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Review

Fibrinogen and Altered Hemostasis in Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) plaques, tau tangles, brain atrophy, and vascular pathology. Vascular defects include cerebrovascular dysfunction, decreased cerebral blood flow, and blood brain barrier (BBB) disruption, among others. Here, we review the evidence that links Aβ with the vascular pathology present in AD, with a specific focus on the hemostatic system and the clotting protein fibrinogen. Fibrinogen is normally found circulating in blood, but in AD it deposits with Aβ in the brain parenchyma and cerebral blood vessels. We found that Aβ and fibrin(ogen) interact, and their binding leads to increased fibrinogen aggregation, Aβ fibrillization, and the formation of degradation-resistant fibrin clots. Decreasing fibrinogen levels not only lessens cerebral amyloid angiopathy and BBB permeability, but it also reduces microglial activation and improves cognitive performance in AD mouse models. Moreover, a prothrombotic state in AD is evidenced by increased clot formation, decreased fibrinolysis, and elevated levels of coagulation factors and activated platelets. Abnormal deposition and persistence of fibrin(ogen) in AD may result from Aβ-fibrin(ogen) binding and altered hemostasis and could thus contribute to Aβ deposition, decreased cerebral blood flow, exacerbated neuroinflammation, and eventual neurodegeneration. Blocking the interaction between fibrin(ogen) and Aβ may be a promising therapeutic target for AD.

Keywords: Blood brain barrier, blood coagulation, cerebral amyloid angiopathy, fibrinogen, hemostasis, thrombosis

Alzheimer's disease (AD), a debilitating and fatal cognitive disorder that currently affects over 26 million people worldwide, is a severe neurodegenerative disease characterized by amyloid-β (Aβ) plaques, tau tangles, and brain atrophy. Accumulating evidence also links AD with vascular risk factors [1]. These correlations, together with profound alterations of cerebrovascular structure and function present in AD [2], suggest a "vascular hypothesis", where vascular pathology eventually leads to neurodegeneration and subsequent cognitive decline [3, 4]. Neuronal dysfunction may also be caused by the accumulation of Aβ, known as the "amyloid hypothesis" [5]. Here, we review the evidence that connects vascular disease and AD, focusing specifically on how the clotting protein fibrin(ogen) could be the missing link between the vascular and amyloid hypotheses [6].

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FIBRIN(OGEN) IN AD

Fibrinogen is a 340 kDa plasma glycoprotein that plays a key role in coagulation. Upon cleavage by thrombin, fibrinogen is converted to fibrin, which polymerizes into a fibrin network, or clot [7]. Fibrinogen further contributes to hemostasis by forming bridges between activated platelets and promoting their aggregation [8]. Fibrin clot lysis is mediated by plasmin, which is generated from plasminogen on the fibrin surface following activation by tissue plasminogen activator (tPA). In addition to its role in coagulation, fibrinogen has adhesive and inflammatory functions

