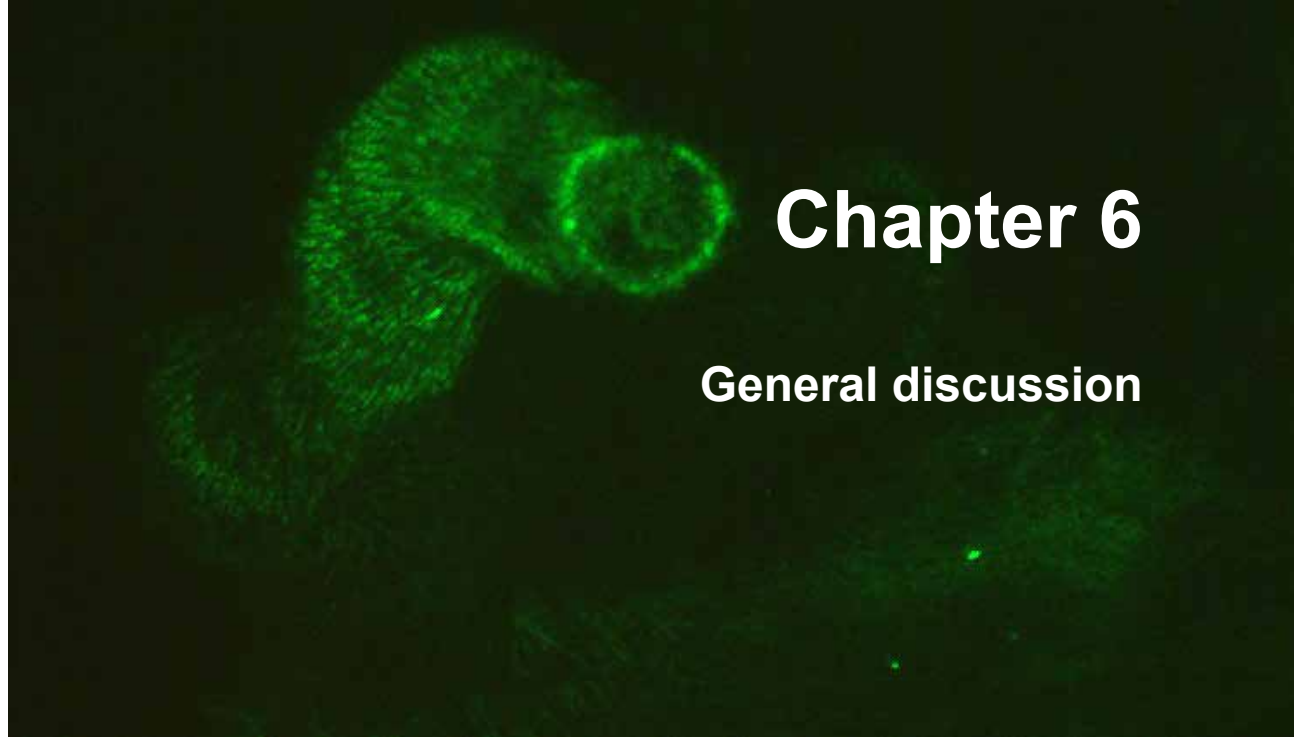

Chapter 6

General discussion

CAA is characterised by progressive disruption of the vessel wall leading to vessel dysfunction and even haemorrhages [1], and its presence is associated with a more rapid cognitive decline in AD patients [2]. Therefore, it is crucial to gain more insight in CAA pathogenesis and identify the key factors that underlie CAA pathology. Previous research of our group provided evidence that tTG is associated with CAA pathogenesis [3], however its exact role in CAA remained unclear.

Results and conclusions of this thesis

The aim of this thesis was to gain more insight into the role of tTG in CAA. As we set out to investigate the distribution of tTG and its activity in CAA, we also not only identified the presence blood-derived transglutaminase FXIIIa in CAA but additionally demonstrated a unique interaction of FXIIIa with Aβ. Furthermore, we demonstrated a tTG-mediated post-translational of ApoE that affected ApoE's bioactivity. Based on our finding on the role of tTG in CAA, we set out to find a suitable animal model to study tTG in CAA. The results of these studies will be summarised and briefly discussed in the first part of this chapter. In the last part of this chapter, the interaction of FXIIIa with Aβ (Chapter 3) and tTG's interaction with ApoE (Chapter 4) will be discussed in more detail because of the novelty of these findings.

