Coagulation factor XIIIa cross-links amyloid β into dimers and oligomers and to blood proteins

Received for publication, August 23, 2018, and in revised form, October 25, 2018 Published, Papers in Press, November 8, 2018, DOI 10.1074/jbc.RA118.005352

Woosuk S. Hur†§1, Nima Mazinani†§1, X. J. David Lu†§, Leesor S. Yefet‡, James R. Byrnes§, Laura Ho†, Ju Hun Yeon†, Sam Filipenko‡, Alisa S. Wolberg§, Wilfred A. Jefferies‡§2, and Christian J. Kastrup‡§1

From the †Michael Smith Laboratories, and Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4, ‡Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4, §Departments of Microbiology & Immunology, Medical Genetics, Zoology, and Urology, the Djavad Mowafaghian Centre for Brain Health, the Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4, and ¶Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

Edited by Paul E. Fraser

In cerebral amyloid angiopathy (CAA) and Alzheimer’s disease (AD), the amyloid β (Aβ) peptide deposits along the vascular lumen, leading to degeneration and dysfunction of surrounding tissues. Activated coagulation factor XIIIa (FXIIIa) covalently cross-links proteins in blood and vasculature, such as in blood clots and on the extracellular matrix. Although FXIIIa co-localizes with Aβ in CAA, the ability of FXIIIa to cross-link Aβ has not been demonstrated. Using Western blotting, kinetic assays, and microfluidic analyses, we show that FXIIIa covalently cross-links Aβ40 into dimers and oligomers (kcat/Km = 1.5 × 10^3 M⁻¹ s⁻¹), as well as to fibrin, platelet proteins, and blood clots under flow in vitro. Aβ40 also increased the stiffness of platelet-rich plasma clots in the presence of FXIIIa. These results suggest that FXIIIa-mediated cross-linking may contribute to the formation of Aβ deposits in CAA and Alzheimer’s disease.

Amyloid β (Aβ) is a 4 kDa intrinsically disordered protein that accumulates along the cerebral vasculature during cerebral amyloid angiopathy (CAA). The accumulation of Aβ leads to the degeneration of surrounding cells and is associated with microhemorrhages (1). Although CAA is present in over 90% of patients with Alzheimer’s disease (AD) (2), the mechanisms underlying Aβ deposition on blood vessels remains unclear.

There are many links between hemostasis and cerebrovascular pathology in AD. Aβ can activate platelets, induce microhemorrhages in the brain, and interact with several coagulation factors (3–6). Aggregates of Aβ can activate coagulation factor XII (FXII) to initiate blood clotting, and can increase fibrin density and resistance to fibrinolysis (5, 7). CAA deposits contain several coagulation factors, and antiplatelet therapy reduces accumulation of CAA deposits and improves cognitive function in mice (8). Currently, the biochemical mechanisms that connect intravascular CAA deposition and hemostasis are not clear.

Aβ is formed from the amyloid β precursor protein (APP), which is expressed by several cells, including platelets, neurons, glial cells, and astrocytes. Platelets account for 95% of circulating APP (9). APP can be cleaved to generate Aβ peptides typically 40 or 42 residues long, Aβ40 and Aβ42, respectively. Platelets cleave APP and release both Aβ40 and Aβ42 when they are activated (10). In both blood and CAA deposits, Aβ40 is more abundant than Aβ42, whereas Aβ42 is more abundant in senile plaques within the brain parenchyma, which is a hallmark of AD (11). Mutations within the Aβ sequence can alter the pathogenicity of the peptide; for example, patients with the Flemish or Italian mutation (A21G and E22K, respectively) have increased CAA deposits, whereas patients with the Arctic mutation (E22G) have more plaque burden (12–16). Aβ40 and Aβ42 can spontaneously aggregate into small, noncovalent oligomers and subsequently large aggregates, both of which are toxic to surrounding cells (17).

