English summary

Transglutaminase activity in Alzheimer's Disease

Alzheimer's Disease

Alzheimer's Disease (AD) is the most common form of dementia. AD is characterised by memory deficits, cognitive decline and behavioural changes that ultimately lead to loss of body functions and death. AD affects 11% of all people above the age of 65, and 32% of all people above the age of 85. Unfortunately, drugs currently on the market for AD can only relieve symptoms but cannot cure or prevent the disease. Diagnosis of AD during life is based on clinical and neurological investigations such as neuropsychological tests, cerebrospinal fluid analysis and neuroimaging; however, the definite diagnosis can still only be made post-mortem by the histological detection of different protein aggregates, i.e. intraneuronal neurofibrillary tangles consisting of accumulation of the hyperphosphorylated tau protein as well as senile plaques and cerebral amyloid angiopathy both consisting of the accumulated amyloid-β protein in the brain parenchyma and blood vessel walls, respectively. Accumulation of Aβ leads to cell death and inflammation. In CAA Aβ deposition results in smooth muscle cells death and vessel wall degeneration. This may lead to haemorrhages and/or increased cognitive decline of patients.

Aβ production and clearance

Aβ is a 4 kDa protein cleaved from the amyloid precursor protein (APP) and is present mainly in two forms: $A\beta_{1-40}$ and $A\beta_{1-42}$. Aβ can interact with itself forming Aβ aggregates. $A\beta_{1-42}$ is more prone to aggregate and is also more prominently present in AD brains. Aβ self-aggregates from monomers and dimers to larger oligomers ultimately forming large mature fibrils with β-pleated sheet conformation. Of these, the dimer and oligomeric Aβ are thought to be the most toxic and related to AD. Aβ is cleared from the brain by enzymatic degradation, as well as via receptor-mediated transport across the blood-brain barrier (BBB). Thirdly, Aβ can be cleared via the interstitial fluid (ISF) drainage that transports Aβ alongside the vessel walls to the peripheral lymph nodes. Especially the first two clearance mechanisms fail with age, resulting in a higher burden of Aβ clearance via the ISF. This may compromise ISF function and thereby increase Aβ deposition in the vessel walls, leading to CAA.

Risk factors for AD

Over 90% of AD cases are sporadic, non-familial, late-onset cases, where the strongest risk factor is age. However, several genetic risk factors, accounting for ~5% of early-onset

(familial) AD, are described that increase Aβ production in the brain. The strongest common genetic variant that is associated with sporadic, late-onset AD is the ApoE gene coding for the plasma protein apolipoprotein E (ApoE). The ApoE gene can be present in three alleles, ε2, ε3 and ε4 with ε4 strongly associated with an increased risk on the development of AD. The ApoE protein is important in transport of lipids and cholesterol in the body and can bind Aβ in the brain. ApoE-Aβ complex formation leads to internalisation and degradation of Aβ by cells or isoform-dependent transport across the BBB. Apart from Aβ aggregation, life style factors such as cardiovascular diseases are risk factors for developing or aggravating AD, highlighting the importance of studying therapeutical targets for AD that improve vascular functioning.

Transglutaminases

Transglutaminases (TGs) are a group of Ca^{2+}-dependent enzymes that catalyse post-translational modifications of proteins including amine incorporation into proteins, deamidation and the formation of stable protein complexes by cross-linking of glutamine and lysine residues. Tissue transglutaminase (tTG) is the most studied TG and is present throughout the body. It can be upregulated by specific signalling pathways involved in cellular stress or tissue damage, is involved in inflammatory processes, cell migration and adhesion, and can function as a signal transduction protein. An important role of tTG is the cross-linking of extracellular matrix (ECM) proteins such as fibronectin and laminin. This process is important in matrix stabilisation and thereby wound healing and angiogenesis. Furthermore, tTG-mediated ECM cross-linking is important in remodelling of blood vessel walls in response to changes in blood flow. With age, tTG activity is increased in the vessel wall, which may lead to increased ECM cross-linking and subsequent vessel wall stiffness and decreased blood flow. In addition, tTG is involved in fibrosis, e.g. kidney and lung fibrosis where increased tTG cross-link activity of ECM proteins results in fibrosis. Moreover, tTG has been implicated in neurodegenerative diseases, such as AD.