To gain more insight in the distribution of tTG in CAA of both AD and HCHWA-D patients, we observed in Chapter 2 that in early forms of CAA, tTG colocalised with the Aβ deposition. In contrast, in end-stage CAA, tTG and cross-links did not colocalise with Aβ but were present in two halos surrounding the Aβ deposition, colocalising with fibronectin and laminin, two major ECM proteins known to be excellent tTG substrates [4]. These results suggest that the role of tTG in early and late stage CAA differs and may change from Aβ cross-linking with subsequent Aβ deposition in early phases to ECM modulation via cross-linking in later stages of CAA. In the latter, tTG-catalysed ECM cross-linking changes the vessel wall structure, leading to alterations in vessel wall remodelling [5]. Previous in vitro studies showed that Aβ is a substrate for tTG-catalysed cross-linking resulting in Aβ dimers, trimers and oligomers [6–10], and as Aβ can upregulate extracellular tTG levels (Chapter 4), this suggests that tTG may catalyse Aβ cross-linking and thereby drive Aβ aggregation in the vessel wall. However, we did not observe cross-link staining in the Aβ deposition itself (Chapter 2) in contrast to Aβ plaques where cross-link staining was found [3]. These data suggest that in CAA, at least in stages of CAA in which extensive Aβ accumulation and vessel wall degeneration is observed, tTG-catalysed cross-linked Aβ is absent or not detectable anymore. Interestingly, the difference between SPs and CAA regarding the cross-link staining suggests that the pathogenesis of SPs and CAA is different with respect to tTG cross-linking. Of importance in this are differences between SPs and CAA related to the Aβ type ($Aβ_{1-42}$ versus $Aβ_{1-40}$ respectively), and presence or absence of post-translational modified Aβ and Aβ-chaperones [11–13]. Together, this suggests that the type of proteins accumulating in the lesions, their post-translational status

and their levels differ between SPs and CAA, and that these differences may determine tTG's cross-linking activity in these AD lesions. As an example for this is the absence of cross-linked protein in late stage CAA, although we cannot rule out that the number of cross-linked Aβ species may simply be too low to detect with immunohistochemistry since the 81D4 antibody is known to detect only high levels of tTG-catalysed cross-linked proteins. Thus, although the data in this thesis demonstrate the presence of tTG protein in CAA, it remains to be investigated if and to what extent tTG cross-linking activity plays a role in Aβ accumulation in the vessel wall and at what stage of CAA development this occurs.

Interestingly, the typical tTG distribution in late stage CAA in both AD and HCHWA-D patients, as well as in CAA of ageing controls, indicates that this is specifically associated with all types of CAA irrespective of the underlying disease. A similar observation has been made for vascular ECM remodelling, as in ageing, AD and HCHWA-D patients, changes in ECM content in vessel walls are described [14, 15] Furthermore, the presence of two halos containing ECM proteins surrounding the actual Aβ deposition in CAA of both AD and HCHWA-D patients has been observed [14, 16, 15, 17]. As tTG covalently modifies its ECM substrates by molecular cross-linking [18], our observations that tTG colocalised with these ECM proteins, indicate that tTG may induce structural changes in ECM proteins in CAA via its cross-linking activity. As ECM cross-linking leads to vascular remodelling and vessel wall stiffening [19, 20], the ability of the vessel wall to expand in response to blood flow will be impaired [20] which may impair blood supply to and in the brain. Alternatively, the tTG-catalysed cross-linking of ECM surrounding the deposited Aβ may also have a protective effect, as it forms a barrier that seals off toxic Aβ from the rest of the brain at the astrocytic side. In addition, at the endothelial side, the barrier may prevent weakening of the endothelial layer and thereby prevent haemorrhages. Indeed, in CAA of AD patients, haemorrhages are not likely to occur in early stages of CAA as the endothelial layer stays intact until late stage CAA [17, 21]. In HCHWA-D patients however, haemorrhages occur frequently, suggesting that the rapid progression of CAA formation in HCHWA-D [22] does not allow sufficient time to form an adequate tTG-catalysed ECM barrier. Thus, although chronological evaluation of the presence and activity of tTG and ECM proteins in CAA is necessary to gain more insight in the role of tTG in ECM cross-linking, the findings in this thesis hint towards the notion that tTG may play a role in the advanced stages of CAA by 'glueing off' the deposited Aβ in the vessel walls in CAA from its environment.