The formation of protein aggregates in CAA and AD may be regulated in part by transglutaminases (TGs). TGs are a family of enzymes that form e-(γ-glutamyl) lysyl isopeptide bonds between their substrates, creating irreversible bonds. TG activity colocalizes with plaques in brains in AD (6). Tissue transglutaminase 2 (TG2) can induce Aβ oligomerization and aggregation in vitro and reduce its clearance (18). However, it is unknown if activated coagulation factor XIIIa (FXIIIa), a transglutaminase in blood plasma and on platelets, can cross-link Aβ in blood. FXIIIa is formed from coagulation factor XIII (FXIII), a protransglutaminase, when it is activated by thrombin in the presence of calcium during blood clotting (19). The primary function of FXIIIa is cross-linking fibrin to itself and to other...
Factor XIIIa covalently cross-links amyloid β

A small molecule substrate of FXIIIa, glycine ethyl ester (GOE), had a similar catalytic efficiency of 2.0 ± 0.7 × 10^4 M^−1 s^−1 (Fig. 1C). The catalytic efficiency of a peptide with the Aβ40 residues scrambled had 10-fold lower catalytic efficiency, indicating that the sequence of Aβ40 is important for FXIIIa activity. We were not able to calculate the catalytic efficiency of Aβ42 because Aβ42 precipitated at the concentrations necessary to perform the assay.

FXIIIa covalently cross-linked Aβ40 to fibrin

Aβ can bind to many proteins in blood, such as FXII, FXIII, and fibrinogen (4, 5, 7). To test if Aβ40 could be covalently cross-linked to other blood proteins, plasma containing Aβ40 was clotted, separated by SDS-PAGE, and immunoblotted against Aβ. Within 10 min, distinct Aβ bands were visible around 50 kDa, 70 kDa, and 100 kDa, and much higher molecular mass (Fig. 2A). The molecular mass of these bands were similar to those of the α and γ chains of fibrin, the main substrates of FXIIIa. Bands with similar molecular mass as fibrin were visible after the γ-γ dimers were formed (Fig. 2C and D). Aβ was still cross-linked when an inhibitor of TG2 (Z006) (20) was added to plasma, but not when T101 was added, indicating that FXIIIa, not TG2, is responsible for cross-linking Aβ40 to fibrin. Aβ40 was incubated with purified fibrinogen, FXIIIa, and thrombin. Bands of Aβ were visible around 50 kDa, 70 kDa, and 100 kDa, correlating to the molecular mass of the α and γ chains of fibrin and cross-linked γ-γ dimers (Fig. 3C and D). Aβ40 was cross-linked to fibrin chains faster than to itself. Aβ40 was not cross-linked to fibrin chains when FXIIIa was inhibited with T101. Lower concentrations of Aβ40 (1 μM) also cross-linked to both purified and plasmatic fibrin by FXIIIa (Fig. S2).

FXIIIa cross-linked Aβ40 to platelet proteins under flow

Because platelets contain the FXIII-A subunit, which can be activated by high concentrations of Ca^{2+}, Aβ40 was incubated with platelets to test if platelet-derived FXIIIa could cross-link Aβ40 to itself or to other proteins. When platelets were activated with ADP, collagen, or thrombin, different patterns of Aβ cross-linking were detected compared with when platelets were not activated (Fig. 3A). The Aβ bands formed with platelets had higher molecular mass than Aβ dimers and trimers, suggesting Aβ was cross-linked to platelet proteins. Both EDTA, which chelates the Ca^{2+} required for FXIIIa activity and platelet activation, and T101 prevented Aβ oligomers from forming. In contrast, Z006 did not prevent Aβ oligomers from forming, indicating that FXIIIa, not TG2, is responsible for cross-linking Aβ in platelets.

To test whether FXIIIa cross-links Aβ40 to blood clots formed under flow, plasma containing platelets and fluorescently tagged Aβ40 were flowed through a microfluidic device.

---

**Table 1.** Table of kinetic parameters for FXIIIa cross-linking Aβ40 to itself or to other proteins. N.D., not determined. n = 3.

| Substrate   | $K_m$ (μM) | $k_{cat}$ (s^−1) | $k_{cat}/K_m$ (M^−1 s^−1) |
|-------------|------------|------------------|--------------------------|
| GOE         | 4.3 ± 2.4  | 0.8 ± 0.2        | 2.0 ± 0.7 × 10^3         |
| Aβ40        | 8.5 ± 1.2  | 1.3 ± 0.5        | 1.0 ± 0.5 × 10^4         |
| Aβ40 Scrambled | 10.9 ± 0.3 | 0.23 ± 0.1 | 2.1 ± 0.1 × 10^4        |
| Aβ42        | N.D.       | N.D.             | N.D.                     |