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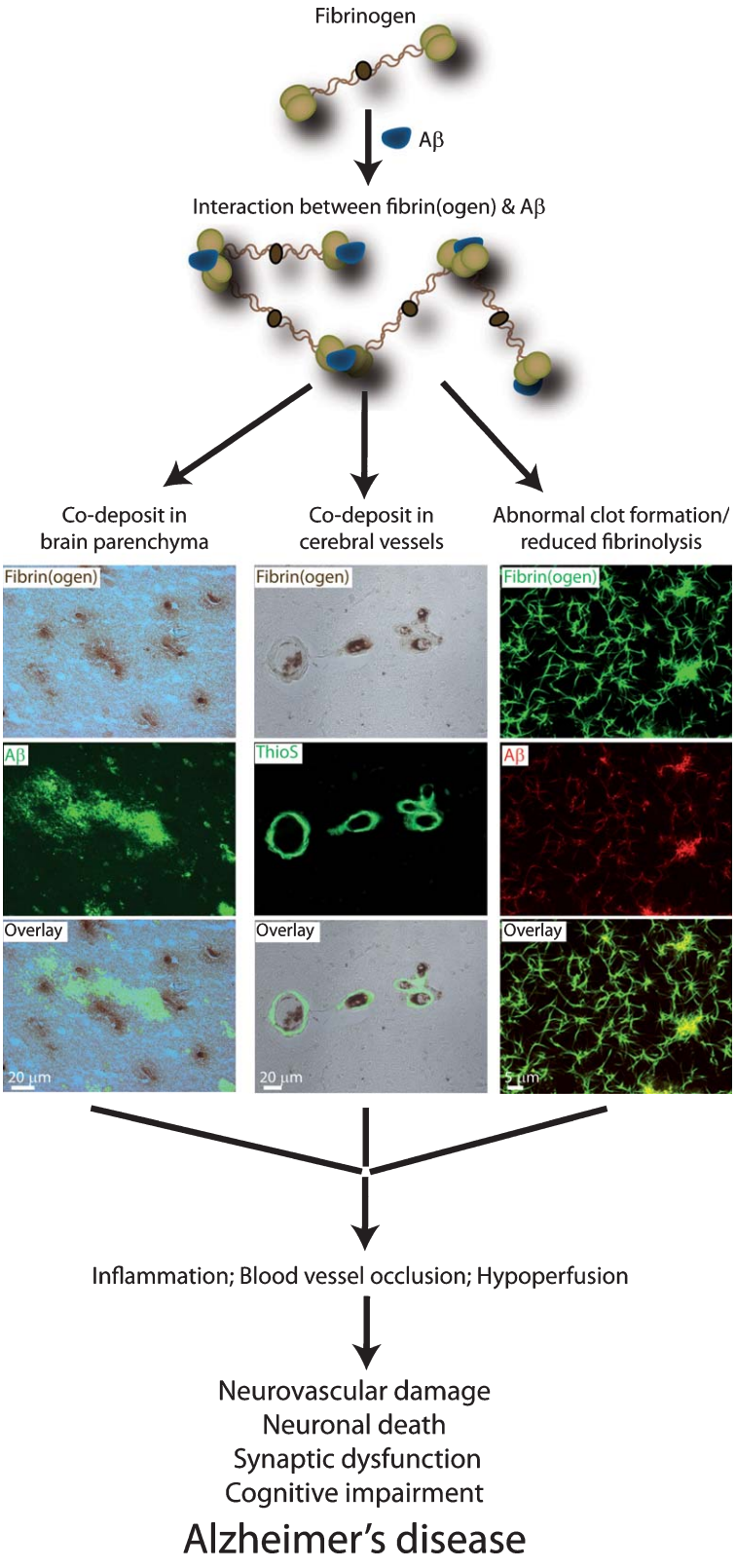
Fibrinogen
Aβ
Interaction between fibrin(ogen) & Aβ
Co-deposit in brain parenchyma
Co-deposit in cerebral vessels
Abnormal clot formation/ reduced fibrinolysis
Fibrin(ogen)
Fibrin(ogen)
Fibrin(ogen)
Aβ
ThioS
Aβ
Overlay
Overlay
Overlay
20 μm
20 μm
5 μm
Inflammation; Blood vessel occlusion; Hypoperfusion
Neurovascular damage
Neuronal death
Synaptic dysfunction
Cognitive impairment
Alzheimer's disease

Fig. 1.

mediated by its interactions with numerous proteins in the circulation and on cell surfaces [7].

Due to its key role in hemostasis, changes in the levels of fibrinogen can have serious pathological consequences. Elevated levels of plasma fibrinogen lead to increased blood viscosity, platelet and endothelial cell activation, erythrocyte aggregation, and reduced blood flow [9], predisposing people to cardiovascular disease and stroke [10]. Interestingly, the analysis of plasma from large cohorts of patients showed that elevated fibrinogen levels are associated with cognitive decline [11], increased risk of AD [12], and brain atrophy in AD patients [13], suggesting a link between an abnormal clotting system and cognitive deficits.

