity [7,11,12,18,23] and A β clearance [29,31,32]. To enhance our understanding of how ApoE ϵ 4 might impact AD development, it is crucial to first elucidate the connections among AD, A β , ApoE and the BBB, along with their potential interactions. In the following, we will describe the postulated effects of ApoE ϵ 4 on BBB integrity via three mechanisms: first, the impact of bApoE via pericytes (Figure 2A); second, the impact of bApoE via endothelial cells (Figure 2B); and third, the impact of pApoE (Figure 2C).

3.1. The Impact of bApoE on BBB Integrity via Pericytes

The following mechanisms are illustrated in Figure 2A. Studies have shown that the low binding affinity between human bApoE ϵ 4 and LRP1, in contrast to bApoE ϵ 2 and bApoE ϵ 3, increases the intracellular CypA level in pericytes. This, in turn, leads to NF κ B activation and finally to a release of MMP9 into the extracellular space (CypA–NF κ B–MMP9 pathway) [7,11]. MMP9 belongs to a family of zinc-dependent extracellular matrix-remodelling endopeptidases and can degrade the capillary basement membrane as well as tight junction proteins (e.g., ZO-1, occludin, claudin-5) of the BBB [11]. Thus, excessive extracellular MMP9 can promote a BBB breakdown that allows the influx of blood-derived neurotoxic proteins, including the coagulation factor thrombin, into the brain [7,11]. Studies *in vitro* demonstrate that pericytes are thrombin-sensitive, MMP9-releasing cell types [33]. Thrombin can bind to pericytic protease-activated receptors (PARs), which triggers a process resulting in the additional release of MMP9. Hence, the influx of thrombin via an already damaged BBB might further promote BBB disruption due to elevated MMP9 release, ultimately favouring the development of a dysfunctional cycle [33].

In addition to these ApoE ϵ 4-driven processes, pericytes might also play an active role in the removal of A β at the BBB through LRP1-mediated clearance (not depicted in the figure) [32]. However, studies reported a degeneration and loss of pericytes, particularly among subjects with AD. This decline seems to be a contributing factor in the progression of AD. Findings in mice suggest that the pericyte loss might result from an intracellular accumulation of A β as researchers observed decreased A β reuptake and diminished loss of pericytes after the inhibition of pericyte LRP1 [32]. Consequently, LRP1 might mediate both A β internalisation and pericyte cell death [32].

3.2. The Impact of bApoE on BBB Integrity via Endothelial Cells

For an illustration, see Figure 2B. In the endothelium, LRP1 regulates the transendothelial clearance of several neurotoxins, including A β . As prior studies have shown, compared with bApoE ϵ 2 and ϵ 3, the binding of A β to bApoE ϵ 4 redirects the rapid clearance of A β from LRP1 to the very low density lipoprotein receptor (VLDLR), which internalises the A β –ApoE ϵ 4 complexes at a reduced pace, resulting in elevated A β levels in the extracellular space [7,11]. A β accumulation, in turn, has been suggested to cause blood–brain barrier dysfunction by influencing multiple properties of endothelial cells [34]. For instance, Shackleton and colleagues postulate that the exposure of brain endothelial cells to A β triggers the release of MMP9 into the extracellular media [29]. Besides its role in breaking down and restructuring the extracellular matrix, MMP9 was also identified as a ligand for the transmembrane endothelial LRP1 and is able to proteolyse it [31,32]. As demonstrated in a prior study, treatment with recombinant MMP9 leads to a dose-dependent increase in proteolytic LDLR and LRP1 receptor shedding in endothelial cells. As studies indicate, bApoE ϵ 4 is less efficient at preventing this proteolytic shedding compared with the other two isoforms [29]. Findings in mice showed that the inactivation and shedding of LRP1 can lead to a disintegration of the BBB via the activation of the self-autonomous CypA–NF κ B–MMP9 pathway, which leads to a loss of important endothelial TJ proteins (ZO-1, occludin, claudin-5) and collagen IV [31]. Consequently, the disruption of the BBB caused by the

reduction in brain endothelial LRP1 might initiate environmental milieu-altering processes that are associated with the influx of blood-derived neurotoxic proteins such as thrombin, albumin or fibrinogen and might result in a loss of neurons and the development of neuronal deficits [31]. Increased fibrinogen levels, in particular, have been suggested to be involved in this process, as studies found that reducing fibrinogen levels can considerably reverse neurodegenerative signatures in mice [23,35] (Figure 2B).

3.3. The Impact of pApoE on BBB Integrity

The following mechanisms are illustrated in Figure 2C. In contrast to bApoE, information about the impact of pApoE on the BBB is scarce. Previous research assumed that bApoE and pApoE act independently and are separated by the BBB. However, a recent and innovative study by Liu and colleagues showed that pApoE $\epsilon 4$ is sufficient to impair brain functions and to aggravate A β pathogenesis by using a conditional mouse model that expressed human ApoE $\epsilon 3$ or $\epsilon 4$ in the liver while lacking brain ApoE [23]. As the authors demonstrated, pApoE $\epsilon 4$ mice had a significantly compromised synaptic plasticity and cognition, increased BBB leakage, cerebrovascular impairments and increased vessel-associated gliosis compared with the pApoE $\epsilon 3$ mice. Via transcriptomic profiling and single-cell RNA sequencing analyses, they also found that the expression of pApoE $\epsilon 4$ impacted the extracellular matrix and BM, resulting in endothelial dysfunctions [23]. Additionally, pApoE $\epsilon 4$ exhibited a detrimental effect on immune responses within the gliovascular unit (extended neurovascular unit supplemented by oligodendrocytes and myelinated axons [19]) and was associated with a downregulation of genes crucial for endothelial cell function as well as maintaining the integrity of the BBB and the extracellular matrix [23]. Furthermore, their plasma proteome profiling revealed that immune-related and stress-related pathways were upregulated in pApoE $\epsilon 4$ mice, while anchoring junction, mitochondrial function and extracellular matrix pathways were downregulated in the brain endothelial cells of mice [23]. Notably, Liu et al. [23] observed that intravenously administering ApoE $\epsilon 3$ young plasma can improve cognitive function and reduce vessel-associated gliosis of aged wild-type mice, whereas exposure to pApoE $\epsilon 4$ young plasma affected BBB integrity without declining cognition. Additionally, they identified an upregulation of the tissue inhibitor of metalloproteinase 3 (TIMP3), an extracellular matrix-binding protein known to modulate the turnover of the extracellular matrix by inhibiting the activity of MMPs, in the plasma of ApoE $\epsilon 3$ mice [36]. They further observed that intravenously administering exogenous TIMP3 led to enhanced TJs in the presence of pApoE $\epsilon 4$ and concluded that ApoE $\epsilon 4$ plasma might have an impaired ability to maintain the integrity of the endothelial barrier via affecting the TIMP3-mediated pathway and/or the organisation of the extracellular matrix in comparison with pApoE $\epsilon 3$ [23].