tTG in AD

In AD brains, the presence and activity of tTG is increased and significantly elevated tTG levels have been reported in the cerebrospinal fluid of AD patients compared to controls. Moreover, the level of the tTG cross-link was significantly elevated in the cerebrospinal fluid of AD patients and a correlation between these cross-links in grey matter and cognitive impairment in AD patients was observed. Previous studies, including by our group, demonstrated the presence of tTG and cross-links in SPs in post-mortem brains of AD patients. As other studies demonstrated the capability of tTG to induce oligomerisation of Aβ *in vitro,* this suggests that tTG can cross-link Aβ *in vivo* leading to Aβ aggregation in the

brain. In CAA, however, tTG and cross-links were associated with the lesion but did not colocalise with the Aβ deposition. This indicates that tTG may be involved in the formation of both SPs and CAA, but that its role may differ in these lesions. Also indirectly, tTG may influence the Aβ cascade i.e. via interaction with proteins that are involved in Aβ deposition, such as ApoE. Interestingly, family members of the ApoE, ApoA-I, ApoA-II, ApoB and ApoC-I are known substrates for tTG-catalysed cross-linking leading to multimerisation of the apolipoproteins. In conclusion, tTG may play an important role in the formation of Aβ aggregates in the brain, both directly via interaction with Aβ or indirectly via interaction with Aβ chaperones.

Factor XIIIa

Another transglutaminase that has been described in this thesis, is Factor XIIIa (FXIIIa). FXIIIa is crucial in the blood coagulation cascade where it cross-links fibrin, the main constituent of the blood clot, into a tight and stable blood clot. FXIIIa is present in the blood, but has been detected in monocytes/macrophages and osteoblasts. In addition to its role in coagulation, FXIIIa is involved in wound healing, bone formation and cell migration and proliferation. Moreover, a mutation in the FXIIIa gene is associated with cerebral haemorrhages and AD. As the BBB in CAA may be compromised, blood proteins may leak into the brain. However, whether also FXIIIa may leak into the brains or whether FXIIIa is involved in the development of AD, is not known.

Goals and results of this thesis

In this thesis we investigated the role of transglutaminases in CAA. As CAA plays an important role in the disease progression of AD and previous evidence indicates a role for tTG in CAA, we studied in **Chapter 2** the distribution pattern of tTG and tTG activity in post-mortem brain tissue of AD cases and in patients with a genetic form of CAA. We found that in early stage of CAA development, tTG protein colocalised with the Aβ deposition in the vessel wall. Possibly, tTG is involved in Aβ aggregation at this stage. However, in end-stage CAA, tTG protein and its cross-linked products did not colocalise with the actual Aβ deposition in CAA, but was present in two halos surrounding the Aβ. Similarly, ECM proteins fibronectin and laminin were also present in such halos, colocalising with tTG. This suggests that tTG may cross-link these ECM proteins which could lead to vessel wall stiffness and hence decreased Aβ clearance via the ISF. However, barrier formation may also protect against continuing damage to the brain vessel wall. Together, these results suggest that tTG may have different roles in early and late stage CAA. Despite the absence of tTG protein in the Aβ deposition in end stage CAA, *in situ* activation of endogenous (t)TG demonstrated clear colocalisation with the deposited Aβ in CAA. These findings hint towards the presence of

