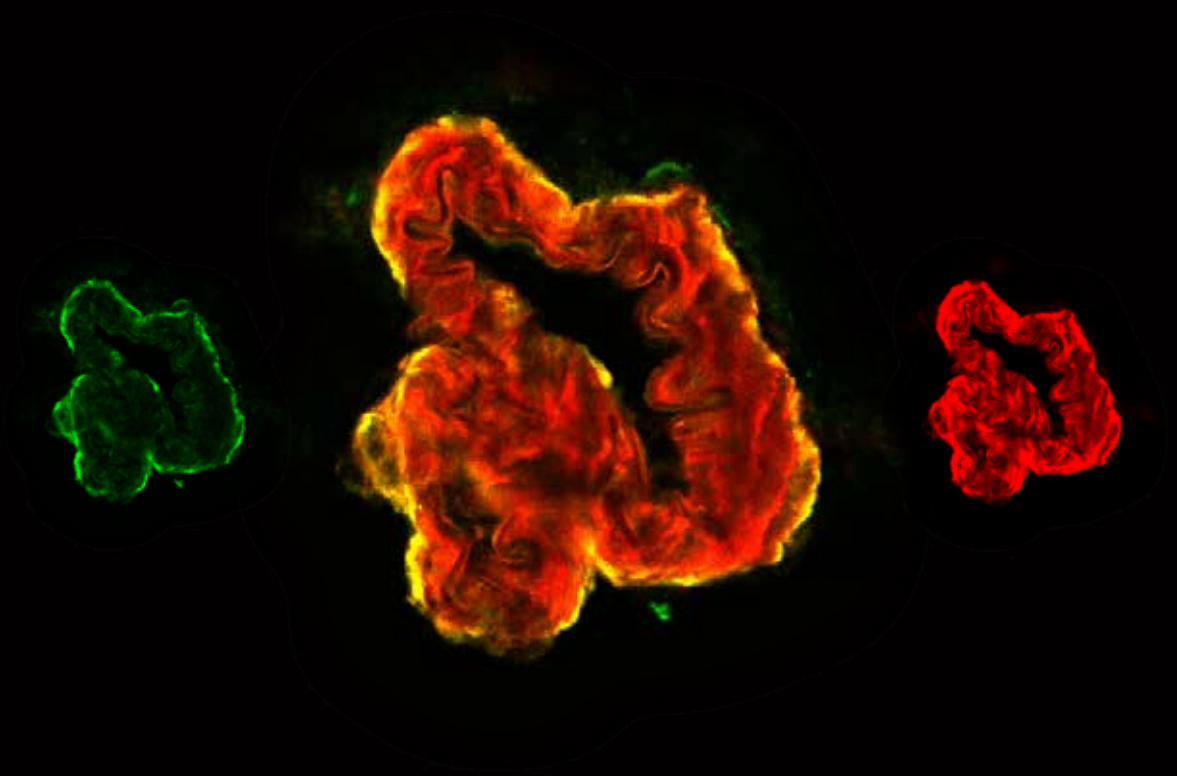
TRANSGLUTAMINASE ACTIVITY IN ALZHEIMER'S DISEASE



MIEKE DE JAGER

Transglutaminase activity in Alzheimer's Disease

Mieke de Jager

The research described in this thesis was conducted at the Department of Anatomy and Neurosciences, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

The project was financially supported by grants from The Brain Foundation of the Netherlands, the ‘International Stichting Alzheimer Onderzoek’ and a Proof-of-Concept fund of the Neuroscience Campus Amsterdam.

ISBN: 978-94-028-0104-0

Cover image: immunofluorescence staining of tTG (green) and tTG activity (red) in a brain blood vessel of the APP23 Alzheimer mouse model as used in Chapter 5. APP23 mice are originally from healthcare company Novartis. Image printed with permission of Novartis.
Chapter images: immunofluorescence stainings of collagen (green) in culture plates after removal of cells, as described in Chapter 4.

Lay-out: Matthijs Ariens - Persoonlijkproefschrift.nl
Formatted by: Matthijs Ariens - Persoonlijkproefschrift.nl
Print: Ipskamp Printing

Printing of this thesis was supported by a grant of the ‘International Stichting Alzheimer Onderzoek’.

VRIJE UNIVERSITEIT

Transglutaminase activity in Alzeimer’s Disease

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op vrijdag 17 juni 2016 om 11.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Anna Maria de Jager

geboren te Zwolle

promotor: prof.dr. J.J.G. Geurts
copromotoren: dr. M.M.M. Wilhelmus
dr. B. Drukarch

Contents

Chapter 1

General introduction

Partly based on:

Wilhelmus MMM, de Jager M, Drukarch B, 2012.
Tissue Transglutaminase: A Novel Therapeutic Target in Cerebral Amloid Angiopathy.
Neurodegener. Dis 10(1-4):317-9.

Wilhelmus MMM, de Jager M, Bakker ENTP, Drukarch B 2014.
Tissue Transglutaminase in Alzheimer's Disease: Involvement in Pathogenesis and its Potential as a Therapeutic Target.
J Alzheimers Dis 42 Suppl 3:S289-303

1. Alzheimer's disease

Over 100 years ago, in 1907, the German psychiatrist and neuropathologist Alois Alzheimer described for the first time a patient with memory problems and typical brain changes that would later become known as Alzheimer's disease (AD) [1]. AD is the most common form of age-related dementia characterised by progressive memory loss, cognitive decline as well as behavioural changes eventually leading to loss of body functions and death [2]. AD affects 11% of all people above the age of 65, and 32% of all people above the age of 85 [3].

Diagnosis of AD during life is based on clinical and neurological investigations such as neuropsychological tests, cerebrospinal fluid analysis and neuroimaging [4]; 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. In addition, other neuropathological characteristics such as brain atrophy, neuronal and synapse loss and inflammation are present [5–7].

Unfortunately, drugs currently on the market for AD can only relieve symptoms but cannot cure or prevent the disease. The most common drugs available are acetylcholine esterase (AChE) inhibitors [8] that temporarily increase acetylcholine concentrations in brain regions that have AD-related cholinergic neuron loss. In addition, N-methyl-D-aspartate (NMDA) antagonists are also broadly prescribed which block the glutamate-mediated over-stimulation of NMDA receptors that is observed in AD [8]. Unfortunately, these drugs do not modify disease progression.

2. Histopathological hallmarks of Alzheimer's disease

Neurofibrillary tangles

In AD and tau-associated dementias, known as tauopathies, the intracellular microtubule-associated protein tau is hyperphosphorylated and accumulates in the brain [9, 10]. In AD, it aggregates in neurons to form neurofibrillary tangles (NFTs) in neurons in several brain regions [5]. The progression and severity of NFTs in the brain have been classified into six stages according to Braak and Braak [7]. At the start of the pathology, NFTs are present in the transentorhinal region and hippocampus, which spreads via the limbic areas throughout the cortex leading to cortical destruction and neuronal loss [7]. The presence of NFTs correlates with the cognitive decline observed in AD patients [11, 12].

Senile plaques

The amyloid-beta protein (Aβ) is cleaved from the amyloid precursor protein (APP, Figure 1) and deposits as senile plaques (SPs) in the brain parenchyma. SPs are present in different areas of the brain such as cortex, hippocampus and thalamus. Classic neuritic plaques are composed of a core of fibrillar Aβ (β-pleated sheet conformation) and dystrophic neurites within or surrounding the Aβ, which can contain hyperphosporylated tau. In addition, synaptic and neuronal loss as well as recruitment of activated microglia and astrocytes are associated with these plaques [12]. Another type of plaques, diffuse SPs, appear as more fine structures and lack the fibrillar Aβ core and dystrophic neurites as in classic plaques. It is thought that diffuse plaques are an early stage of plaque formation developing into classic plaques [5].