Despite the absence of tTG and TG cross-links in the Aβ deposition itself in end-stage CAA (Chapter 2), we did find in situ TG activity colocalising with the Aβ deposition. Although we could inhibit this TG activity with the tTG-specific inhibitor Z-DON, the concentration we used was in micromolar range and may also (partly) block other TGs [23]. As in late stage CAA the BBB can be impaired and blood proteins may enter the vessel wall, a likely candidate for another TG in CAA is the blood-derived FXIIIa. Therefore, in Chap-

ter 3, we investigated the presence of FXIIIa in CAA. Using the FXIIIa specific substrate F11, we demonstrated FXIIIa activity in CAA, indicating that FXIIIa is not only present but also active in the Aβ deposition in CAA. The presence of the FXIIIa protein in CAA in AD was confirmed by immunohistochemical staining. Interestingly, a similar FXIIIa distribution pattern and colocalisation with Aβ was found in CAA in HCHWA-D cases, indicating that the presence of FXIIIa is a general phenomenon observed in CAA (unpublished data). Furthermore, in vitro, FXIIIa formed complexes with Aβ, independent of the cross-link activity. Thus, in initial stages of CAA development, tTG is present in the Aβ deposition (Chapter 2) and might play a role in CAA formation as described above, whereas when the BBB becomes impaired with ongoing vessel wall degeneration in CAA, FXIIIa may enter the vessel wall and be of greater importance in the progression of CAA.

During the process of Aβ aggregation and deposition in the vessel wall, SMCs gradually die, probably as a consequence of increasing levels of Aβ or the presence of toxic Aβ species. The major Aβ chaperone, ApoE, is known to protect cerebrovascular cells from Aβ toxicity [24–28], however, in CAA this protection apparently fails which raises the question whether ApoE may be structurally modified resulting in loss of its protective function. Apolipoprotein family members are known substrates for tTG-catalysed cross-linking [29, 30], and in Chapter 4 we indeed found that tTG-catalysed cross-linking of ApoE leads to impaired function of ApoE and hence impaired protection of SMCs towards Aβ-induced cytotoxicity. This previously unknown modification of ApoE is an attractive explanation for the observed SMC death in CAA and will be discussed in more detail later.

To design therapies that may prevent or delay CAA development in vivo, chronological evaluation of tTG and FXIIIa expression and activity during the course of CAA progression is essential. This can be studied in animal models and in our quest to find a suitable model we did find clear association of tTG with Aβ in two AD mouse models, i.e. the APP23 and $APP_{SWE}/PS1_{\Delta E9}$ mouse models (Chapter 5). Interestingly, both tTG and in situ active tTG where associated with Aβ plaques and vascular Aβ. In addition, tTG staining colocalised with Aβ-associated reactive astrocytes. Thus, alike the human situation, tTG was associated with Aβ depositions in these mice. Unfortunately, however, in these models, tTG distribution was not similar to the distribution in human AD. However, mouse models, in which genetic over-expression of Aβ occurs, represent only in a minority of the AD patients [31] as age-related changes and cardio-vascular diseases contribute to AD and CAA [32–36] as well as posttranslational modifications of Aβ that affect its aggregation. These factors may not be as substantial or important in AD mouse models that develop CAA [37–39] and thus vascular Aβ deposits in mice differ from human CAA which may affect the tTG expression. In addition, tTG levels in mice brains are 3-4 times lower compared to humans [40]. We also did not find the age-related increase in tTG activity that occurs in humans [20, 41, 42] in these mice (not shown), in contrast to a recent study that found increased tTG and cross-links in the APP/PS1 mice [43]. However, differences in protocol and analysis methods may explain this discrepancy, indicating that more research