another TG family member in the Aβ part of CAA. As CAA is associated with blood-brain barrier disruption, blood-derived proteins could leak into the vessel wall. The TG family member FXIIIa is present in the blood and plays a crucial role in the blood-clotting cascade by cross-linking fibrin molecules. In fact, as association of fibrin with CAA has been reported, we hypothesised that FXIIIa leaks into the blood vessel wall in CAA. Therefore in **Chapter 3** we studied the distribution and *in situ* activity of FXIIIa in CAA. Indeed, we observed both the presence of FXIIIa protein and FXIIIa activity in the Aβ part of CAA. In addition, we demonstrated *in vitro* that FXIIIa and Aβ form complexes, independent of the cross-link activity of FXIIIa. These complexes protected smooth muscle cells against Aβ-induced cell death, which is an important phenomenon in CAA. We conclude from this study that FXIIIa forms unique complexes with Aβ, which may play an important role in Aβ aggregation in the vessel wall.

An important hallmark of early stage CAA, is Aβ-induced smooth muscle cell (SMC) death in the vessel wall. ApoE, the major Aβ chaperone, not only has a role in Aβ binding and aggregation, but also protects smooth muscle cells against Aβ-induced SMC death. This raises the question whether ApoE may be structurally changed and therefore cannot offer protection against Aβ cytotoxicity in CAA. As tTG is present in the vessel walls in early stage CAA, and tTG is known to cross-link ApoE family members, we tested in **Chapter 4** the hypothesis that ApoE is an *in vitro* substrate for tTG-catalysed cross-linking leading to a non-functional ApoE protein. Indeed, we showed that *in vitro* tTG can cross-link ApoE, resulting in ApoE multimers. *In vitro* incubation of SMCs with Aβ resulted in increased secretion of both tTG and ApoE. In addition, incubation of SMCs with cross-linked ApoE did not protect the cells against Aβ toxicity, in contrast to non-cross-linked ApoE. Thus, in CAA the presence of both tTG and ApoE may be increased leading to tTG-catalysed cross-linking of ApoE into a non-functional protein. This could explain the SMC death in CAA.

Finally, we set out to identify a suitable animal model that mimics tTG's association with human CAA and to obtain more insight into the role of tTG in the pathogenesis of CAA. In **Chapter 5,** we investigated the distribution pattern of both tTG and its activity in two well-known AD mouse models. For this purpose, we used the APP$_{swe}$/PS1$_{\Delta E9}$ (APP/PS1) mice that show early onset and fast progressing Aβ pathology and the APP23 mouse model that displays a later onset in age and slower progression of pathology. We observed that tTG and tTG activity were present in both mouse models in SPs and associated astrocytes as well as in CAA, already early in the development of pathology. However, the distribution of tTG in mice was different from the distribution in human AD tissue. Therefore, we conclude that although tTG seems to have a role from the start of Aβ pathology, these results cannot be directly translated to the human situation.

In **Chapter 6** the results of this thesis are summarised and discussed. In this thesis we demonstrated that both tTG and FXIIIa can play a role in different aspects of the development of CAA. In early stage CAA, locally elevated levels of Aβ can lead to increased secretion of tTG and ApoE. Subsequently, tTG may cross-link ApoE and decrease the protective activity of ApoE against Aβ-induced cell death. Furthermore, tTG may be involved in ECM cross-linking, which may lead to vessel wall remodelling. Future studies are required to determine the effects of vessel wall remodelling on the onset and/or development of CAA. The vessel wall degeneration in CAA may result in BBB leakage, and FXIIIa leakage from the blood into the vessel wall. FXIIIa may then form complexes with Aβ, contributing to Aβ deposition in the vessel wall. More research is necessary to unravel the exact roles of both tTG and FXIIIa activity in CAA and at what stage of CAA pathology the TGs come into play. Unfortunately, the mouse models studied in this thesis are not sufficient to study these questions. Other models, both *in vitro* and *in vivo,* are needed for future studies to evaluate the use of tTG and FXIIIa as therapeutically targets and design therapies that may prevent or delay CAA development in vivo.