Cerebral amyloid angiopathy

Aβ also deposits in the cerebral blood vessel walls as cerebral amyloid angiopathy (CAA). The vessel wall consists of an intimal layer with endothelial cells, a medial layer with smooth muscle cells important in contraction and relaxation of the blood vessels and the outer adventitia layer with fibroblasts [13]. CAA development starts with Aβ deposition in the medial layer and progresses into all layers of the vessel wall. CAA is present most prominently in occipital and parietal areas of the brain, whereas veins as well as vessels in the white matter are hardly involved [12, 14]. Two types of CAA have been described; type 1 CAA exhibits Aβ deposition in capillary cerebral vessels (capCAA) as well as larger parenchymal and leptomeningeal vessels and is most strongly associated with cognitive decline [14, 15]. Type 2 CAA however, is defined as Aβ deposition in larger vessels only without involvement of capillaries.

CAA is present in about 30% of non-demented elderly, but occurs in 80% to 100% of AD patients and contributes to the cognitive decline of these patients [14, 16–19]. CAA leads to smooth muscle cell (SMC) death and vascular remodelling, characterised by altered expression and distribution of extracellular matrix (ECM) proteins. These changes lead to degeneration and weakening of the vessel wall. This may result in blood brain barrier permeability, eventually leading to haemorrhages [20–24]. Furthermore, CAA leads to impaired vascular autoregulation and hypoperfusion [14], as well as an inflammatory reaction [25, 26].

A possible underlying mechanism for CAA formation is thought to be impaired clearance of Aβ via the interstitial fluid (ISF) drainage. This is the so-called lymphatic drainage of the brain where solutes and molecules drain alongside the blood vessels to the lymph nodes at the base of the skull and are thereby removed from the brain [27–29]. The ISF depends on the pulsations of the blood flow and flows in the opposite direction of the blood flow [30]. Several animal and human studies suggested that Aβ can be transported via this route as well [31]. With age, Aβ clearance becomes more dependent on the ISF [32]. The reason behind this will be explained later on in paragraph 4. However, as vessels stiffen with age [33, 34], the ISF drainage of Aβ will be less and may result in increased Aβ deposition in the brain [29]. This is thought to be important for CAA development where Aβ will get entrapped in the vessel wall and forms aggregates [32, 35].

In addition to CAA associated with AD, familial forms of CAA have also been described

such as Hereditary Cerebral Haemorrhages with Amyloidosis of the Dutch type (HCHWA-D). These patients bear the E693Q mutation (glutamate to glutamine substitution) in the APP gene (Figure 1), which was reported in Dutch families [36]. The mutation enhances the oligomerisation and fibril formation of Aβ and increases fibril stability and Aβ toxicity. This leads to severe CAA, clinically diagnosed by stroke due to haemorrhages at a young age (mean 50 years) and cognitive decline in most of the patients. Eventually, patients die at a mean age of 60 years [36].

3. Aβ production and aggregation

According to the amyloid-cascade hypothesis, Aβ production and deposition starts a cascade of events leading to neuronal loss and cognitive decline (Figure 2). Aβ is a 4 kDa protein cleaved from the amyloid precursor protein (APP) (Figure 1). APP is a transmembrane protein that is present in most cells and tissues as well as in the brain. Its function is not completely clear, although it may have neuroprotective effects and be involved in cell-cell interactions [5]. APP is proteolytically cleaved by α-, β- and γ-secretase. Cleavage by α-secretase results in a non-amyloidogenic product [5, 37, 38] whereas consecutive cleavage by the β- and γ-secretase results in release of Aβ. Aβ can vary in length between 38 and 43 amino acids, of which the 40 and 42 amino acid lengths are most common. Aβ self-aggregates from monomers and dimers to larger oligomers ultimately forming large mature fibrils with β-pleated sheet conformation [39]. Of these, the dimer and oligomeric Aβ are thought to be the most toxic and related to AD [40–42].

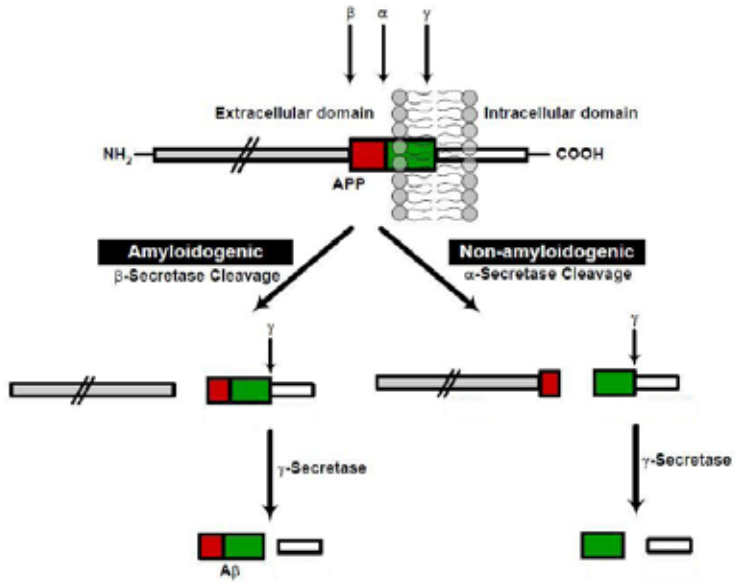


Figure 1 The amyloid precursor protein (APP) is cleaved by either α-secretase resulting in a non-amyloidogenic product, or by β-secretase and γ-secretase, resulting in Aβ. Figure modified from "Kumar J et al., 2011. Aging 3:803-812." [43].

Aβ-interacting proteins and post-translational modifications

Importantly, several Aβ-interacting proteins, so-called Aβ chaperones, interact with Aβ and influence the Aβ cascade. Examples of such chaperones are the extracellular matrix proteins heparan sulphate proteoglycans (HSPGs) as well as heat shock proteins (Hsps) and apolipoprotein E (ApoE, see below). These chaperones are found in the Aβ deposits in the brain and influence Aβ aggregation and clearance [44]. In addition to chaperones, post-translational modifications of Aβ, such as metal-oxidation of Aβ and the formation of pyroglutamate-modified Aβ, also influence the Aβ cascade and enhance Aβ aggregation and/or protect Aβ against degradation [45, 46]. Another post-translational modification is cross-linking of Aβ by the enzyme tissue transglutaminase that results in the formation of stable Aβ dimers and trimers and might play an important role in Aβ aggregation and/or the persistence of intermediate forms of Aβ [47]. Taken together, Aβ chaperones as well as post-translational modifications of Aβ are crucial in the onset or progression of both the Aβ cascade and Aβ deposition in AD.

Differences between SPs and CAA in Aβ and Aβ-associated proteins

The Aβ cascade results in the formation of both SPs and CAA. However, clear differences are observed between these lesions. In SPs, the 42 amino acid form of Aβ ($Aβ_{1-42}$) is the major form, whereas CAA consists mainly of $Aβ_{1-40}$ [14]. In addition, N-terminal post translational modifications such as pyroglutamylation and racemisation of Aβ are found both in SPs and in CAA, however the detection of modified Aβ species in CAA is variable and minimal compared to SPs [48, 49]. Furthermore, in both SPs and CAA, Aβ-chaperones are found, such as ApoE, HSPGs, and members of the complement system. However, several inflammatory proteins such as α1-antichymotrypsin, α2-macroglobulin and intercellular adhesion molecule-1 (ICAM-1) are only present in SPs indicating that other processes underlie the inflammatory reaction in both lesions [50]. Taken together, the differences in Aβ type and modifications as well as Aβ-chaperones present in SPs and CAA suggests differences in the pathogenesis of both lesions.