---

**Results**

**Aβ40 is a substrate of FXIIIa**

To test if Aβ could be covalently cross-linked by FXIIIa, Aβ was incubated with FXIIIa and changes in molecular mass were detected using Western blotting. FXIIIa cross-linked monomeric Aβ40 into dimers and oligomers, and to FXIIIa itself (Fig. 1A). Cross-linked Aβ oligomers did not form when FXIIIa was inhibited by chelating calcium ions with EDTA or with T101, an irreversible inhibitor of FXIIIa transglutaminase activity. However, with T101, there was a distinct band near 85 kDa, corresponding to the molecular mass of FXIIIa attached to Aβ40, which has been reported previously (6). Similar trends were observed with Aβ42, although low concentrations of SDS-resistant oligomers formed without FXIIIa. When the only glutamine of Aβ40 was mutated to asparagine (Aβ40 Q15N), FXIIIa did not generate Aβ oligomers, indicating that the oligomerization depended on the glutamine residue of Aβ40.

To determine the kinetic constants of FXIIIa-mediated Aβ cross-linking, the release of ammonia, a product of the transglutaminase reaction (Fig. 1B), was measured using a photometric assay. FXIIIa-mediated cross-linking of Aβ40 had $K_m$ of 8.5 ± 1.2 μM and $k_{cat}$ of 1.3 ± 0.5 s^−1, resulting in a catalytic efficiency of 1.5 ± 0.5 × 10^5 M^−1 s^−1. A small molecule substrate of FXIIIa, glycine ethyl ester (GOE), had a similar catalytic efficiency of 2.0 ± 0.7 × 10^4 M^−1 s^−1 (Fig. 1C). The catalytic efficiency of a peptide with the Aβ40 residues scrambled had 10-fold lower catalytic efficiency, indicating that the sequence of Aβ40 is important for FXIIIa activity. We were not able to calculate the catalytic efficiency of Aβ42 because Aβ42 precipitated at the concentrations necessary to perform the assay.
Aβ40 accumulated on the clot, and colocalized directly on platelet aggregates and fibrin fibers. The co-localization of Aβ40 with platelets, measured by the ratio of Aβ40 fluorescence to platelet fluorescence, was significantly decreased when T101 was added, indicating that FXIIIa can cross-link Aβ40 to blood clots under flow (Fig. 3, B and C).

**Aβ40 increases clot stiffness of PRP and PPP via FXIIIa**

Because cross-linking increases fibrin stiffness (19), we tested the effect of Aβ40 on fibrin clot stiffness using thromboelastography (TEG). When whole blood was clotted in the presence of Aβ40, no significant difference in clot stiffness was observed (Fig. 4A). Because the contribution of red blood cells may have masked subtle differences of Aβ on clot stiffness, we tested if the influence of Aβ40 on fibrin could be detected when red blood cells were removed (21). Aβ40 increased the stiffness of clots formed in platelet-rich plasma (PRP) by 27 and 39%, respectively (Fig. 4, B and C). The increase in clot stiffness depended on both cross-linking by FXIIIa and platelet-platelet interactions, since inhibitors of FXIIIa (T101) or integrin IIb/IIIa (eptifibatide) abrogated the increase of clot stiffness induced by Aβ40.

**Aβ40 mutants are differentially cross-linked by FXIIIa**

Certain point mutations of Aβ increase the probability of developing CAA (12–16). To test whether FXIIIa cross-links...
The results show that Aβ40 is covalently cross-linked by FXIIIa, both to itself and to fibrin and platelet proteins by FXIIIa under flow. Although the reaction occurs in vitro, the physiological relevance and significance of these reactions in vivo must be further investigated.

The cross-linking of Aβ40 to fibrin chains was visible only after the γ-γ dimers were formed. This is consistent with the kinetic data, where the catalytic efficiency of FXIIIa and Aβ ($k_{cat}/K_m = 1.5 \pm 0.5 \times 10^5 \text{M}^{-1}\text{s}^{-1}$) was lower than that of fibrin γ-chains ($5.1 \times 10^7 \text{M}^{-1}\text{s}^{-1}$) (22). Aβ was cross-linked to fibrin at an Aβ concentration of 1 μM, which is a concentration that may occur at sites of cerebrovascular damage (23).