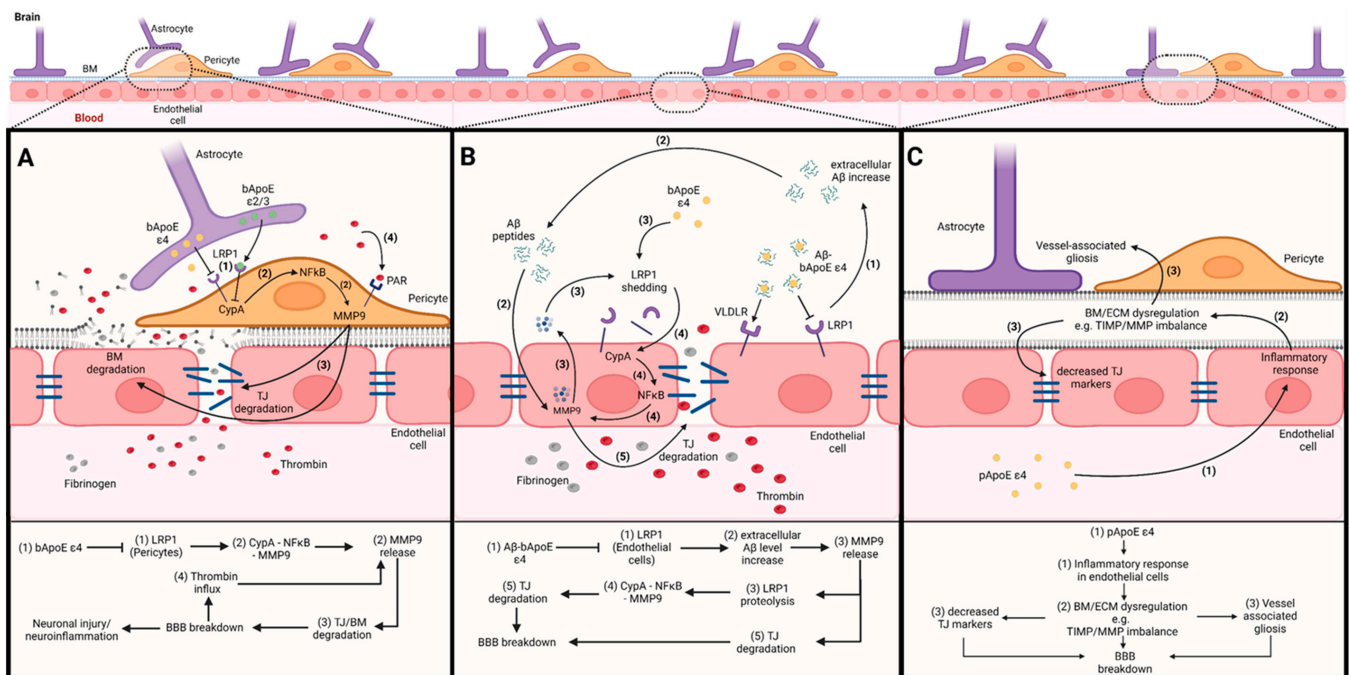


Figure 2. Effect of ApoE $\epsilon 4$ on the BBB via three different mechanisms. **(A) (bApoE $\epsilon 4$ via pericytes):** In contrast to bApoE $\epsilon 2$ and $\epsilon 3$, (1) bApoE $\epsilon 4$ is not able to bind to LRP1, which activates a CypA–NF κ B–MMP9 pathway in pericytes and leads (2) to a release of MMP9 into the extracellular space. Here, MMP9 enzymatically degrades components of the ECM, which leads to (3) a BM/TJ breakdown resulting in an influx of neurotoxic proteins such as the coagulation factor thrombin. (4) Elevated levels of thrombin, in turn, might contribute to a further BBB leakage through MMP9 production via protease-activated receptors (PARs). **(B) (bApoE $\epsilon 4$ via endothelial cells):** (1) A β –bApoE $\epsilon 4$ compounds only interact weakly with LRP1 and are redirected to VLDLR, which internalizes A β –bApoE $\epsilon 4$ compounds at a reduced pace leading to increased A β levels (2). The exposure of endothelial cells to A β (3) promotes the release of MMP9 into the extracellular media. Subsequently, MMP9 impairs TJ maintenance via two different pathways. On the one hand, as bApoE $\epsilon 4$ (3) is less effective in the prevention of LRP1 shedding, MMP9 is able to proteolyse LRP1, which, in turn, promotes (4) the activation of the CypA–NF κ B–MMP9 pathway in endothelial cells. On the other hand, elevated A β levels (2) lead to an elevated MMP9 release resulting in BM/TJ breakdown (5). **(C) (pApoE $\epsilon 4$):** (1) pApoE $\epsilon 4$ may influence blood plasma factors, which contribute to inflammatory responses in endothelial cells. The resulting BM/ECM dysregulation (2), e.g., via TIMP/MMP imbalance, leads to (3) decreased TJ markers as well as (3) vessel-associated gliosis and promotes BBB breakdown. **Abbreviations:** A β = amyloid beta; BBB = blood–brain barrier; BM = basement membrane; bApoE = brain ApoE; CypA = cyclophilin A; ECM = extracellular matrix; LRP1 = low-density lipoprotein receptor-related protein 1; MMP9 = metalloproteinase 9; NF κ B = nuclear factor- κ B; pApoE = peripheral ApoE; PAR = protease-activated receptor; TIMP = tissue inhibitors of metalloproteinases; TJ = tight junction; VLDLR = very low density lipoprotein receptor. Created with [BioRender.com](https://www.biorender.com) accessed on 1 September 2023.