4. Aβ clearance

Aβ is cleared from the brain via several routes. An important way is by enzymatic degradation where insulin degrading enzyme (IDE) and neprilysin (NEP) are the most important Aβ-degrading enzymes [27]. In addition, uptake and degradation of Aβ by glial cells is another clearance mechanism [51, 52]. Furthermore, Aβ may be cleared via receptor-mediated transport over the blood brain barrier (BBB) into the blood by Low density lipoprotein receptor-related protein 1 (LRP-1). LRP-1 transports Aβ via direct binding of Aβ or in a complex with the Aβ chaperone ApoE [53]. However, in AD, lower endothelial LRP-1 levels are found, as well as a damaged oxidised LRP-1 [54, 55]. A fourth clearance pathway is via the ISF drainage that transports Aβ alongside the vessel walls to the peripheral lymph nodes [56]. With age, the first described clearance mechanisms fail, increasing the pres-

sure on the ISF drainage, which might lead the development of CAA and late-onset AD [32]. However, other factors can contribute to AD development as well, including genetic mutations and vascular diseases.

5. 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 are described that can increase the risk of sporadic AD or are causative for familial AD. Rare mutations accounting for ~5% of early-onset (familial) AD are found in three genes: APP, PSEN1, PSEN2. Mutations in the APP gene influence APP proteolytic processing and/or aggregation and thereby Aβ production. Mutations in PSEN1 and PSEN2, coding for the presinillin 1 and 2, components of the γ-secretase, alter the γ-secretase-mediated cleavage of APP, which results in increased levels of $A\beta_{1-42}$ and its aggregation and earlier onset of the disease [5, 57, 58].

ApoE

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 that differ in one or two amino acids [5, 57, 58]. The ε4 allele is considered the high risk allele for development of AD and type 1 CAA, whereas ε2 is protective for AD, but associated with type 2 CAA and increases the risk on CAA-associated haemorrhages [59]. The ε3 allele is neutral and the most common form as well [20, 57].

The function of ApoE is to transports cholesterol and other lipids throughout the body [53, 60]. In addition, it has a role in Aβ binding, aggregation and transport. In the CNS, the major source of ApoE are astrocytes and microglia [61], although cerebrovascular cells are also known to produce ApoE [62]. ApoE has been immunohistochemically detected in SPs, CAA and NFTs [63, 64] suggesting an interaction with Aβ. In vitro, the binding of Aβ to ApoE is isoform dependent (ε2>ε3>>ε4) and also depends on the lipidation status of ApoE [65, 66]. ApoE-Aβ complex formation leads to internalisation and degradation of Aβ by cells such as astrocytes [67] or an isoform dependent (ε4<ε3<ε2) BBB-mediated clearance via LRP-1 [53].

Not only results the ApoE4 genotype in impaired Aβ binding and clearance from the brain, ApoE4 also accelerates the fibrillation and deposition of Aβ leading to more SPs and CAA [53, 68–71]. Interestingly, in APP transgenic mice deficient of ApoE, β-pleated sheet amyloid deposition was decreased compared to APP mice with ApoE [72], whereas overexpression of ApoE4 resulted in more Aβ deposition than ApoE3 [73]. In addition, in vitro, ApoE protected cells from Aβ-induced toxicity, whereas ApoE4 resulted in less protection [74–76]. Taken together, although the exact role of ApoE in AD is still unclear, increasing evidence points towards an important role of ApoE in AD pathogenesis.

6. Vascular and life-style factors in AD

For long, the amyloid-cascade hypothesis explained the neurodegeneration and cognitive decline in AD as a consequence of Aβ deposition. However, environmental and life-style factors such as smoking and diet (trans-unsaturated and saturated fatty acids), traumatic brain injury, diabetes mellitus type II and low or high bodyweight may also increase the risk of developing AD [57, 77–79]. In addition, vascular dysfunction that occurs in normal ageing, is more pronounced in AD, and may worsen or even precede neurodegeneration [80–82] (Figure 2). This vascular dysfunction can be caused by (neuro) vascular diseases such as stroke, cerebral haemorrhage, white matter changes, CAA, atherosclerosis and hypertension and are associated with AD and dementia in general [5, 14, 57, 78, 83]. In addition, changes in the microvasculature are observed in AD and ageing. To illustrate this, variations in capillary diameter, fragmented and irregular shaped capillaries and increased vessel tortuosity are all found in AD brains [84–87]. Furthermore, alterations in vascular membrane components and extracellular matrix (ECM) proteins are observed in AD and CAA [23, 24]. The observed changes may be due to the toxic effects of Aβ and its deposition but are also found to be independent of Aβ deposition [80]. Taken together, these vessel wall changes may lead to hypoperfusion, ischemia, BBB breakdown and increased Aβ production and can thereby initiate or contribute to AD pathology [82, 88–90]. Moreover, the impaired Aβ clearance from the brain resulting in Aβ deposition will even more impair the brain's function [89, 90]. Therefore, vascular dysfunction, including CAA, is now considered as an important initiator/contributor to the development of AD pathology, highlighting the importance of studying therapeutical targets for AD that improve vascular functioning.

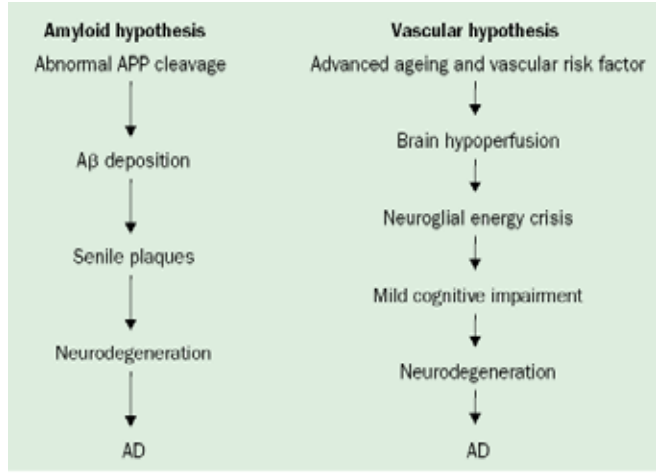


Figure 2 The amyloid and vascular hypothesis leading to the development of AD. Modified from "De la Torre J, 2004. Lancet Neurol 3(3):184-90." [91].

7. Transglutaminases

Overview

Transglutaminases (TGs, EC 2.3.2.13) 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 [92]. Nine mammal TGs have been described, TG1-TG7, Factor XIIIa (FXIIIa) and the inactive Band 4.2 [92]. TG1, also known as the keratinocyte TG, TG3 and TG5 are expressed in the skin and important in terminal differentiation of keratinocytes and formation of the cornified cell envelope, a highly cross-linked layer of proteins in the skin that provides a physical and water barrier function [93–95]. TG4 is expressed in the prostate and involved in semen coagulation in rodents [92, 94]. The function of TG6 and TG7 is still largely unknown [92]. Band 4.2, or the ATP-binding erythrocyte membrane protein band 4.2, lacks enzymatic activity but is present among others in erythrocytes and important in cytoskeleton integrity [95]. The two remaining TGs, tissue transglutaminase or TG2 and the blood-derived Factor XIIIa, are described in more detail below as they are the main focus of this thesis.