The cross-linking of Aβ may potentially be influenced by Aβ–albumin interactions. Albumin sequesters ~90% of Aβ in plasma and preferentially binds oligomeric Aβ to monomeric Aβ (24, 25). FXIIIa cross-linked Aβ to fibrin both in buffer and in plasma at similar rates, suggesting that albumin does not play a significant role in influencing the rate of cross-linking in these conditions. However, how albumin affects the clearance of cross-linked oligomeric Aβ requires further examination.

Aβ and FXIIIa can form stable complexes in vitro, and FXIIIa is catalytically active in vessels with CAA (6). Although isopeptide bonds formed by transglutaminases have been discovered in CAA, covalent cross-linking of Aβ by FXIIIa was not detected ex vivo previously (6). The discrepancy with the data here may be because of the higher, although physiological, FXIIIa concentrations and longer reaction times used, and potentially higher specific activity of FXIIIa. Although the cross-linking of Aβ to itself was visible only after 3 h, cross-linking to fibrin occurred within minutes.

Cross-linking of Aβ may have implications in at least two scenarios. First, Aβ may modify clot structure at sites of damage in the cerebral vasculature or at platelet aggregates. It remains to be determined what the effect on clotting might be, but it may contribute to fibrinolysis because non–cross-linked aggregates of Aβ increase resistance to fibrinolysis and activate the coagulation cascade through FXII (5, 26). Cross-linking Aβ to fibrin could enhance clotting by localizing the platelet activating sequence (Aβ25–Aβ35) to fibrin (3). Cross-linking of Aβ may have a greater significance in arteries than veins, as arterial clots have fewer red blood cells, because Aβ increased the stiffness of PRP, but not whole blood clots.

Second, FXIIIa-mediated activity may contribute to CAA and AD pathology. Given that blocking the binding between Aβ and fibrin with a small molecule can improve cognitive impairment in mouse models of AD, covalent cross-linking of Aβ and fibrin may exacerbate CAA pathology (26). In patients with AD, there is a higher frequency of a FXIII allele (V34L) that underlies the more prominent form of Aβ within CAA. AD patients with the Flemish mutation (Aβ40 A21G) have increased CAA phenotype, and Aβ40 with the Flemish mutation was cross-linked to a higher extent compared with WT Aβ40 (17). An alternative hypothesis is that cross-linking of Aβ by FXIIIa is a physiological process that is separate from aggregation and amyloid accumulation.

In conclusion, synthetic Aβ40 can be covalently cross-linked to itself, and to fibrin and platelet proteins by FXIIIa under flow. Given that Aβ and FXIIIa colocalize within CAA, these results...
provide motivation to test if FXIIIa contributes to the accumulation of intravascular deposits of Aβ in CAA.

**Experimental procedures**

**Platelet preparation**

This study was approved by the Research Ethics Board of the University of British Columbia (H12–01516), and written informed consent was obtained from all healthy volunteers in accordance with the Declaration of Helsinki. Platelets and PRP were isolated as described previously (28). Platelets were resuspended in Tyrode’s buffer (119 mM NaCl, 5 mM KCl, 25 mM HEPES, 2 mM CaCl₂, 2 mM MgCl₂, 6 g/liter glucose, pH 6.5) or in plasma at a final concentration of 2 × 10^9 platelets/ml or as otherwise specified.

**Cross-linking of Aβ**

Aβ peptides (AnaSpec, Fremont, CA) were initially dissolved in dimethyl sulfoxide at 20 mg/ml and diluted with 25 mM HEPES buffer to 1 mg/ml. To test if Aβ is cross-linked by purified FXIIIa, Aβ (25 μM) was incubated with purified FXIIIa (200 nM, Haematologic Technologies, Essex Junction, VT), CaCl₂ (4 mM), DTT (200 μM), human thrombin (70 nM, Haematologic...
pared and devices were coated as described previously (device was coated with inert PC vesicles. The vesicles were pre-
dyserine (PS) and phosphatidylcholine (PC); the rest of the coated with lipid vesicles containing tissue factor, phosphati-
c on 37 °C. The samples were treated with a reaction-quenching buffer (8 m urea, 50 mM DTT, 12.5 EDTA) for at least 1 h at 60 °C to dissolve the clot. To test if Aβ was cross-linked to platelet proteins, platelets were incubated with Aβ40 (25 μM), CaCl$_2$ (4 mM), and human thrombin (70 nM), ADP (50 μM), rat tail collagen (10 nM, Sigma), EDTA (12.5 mM), or T101 (2.5 mM).