Previous studies have already shown that the balance between MMPs and TIMPs plays a key role in the stability and normal function of the extracellular matrix [36], whereas imbalances were associated with vessel wall impairments [37]. Moreover, TIMP3 directly binds to LRP1, which mediates its endocytosis and degradation, as well as to a soluble form of LRP1 (sLRP1) [36], which is generated through the proteolytic shedding of LRP1 by MMP9 at the cell surface [29]. Although sLRP1 is still capable of binding ligands, such as TIMP3, intracellularly [29], leading to elevated extracellular TIMP3 levels [36]. As an inhibitory effect of TIMP3 on MMP9 activity has been demonstrated [37], the impairment of the TIMP3-

mediated pathway by pApoE ϵ 4 and TIMP3 binding to sLRP1 might hinder the inhibition of MMP9. This, in turn, promotes the degradation of tight junctions and results in damage to the BBB. Although this is in line with observations of increased TIMP3 protein levels in the brains of AD patients and mouse models of AD [37,38], also contrary results have been reported [39]. As TIMP3 interacts with multiple other substrates in addition to MMP9 with reports indicating that elevated TIMP3 may contribute to AD by increasing A β production via the APP pathway [38], it might exert a much more complex role in AD, which has to be further clarified.

4. Pathway Model

Building upon the above-mentioned findings, we now present a pathway model integrating the three mechanisms by which ApoE might impact BBB stability. An illustration of the model is presented in Figure 3.

To begin with, astrocytic-expressed bApoE ϵ 4 is ineffective at suppressing the pro-inflammatory CypA–NF κ B–MMP9 pathway in pericytes (Figure 3, Path I). More specifically, the inability of bApoE ϵ 4 to bind to LRP1 in pericytes cannot prevent the activation of CypA. Consequently, activated CypA leads to NF κ B-mediated MMP9 release, which impairs the maintenance of TJs and the BM and results in BBB breakdown [7,11]. Thereby, the resulting influx of blood-derived neurotoxic proteins (e.g., fibrinogen, thrombin, albumin) accelerates inflammatory and neurodegenerative processes in the brain, which might contribute to the pathogenesis of AD [33,40]. While fibrinogen is considered to particularly stimulate microglial activation [40], elevated thrombin levels might further promote BBB disruption via binding to pericytic PARs and increasing MMP9 release [33]. Hence, it is conceivable that the influx of thrombin ultimately promotes the initiation of a self-perpetuating dysfunctional cycle [33], which persistently diminishes the BBB in the long term (Cycle A). Thereby, the pericyte-driven release of MMP9 might represent the start of a second dysfunctional cycle (Cycle B). As MMP9 is able to proteolyse LRP1 in endothelial cells [31], it can be assumed that both BBB impairment due to deficient CypA inhibition in pericytes and the resulting influx of thrombin [33] promote MMP9-driven shedding of LRP1 in endothelial cells, which finally leads to impaired A β clearance and increased A β levels (Path III) [29,31]. Thereby, a loss of LRP1 might initiate the activation of the CypA–NF κ B–MMP9 pathway in the endothelium leading to an additional MMP9 release [31]. Given the already weak interaction of A β –bApoE ϵ 4 compounds with endothelial LRP1 (Path II), the additional LRP1 loss and elevated MMP9 release (Cycle B) further increase A β accumulation contributing to the formation of toxic A β oligomers and plaques [29,31], which finally promote inflammatory and neurodegenerative processes related to AD [3,7,27,28]. These processes might be accelerated via the Path II-driven extracellular accumulation of A β , which might also initiate MMP9 release in endothelial cells (Path IV) [29].

Furthermore, the presence of pApoE ϵ 4 from the liver may influence blood plasma-based factors, which contribute to inflammatory responses in both endothelial cells and cells of the gliovascular unit leading to BBB breakdown (Path V). This modulation additionally contributes to the degradation of TJs and a dysregulation of the BM and extracellular matrix due to TIMP/MMP9 imbalance as well as increased vessel-associated gliosis [23].

In summary, our proposed model indicates that the described pathways might directly or indirectly contribute to inflammatory and neurodegenerative processes promoting the pathogenesis of AD. They operate predominantly via the development and maintenance of a persistent MMP9 release through Cycles A and B via bApoE ϵ 4 as well as the peripheral modulation of plasma factors via pApoE ϵ 4 [23].