Tissue transglutaminase (tTG) – structure and function

tTG or TG2 is a 78 kDa protein composed of four domains (Figure 3): an N-terminal β-sandwich (for fibronectin and integrin binding), the catalytic core important in cross-linking activity present in a α-helical structure and two β-barrels at the C-terminal [96]. The transamidating activity of the enzyme is regulated by the catalytic site in which Cys277, His335 and Asp358 play an essential role [97]. Upon calcium binding, a conformational change in the protein is induced (Figure 3), creating an open access to the active site, and thus inducing the transamidating activity. The calcium-induced transamidating activity is counteracted by binding of guanosine-5-triphosphate (GTP) to the enzyme [94]. tTG can also be regulated by the interaction of tTG with phospholipids as well as nitrosylation of the cysteine residue involved in activation and inhibition respectively [94]. After calcium activation, the active site cysteine Cys277 binds a protein-bound glutamine residue, resulting in the liberation of ammonia and the formation of a thioester intermediate between tTG and the glutamine bearing protein substrate. Accordingly, the thioester intermediate is attacked by a nucleophilic primary amine, either a small molecule amine such as putrescine or the ε-amino group of a protein-bound lysine residues [98]. This results in the formation of a relatively stable isopeptide bond. This reaction can induce a bridge, cross-link, between a lysine donor residue of one protein with an acceptor glutamine residue of another protein, creating a cross-link between two proteins (Figure 4). In contrast, if both a lysine donor and a glutamine acceptor are present within a protein, this cross-link induces an intramolecular bridge. tTG-catalysed intermolecular cross-links induce stable, rigid, and insoluble protein complexes [99], whereas intramolecular cross-links change the conformation of proteins. In addition to cross-linking, tTG can also deamidate proteins and have isopeptidase activity (Figure 4). To block tTG activity, several types of inhibitors have been developed and can be divided into three classes: competitive amine inhibitors, reversible inhibitors, and irreversible inhibitors, respectively. Amine inhibitors function by competing with other natural amine substrates for tTG in the transamidation reaction. tTG is therefore still active yet the isodipeptide cross-link is now formed between the natural glutamine substrate and the competitive amine inhibitor rather than between the natural glutamine substrate and natural amine substrates. Well-known competitive amine inhibitors are putrescine, cystamine, spermidine, histamine and cadaverine analogues like monodansylcadaverine [98]. Reversible tTG inhibitors inhibit substrate access to the active site of tTG, such as GTP that prevents the binding of calcium to its binding site on tTG. Irreversible tTG inhibitors act via blockade of enzyme activity by covalently modifying the catalytic site of the enzyme and thereby prevent substrate binding.

tTG-activity independent functions

tTG has also cross-link activity independent functions. By binding to GTP, tTG can function as a G protein in signal transduction for receptors such as the α1 adrenergic receptor and the oxytocin receptor. However, the physiological function of tTG as a G-protein is not completely clear yet [97]. In vitro kinase activity of tTG has been described as well, but in vivo relevance is not yet known [97]. In addition, tTG can be translocated to the cell membrane through a yet largely unknown mechanism, and mediate the interaction of β-integrins with fibronectin via tTG's fibronectin and integrin binding domain independent of the cross-link activity [96].

Roles of tTG in health and disease

tTG is ubiquitously expressed and mainly localised in the cytosol, but can also be present at the plasma membrane as well as in the nucleus. Some cell types e.g. endothelial cells and smooth muscle cells constitutively express tTG at high levels, however, in general, tTG inside the cell is inactive due to low calcium concentrations. tTG can be upregulated by specific signalling pathways involved in cellular stress or tissue damage. An important process when tTG is upregulated is during apoptosis where tTG cross-links intracellular proteins before the cell will be cleared by phagocytosis, thereby limiting the release of harmful cell components into the extracellular space [96, 97]. Inflammatory mediators such as cytokines, can also increase the expression of tTG which in turn activates NF-κB, thus leading to more inflammation [95].

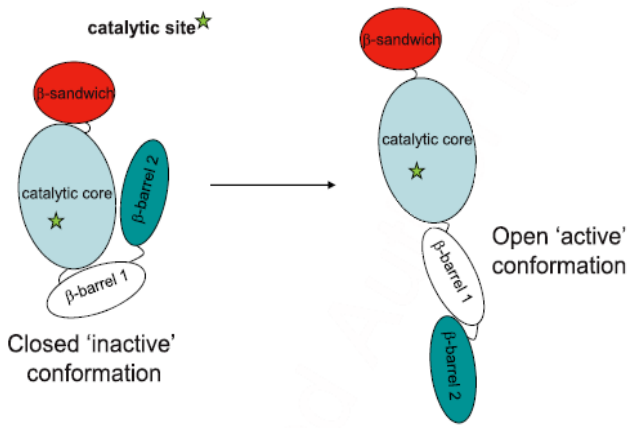


Figure 4 Inter- and intramolecular cross-linking of peptides induced by tissue transglutaminase (tTG) cross-linking activity. The main activity of tTG is to catalyse a calcium-dependent acyl transfer reaction between the γ-carboxamide group of a polypeptide-bound glutamine and the ε-amino group of a polypeptide bound lysine residue to form an ε-(γ-glutamyl)lysine isopeptide bond, also known as a cross-link. This reaction can occur either through the formation of an intermolecular cross-link between two proteins, or as an intramolecular cross-link within a protein. Figure from "Wilhelmus MMM et al., 2014. J Alzheimers Dis 42 Suppl 3:S289-303" [100].

An important mediator of tTG expression and inflammation is transforming growth factor beta (TGF-β) that can be activated by tTG and in turn induces tTG expression [94]. At the cell surface, binding of tTG to β-integrin and fibronectin facilitates cell adhesion and migration independent of the cross-link activity leading to downstream signalling pathways important in cell survival [97]. In addition, tTG can be transported out of the cell where it can cross-link extracellular matrix (ECM) proteins such as fibronectin and collagens. This process is important in matrix stabilisation and thereby wound healing and angiogenesis [95]. Furthermore, tTG-mediated ECM cross-linking is important in remodelling of blood vessel walls in response to changes in blood flow [101]. Of importance in vascular biology is the ability of nitric oxide (NO) species to inhibit tTG activity; however, as vascular NO diminishes with age, this can lead to increased tTG activity and ECM cross-linking leading to vascular stiffness and decreased vascular compliance [102]. In addition, it has been described that with age tTG and its activity are increased [102–104]. Also in other pathological conditions, such as rheumatoid arthritis, tissue fibrosis and scarring, increased levels of tTG are observed [92]. In kidney and lung fibrosis for example, increased levels of tTG lead to ECM cross-linking resulting in renal or lung failure due to fibrotic scar formation [105, 106]. In addition, tTG is also associated with cancer metastasis and celiac disease [97, 107] as well as neurodegenerative disorders such as AD (see below). To study the role of tTG in more detail, tTG knock-out mice are generated; however, these mice are viable and only display a mild phenotype including impaired wound healing and delayed

blood vessel wall remodelling, despite the many roles of tTG described. However, other TGs such as FXIIIa may compensate for the absence of tTG [108, 109].

Factor XIIIa – structure and function

The blood-derived clotting factor XIII (FXIII) is a tetramer that circulates in the blood and consists of two A subunits (FXIIIA) and two carrier B subunits (FXIIIB) and has a crucial role in the blood clotting cascade. The A subunits are derived from bone-marrow cells, whereas the B-subunits are produced in the liver. The B subunits protect the A subunits by formation of a FXIIIA2B2 tetramer in the blood plasma [110]. In cells, mainly from bone-marrow origin such as platelets, FXIIIA subunits can also be present without the B subunit, called cellular FXIIIA (cFXIIIA). FXIIIA has also been detected in megakaryotes, monocytes and macrophages, chondrocytes, osteoblasts and osteocytes [110]. Similar to tTG, the FXIIIA subunit consists of four domains, the β-sandwich domain, the catalytic core and two β-barrels plus a NH2-terminal activation peptide. In the catalytic core, the amino acid residues Cys314, His373 and Asp396 are of importance in the cross-linking of glutamine and lysine residues. Upon vascular trauma, the coagulation cascade is activated. In the last step, the inactive FXIII is activated by thrombin-mediated cleavage of the activation peptide together with a calcium-induced conformational change resulting in release of the B subunits and the formation of the active transglutaminase FXIIIa. Under high calcium concentrations, however, thrombin-mediated cleavage is not necessary for FXIII activation [110]. The active FXIIIa (83kDa) has a crucial role in catalysing the final step in the blood coagulation cascade by cross-linking fibrin molecules together into a tight blood clot that is difficult to degrade. FXIIIa also cross-links α2-plasmin inhibitor into the clot, thereby delaying clot degradation by plasmin. In addition FXIIIa has other substrates such as adhesive/matrix proteins (e.g. fibronectin) and cytoskeletal proteins (e.g. actin) and has also been implicated in wound healing by cross-linking ECM, migration and proliferation of monocytes/macrophages and angiogenesis. Furthermore, FXIIIa is important in maintaining pregnancy, reducing vascular permeability and bone development [110]. Pathophysiologically, FXIIIa has been linked to several vascular pathologies such as hypertension and atherosclerosis as well as ageing [57, 83, 111, 112]. For instance, increased levels of the FXIIIA-subunit are associated with age [113], and the expression of the FXIIIA-subunit is increased in both monocytes of hypertensive patients [114] and in plasma cells of patients with atherosclerosis in the coronary arteries [115].