**Western blotting**

Samples were reduced, boiled, and separated on 10% or 4–15% Tris-glycine gradient gels (Bio-Rad). After electrophoresis, the samples were transferred to a nitrocellulose membrane (GE Healthcare) and blocked with Odyssey Blocking Buffer (LI-COR, Lincoln, NE). The membranes were treated with a primary antibody against Aβ (1:1000; 6E10, Covance, Princeton, NJ), FXIII-A (1:1000; SAF13A-AP, Affinity Bionicals, Ancaster, Ontario, Canada), or fibrin (1:50,000; A0080, Dako, Santa Clara, CA), washed, and treated with HRP-labeled anti-host secondary antibody (1:15,000; Abcam, Cambridge, MA).

**Kinetics assay**

The rate of cross-linking of Aβ was determined by measuring the rate at which ammonia was produced during the TG reaction, using steady-state kinetics at 37 °C. A calibration curve of ammonia concentration and absorbance at 570 nm was determined with a Tecan M200 plate reader (BioVision Inc., Milpitas, CA). FXIIIa was mixed with Aβ/H9252 and Aβ/H9262 at a rate of 0.33 ml/min, which corresponds to venous shear rates (20 s$^{-1}$). Clotting was monitored using an epifluorescence microscope (Leica DMI6000B). The clots were then washed with calcium-saline solution (40 mM CaCl$_2$, 90 mM NaCl) at a rate of 5 μl/min for 10 min and imaged. For statistical analysis, fluorescence intensities were measured at five equally distributed locations along the length of the channel.

**Thromboelastography**

The shear elastic moduli were evaluated at 37 °C using a TEG Hemostasis Analyzer System 5000 (Haemoscope Corp., Niles, IL). Citrated whole blood, PRP, or PPP (Affinity Bionicals) was combined with CaCl$_2$ (10 mM) and thrombin (200 nM), with or without Aβ40 (15 μM), T101 (800 μM), or eptifibatide (1.4 mM, Sigma) over 3 h.

**Statistical evaluation**

Statistical analyses were performed using GraphPad Prism 7.0. All results presented in graphs are the mean ± S.E. N indicates number of independent experiments, performed on separate days. A two-tailed unpaired Student’s t test was used for all analyses. Significance was designated at p values <0.05.

**Acknowledgments**—We thank the University of British Columbia Centre for Blood Research for support with blood collection. We also thank Dr. Cheryl Pfeifer for advice.

**References**

1. Alonzo, N. C., Hyman, B. T., Rebeck, G. W., and Greenberg, S. M. (1998) Progression of cerebral amyloid angiopathy: Accumulation of amyloid-beta 40 in affected vessels. *J. Neuropathol. Exp. Neurol.* 57, 353–359
2. Love, S., Miners, S., Palmer, J., Chalmers, K., and Kehoe, P. (2009) Insights into the pathogenesis and pathogenicity of cerebral amyloid angiopathy. *Front. Biosci.* 14, 4778–4792
3. Sonkar, V. K., Kulkarni, P. P., and Dash, D. (2014) Amyloid beta peptide stimulates platelet activation through RhoA-dependent modulation of actomyosin organization. *FASEB J.* 28, 1819–1829
4. Ahn, H. J., Zamolodchikov, D., Cortes-Cantelli, M., Norris, E. H., Glickman, J. F., and Strickland, S. (2010) Alzheimer’s disease peptide beta-amyloid interacts with fibrinogen and induces its oligomerization. *Proc. Natl. Acad. Sci. USA.* 107, 21812–21817
5. Zamolodchikov, D., Renne, T., and Strickland, S. (2016) The Alzheimer’s disease peptide beta-amyloid promotes thrombin generation through activation of coagulation factor XII. *J. Thromb. Haemost.* 14, 995–1007
6. de Jager, M., Boot, M. V., Bol, J. G., Brevé, J. J. P., Jongenelen, C. A. M., Drukarch, B., and Wilhelmus, M. M. M. (2016) The blood clotting Factor XIIIa forms unique complexes with amyloid-beta (A) and colocalizes with deposited A in cerebral amyloid angiopathy. *Neuropathol. Appl. Neurobiol.* 42, 255–272
7. Zamolodchikov, D., and Strickland, S. (2012) A beta delays fibrin clot lysis by altering fibrin structure and attenuating plasminogen binding to fibrin. *Blood* 119, 3342–3351