Fibrin(ogen), blood brain barrier dysfunction, and cerebral amyloid angiopathy formation

Fibrin(ogen) is normally excluded from the brain by the blood brain barrier (BBB), but it has been detected in the brains of AD patients and mouse models [14–20]. BBB dysfunction has been reported in brains of AD mice [18, 21] and humans [19, 20, 22], which may explain the extravasation of fibrin(ogen), and thus its presence, in the brain parenchyma. This movement of fibrin(ogen) through the BBB appears to be rather specific, since some smaller molecules are not BBB permeable in this disorder [23, 24]. BBB dysfunction may be initially induced by vascular factors currently connected with AD [1], including ischemia [25], ingestion of saturated fats or cholesterol [26], inflammation processes in the brain [27], or hypertensive crises [28]. Another possible mechanism for BBB malfunction in AD could be the increased deposition of fibrin(ogen). *In vitro,* pathologically high levels of fibrinogen can promote microvascular permeability by decreasing levels of endothelial tight junction proteins [29, 30], which could in turn contribute to the accumulation of fibrin(ogen) outside the circulation. A third mechanism for BBB disruption in AD could be the presence of cerebral amyloid angiopathy (CAA), deposits of Aβ in cortical and leptomeningeal vessel walls of arteries, arterioles, and capillaries, which can result from the incomplete or improper clearance of Aβ from the brain parenchyma [31, 32]. CAA-positive vessels exhibit smooth muscle cell loss, vessel wall remodeling [33, 34], and occlusions [35]. These changes could lead to blood vessel weakening and BBB dysfunction, which may contribute to the increased occurrence of cerebral microbleeds, hemorrhages, and infarcts typical of CAA [33, 34, 36]. These processes can in turn impede cerebral blood flow and reduce blood supply to the brain, leading to the hypoperfusion observed in AD patients [37, 38]. This evidence clearly suggests that the CAA-impaired vasculature can significantly impact brain function.

We and others have reported that CAA-positive vessels in the brains of AD mice and patients also contain fibrin(ogen) deposits [14, 39] (Fig. 1). To determine if fibrin(ogen) affects the accumulation of Aβ in the vasculature, we decreased fibrinogen levels in two AD mouse models either genetically or pharmacologically. The total amount of CAA was significantly diminished [14], suggesting that fibrinogen directly affects the deposition and clearance of Aβ deposits in the brain vasculature. More importantly, we also found that AD mice with reduced fibrinogen levels in their blood showed significant improvement in spatial memory [14].

Interaction between fibrin(ogen) and Aβ

Since fibrin(ogen) and Aβ co-deposit in the AD brain [14, 16, 18–20, 39] (Fig. 1), we hypothesized that Aβ and fibrinogen could physically interact. We found that fibrinogen binds to Aβ *in vitro* ($K_d = 26.3 \pm 6.7$ nM), which enhances fibrinogen aggregation and increases Aβ fibrillization [40]. It is thus possible that the development of CAA is accelerated when Aβ encounters fibrin(ogen) in the vessel wall. In agreement with this hypothesis, mice subjected to ischemic lesioning showed rapid increases in Aβ deposition in the vessels and parenchyma near the infarcted area [41], which could be due to improper clearance of Aβ through the vasculature. These lesions, which involve the production of fibrin, may also induce

Fig. 1. The scheme represents how the amyloid-β (Aβ)-fibrin(ogen) interaction may affect Alzheimer's disease (AD) pathology. In an AD patient, Aβ interacts with fibrin(ogen) in the brain parenchyma (left panels) and cerebral blood vessels (middle panels). The Aβ-fibrin(ogen) interaction could also alter blood clot formation and degradation (right panels), which could be exacerbated by the prothrombotic environment present in AD. Together, these abnormalities would enhance inflammation and lead to vessel occlusion and hypoperfusion. These deleterious alterations would be sustained, since fibrin(ogen) persists longer in the AD brain due to its interaction with Aβ, and could eventually lead to neurovascular damage, neuronal dysfunction, and cognitive decline. Left panels show fibrin(ogen) and Aβ immunohistochemistry on frozen sections of the frontal cortex of an AD patient. Middle panels show fibrin(ogen) immunohistochemistry and Thioflavin S staining on paraffin-embedded sections of the frontal cortex of an AD patient [14]. Right panels show an *in vitro* fibrin clot in the presence of Aβ [14, 42].

Aβ aggregation and deposition following fibrin(ogen) extravasation through a dysfunctional BBB.