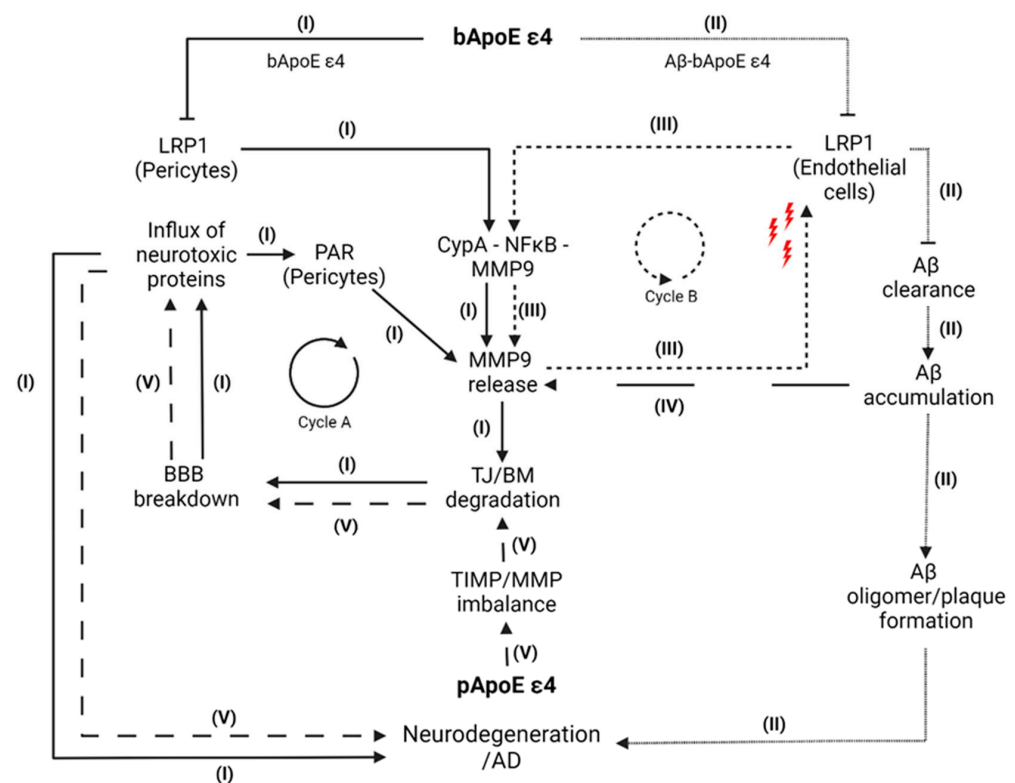


Figure 3. Pathway model. **Path I:** bApoE ε4 cannot prevent the activation of the CypA-NFκB-MMP9 pathway in pericytes resulting in elevated MMP9 levels. Subsequently, MMP9 impairs the maintenance of BM and TJ leading to a BBB breakdown and influx of neurotoxic proteins such as thrombin and fibrinogen, which promote neurodegenerative processes in the brain. Additionally, the elevated thrombin influx might further accelerate BBB disruption via binding to pericytic PARs leading to an MMP9 releasing dysfunctional cycle (Cycle A). **Path II:** In endothelial cells, Aβ-ApoE ε4 complexes redirect the clearance of Aβ from LRP1 to slower VLDLR, which leads to an increase in Aβ molecules resulting in the formation of toxic Aβ oligomers and plaques, which finally promote inflammatory and neurodegenerative processes related to AD. **Path III:** Pericyte-driven release of MMP9 might promote MMP9-driven shedding of LRP1 in endothelial cells leading to an impaired Aβ clearance and increased Aβ levels. The loss of LRP1 might initiate the activation of the CypA-NFκB-MMP9 in endothelial cells leading to an additional MMP9 release, which, in turn, promotes the further shedding of LRP1 (Cycle B). **Path IV:** Paths I and III might be accelerated via the Path II-driven extracellular accumulation of Aβ, which might initiate MMP9 release in endothelial cells leading to BM/TJ degradation (Path I) as well as LRP1 shedding (Path III) resulting in neurodegenerative processes. **Path V:** pApoE ε4 may influence blood-plasma-based factors, which contribute to inflammatory responses in endothelial cells. These contribute to a TIMP/MMP9 imbalance resulting in a BM/ECM dysregulation as well as degradation of TJs. The resulting BBB breakdown enables an influx of a wide range of neurotoxic proteins, which promote AD-related neurodegenerative processes. **Abbreviations:** Aβ = amyloid beta; BBB = blood-brain barrier; BM = basement membrane; bApoE = brain ApoE; CypA = cyclophilin A; ECM = extracellular matrix; LRP1 = low-density lipoprotein receptor-related protein 1; MMP9 = metalloproteinase 9; NFκB = nuclear factor-κB; pApoE = peripheral ApoE; PAR = protease-activated receptor; TIMP = tissue inhibitors of metalloproteinases; TJ = tight junction; VLDLR = very low density lipoprotein receptor. Modified from Zlokovic [7]. Created with BioRender.com accessed on 1 September 2023.

However, this model has to be seen in light of some limitations. Since the underlying biological processes have mainly been studied in cell and mouse models, we cannot make any assertions as to whether the described mechanisms by which ApoE ε4 may impact BBB integrity are transferable to humans. Also, as we were not able to test our

model with mechanistic wet lab experiments, additional studies are needed for verification. Moreover, this model focuses on the role of MMP9 and represents only an excerpt of the processes affecting BBB stability. For instance, LRP1 [41] and TIMP3 [36] exert much more wide-ranging physiological roles and might be involved in additional pathophysiological processes. Therefore, further studies are warranted to bridge this gap. Furthermore, since this is not a systematic review, we did not perform a structured, systematic literature search. Instead, we have concentrated on the most recent and robust studies regarding the influence of ApoE ϵ 4 on BBB integrity, which have offered a deeper understanding of the current state of knowledge. We have made every effort to include all available evidence, but we cannot ensure absolute comprehensiveness.

5. Future Perspectives

As visualised by our supposed model, both bApoE ϵ 4 and pApoE ϵ 4 are likely involved in neurodegenerative processes via BBB integrity impairment or A β accumulation, contributing to the pathogenesis of AD. Disrupting the balance of the BBB leads to neuronal damage and impedes A β clearance at the gliovascular unit, potentially creating a harmful neurodegenerative environment via a persistent MMP9 release. Although cell and mouse models suggest that AD-related pathologies have a significant impact on each component of the gliovascular unit, additional clinical studies are needed to determine whether BBB disruption is an essential characteristic of AD development and progression.