**Author contributions**—W. S. H., N. M., L. H., J. H. Y., S. F., W. A. J., and C. J. K. conceptualization; W. S. H., N. M., X. J. D. L., L. S. Y., J. R. B., J. H. Y., A. S. W., and W. A. J. data curation; W. S. H., N. M., X. J. D. L., L. S. Y., J. R. B., A. S. W., W. A. J., and C. J. K. formal analysis; W. S. H., N. M., X. J. D. L., L. S. Y., J. R. B., and J. H. Y. investigation; W. S. H., N. M., and C. J. K. methodology; W. S. H., N. M., A. S. W., W. A. J., and C. J. K. writing-original draft; W. S. H., N. M., X. J. D. L., L. S. Y., J. R. B., L. H., J. H. Y., S. F., A. S. W., W. A. J., and C. J. K. writing-review and editing; A. S. W., W. A. J., and C. J. K. funding acquisition; C. J. K. resources; C. J. K. supervision; C. J. K. project administration.
Factor XIIIa covalently cross-links amyloid β

8. Donner, L., Falk, E., Gremer, L., Klinker, S., Pagani, G., Ljungberg, L. U., Lothmann, K., Bizzi, F., Schaller, M., Gohlke, H., Willbold, D., Grenegard, M., and, Elvers, M. (2016) Platelets contribute to amyloid β-aggregation in cerebral vessels through integrin αIIbβ3-induced outside-in signaling and clusterin release. Sci. Signal. 9, ra52 CrossRef Medline

9. Li, Q. X., Berndt, M. C., Bush, A. I., Rumble, B., Mackenzie, I., Friedhuber, A., Beyreuther, K., and Masters, C. L. (1994) Membrane-associated forms of the βA4 amyloid protein-precursor of Alzheimer’s disease in human platelet and brain-surface expression on the activated human platelet. Blood 84, 133–142 Medline

10. Canobbio, I., Abubaker, A. A., Visconte, C., Torti, M., and Pula, G. (2015) Role of amyloid peptides in vascular dysfunction and platelet dysregulation in Alzheimer’s disease. Front. Cell. Neurosci. 9, 65 CrossRef Medline

11. Murphy, M. P., and LeVine, H. (2010) Alzheimer’s disease and the amyloid-beta peptide. J. Alzheimer’s Dis. 19, 311–323 CrossRef Medline

12. Nilsberth, C., Westlind-Danielsson, A., Eckman, C. B., Condron, M. M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D. B., Yonkin, S. G., Nislund, J., and Lannfelt, L. (2001) The ‘Arctic’ APP mutation (E693G) causes Alzheimer’s disease by enhanced Aβ protofibril formation. Nat. Neurosci. 4, 887–893 CrossRef Medline

13. Bagiani, O., Giaccone, G., Rossi, G., Mangieri, M., Capobianco, R., Morbin, M., Mazzoleni, G., Cupidi, C., Marcon, G., Giovagnoli, A., Bizzi, A., Di Fede, G., Puoti, G., Carella, F., Salmaggi, A., Romorini, A., Patruno, G. M., Magoni, M., Padovani, A., and Tagliavini, F. (2010) Hereditary cerebral hemorrhage with amyloidosis associated with the E693K mutation of APP. Arch. Neurol. 67, 987–995 CrossRef Medline

14. Fernandez-Madrid, I., Levy, E., Marder, K., and Frangione, B. (1991) Codon-618 variant of Alzheimer amyloid gene associated with inherited cerebral-hemorrhage. Ann. Neurol. 30, 730–733 CrossRef Medline

15. Grabowski, T., Cho, H. S., Vonsattel, J. P. G., Rebeck, G. W., and Greenberg, S. M. (2001) Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. Ann. Neurol. 49, 697–705 CrossRef Medline

16. Hendriks, L., van Duijn, C. M., Cras, P., Cruts, M., Van Hul, W., van Harsskamp, F., Warren, A., McInnis, M. G., Antonarakis, S. E., Martin, J.-J., Hofman, A., and Van Broeckhoven, C. (1992) Presenile-dementia and cerebral-hemorrhage linked to a mutation at codon-692 of the β-amyloid precursor protein gene. Nat. Genet. 1, 218–221 CrossRef Medline