8. TGs in the brain and in Alzheimer's disease

Thus far in the human brain, expression of TG1, tTG, TG3 and FXIIIa has been observed. TG1 and TG3 are present in neurons in different brain areas [116] and additional TG1 is found in astrocytes and microglia [117]. In AD brains, TG1 levels are increased [116] and TG1 colocalised with neurofibrillary tangles [117] suggesting a role for TG1 in NFT formation.

Immunohistochemical detection of FXIIIa was observed in the vasculature of both control and AD brains as well as in microglia in AD [118]. Interestingly, a Val34Leu polymorphism in the FXIIIA gene was associated with an increased risk on cerebral haemorrhages and AD [119, 120]. Furthermore, FXIIIa's main substrate fibrin(ogen) has been observed in AD and CAA [121]. However, whether FXIIIa is present in the AD hallmarks and plays a role in AD pathogenesis has not been investigated.
The role of tTG in AD has been studied most extensively, and evidence is mounting that tTG plays an important role in AD pathogenesis.

tTG in control and AD brains
tTG is present in neurons in many brain regions [116], in the vessel walls and in astrocytes [117]. In AD, both tTG levels and TG activity are elevated in the cortex compared to control patients [122]. Significantly elevated tTG levels have been reported in the cerebrospinal fluid of AD patients compared to controls [123]. Moreover, the level of ε-(γ-glutamyl)lysine isopeptides was significantly elevated in the cerebrospinal fluid of AD patients [124] and a correlation between these cross-links in grey matter and cognitive impairment in AD patients was observed [125]. In addition, immunohistochemical studies on postmortem tissue sections have shown that tTG is present in difuse and classic SPs [117, 126], as well as in NFTs [117]. More interestingly, our group reported the presence of TG-catalysed cross-links in both diffuse and classic SPs [117], indicating that tTG is not only present but also catalytically active within these lesions. Together these data point towards a role for tTG in SPs formation and/or stabilisation [117]. In CAA, tTG and its cross-links did not colocalise with the Aβ deposition itself, but were present in the luminal and abluminal layer of the vessel wall surrounding the Aβ deposition [117], thus hinting towards different roles of tTG in SPs and CAA. Together, the association of tTG with the pathological hallmarks of AD suggests an important role of tTG in AD pathogenesis possibly by the cross-linking of Aβ and tau and/or other pathology-associated proteins.

tTG-catalysed cross-linking of Aβ and Aβ-associated proteins
The above-described immunohistochemical findings suggest that tTG may be key in the development of AD perhaps by the direct interaction with and/or modification of Aβ and tau. Indeed, in vitro data provide proof that tTG is able to affect both tau [127] and Aβ aggregation. In early studies, both wild-type $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}40}$ with the Dutch mutation (Glu22 to Gln) were found to be good substrates for tTG cross-linking [128]. Moreover, tTG-catalysed cross-linking resulted in the formation of Aβ oligomers [128]. In addition, APP is a substrate for tTG-catalysed cross-linking resulting in APP dimers and multimers [129], and tTG also induces intramolecular cross-links in Aβ itself [130]. Intriguingly, tTG-catalysed intermolecular cross-linking of Aβ induces the formation of stable Aβ oligomers that are resistant towards Aβ degrading enzymes, in particular insulin degrading enzyme and neprilysin [47]. In addition, tTG-catalysed cross-linking of Aβ lowers its oligomerisation threshold for self aggregation, suggesting that tTG is capable of driving the aggregation process of Aβ at physiological Aβ levels. Furthermore, these tTG-mediated Aβ oligomers and protofibrils are toxic in that they inhibit long term potentiation in the CA1 region of the hippocampus [47]. Earlier work on the effects of tTG-catalysed cross-linking of Aβ showed that when tTG activity is blocked in cultured neuroblastoma cells, Aβ-induced cell death is reduced, whereas induction of tTG enhances Aβ-driven neurotoxicity [131]. Moreover, $A\beta_{1\text{-}42}$ treatment of monocytes induces tTG expression in vitro [132]. Together, these data indicate that tTG is a likely candidate responsible for initiating the Aβ cascade in AD brains by the formation of stable Aβ dimers, oligomers, and protofibrils.
Also indirectly, tTG may influence the Aβ cascade i.e. via interaction with Aβ-chaperones. Interestingly, family members of the Aβ-chaperone ApoE, ApoA-I, ApoA-II, ApoB and ApoC-I are known substrates for tTG-catalysed cross-linking leading to multimerisation of the apolipoproteins [133, 134]. As both tTG and ApoE are observed in the Aβ depositions in AD, it would be worthwhile to study whether ApoE itself is a tTG substrate and whether this may influence the Aβ chaperone function of ApoE.

tTG in vascular alterations in CAA
In CAA, tTG did not colocalise with the Aβ deposition but was present in two halos surrounding the Aβ deposition [117]. In addition, resembling tTG staining, the distribution and expression of several ECM proteins, tTG substrates, are altered [23, 24]. Thus, as tTG is important in ECM remodelling in the vessel wall [101], it may play a role in ECM changes and remodelling in CAA. This could in turn affect the composition and function of the vessel wall as well as the Aβ clearance via the ISF drainage alongside the vessel wall.

9. Aims and outline of the thesis
CAA, one of the key hallmarks of AD, results in progressive disruption of the cerebral vessel wall and plays an important role in the disease progression during AD. Unfortunately, mechanisms underlying CAA remain largely unknown. Previous work of our group provided first evidence of a role for tTG in the pathogenesis of CAA.

As a follow up to these initial results, the aims of the studies described in this thesis were:
1. To gain more insight into the distribution pattern of both tTG and its activity, as well as its cellular source in CAA. In addition, to investigate possible colocalisation of tTG and its in situ activity with ECM proteins in CAA (Chapter 2)
2. To investigate the distribution pattern of FXIIIa and its in situ activity in CAA, and study both the interaction of FXIIIa with Aβ and whether Aβ is a substrate for FXIIIa-catalysed cross-linking in vitro (Chapter 3)
3. To study the interaction of tTG with ApoE and to investigate the consequences of tTG-catalysed cross-linking on ApoE's protective role in Aβ-mediated cytotoxicity towards cerebrovascular cells (Chapter 4)

4. To investigate the suitability of AD mouse models to study the role of tTG in CAA (Chapter 5)

Outline thesis

In Chapter 2 we determined the distribution pattern of tTG and tTG activity in post-mortem brain tissue of both AD and HCHWA-D cases. In addition, we identified the cellular source of tTG as well as the colocalisation of tTG with ECM proteins in CAA. Surprisingly, however, we found that, in contrast to SPs, tTG protein and its cross-linked products did not colocalise with the actual Aβ deposition in CAA, whereas in situ activation of endogenous (t)TG in the post-mortem tissue demonstrated clear colocalisation with the deposited Aβ in CAA. These findings hinted 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, together with its activator thrombin in AD, especially in CAA. In addition we investigated in vitro if FXIIIa binds Aβ and whether Aβ could act as a substrate for FXIIIa-catalysed cross-linking.