Despite the extensive research in the field, several open questions and scientific challenges persist. These include a more detailed investigation of blood-derived proteins regarding their neurotoxicity and their specific roles in the pathogenesis of AD. In addition to thrombin [33,42], it is assumed that fibrinogen isoforms exert a significant role in the vascular pathology of AD and neuroinflammation [43]. With regard to BBB breakdown, fibrinogen induces a pathogenic response in microglia, contributing to oxidative stress, inflammation and neurodegeneration [35], leading to dendrite loss as well as dendritic spine elimination and cognitive decline in an AD-related manner [40]. This is underpinned by the observation that the removal of fibrinogen can largely reverse such blood-induced microglia neurodegenerative signatures [35].

In addition, it remains elusive whether an ApoE ϵ 4-driven neurotoxic protein influx promotes neurodegenerative processes in general or if it is restricted to AD-specific brain areas. According to previous research, the initial manifestation of AD pathology occurs in the transentorhinal cortex and progressively extends into the entorhinal cortex and hippocampus, two integral parts of the limbic system [44]. Cross-sectional and longitudinal analyses of regional grey matter volumes and grey matter atrophies showed that hippocampal and entorhinal cortex volumes were the most vulnerable areas in human non-demented ApoE ϵ 4 carriers [45,46]. Research findings also indicate that ApoE ϵ 4 mice show restricted neuronal dendritic arborization in the hippocampus, characterised by reduced branching and spine density, as well as a decrease in their complexity within the entorhinal cortex [47]. Additionally, the examination of BBB permeability revealed heightened BBB disruption in the hippocampus and parahippocampal gyrus among cognitively healthy individuals carrying the ApoE ϵ 4 allele (ϵ 3/ ϵ 4 and ϵ 4/ ϵ 4) in comparison with cognitively healthy individuals with the ApoE ϵ 3 homozygous genotype (ϵ 3/ ϵ 3) [13,14]. Although these results suggest a brain area-specific effect of ApoE ϵ 4 on BBB integrity, more research is needed to confirm these observations.

Another open question is how such an ApoE ϵ 4-driven BBB breakdown can be avoided. Possibly, the inhibition of MMP9 release might be a promising target to prevent TJ and BM degradation as well as LRP1 shedding. For instance, treatment of ApoE ϵ 4 mice with the immunosuppressive intracellular CypA-binding drug cyclosporine A indicated that BBB changes are reversible and cyclosporine A might be a promising drug approach to prevent bApoE ϵ 4-induced BBB breakdown [11]. A similar effect was reported for the non-immunosuppressive CypA inhibitor Debio-25, which was shown to protect against neurodegeneration even in the presence of LRP1 endothelial deficiency [31]. Besides

CypA inhibitors, NF κ B inhibitors such as pyrrolidine dithiocarbamate (PDTC) might be interesting targets to prevent BBB breakdown. Experiments indicate that PDTC can reduce MMP9 activation and reverse BBB breakdown in mice [11]. Additionally, Shackleton and colleagues showed that the treatment of human brain microvascular endothelial cells and ApoE ϵ 4 mice with the MMP9 inhibitor SB-3CT can significantly improve A β removal through the prevention of LRP1 shedding [29]. Although these drugs targeting the CypA–NF κ B–MMP9 pathway represent promising treatment approaches, additional studies are needed to verify their efficacy related to AD in clinical studies.

Regarding pApoE, Liu and colleagues showed that the addition of exogenous TIMP3 led to enhanced TJs in the presence of pApoE ϵ 4 [23]. Hence, TIMP3 might play a key role in BBB maintenance via MMP inhibition. It is conceivable that a TIMP3 replenishment or plasma exchange in ApoE ϵ 4 carriers contributes to the maintenance of BBB integrity by affecting the TIMP3-mediated pathway or restoring the TIMP3/MMP9 balance, respectively. TIMP3 might, therefore, represent an interesting drug target in preventing TJ and BM disruption and protecting BBB integrity. However, as reports on the application of TIMP3 in neurodegenerative diseases are limited, so far, treatment approaches remain elusive and should be further explored.

In addition to BBB-stabilising medications, the selective opening of the BBB to systematically administer therapeutic drugs into the parenchyma of neural tissues has been proposed as another interesting treatment approach [22]. However, considering the impact of ApoE ϵ 4 on BBB integrity, such approaches should be handled with care, especially for ApoE ϵ 4 carriers. It is essential to ensure that unintended neurotoxin influx is prevented and that the BBB can be restored. For instance, increased BBB permeability is implicated in postoperative neurocognitive impairments following peripheral surgery with inhalation anaesthetics such as sevoflurane or isoflurane [48]. Similar findings were reported in animal research, where particularly older sevoflurane-treated rats showed increased BBB permeability and failed to regain BBB integrity within 24 h of exposure to anaesthetics [49]. Consequently, since BBB integrity in older ApoE ϵ 4 carriers might already be weakened due to a bApoE ϵ 4-driven elevated MMP9 release as well as pApoE ϵ 4-driven TIMP/MMP imbalance, drug approaches that result in targeted or unintended alteration of BBB permeability might increase the risk of advanced neurodegenerative processes.

6. Conclusions

This work presents an overview of how brain-derived and peripheral ApoE ϵ 4 might be involved in blood–brain barrier breakdown and promote neurodegenerative processes possibly leading to Alzheimer’s disease. To the best of our knowledge, this is the first model that outlines the impact of both bApoE ϵ 4 and pApoE ϵ 4 on BBB integrity, focusing on detrimental neurodegenerative cycles triggered by MMP9 release and LRP1 shedding. Hence, our model can provide valuable insights for researchers studying neurodegeneration and the role of ApoE ϵ 4 in Alzheimer’s disease. However, future work is needed to verify the herein outlined impact of bApoE ϵ 4 and pApoE ϵ 4 on the pathogenesis of AD. Lastly, further research is warranted to unravel AD-specific effects of neurotoxic proteins, their potential interactions with ApoE ϵ 4/A β , the possible wide-ranging physiological and pathological roles of LRP1 and TIMP3 in AD pathogenesis as well as the development of potential treatment approaches to prevent or repair ApoE ϵ 4-related BBB impairments.