is required to unravel this discrepancy between studies. Nevertheless, it is clear that the differences between mouse and human Aβ pathology and their respective tTG expression will affect the results when studying the role of tTG in vascular Aβ deposits in these models. With respect to FXIIIa, we could detect FXIIIa activity in the same mouse models used in Chapter 5 (not shown), although expression and distribution was not different in CAA vessels when compared to non-CAA vessels. Thus, studying the exact role of tTG and FXIIIa in CAA using animal models may be difficult, although these models may be useful to clarify at least some of the aspects of the role of TGs in the Aβ cascade and Aβ deposition in the brain. For this purpose, specific tTG and FXIIIa inhibitors in AD mice may be used or tTG or FXIIIa knock-out mice cross-bred with AD mice. Alternatively, mouse models that do show age-related changes and age-related Aβ deposition without APP over-expression, such as the senescence accelerated mouse prone 8 (SAMP8) model or spontaneously hypertensive stroke prone rats (SHRSP), may be more useful. These models show Aβ accumulation, although they lack either the characteristic β-pleated sheet formation of Aβ or vascular Aβ in the SAMP8 and SHRSP models respectively [44–48]. Thus, these models are not ideal to study TGs in CAA either, although they may provide an additional piece of the puzzle on the role of TGs in Aβ deposition.

Another approach, circumventing the difficulty of extrapolating animal data to humans, is to use positron emission tomography (PET) imaging of TGs in humans. In fact, in our group PET tracers to detect active tTG are under development. Low, picomolar to nanomolar, concentrations of radio-tracers are already sufficient for imaging and will not affect the physiological function of tTG. In this way, PET imaging may be used to study the localisation of active tTG and compare this between AD patients and healthy controls. Furthermore, it should be established whether PET imaging of active tTG could be used as a biomarker to identify patients at risk of developing AD and/or CAA.

To summarise, in this thesis we showed that TGs are differentially present in early and later stages of CAA (see Figure). In early stages both tTG-catalysed cross-linking of Aβ leading to Aβ aggregation as well as tTG-catalysed cross-linking of ApoE leading to SMC death may occur. In later stages, tTG may cross-link ECM proteins leading to vessel wall remodelling which may affect vessel wall integrity and brain functioning. In addition, when the BBB integrity is hampered, FXIIIa may enter the vessel wall and form complexes with Aβ and affect CAA development. As specific inhibitors for the cross-linking activity of both tTG and FXIIIa are available, our findings suggest therapeutic possibilities to counteract CAA by targeting tTG and/or FXIIIa cross-linking activity or protein-protein interaction. It is to be expected that this will be most relevant in early stages of lesion development, as in later stages the vessel wall is already degenerated and these processes will most likely not be reversible. Clearly, for a therapy to become a real option, more research is necessary to gain insight into the exact role of both tTG and FXIIIa in CAA, in particular at what stage of CAA pathology both TGs come into play.

In the remainder of this chapter I will focus on the by us, in this thesis described, discovered interactions, i.e. the tTG-catalysed modification of ApoE and the FXIIIa-Aβ complex formation, and their possible implications for therapy.

tTG-catalysed post-translational modifications of ApoE in CAA

Post-translational modifications of proteins are known to impact protein function significantly by affecting their bioactivity, localisation and interaction with other proteins [49]. With respect to TGs for instance, transglutaminase was shown to catalyse the dimerisation of interleukin-2 (IL-2). This resulted in a highly cytotoxic IL-2 dimer, in contrast to its monomeric form [50]. The most prominent post-translational modification known for ApoE is its lipidation state, that directly affects ApoE's role in Aβ fibrillisation and clearance as well as ApoE's binding to its receptor, the low-density lipoprotein receptor-related protein-1 (LRP-1) [51]. Thus, post-translational modifications of ApoE have great impact on ApoE's bioactivity. Interestingly, post-translational modification via tTG-catalysed cross-linking of apolipoproteins has been described earlier [29, 30], although the functional consequences for these proteins were not investigated. We now showed here that tTG can modify ApoE as well, and that this post-translational modification results in loss of function of ApoE regarding its protective role against Aβ toxicity. In earlier studies, this protection of ApoE was suggested to mainly depend on the isoform of ApoE [24, 25, 52], while in later studies it was found that, at least for cerebrovascular cells, the level of extracellular ApoE associated with the ApoE genotype is crucial, with cerebrovascular cells expressing the ε4 genotype producing lower levels compared to non-ε4 carriers. Thus, lower levels of available ApoE impaired the extent of protection [26, 27]. However, as not all CAA patients have an ε4 genotype, other mechanisms might explain the cerebrovascular smooth muscle cell loss in CAA. Based on the results described in this thesis, we propose the following new hypothesis: in vivo, in early stages of CAA development, increasing levels of Aβ in the cerebral vessel wall may trigger SMCs to increased secretion of ApoE [27] (Chapter 4), as a protective mechanism against Aβ-mediated toxicity. In addition, the locally elevated Aβ levels induce the secretion of active tTG into the extracellular environment (Chapter 4). tTG may become activated by cell stress [53], a process that is induced by Aβ [54]. Once active, tTG can bind and cross-link ApoE. This process is likely to occur extracellularly, as ApoE and tTG have different secretion routes and may therefore not interact within the cell. ApoE is secreted via the classical secretion pathway through the ER and Golgi network [55, 56]. In contrast, tTG is secreted via non-classical pathways, which is still not completely understood. So far, studies have shown that tTG can be transported via recycling endosomes [57]. In addition, tTG translocation to the extracellular space can be regulated by nitric oxide [58] and binding of tTG to cell surface fibronectin [59]. Thus, although the mechanism tTG secretion is still unclear, Aβ treatment of neuronal cells has been shown to result in translocation of tTG to the cell membrane [60]. Once secreted in the extracellular space, active tTG can interact with ApoE, leading to cross-linking of ApoE