The above-described chapters indicate that in CAA tTG activity might not only be involved in the cross-linking of Aβ, but also with other Aβ-associated proteins, known as Aβ chaperones. One of the major Aβ chaperones known to be involved in the pathogenesis of CAA is the AD risk factor ApoE. Interestingly, other apolipoproteins are already known to be substrates of TGs. Therefore, in Chapter 4 we questioned whether ApoE is an in vitro substrate for tTG-catalysed cross-linking. In addition, given the protective role of ApoE in Aβ-mediated cytotoxicity towards smooth muscle cell in CAA, we studied the effect of ApoE cross-linking on its protective activity to counteract Aβ-mediated toxicity in primary human cerebral smooth muscle cells.

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 therefore, we investigated the distribution pattern of both tTG and its activity in two well-known AD mouse models. For this purpose, we used the APPswe/PS1ΔE9 (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.

In Chapter 6 the results of this thesis are summarised and discussed, and possible directions for future research are given.

References

1. Alzheimer A (1907) Uber eine eigenartige Erkrankung der Hirnrinde. Allg Zeitschrife Psychiatr 64:146–148.
2. Förstl H, Kurz A (1999) Clinical features of Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci 249:288–90.
3. (2014) 2014 Alzheimer's disease facts and figures. Alzheimer's Dement 10:e47–e92. doi: 10.1016/j.jalz.2014.02.001
4. Dubois B, Feldman HH, Jacova C, et al. (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6:734–46. doi: 10.1016/S1474-4422(07)70178-3
5. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741–66.
6. Hyman BT, Phelps CH, Beach TG, et al. (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 8:1–13. doi: 10.1016/j.jalz.2011.10.007
7. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239–259.
8. Lukiw W (2012) Amyloid beta (Aβ) peptide modulators and other current treatment strategies for Alzheimer's disease (AD). Expert Opin Emerg Drugs 17:43–60.
9. Selkoe D (1991) The molecular pathology of Alzheimer's disease. Neuron 6:487–498.
10. Yoshida M (2006) Cellular tau pathology and immunohistochemical study of tau isoforms in sporadic tauopathies. Neuropathology 26:457–70.
11. Arriagada P V, Growdon JH, Hedley-Whyte ET, Hyman BT (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 42:631–9.
12. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1:a006189. doi: 10.1101/cshperspect.a006189
13. Lee RM (1995) Morphology of cerebral arteries. Pharmacol Ther 66:149–73.
14. Attems J (2005) Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. Acta Neuropathol 110:345–59. doi: 10.1007/s00401-005-1074-9
15. Jellinger KA, Attems J (2006) Prevalence and impact of cerebrovascular pathology in Alzheimer's disease and parkinsonism. Acta Neurol Scand 114:38–46. doi: 10.1111/j.1600-0404.2006.00665.x
16. Attems J, Quass M, Jellinger K a, Lintner F (2007) Topographical distribution of cerebral amyloid angiopathy and its effect on cognitive decline are influenced by Alzheimer disease pathology. J Neurol Sci 257:49–55. doi: 10.1016/j.jns.2007.01.013
17. Keage H a D, Carare RO, Friedland RP, et al. (2009) Population studies of sporadic cerebral amyloid angiopathy and dementia: a systematic review. BMC Neurol 9:3. doi: 10.1186/1471-2377-9-3
18. Jellinger KA, Attems J (2005) Prevalence and pathogenic role of cerebrovascular lesions in Alzheimer disease. J Neurol Sci 229-230:37–41. doi: 10.1016/j.jns.2004.11.018
19. Thal DR, Ghebremedhin E, Orantes M, Wiestler OD (2003) Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. J Neuropathol Exp Neurol 62:1287–301.
20. Attems J, Jellinger K, Thal DR, Van Nostrand W (2011) Review: sporadic cerebral amyloid angiopathy. Neuropathol Appl Neurobiol 37:75–93. doi: 10.1111/j.1365-2990.2010.01137.x
21. Hartz AMS, Bauer B, Soldner ELB, et al. (2012) Amyloid-β contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. Stroke 43:514–23. doi: 10.1161/STROKEAHA.111.627562
22. Zipfel GJ, Han H, Ford AL, Lee J-M (2009) Cerebral amyloid angiopathy: progressive disruption of the neurovascular unit. Stroke 40:S16–9. doi: 10.1161/STROKEAHA.108.533174
23. Van Duinen SG, Maat-Schieman ML, Bruijn JA, et al. (1995) Cortical tissue of patients with hereditary cerebral hemorrhage with amyloidosis (Dutch) contains various extracellular matrix deposits. Lab Invest 73:183–9.
24. Zhang WW, Lempessi H, Olsson Y (1998) Amyloid angiopathy of the human brain: immunohistochemical studies using markers for components of extracellular matrix, smooth muscle actin and endothelial cells. Acta Neuropathol 96:558–63.
25. Richard E, Carrano A, Hoozemans JJ, et al. (2010) Characteristics of dyshoric capillary cerebral amyloid angiopathy. J Neuropathol Exp Neurol 69:1158–67. doi: 10.1097/NEN.0b013e3181fab558
26. Bruinsma IB, de Jager M, Carrano A, et al. (2011) Small heat shock proteins induce a cerebral inflammatory reaction. J Neurosci 31:11992–2000. doi: 10.1523/JNEUROSCI.0945-11.2011
27. Wang Y-J, Zhou H-D, Zhou X-F (2006) Clearance of amyloid-beta in Alzheimer's disease: progress, problems and perspectives. Drug Discov Today 11:931–8. doi: 10.1016/j.drudis.2006.08.004

28. Carare RO, Bernardes-Silva M, Newman T a, et al. (2008) Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol 34:131–44. doi: 10.1111/j.1365-2990.2007.00926.x
29. Weller RO, Djuanda E, Yow H-Y, Carare RO (2009) Lymphatic drainage of the brain and the pathophysiology of neurological disease. Acta Neuropathol 117:1–14. doi: 10.1007/s00401-008-0457-0
30. Schley D, Carare-Nnadi R, Please CP, et al. (2006) Mechanisms to explain the reverse perivascular transport of solutes out of the brain. J Theor Biol 238:962–74. doi: 10.1016/j.jtbi.2005.07.005
31. Weller R, Massey A (2000) Cerebral amyloid angiopathy: accumulation of Aβ in interstitial fluid drainage pathways in Alzheimer's disease. Ann N Y Acad Sci 903:110–117.
32. Weller RO, Subash M, Preston SD, et al. (2008) Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. Brain Pathol 18:253–66. doi: 10.1111/j.1750-3639.2008.00133.x
33. Greenwald SE (2007) Ageing of the conduit arteries. J Pathol 211:157–72. doi: 10.1002/path.2101
34. Shin HK, Jones PB, Garcia-Alloza M, et al. (2007) Age-dependent cerebrovascular dysfunction in a transgenic mouse model of cerebral amyloid angiopathy. Brain 130:2310–9. doi: 10.1093/brain/awm156
35. Hawkes C a, Härtig W, Kacza J, et al. (2011) Perivascular drainage of solutes is impaired in the ageing mouse brain and in the presence of cerebral amyloid angiopathy. Acta Neuropathol 121:431–43. doi: 10.1007/s00401-011-0801-7
36. Maat-Schieman M, Roos R, Duinen S Van (2005) Hereditary cerebral hemorrhage with amyloidosis of the Dutch type. Neuropathology 25:288–297.
37. Chow VW, Mattson MP, Wong PC, Gleichmann M (2010) An overview of APP processing enzymes and products. Neuromolecular Med 12:1–12. doi: 10.1007/s12017-009-8104-z
38. Haass C, Kaether C, Thinakaran G, Sisodia S (2012) Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2:a006270. doi: 10.1101/cshperspect.a006270
39. Walsh D, Hartley D, Kusumoto Y (1999) Amyloid β-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J Biol Chem 274:25945–25952.
40. Selkoe DJ (2005) Defining molecular targets to prevent Alzheimer disease. Arch Neurol 62:192–5. doi: 10.1001/archneur.62.2.192
41. Dahlgren KN, Manelli AM, Stine WB, et al. (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J Biol Chem 277:32046–53. doi: 10.1074/jbc.M201750200
42. Mc Donald JM, Savva GM, Brayne C, et al. (2010) The presence of sodium dodecyl sulphate-stable Abeta dimers is strongly associated with Alzheimer-type dementia. Brain 133:1328–41. doi: 10.1093/brain/awq065
43. Kumar S, Walter J (2011) Phosphorylation of amyloid beta (Aβ) peptides - a trigger for formation of toxic aggregates in Alzheimer's disease. Aging (Albany NY) 3:803–12.
44. Wilhelmus MMM, Waal RMW, Verbeek MM (2007) Heat Shock Proteins and Amateur Chaperones in Amyloid-Beta Accumulation and Clearance in Alzheimer's Disease. Mol Neurobiol 35:203–216. doi: 10.1007/s12035-007-0029-7
45. Jawhar S, Wirths O, Bayer TA (2011) Pyroglutamate amyloid-β (Aβ): a hatchet man in Alzheimer disease. J Biol Chem 286:38825–32. doi: 10.1074/jbc.R111.288308
46. Atwood CS, Martins RN, Smith MA, Perry G (2002) Senile plaque composition and posttranslational modification of amyloid-beta peptide and associated proteins. Peptides 23:1343–50.
47. Hartley DM, Zhao C, Speier AC, et al. (2008) Transglutaminase induces protofibril-like amyloid beta-protein assemblies that are protease-resistant and inhibit long-term potentiation. J Biol Chem 283:16790–800. doi: 10.1074/jbc.M802215200
48. Prelli F, Castano E, Glenner GG, Frangione B (1988) Differences Between Vascular and Plaque Core Amyloid in Alzheimer's Disease. J Neurochem 51:648–651. doi: 10.1111/j.1471-4159.1988.tb01087.x
49. Tekirian TL, Saido TC, Markesbery WR, et al. (1998) N-terminal heterogeneity of parenchymal and cerebrovascular Abeta deposits. J Neuropathol Exp Neurol 57:76–94.
50. Verbeek MM, Otte-Höller I, Veerhuis R, et al. (1998) Distribution of A beta-associated proteins in cerebrovascular amyloid of Alzheimer's disease. Acta Neuropathol 96:628–36.
51. Rogers J, Strohmeyer R, Kovelowski CJ, Li R (2002) Microglia and inflammatory mechanisms in the clearance of amyloid beta peptide. Glia 40:260–9. doi: 10.1002/glia.10153
52. Nielsen HM, Veerhuis R, Holmqvist B, Janciauskiene S (2009) Binding and uptake of A beta1-42 by primary human astrocytes in vitro. Glia 57:978–88. doi: 10.1002/glia.20822