THROMBOTIC/FIBRINOLYTIC SYSTEM IN AD

Abnormal clot formation and degradation in AD

We have shown that the presence of Aβ affects the structure of fibrin clots and interferes with clot lysis *in vitro* [14, 42]. Our *in vivo* experiments also demonstrate that AD mice have an increased propensity to clot, and the resulting thrombi are more resistant to degradation than clots formed in controls [14]. Similar results were obtained by Klohs et al. who examined vascular function in a mouse model of CAA [39]. A significant reduction in the number of functional intracortical microvessels in aged CAA mice compared to control littermates was observed, which was attributed to obstructed perfusion resulting from abnormal fibrin clot lysis. Fibrinogen and CAA co-deposited in the vessels of these mice, which could suggest that vessel obstruction results from the formation of persistent Aβ-laden fibrin clots. *In vitro* studies suggest that the delay in fibrin clot lysis in AD could partially be due to the formation of a tighter fibrin network in the presence of Aβ and to the interference of Aβ with the binding of plasminogen to fibrin [42]. Another possibility could be a delay in the *in vivo* recanalization process that involves extravasation of emboli into the perivascular parenchyma, which slows with age [43]. Fibrin(ogen) deposits and clots in AD cerebral vessels could thus be rendered more persistent through their interaction with Aβ, which could initiate and/or aggravate brain hypoperfusion and inflammation.

Anticoagulant therapy as treatment for AD?

Studies of anticoagulant treatment in AD mice and patients provide another link between AD and the hemostatic abnormalities observed in this disease. Aβ accumulation and Aβ-induced cytotoxicity were reduced [44] and spatial memory was improved [45] after treatment of AD mice with enoxaparin, a low molecular weight heparin. In human studies, placebo-treated dementia patients showed a significant deterioration in cognition, while warfarin-treated patients did not show any change in cognitive decline [46]. In addition, unregulated studies carried out several decades ago showed that the majority of dementia patients treated with warfarin presented with improved cognition compared to untreated patients [47, 48]. Furthermore, an epidemiological study on atrial fibrillation patients showed that long-term warfarin treatment is protective against dementia [49]. These results suggest that repurposing existing anticoagulants for the treatment of AD may be beneficial. However, it is well known that anticoagulant treatment may increase the incidence of major systemic bleeding in elderly patients and that the AD brain is prone to hemorrhage due to the presence of CAA [32, 34, 36]. Recent advances in the development of effective anticoagulants with a lower risk of intracranial bleeding [50] are encouraging and may be safer for AD patients. Since it may be challenging to completely eliminate the risk of bleeding during anticoagulant use, identifying a drug that blocks the Aβ-fibrin(ogen) interaction, thereby preventing abnormal Aβ-related clot formation and degradation without affecting normal coagulation, may be an interesting alternative therapy for AD patients.

Relationship between AD and stroke

Based on our hypothesis, the Aβ-fibrin(ogen) interaction contributes to blood vessel occlusion, resulting in decreased cerebral blood flow and compromised neuronal viability (Fig. 1). This model implies the existence of a close relationship between AD physiopathology and stroke/microinfarcts. Clinical studies indicate that asymptomatic spontaneous cerebral emboli are highly correlated with AD [51] and a rapid cognitive decline [52]. Furthermore, microinfarcts are more common in AD patients compared with non-demented controls [53]. Another group has shown that cerebral infarcts significantly decrease the cognitive function of patients [54]. Thus, not only may stroke increase the risk of developing dementia [55], but AD patients also demonstrate a greater risk for stroke [56]. It is possible that the overlap between these two disorders could be explained by the increased formation and persistence of occlusive fibrin clots, resulting from the Aβ-fibrin(ogen) interaction and altered levels of prothrombotic and fibrinolytic factors (discussed below).

Prothrombotic state in AD

A number of studies suggest that AD patients may have an enhanced potential for thrombosis in the circulation. The coagulation cascade involves the sequential activation of a series of factors, leading to the production of thrombin which mediates the final step of the

coagulation cascade: the conversion of fibrinogen to fibrin. A prothrombotic state is induced when excessive quantities of thrombin are formed as a result of dysregulated hemostasis [57]. Elevated levels of activated factors VII and V (among others) contribute to a hypercoagulable state by promoting thrombin generation. Increased levels of activated factor VII were found in the plasma [58] and serum [59] of AD patients. Furthermore, carriers of factor V Leiden, a mutant form of factor V that is more resistant to inactivation and thus has increased thrombotic tendency, have an increased risk for dementia [60]. Elevated levels of von Willebrand Factor, which mediates the adhesion of platelets to wound sites and activates platelets [61], have been found in AD patient plasma [58] and serum [59]. Older adults with increased D-dimer and prothrombin fragment 1 + 2 levels, both markers of increased thrombin generation, are at higher risk for future cognitive decline [62], and prothrombin fragment 1 + 2 levels are increased in AD patient serum [59]. Increased thrombin generation in AD patients could result in unnecessary coagulation and could also contribute to the formation of abnormally structured fibrin clots that are resistant to degradation [63].