which results in a non-functional ApoE that may thus play a key role in loss of protection against Aβ-induced cell death.

Until now, the mechanisms underlying the impaired ApoE protection of cerebral cells in CAA are unknown. ApoE is known to affect the Aβ cascade by modulating the aggregation pathway, yet it remains unclear whether ApoE induces Aβ aggregation or actually inhibits this aggregation pathway as both in vitro and in vivo studies report contradictive findings [61]. Alternatively, ApoE might modulate the interaction and/or internalisation of Aβ by vascular cells [24, 26, 62]. Aβ-mediated cerebrovascular cell death is directed via LRP-1 receptor [62] as prevention of Aβ/LRP-1 interaction, using the LRP-1 chaperone receptor-associated protein (RAP), blocks Aβ-mediated toxicity towards cerebrovascular cells [62]. Both ApoE and Aβ act or even compete for this receptor. Our data hint towards impaired binding of cross-linked ApoE to LRP-1, which may result in increased Aβ internalisation and consequent cell death [24, 26, 62]. Thus, although the underlying mechanisms require further studies, tTG-catalysed cross-linking and consequent inactivation of ApoE may explain the characteristic SMC death found in CAA.

Importantly, the presence of cross-linked ApoE in vivo has not been demonstrated, but would provide direct evidence for an in vivo role of tTG cross-linking activity in modifying ApoE. Until now, extraction of actual cross-linked (t)TG substrates from tissues has been proven difficult, probably due to low in vivo levels of cross-linked material and high levels of cross-linked proteins necessary for a successful immunoprecipitation (IP) to detect cross-links with the 81D4 antibody. Until now, only cross-linked tau has been successfully extracted from human AD and other tauopathies [63–65]. Unfortunately, most studies demonstrate tTG substrates indirectly by measuring general cross-link levels in tissues, e.g. brains, or CSF [66, 67], as well as by in vitro or in situ incorporation of amine- or glutamine-rich peptides [4, 68], similar to our approach. However, if technical issues may be solved and in future studies the presence of in vivo cross-linked ApoE has been demonstrated, it would be interesting to study whether tTG-catalysed cross-linking of ApoE is specific for CAA in AD cases or whether it also occurs in CAA of ageing controls, which would suggest an ageing effect. The latter would be likely, because we did not find differences in localisation or intensity of tTG and ApoE staining in CAA of AD and CAA in control cases, suggesting that tTG-catalysed cross-linking of ApoE may occur in all CAA. When therapies such as locally inhibiting interaction of tTG with ApoE would be available, this may benefit not only patients with AD and CAA, but also ageing people. Thus, SMC death and vessel wall degeneration may be prevented which may prevent and/or slow down the development of CAA and AD. For this to become an option, the binding site of tTG to ApoE and the residues involved in cross-linking need to be clarified. This can be determined by using different truncated or mutated forms of tTG and ApoE and analyse the binding of tTG to ApoE as well as tTG-catalysed multimerisation of ApoE. This would provide information how to interfere with the interaction, which may be beneficial in vivo by inhibiting tTG-catalysed ApoE cross-linking. For this purpose, ideally the physiological

cross-link activity of tTG should be maintained e.g. for its role in wound healing and apoptosis [69–71]. It has been shown that for the tTG-fibronectin interaction, specific peptides could block protein-protein interaction [72]. Thus, for the tTG-ApoE binding a similar strategy may be followed to block the interaction without inhibiting tTG transamidation activity.