53. Holtzman DM, Herz J, Bu G (2012) Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb Perspect Med 2:a006312. doi: 10.1101/cshperspect.a006312
54. Deane R, Sagare A, Zlokovic B (2008) The role of the cell surface LRP and soluble LRP in blood-brain barrier AÎ2 clearance in Alzheimer's disease. Curr Pharm Des 14:1601–1605.
55. Owen JB, Sultana R, Aluise CD, et al. (2010) Oxidative modification to LDL receptor-related protein 1 in hippocampus from subjects with Alzheimer disease: implications for Aβ accumulation in AD brain. Free Radic Biol Med 49:1798–803. doi: 10.1016/j.freeradbiomed.2010.09.013
56. Weller RO, Preston SD, Subash M, Carare RO (2009) Cerebral amyloid angiopathy in the aetiology and immunotherapy of Alzheimer disease. Alzheimers Res Ther 1:6. doi: 10.1186/alzrt6
57. Mayeux R, Stern Y (2012) Epidemiology of Alzheimer disease. Cold Spring Harb Perspect Med. doi: 10.1101/cshperspect.a006239
58. Obulesu M, Somashekhar R, Venu R (2011) Genetics of Alzheimer's disease: an insight into presenilins and apolipoprotein E instigated neurodegeneration. Int J Neurosci 121:229–36. doi: 10.3109/00207454.2010.551432
59. Thal DR, Ghebremedhin E, Rüb U, et al. (2002) Two types of sporadic cerebral amyloid angiopathy. J Neuropathol Exp Neurol 61:282–93.
60. Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622–30.
61. Strittmatter WJ, Saunders AM, Schmechel D, et al. (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A 90:1977–81.
62. Wilhelmus MMM, Otte-Höller I, van Triel JJJ, et al. (2007) Lipoprotein receptor-related protein-1 mediates amyloid-beta-mediated cell death of cerebrovascular cells. Am J Pathol 171:1989–99. doi: 10.2353/ajpath.2007.070050
63. Namba Y, Tomonaga M, Kawasaki H, et al. (1991) Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res 541:163–6.
64. Wisniewski T, Frangione B (1992) Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 135:235–8.
65. LaDu MJ, Falduto MT, Manelli AM, et al. (1994) Isoform-specific binding of apolipoprotein E to beta-amyloid. J Biol Chem 269:23403–6.
66. Strittmatter WJ, Weisgraber KH, Huang DY, et al. (1993) Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci U S A 90:8098–102.
67. Koistinaho M, Lin S, Wu X, et al. (2004) Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. Nat Med 10:719–26. doi: 10.1038/nm1058
68. Wisniewski T, Castaño EM, Golabek A, et al. (1994) Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. Am J Pathol 145:1030–5.
69. Sanan DA, Weisgraber KH, Russell SJ, et al. (1994) Apolipoprotein E associates with beta amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. J Clin Invest 94:860–9. doi: 10.1172/JCI117407
70. Premkumar DR, Cohen DL, Hedera P, et al. (1996) Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. Am J Pathol 148:2083–95.
71. Schmechel DE, Saunders AM, Strittmatter WJ, et al. (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci U S A 90:9649–53.
72. Bales KR, Verina T, Cummins DJ, et al. (1999) Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 96:15233–8.
73. Holtzman DM, Bales KR, Tenkova T, et al. (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 97:2892–7. doi: 10.1073/pnas.050004797
74. Bruinsma IB, Wilhelmus MMM, Kox M, et al. (2010) Apolipoprotein E protects cultured pericytes and astrocytes from D-Abeta(1-40)-mediated cell death. Brain Res 1315:169–80. doi: 10.1016/j.brainres.2009.12.039
75. Wilhelmus MMM, Otte-Höller I, Davis J, et al. (2005) Apolipoprotein E genotype regulates amyloid-beta cytotoxicity. J Neurosci 25:3621–7. doi: 10.1523/JNEUROSCI.4213-04.2005
76. Verbeek MM, Van Nostrand WE, Otte-Höller I, et al. (2000) Amyloid-beta-induced degeneration of human brain pericytes is dependent on the apolipoprotein E genotype. Ann N Y Acad Sci 903:187–99.