The AD brain parenchyma can also be characterized as a prothrombotic environment. Elevated levels of prothrombin [22] and thrombin [64, 65] have been found in microvessel walls and the surrounding neuropil in the AD brain. Thus, increased levels of fibrinogen extravasating into the AD vessel wall and brain parenchyma through a dysfunctional BBB would be converted to fibrin, which could be abnormally stable due to increased levels of thrombin found in AD. Fibrin stability may be further enhanced by the deficiency of fibrinolytic enzymes in the AD brain parenchyma. Decreased levels of plasmin [66], decreased tPA activity [67], and increased levels of plasminogen activation inhibitors [67, 68] have been detected in the brains of AD mice and patients. The elevated levels of Aβ found in the AD brain could also contribute to fibrin persistence, since Aβ intercalates into polymerizing fibrin and slows clot degradation [14, 42]. Thus, it is possible that Aβ contributes to the prothrombotic state in AD by binding to fibrinogen and altering fibrin clotting. It remains to be determined if Aβ affects other hemostatic factors, which may further contribute to the hypercoagulable state observed in this disorder.

Platelets in AD

Platelets are an essential part of the hemostatic response to injury, forming a platelet plug in the presence of fibrin in response to vascular damage. Platelets circulate in an inactive form, but can aggregate following activation by various signals. Increased levels of activated platelets have been found in AD patients [69, 70]. Furthermore, levels of coated platelets, a highly pro-coagulant subset of activated platelets [71], correlate with AD severity [72, 73]. Platelets of AD patients produce more Aβ compared to controls [74], which is released into the circulation upon platelet activation. Because platelets become activated at sites of injury and clot formation, AD patients' platelets might deliver Aβ directly to clots as they are forming. *In vitro* and *in vivo* experiments have demonstrated that fibrin clots are more difficult to degrade in the presence of Aβ [14, 42], suggesting a mechanism by which platelets releasing increased levels of Aβ in AD could contribute to enhanced thrombosis.

CROSS-TALK BETWEEN INFLAMMATION AND HEMOSTASIS IN AD

Many neurodegenerative diseases present a strong inflammatory component associated with their pathology [75]. The immune response in these pathologies is considered a double-edged sword; it is a way for the body to clear unwanted material, yet deleterious effects can occur as a result of a sustained and chronic inflammatory response. Since the cross-talk between coagulation and inflammation plays a fundamental role *in vivo* [76], we focus our discussion on the AD-related associations between hemostasis, the activation of the complement system, and microglial cells.

Relationship between the complement and coagulation cascades in AD

The complement system helps antibodies and phagocytic cells clear pathogens from the body and is thought to play an important role in the pathogenesis of AD [77, 78], since *in vitro* as well as *in situ* evidence shows that Aβ [79] and tau [80] are potent activators of the complement pathway. Complement proteins co-localize in the human AD brain with neurofibrillary tangles, plaques [77, 81], and dystrophic neurites [82]. There is bidirectional cross-talk between the complement and hemostatic systems. On one hand, since some complement proteins are substrates for several coagulation factors [83], increased levels of coagulation factors present in the AD brain and circulation could enhance the activation of the complement cascade. On the other hand, complement activation might lead to coagulation and fibrin deposition. *Ex vivo*

as well as *in vitro* data show how complement proteins influence fibrin clot characteristics [84] and induce a prothrombotic/anti-fibrinolytic state [85–87]. Therefore, after the initial activation of the complement cascade by Aβ and tau, complement proteins could affect fibrin clot formation and induce a prothrombotic state in the AD brain. These mechanisms could be mutually reinforcing, and the bidirectional cross-talk between coagulation and the complement system could synergistically exacerbate AD pathology.