FXIIIa-Aβ complex formation in CAA

Until now, the focus of research into a role of TGs in CAA has been limited to tTG, as earlier studies did not demonstrate the presence of other TGs, such as TG1 and TG3, in the brain vessel wall and in CAA [3, 73]. We now identified FXIIIa in the Aβ deposition in CAA, as well as FXIIIa-Aβ complex formation in vitro, pointing to a role of FXIIIa in CAA pathology.

The presence of blood-derived proteins, including IgG and albumin, is not an uncommon finding and has been described before in the vessel walls of CAA in humans and mice [46, 74, 75]. In addition, the main blood clot constituent fibrinogen was also found in the Aβ deposition in CAA [76, 77]. Importantly, however, none of these proteins were exclusively present in CAA, but were also found in non-CAA vessels in both control and CAA cases [46, 74–77]. We confirmed these findings for fibrinogen, in that it was present in both CAA and non-CAA vessels (not shown). Studies did, however, show that fibrinogen may play an important role in AD in that Aβ altered the fibrinogen clot structure and depletion of fibrinogen in AD mice decreased CAA burden [76]. Although fibrinogen may affect AD and CAA, the presence of FXIIIa and thrombin exclusively in CAA indicates a more specific association of FXIIIa with CAA pathology compared to other blood-derived proteins, including fibrinogen. FXIIIa would therefore be a more attractive therapeutic target to specifically counteract CAA compared to other blood-derived proteins that are observed in blood vessel walls. As mentioned, also thrombin was present exclusively in CAA. This finding indicates that FXIII is converted to FXIIIa by thrombin [78] within the vessel wall. We thus hypothesise that when BBB permeability occurs in later stages of CAA development FXIII and thrombin leak into the vessel wall, FXIII is converted into its active form FXIIIa and ends up in the Aβ part of CAA where it can interact with Aβ. However, we cannot completely rule out local production of FXIIIa by cells during CAA, as FXIIIa has been observed in microglia [79] and CAA-associated astrocytes by us (Chapter 3).

The FXIIIa-Aβ complex formation is a cross-link independent interaction which is in line with the absence of cross-link staining in the Aβ deposition (Chapter 2). Interestingly, our findings on the FXIIIa-Aβ complex are different from the interactions observed between tTG and Aβ, in which primarily Aβ cross-linking plays a role [10]. Although both TGs require the glutamine at position 15 in the Aβ protein for interaction (Chapter 3 and [10]), FXIIIa appears to be more prone to form cross-link independent stable complexes with its substrates. For instance, FXIIIa is known to form cross-link independent complexes with other proteins, e.g. the complex of thrombin, fibrinogen and FXIII is suggested to be required for activation of FXIII [80] and the zymogen FXIII itself is arranged in a non-covalent

complex of two A and two B subunits [78]. Moreover, fibrinogen type 2 binds the B-subunit of the FXIII tetramer, thereby slowing the cross-link activity [81, 82]. Together, these data suggest that cross-link independent interaction of FXIII(a) with other proteins is a physiological phenomenon. However, for tTG several cross-link independent interactions are described as well, such as its GTPase activity and interaction of tTG with integrins and fibronectin at the cell surface to promote cell adhesion [71]. Thus, presently, it remains unclear why FXIIIa and tTG interact differently with Aβ. Studying the binding and recognition sites of both TGs for Aβ, using truncated or mutated forms of TGs, will provide more insight into the exact binding sites of Aβ to FXIIIa and tTG and the underlying mechanism of the differences in their interaction.