Author Contributions: Conceptualization, K.K.; writing—original draft preparation, K.K.; writing—review and editing, L.G., L.K., S.B., H.J.G. and S.V.d.A.; project administration, S.V.d.A.; visualisation, K.K.; supervision, S.V.d.A. and H.J.G.; funding acquisition, S.V.d.A. and H.J.G. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by the German Federal Ministry of Education and Research (BMBF, gr. No. 01KU2004) under the frame of ERA PerMed (Trajectome project, ERAPERMED2019-108).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: H.J.G. has received travel grants and speakers honoraria from Fresenius Medical Care, Neuraxpharm, Servier and Janssen Cilag, as well as research funding from Fresenius Medical Care. All other authors have no conflict to declare.

References

- Passeri, E.; Elkhoury, K.; Morsink, M.; Broersen, K.; Linder, M.; Tamayol, A.; Malaplate, C.; Yen, F.T.; Arab-Tehrany, E. Alzheimer's Disease: Treatment Strategies and Their Limitations. *Int. J. Mol. Sci.* **2022**, *23*, 13954. [\[CrossRef\]](#) [\[PubMed\]](#)
- Skaria, A.P. The economic and societal burden of Alzheimer disease: Managed care considerations. *Am. J. Manag. Care* **2022**, *28*, S188–S196. [\[CrossRef\]](#)
- Sengupta, U.; Nilson, A.N.; Kaye, R. The Role of Amyloid- β Oligomers in Toxicity, Propagation, and Immunotherapy. *EBioMedicine* **2016**, *6*, 42–49. [\[CrossRef\]](#)
- WHO. *World Report on Ageing and Health*; World Health Organization: Geneva, Switzerland, 2015; ISBN 9789240694811.
- Alzheimers Association. 2021 Alzheimer's disease facts and figures. *Alzheimers. Dement.* **2021**, *17*, 327–406. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kunkle, B.W.; Grenier-Boley, B.; Sims, R.; Bis, J.C.; Damotte, V.; Naj, A.C.; Boland, A.; Vronskaya, M.; van der Lee, S.J.; Amlie-Wolf, A.; et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates A β , tau, immunity and lipid processing. *Nat. Genet.* **2019**, *51*, 414–430. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zlokovic, B.V. Cerebrovascular effects of apolipoprotein E: Implications for Alzheimer disease. *JAMA Neurol.* **2013**, *70*, 440–444. [\[CrossRef\]](#)
- Ratan, Y.; Rajput, A.; Maleysm, S.; Pareek, A.; Jain, V.; Pareek, A.; Kaur, R.; Singh, G. An Insight into Cellular and Molecular Mechanisms Underlying the Pathogenesis of Neurodegeneration in Alzheimer's Disease. *Biomedicines* **2023**, *11*, 1398. [\[CrossRef\]](#)
- Huang, Y.-W.A.; Zhou, B.; Wernig, M.; Südhof, T.C. ApoE2, ApoE3, and ApoE4 Differentially Stimulate APP Transcription and A β Secretion. *Cell* **2017**, *168*, 427–441.e21. [\[CrossRef\]](#)
- Montagne, A.; Nikolakopoulou, A.M.; Huuskonen, M.T.; Sagare, A.P.; Lawson, E.J.; Lazic, D.; Rege, S.V.; Grond, A.; Zuniga, E.; Barnes, S.R.; et al. APOE4 accelerates advanced-stage vascular and neurodegenerative disorder in old Alzheimer's mice via cyclophilin A independently of amyloid- β . *Nat. Aging* **2021**, *1*, 506–520. [\[CrossRef\]](#)
- Bell, R.D.; Winkler, E.A.; Singh, I.; Sagare, A.P.; Deane, R.; Wu, Z.; Holtzman, D.M.; Betsholtz, C.; Armulik, A.; Sallstrom, J.; et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* **2012**, *485*, 512–516. [\[CrossRef\]](#)
- Halliday, M.R.; Rege, S.V.; Ma, Q.; Zhao, Z.; Miller, C.A.; Winkler, E.A.; Zlokovic, B.V. Accelerated pericyte degeneration and blood-brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **2016**, *36*, 216–227. [\[CrossRef\]](#) [\[PubMed\]](#)
- Montagne, A.; Nation, D.A.; Sagare, A.P.; Barisano, G.; Sweeney, M.D.; Chakhoyan, A.; Pachicano, M.; Joe, E.; Nelson, A.R.; D'Orazio, L.M.; et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. *Nature* **2020**, *581*, 71–76. [\[CrossRef\]](#) [\[PubMed\]](#)
- Moon, W.-J.; Lim, C.; Ha, I.H.; Kim, Y.; Moon, Y.; Kim, H.-J.; Han, S.-H. Hippocampal blood-brain barrier permeability is related to the APOE4 mutation status of elderly individuals without dementia. *J. Cereb. Blood Flow Metab.* **2021**, *41*, 1351–1361. [\[CrossRef\]](#)
- Alata, W.; Ye, Y.; St-Amour, I.; Vandal, M.; and Calon, F. Human Apolipoprotein E ϵ 4 Expression Impairs Cerebral Vascularization and Blood—Brain Barrier Function in Mice. *J. Cereb. Blood Flow Metab.* **2015**, *35*, 86–94. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wang, H.-L.; Zhang, C.-L.; Qiu, Y.-M.; Chen, A.-Q.; Li, Y.-N.; Hu, B. Dysfunction of the Blood-brain Barrier in Cerebral Microbleeds: From Bedside to Bench. *Aging Dis.* **2021**, *12*, 1898–1919. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lin, X.; Fan, Y.; Zhang, F.; Lin, Y. Cerebral microinfarct is emergency consequence of Alzheimer's disease: A new insight into development of neurodegenerative diseases. *Int. J. Biol. Sci.* **2022**, *18*, 1569–1579. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jackson, R.J.; Meltzer, J.C.; Nguyen, H.; Commings, C.; Bennett, R.E.; Hudry, E.; Hyman, B.T. APOE4 derived from astrocytes leads to blood-brain barrier impairment. *Brain* **2022**, *145*, 3582–3593. [\[CrossRef\]](#)
- Alonso-Alonso, M.L.; Sampedro-Viana, A.; Fernández-Rodicio, S.; Bazarra-Barreiros, M.; Ouro, A.; Sobrino, T.; Campos, F.; Castillo, J.; Hervella, P.; Iglesias-Rey, R. Need for a Paradigm Shift in the Treatment of Ischemic Stroke: The Blood-Brain Barrier. *Int. J. Mol. Sci.* **2022**, *23*, 9486. [\[CrossRef\]](#)
- Xu, L.; Nirwane, A.; Yao, Y. Basement membrane and blood-brain barrier. *Stroke Vasc. Neurol.* **2019**, *4*, 78–82. [\[CrossRef\]](#)
- Huffels, C.F.M.; Middeldorp, J.; Hol, E.M. A β Pathology and Neuron-Glia Interactions: A Synaptocentric View. *Neurochem. Res.* **2023**, *48*, 1026–1046. [\[CrossRef\]](#)
- Nakada-Honda, N.; Cui, D.; Matsuda, S.; Ikeda, E. Intravenous injection of cyclophilin A realizes the transient and reversible opening of barrier of neural vasculature through basigin in endothelial cells. *Sci. Rep.* **2021**, *11*, 19391. [\[CrossRef\]](#) [\[PubMed\]](#)
- Liu, C.-C.; Zhao, J.; Fu, Y.; Inoue, Y.; Ren, Y.; Chen, Y.; Doss, S.V.; Shue, F.; Jeevaratnam, S.; Bastea, L.; et al. Peripheral apoE4 enhances Alzheimer's pathology and impairs cognition by compromising cerebrovascular function. *Nat. Neurosci.* **2022**, *25*, 1020–1033. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ghebranious, N.; Ivacic, L.; Mallum, J.; Dokken, C. Detection of ApoE E2, E3 and E4 alleles using MALDI-TOF mass spectrometry and the homogeneous mass-extend technology. *Nucleic Acids Res.* **2005**, *33*, e149. [\[CrossRef\]](#) [\[PubMed\]](#)