Fibrin(ogen) and the inflammatory response in AD

Microglial cells are the resident macrophages of the brain that become activated after an insult and release cytokines and free radicals. The presence of activated microglia progressively increases with AD pathogenesis [16]. They are found in and around Aβ plaques [19, 77], releasing a variety of cytokines and chemokines [88]. Although microglial cells are clearly involved in AD, their exact contribution to neuroprotection versus neurodegeneration is not clear. The activation of microglia might have a beneficial effect aiding in the clearance of plaques. However, as the disease progresses, the clearance system may be overwhelmed as microglia become dysfunctional and detrimental through the continuous release of inflammatory molecules [88].

In addition to its well-known function in hemostasis, fibrinogen also acts as a pro-inflammatory molecule. Fibrin(ogen) binds the integrin $\alpha_M\beta 2$ present in activated leukocytes, which is essential for the inflammatory response [89]. This role of fibrinogen is crucial in diseases such as atherosclerosis and rheumatoid arthritis, but it has also been implicated in the inflammatory pathology related to neurological diseases, such as stroke, spinal cord injury, and multiple sclerosis [90].

Several pieces of evidence demonstrate a tight relationship between fibrinogen and inflammation in AD. A close association between fibrin(ogen) deposits and microglial clusters has been observed in the human AD brain [16, 19]. Moreover, results from our laboratory show that modulating fibrinogen levels in an AD mouse model dramatically influences not only the inflammatory response associated with this disorder, but also the neurovascular damage and BBB dysfunction [18, 91]. Ryu and colleagues demonstrated that decreasing fibrinogen levels prevents microglial activation and neuronal loss after hippocampal injection of Aβ in rats. They also showed that fibrinogen injection alone into the hippocampus induces microgliosis, BBB permeability, and neuronal death, which were all exacerbated when Aβ was co-injected with fibrinogen [19].

In AD, fibrin(ogen) is found deposited in the cerebral vessels and brain parenchyma, which may contribute to and promote neuroinflammation. Moreover, this inflammatory response in the brain is sustained, since fibrin(ogen) is not efficiently cleared from the brain when bound to Aβ and because hemostasis is altered in AD. This persistent fibrin(ogen) would therefore ultimately exacerbate an already active inflammatory response. It remains to be determined whether increased fibrinogen extravasation and deposition in the AD brain contribute to the neuronal damage associated with AD, or whether fibrinogen deposition is affected by genetic risk factors such as ApoE isoform. Another interesting question is whether blocking the Aβ-fibrinogen interaction would affect the various pathologies observed in AD.

CONCLUSION

We provide evidence suggesting that fibrin(ogen) and altered hemostasis play a fundamental role in AD physiopathology. In our model, which is summarized in the figure, the Aβ-fibrin(ogen) interaction promotes the deposition of both fibrin(ogen) and Aβ in the AD brain parenchyma and vessels and induces the formation of abnormal fibrin clots that are more resistant to degradation. Altered hemostasis further contributes to a prothrombotic environment in the AD brain and circulation, leading to persistent fibrin(ogen) deposits that promote neuroinflammation, blood vessel occlusion, and hypoperfusion. These events could eventually result in the vascular damage, synaptic dysfunction, neuronal death, and cognitive impairment observed in AD. Several lines of evidence support our hypothesis that fibrin(ogen) plays a critical role in AD pathogenesis: 1) High levels of fibrinogen in plasma increase the risk for dementia in humans; 2) pharmacological or genetic depletion of fibrinogen in AD mouse models lessens BBB dysfunction, neurovascular damage, neuroinflammation, and CAA pathology and improves cognitive function; 3) anticoagulant treatment in patients and AD mice showed improvement in memory or a slower cognitive decline compared to controls; 4) there is an association between stroke/microinfarcts and AD; and 5) fibrinogen in cerebrospinal fluid has been identified as a useful biomarker to distinguish between AD

and cognitively-normal or mildly demented patients [92, 93] and correlates with disease progression [94]. Given this evidence, it may be possible to use the presence of the Aβ-fibrinogen complex in plasma or cerebrospinal fluid as a biomarker for AD. Additionally, an inhibitor of the Aβ-fibrin(ogen) interaction might prove successful in slowing or reversing vascular deficits that contribute to AD pathogenesis. One of the advantages of this specific approach is that it might prevent the abnormal clotting provoked by Aβ without affecting physiological coagulation. Since AD is a multifactorial disease, we believe this approach could be used in combination with other strategies to treat the various pathologies associated with this disorder.

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