17. Benilova, I., Karran, E., and De Strooper, B. (2012) The toxic Aβ oligomer and Alzheimer’s disease: An emperor in need of clothes. Nat. Neurosci. 15, 349–357 CrossRef Medline

18. Hartley, D. M., Zhao, C. H., Speier, A. C., Woodard, G. A., Li, S. M., Li, Z. L., and Walz, T. (2008) Transglutaminase induces protofibril-like amyloid β-protein assemblies that are protease-resistant and inhibit long-term potentiation. J. Biol. Chem. 283, 16790–16800 CrossRef Medline

19. Muszbek, L., Bereczky, Z., Bagoly, Z., Komáromi, I., and Katona, E. (2011) Factor XIII: A coagulation factor with multiple plasmatic and cellular functions. Physiol. Rev. 91, 931–972 CrossRef Medline

20. Dale, G. L., Friese, P., Batar, P., Hamilton, S. F., Reed, G. L., Jackson, K. W., Clemenson, K. J., and Alberio, L. (2002) Stimulated platelets use serotonin to enhance their retrieval of procoagulant proteins on the cell surface. Nature 415, 175–179 CrossRef Medline

21. Bochsen, L., Johansson, P. I., Kristensen, A. T., Daugaard, G., and Ostrowski, S. R. (2011) The influence of platelets, plasma and red blood cells on functional haemostatic assays. Blood Coagul. Fibrinolysis 22, 167–175 CrossRef Medline

22. Lewis, K. B., Teller, D. C., Fry, J., Lasser, G. W., and Bishop, P. D. (1997) Crosslinking kinetics of the human transglutaminase, factor XIII A(2), acting on fibrin gels and gamma-chain peptides. Biochemistry 36, 995–1002 CrossRef Medline

23. Kucheryavyykh, L. Y., Dávila-Rodríguez, J., Rivera-Aponte, D. E., Zueva, L. V., Washington, A. V., Sanabria, P., and Inyushin, M. Y. (2017) Platelets are responsible for the accumulation of β-amyloid in blood clots inside and around blood vessels in mouse brain after thrombosis. Brain Res. Bull. 128, 98–105 CrossRef Medline

24. Bier, A. L., Ostaszewski, B., Stimson, E. R., Hyman, B. T., Maggio, J. E., and Selkoe, D. J. (1996) Amyloid β-peptide is transported on lipoproteins and albumin in human plasma. J. Biol. Chem. 271, 32916–32922 CrossRef Medline

25. Milojevic, J., Costa, M., Ortiz, A. M., Jorquera, J. I., and Melacini, G. (2014) In vitro amyloid-β binding and inhibition of amyloid-β self-association by therapeutic albumin. J. Alzheimer’s Dis. 38, 753–765 CrossRef Medline

26. Ahn, H. J., Glickman, J. F., Poone, K. L., Zamolodchikov, D., Jno-Charles, O. C., Norris, E. H., and Strickland, S. (2014) A novel α fibrinogen interaction inhibitor rescues altered thrombosis and cognitive decline in Alzheimer’s disease mice. J. Exp. Med. 211, 1049–1062 CrossRef Medline

27. Gerardino, L., Papaleo, P., Flex, A., Gaetani, E., Fioroni, G., Pola, G., and Pola, R. (2006) Coagulation factor XIII Val34Leu gene polymorphism and Alzheimer’s disease. Neuro. Res. 28, 807–809 CrossRef Medline

28. Hur, W. S., Mazini, N., Lu, X. J. D., Britton, H. M., Byrnes, J. R., Wolberg, A. S., and Kastrup, C. J. (2015) Coagulation factor XIIIa is inactivated by plasmin. Blood 126, 2329–2337 CrossRef Medline

29. Whitesides, G. M. (2006) The origins and the future of microfluidics. Nature 442, 368–373 CrossRef Medline

30. Yeon, J. H., Mazinani, N., Schlappi, T. S., Chan, K. Y. T., Baylis, J. R., Smith, S. A., Donovan, A. J., Kudela, D., Stucky, G. D., Liu, Y., Morrissey, J. H., and Kastrup, C. J. (2017) Localization of short-chain polyphosphate enhances its ability to clot flowing blood plasma. Sci. Rep. 7, 42119 CrossRef Medline