25. El Haj, M.; Antoine, P.; Amouyel, P.; Lambert, J.-C.; Pasquier, F.; Kapogiannis, D. Apolipoprotein E (APOE) ϵ 4 and episodic memory decline in Alzheimer's disease: A review. *Ageing Res. Rev.* **2016**, *27*, 15–22. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Kepp, K.P.; Robakis, N.K.; Høilund-Carlsen, P.F.; Sensi, S.L.; Vissel, B. The amyloid cascade hypothesis: An updated critical review. *Brain* **2023**. [\[CrossRef\]](#)
27. Hashimoto, T.; Serrano-Pozo, A.; Hori, Y.; Adams, K.W.; Takeda, S.; Banerji, A.O.; Mitani, A.; Joyner, D.; Thyssen, D.H.; Bacskai, B.J.; et al. Apolipoprotein E, especially apolipoprotein E4, increases the oligomerization of amyloid β peptide. *J. Neurosci.* **2012**, *32*, 15181–15192. [\[CrossRef\]](#)
28. Fontana, I.C.; Zimmer, A.R.; Rocha, A.S.; Gosmann, G.; Souza, D.O.; Lourenco, M.V.; Ferreira, S.T.; Zimmer, E.R. Amyloid- β oligomers in cellular models of Alzheimer's disease. *J. Neurochem.* **2020**, *155*, 348–369. [\[CrossRef\]](#)
29. Shackleton, B.; Ringland, C.; Abdullah, L.; Mullan, M.; Crawford, F.; Bachmeier, C. Influence of Matrix Metalloproteinase 9 on Beta-Amyloid Elimination Across the Blood-Brain Barrier. *Mol. Neurobiol.* **2019**, *56*, 8296–8305. [\[CrossRef\]](#)
30. Rhea, E.M.; Banks, W.A. Interactions of Lipids, Lipoproteins, and Apolipoproteins with the Blood-Brain Barrier. *Pharm. Res.* **2021**, *38*, 1469–1475. [\[CrossRef\]](#)
31. Nikolakopoulou, A.M.; Wang, Y.; Ma, Q.; Sagare, A.P.; Montagne, A.; Huuskonen, M.T.; Rege, S.V.; Kisler, K.; Dai, Z.; Körbelin, J.; et al. Endothelial LRP1 protects against neurodegeneration by blocking cyclophilin A. *J. Exp. Med.* **2021**, *218*, e20202207. [\[CrossRef\]](#)
32. Ma, Q.; Zhao, Z.; Sagare, A.P.; Wu, Y.; Wang, M.; Owens, N.C.; Vergheze, P.B.; Herz, J.; Holtzman, D.M.; Zlokovic, B.V. Blood-brain barrier-associated pericytes internalize and clear aggregated amyloid- β 42 by LRP1-dependent apolipoprotein E isoform-specific mechanism. *Mol. Neurodegener.* **2018**, *13*, 57. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Machida, T.; Takata, F.; Matsumoto, J.; Takenoshita, H.; Kimura, I.; Yamauchi, A.; Dohgu, S.; Kataoka, Y. Brain pericytes are the most thrombin-sensitive matrix metalloproteinase-9-releasing cell type constituting the blood-brain barrier in vitro. *Neurosci. Lett.* **2015**, *599*, 109–114. [\[CrossRef\]](#)
34. Yamazaki, Y.; Shinohara, M.; Shinohara, M.; Yamazaki, A.; Murray, M.E.; Liesinger, A.M.; Heckman, M.G.; Lesser, E.R.; Parisi, J.E.; Petersen, R.C.; et al. Selective loss of cortical endothelial tight junction proteins during Alzheimer's disease progression. *Brain* **2019**, *142*, 1077–1092. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Mendiola, A.S.; Yan, Z.; Dixit, K.; Johnson, J.R.; Bouhaddou, M.; Meyer-Franke, A.; Shin, M.-G.; Yong, Y.; Agrawal, A.; MacDonald, E.; et al. Defining blood-induced microglia functions in neurodegeneration through multiomic profiling. *Nat. Immunol.* **2023**, *24*, 1173–1187. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Fan, D.; Kassiri, Z. Biology of Tissue Inhibitor of Metalloproteinase 3 (TIMP3), and Its Therapeutic Implications in Cardiovascular Pathology. *Front. Physiol.* **2020**, *11*, 661. [\[CrossRef\]](#)
37. Jäkel, L.; Kuiperij, H.B.; Gerding, L.P.; Custers, E.E.M.; van den Berg, E.; Jolink, W.M.T.; Schreuder, F.H.B.M.; Küsters, B.; Klijn, C.J.M.; Verbeek, M.M. Disturbed balance in the expression of MMP9 and TIMP3 in cerebral amyloid angiopathy-related intracerebral haemorrhage. *Acta Neuropathol. Commun.* **2020**, *8*, 99. [\[CrossRef\]](#)