Nevertheless, our finding of the FXIIIa-Aβ complex formation implicates a so far novel role for FXIIIa. Although the in vivo presence of FXIIIa-Aβ complexes needs to be confirmed, FXIIIa may affect Aβ's aggregation pathway upon binding to Aβ. This could have a protective function by preventing Aβ to interact with itself forming toxic species that cause cell death via LRP-1 uptake [62]. Indeed, we found that these FXIIIa-Aβ complexes protected against Aβ-induced SMC death, possibly by interfering with Aβ self-interaction or cellular uptake. However, in late stages of CAA, SMCs are not present anymore suggesting that either FXIIIa failed in its protective function or FXIIIa is only present in the Aβ deposition when SMCs loss is already complete. Another effect of the FXIIIa-Aβ complex formation may be that these complexes seal off the vessel wall thereby preventing entry of blood into the vessel wall, and thus haemorrhages, that may occur in CAA lesions [2]. On the other hand, more likely the FXIIIa-Aβ complex formation has detrimental consequences in the vessel wall, by contributing to protein deposition in the vessel wall. For instance, persistence of these complexes with possible entrapment of other proteins may occur. This may promote CAA progression. Indeed, in AD mice, the normal clearance route of Aβ alongside the vessel walls via the interstitial fluid drainage was altered, and associated with the burden of CAA in these mice [83]. Thus, blockade of this clearance pathway by deposition of Aβ or other proteins, such as FXIIIa, may limit clearance of Aβ and thus promote protein deposition in the vessel wall and thus CAA development [84]. In addition, complex formation of FXIIIa with Aβ may impair FXIIIa's physiological role in blood-clotting if haemorrhages in these areas occur. This process could, especially in patients with severe CAA-related haemorrhages such as in HCHWA-D exacerbate the haemorrhages. Clearly, the role of FXIIIa and the effect of FXIIIa-Aβ complexes in CAA require more research.

Future studies need to confirm if the specific association of FXIIIa with CAA points towards FXIIIa as an attractive therapeutic target for CAA. First of all, the in vivo presence of FXIIIa-Aβ complexes needs to be confirmed. We attempted to extract FXIIIa-Aβ complexes using an FXIIIa antibody, (Chapter 3), but found no complexes. As described for the putative presence of cross-linked ApoE in the brain, extraction of protein complexes may technically be difficult and the amount of complexes may be too low to detect. When

detection would be possible, several follow-up studies need to be performed. First of all, the exact site of interaction of FXIIIa with Aβ needs to be determined. This can be done by using truncated and mutated forms of FXIIIa and Aβ to analyse the binding and complex formation. A promising finding is the cross-link independent interaction of FXIIIa with Aβ which will open up possibilities to design peptides to inhibit or alter this protein-protein interaction similar to peptides that block the tTG-fibronectin interaction [72]. Thus inhibiting cross-link activity is not necessary, and FXIIIa's physiological cross-link function in coagulation can be maintained. In addition, it is interesting to study whether the blood-derived FXIIIa and/or the FXIIIa-Aβ complexes might be used as a blood biomarker to diagnose CAA. Although we suggested that FXIIIa involvement may be a late effect in CAA, future studies need to determine when exactly FXIIIa is involved in CAA and whether changes in FXIIIa levels occur in the blood.

Despite remaining uncertainties, FXIIIa is increasingly recognised as an important player in different diseases and physiological processes such as angiogenesis and several vascular diseases [78]. Our study now suggests the involvement of FXIIIa in CAA as well. Future studies are expected to unravel the contribution of FXIIIa to CAA development.

Concluding Figure

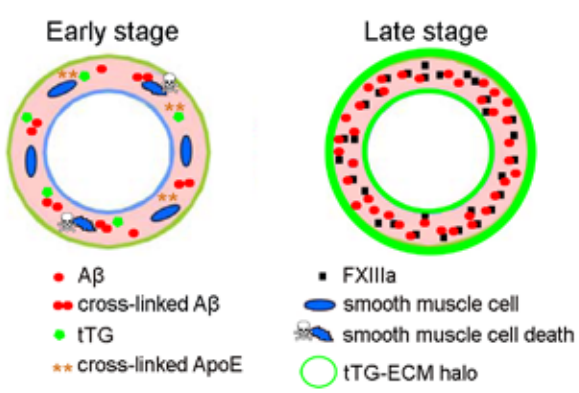

Figure Hypothesis on the role of TGs in CAA development based on the results of this thesis. In early stages of CAA, increased tTG levels, likely induced by locally elevated Aβ levels in the vessel wall, may lead to cross-linking of Aβ, leading to Aβ aggregation in the vessel wall. In addition, Aβ induces the secretion of ApoE, and tTG-catalysed cross-linking of ApoE results in a non-functional ApoE and thereby impaired protection of smooth muscle cells (SMCs) against Aβ-induced cell death. In later stages of CAA, tTG may catalyse ECM cross-linking resulting in vessel wall remodelling and barrier formation. Moreover, leakage of FXIIIa from the blood into the vessel wall may lead to FXIIIa-Aβ complex formation and subsequent protein deposition in the vessel wall. Although the chronological order of events is yet unclear, these processes may underlie or contribute to CAA development and/or progression.

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