38. Hoe, H.-S.; Cooper, M.J.; Burns, M.P.; Lewis, P.A.; van der Brug, M.; Chakraborty, G.; Cartagena, C.M.; Pak, D.T.S.; Cookson, M.R.; Rebeck, G.W. The metalloprotease inhibitor TIMP-3 regulates amyloid precursor protein and apolipoprotein E receptor proteolysis. *J. Neurosci.* **2007**, *27*, 10895–10905. [\[CrossRef\]](#)
39. Park, J.H.; Cho, S.-J.; Jo, C.; Park, M.H.; Han, C.; Kim, E.-J.; Huh, G.Y.; Koh, Y.H. Altered TIMP-3 Levels in the Cerebrospinal Fluid and Plasma of Patients with Alzheimer's Disease. *J. Pers. Med.* **2022**, *12*, 827. [\[CrossRef\]](#)
40. Merlini, M.; Rafalski, V.A.; Rios Coronado, P.E.; Gill, T.M.; Ellisman, M.; Muthukumar, G.; Subramanian, K.S.; Ryu, J.K.; Syme, C.A.; Dávalos, D.; et al. Fibrinogen Induces Microglia-Mediated Spine Elimination and Cognitive Impairment in an Alzheimer's Disease Model. *Neuron* **2019**, *101*, 1099–1108.e6. [\[CrossRef\]](#)
41. Shinohara, M.; Tachibana, M.; Kanekiyo, T.; Bu, G. Role of LRP1 in the pathogenesis of Alzheimer's disease: Evidence from clinical and preclinical studies. *J. Lipid Res.* **2017**, *58*, 1267–1281. [\[CrossRef\]](#)
42. Iannucci, J.; Grammas, P. Thrombin, a Key Driver of Pathological Inflammation in the Brain. *Cells* **2023**, *12*, 1222. [\[CrossRef\]](#)
43. Rehiman, S.H.; Lim, S.M.; Lim, F.T.; Chin, A.-V.; Tan, M.P.; Kamaruzzaman, S.B.; Ramasamy, K.; Abdul Majeed, A.B. Fibrinogen isoforms as potential blood-based biomarkers of Alzheimer's disease using a proteomics approach. *Int. J. Neurosci.* **2022**, *132*, 1014–1025. [\[CrossRef\]](#)
44. Lazarov, O.; Hollands, C. Hippocampal neurogenesis: Learning to remember. *Prog. Neurobiol.* **2016**, *138–140*, 1–18. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Régy, M.; Dugravot, A.; Sabia, S.; Fayosse, A.; Mangin, J.-F.; Chupin, M.; Fischer, C.; Bouteloup, V.; Dufouil, C.; Chêne, G.; et al. Association of APOE ϵ 4 with cerebral gray matter volumes in non-demented older adults: The MEMENTO cohort study. *Neuroimage* **2022**, *250*, 118966. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Kirchner, K.; Garvert, L.; Wittfeld, K.; Ameling, S.; Bülow, R.; Meyer Zu Schwabedissen, H.; Nauck, M.; Völzke, H.; Grabe, H.J.; van der Auwera, S. Deciphering the Effect of Different Genetic Variants on Hippocampal Subfield Volumes in the General Population. *Int. J. Mol. Sci.* **2023**, *24*, 1120. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Flowers, S.A.; Rebeck, G.W. APOE in the normal brain. *Neurobiol. Dis.* **2020**, *136*, 104724. [\[CrossRef\]](#)

48. Spieth, L.; Berghoff, S.A.; Stumpf, S.K.; Winchenbach, J.; Michaelis, T.; Watanabe, T.; Gerndt, N.; Düking, T.; Hofer, S.; Ruhwedel, T.; et al. Anesthesia triggers drug delivery to experimental glioma in mice by hijacking caveolar transport. *Neurooncol. Adv.* **2021**, *3*, vdab140. [[CrossRef](#)]
49. Acharya, N.K.; Goldwaser, E.L.; Forsberg, M.M.; Godsey, G.A.; Johnson, C.A.; Sarkar, A.; DeMarshall, C.; Kosciuk, M.C.; Dash, J.M.; Hale, C.P.; et al. Sevoflurane and Isoflurane induce structural changes in brain vascular endothelial cells and increase blood-brain barrier permeability: Possible link to postoperative delirium and cognitive decline. *Brain Res.* **2015**, *1620*, 29–41. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.