77. Altman R, Rutledge JC (2010) The vascular contribution to Alzheimer's disease. Clin Sci (Lond) 119:407–21. doi: 10.1042/CS20100094
78. Dickstein DL, Walsh J, Brautigam H, et al. (2010) Role of vascular risk factors and vascular dysfunction in Alzheimer's disease. Mt Sinai J Med 77:82–102. doi: 10.1002/MSJ
79. Vagelatos NT, Eslick GD (2013) Type 2 Diabetes as a Risk Factor for Alzheimer's Disease: The Confounders, Interactions, and Neuropathology Associated With This Relationship. Epidemiol Rev. doi: 10.1093/epirev/mxs012
80. Kalaria R (1996) Cerebral vessels in ageing and Alzheimer's disease. Pharmacol Ther 72:193–214.
81. Kalaria RN (1999) The Blood-Brain Barrier and Cerebrovascular Pathology in Alzheimer's Disease. Ann N Y Acad Sci 893:113–125. doi: 10.1111/j.1749-6632.1999.tb07821.x
82. Zlokovic B V (2005) Neurovascular mechanisms of Alzheimer's neurodegeneration. Trends Neurosci 28:202–8. doi: 10.1016/j.tins.2005.02.001
83. Breteler MM. (2000) Vascular risk factors for Alzheimer's disease: Neurobiol Aging 21:153–160. doi: 10.1016/S0197-4580(99)00110-4
84. Perlmutter LS (1994) Microvascular pathology and vascular basement membrane components in Alzheimer's disease. Mol Neurobiol 9:33–40. doi: 10.1007/BF02816103
85. Perlmutter LS (1990) Microangiopathy, the vascular basement membrane and Alzheimer's disease: a review. Brain Res Bull 24:677–686. doi: 10.1016/0361-9230(90)90007-M
86. Kalaria RN, Pax AB (1995) Increased collagen content of cerebral microvessels in Alzheimer's disease. Brain Res 705:349–352. doi: 10.1016/0006-8993(95)01250-8
87. Farkas E, Jong G De (2000) Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease. Acta Neuropathol 100:395–402.
88. Kalaria RN (2010) Vascular basis for brain degeneration: faltering controls and risk factors for dementia. Nutr Rev 68 Suppl 2:S74–87. doi: 10.1111/j.1753-4887.2010.00352.x
89. Zlokovic B V (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 12:723–38. doi: 10.1038/nrn3114
90. Sagare AP, Bell RD, Zlokovic B V (2012) Neurovascular Dysfunction and Faulty Amyloid β-Peptide Clearance in Alzheimer Disease. Cold Spring Harb Perspect Med. doi: 10.1101/cshperspect.a011452
91. De la Torre JC (2004) Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neurol 3:184–90. doi: 10.1016/S1474-4422(04)00683-0
92. Lorand L, Graham RM (2003) Transglutaminases: crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol 4:140–56. doi: 10.1038/nrm1014
93. Eckert RL, Sturniolo MT, Broome A-M, et al. (2005) Transglutaminase function in epidermis. J Invest Dermatol 124:481–92. doi: 10.1111/j.0022-202X.2005.23627.x
94. Griffin M, Casadio R, Bergamini C (2002) Transglutaminases: nature's biological glues. Biochem J 396:377–396.
95. Iismaa S, Mearns B (2009) Transglutaminases and disease: lessons from genetically engineered mouse models and inherited disorders. Physiol Rev 89:991–1023. doi: 10.1152/physrev.00044.2008.
96. Fesus L, Piacentini M (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. Trends Biochem Sci 27:534–539.
97. Gundemir S, Colak G, Tucholski J, Johnson GVW (2012) Transglutaminase 2: a molecular Swiss army knife. Biochim Biophys Acta 1823:406–19. doi: 10.1016/j.bbamcr.2011.09.012
98. Lorand L, Conrad SM (1984) Transglutaminases. Mol Cell Biochem 58:9–35.
99. Folk JE, Finlayson JS (1977) The epsilon-(gamma-glutamyl)lysine crosslink and the catalytic role of transglutaminases. Adv Protein Chem 31:1–133.
100. Wilhelmus MMM, de Jager M, Bakker ENTP, Drukarch B (2014) Tissue Transglutaminase in Alzheimer's Disease: Involvement in Pathogenesis and its Potential as a Therapeutic Target. J Alzheimers Dis. doi: 10.3233/JAD-132492
101. Bakker ENTP, Buus CL, Spaan J a E, et al. (2005) Small artery remodeling depends on tissue-type transglutaminase. Circ Res 96:119–26. doi: 10.1161/01.RES.0000151333.56089.66
102. Santhanam L, Tuday EC, Webb AK, et al. (2010) Decreased S-nitrosylation of tissue transglutaminase contributes to age-related increases in vascular stiffness. Circ Res 107:117–25. doi: 10.1161/CIRCRESAHA.109.215228
103. Lu T, Pan Y, Kao S-Y, et al. (2004) Gene regulation and DNA damage in the ageing human brain. Nature 429:883–91. doi: 10.1038/nature02661
104. Park SC, Yeo EJ, Han JA, et al. (1999) Aging process is accompanied by increase of transglutaminase C. J Gerontol A Biol Sci Med Sci 54:B78–83.

105. Johnson TS, Griffin M, Thomas GL, et al. (1997) The role of transglutaminase in the rat subtotal nephrectomy model of renal fibrosis. J Clin Invest 99:2950–60. doi: 10.1172/JCI119490
106. Olsen KC, Sapinoro RE, Kottmann RM, et al. (2011) Transglutaminase 2 and its role in pulmonary fibrosis. Am J Respir Crit Care Med 184:699–707. doi: 10.1164/rccm.201101-0013OC
107. Lindfors K, Kaukinen K, Mäki M (2009) A role for anti-transglutaminase 2 autoantibodies in the pathogenesis of coeliac disease? Amino Acids 36:685–91. doi: 10.1007/s00726-008-0127-5
108. Bakker ENTP, Pistea A, VanBavel E (2008) Transglutaminases in vascular biology: relevance for vascular remodeling and atherosclerosis. J Vasc Res 45:271–8. doi: 10.1159/000113599
109. Bakker ENTP, Pistea A, Spaan J a E, et al. (2006) Flow-dependent remodeling of small arteries in mice deficient for tissue-type transglutaminase: possible compensation by macrophage-derived factor XIII. Circ Res 99:86–92. doi: 10.1161/01.RES.0000229657.83816.a7
110. Muszbek L, Bereczky Z, Bagoly Z, et al. (2011) Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. Physiol Rev 91:931–72. doi: 10.1152/physrev.00016.2010
111. Iadecola C (2010) The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta Neuropathol 120:287–96. doi: 10.1007/s00401-010-0718-6
112. Polidori MC, Pientka L, Mecocci P (2012) A review of the major vascular risk factors related to Alzheimer's disease. J Alzheimers Dis 32:521–30. doi: 10.3233/JAD-2012-120871
113. Ariëns RA, Kohler HP, Mansfield MW, Grant PJ (1999) Subunit antigen and activity levels of blood coagulation factor XIII in healthy individuals. Relation to sex, age, smoking, and hypertension. Arterioscler Thromb Vasc Biol 19:2012–6.
114. AbdAlla S, Lother H, Langer A, et al. (2004) Factor XIIIA transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis. Cell 119:343–54. doi: 10.1016/j.cell.2004.10.006
115. Ma J, Liew C-C (2003) Gene profiling identifies secreted protein transcripts from peripheral blood cells in coronary artery disease. J Mol Cell Cardiol 35:993–8.
116. Kim S, Grant P, Lee J (1999) Differential expression of multiple transglutaminases in human brain. J Biol Chem 274:30715–30721.
117. Wilhelmus MMM, Grunberg SCS, Bol JGJM, et al. (2009) Transglutaminases and transglutaminase-catalyzed cross-links colocalize with the pathological lesions in Alzheimer's disease brain. Brain Pathol 19:612–22. doi: 10.1111/j.1750-3639.2008.00197.x
118. Akiyama H, Kondo H, Ikeda K, Arai T (1995) Immunohistochemical detection of coagulation factor XIIIa in postmortem human brain tissue. Neurosci Lett 202:29–32.
119. Catto AJ, Kohler HP, Bannan S, et al. (1998) Factor XIII Val 34 Leu: a novel association with primary intracerebral hemorrhage. Stroke 29:813–6.
120. Gerardino L, Papaleo P, Flex A, et al. (2006) Coagulation factor XIII Val34Leu gene polymorphism and Alzheimer's disease. Neurol Res 28:807–9. doi: 10.1179/016164106X110454
121. Cortes-Canteli M, Paul J, Norris EH, et al. (2010) Fibrinogen and β-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. Neuron 66:695–709. doi: 10.1016/j.neuron.2010.05.014.Fibrinogen
122. Johnson GV., Cox TM, Lockhart JP, et al. (1997) Transglutaminase activity is increased in Alzheimer's disease brain. Brain Res 751:323–329. doi: 10.1016/S0006-8993(96)01431-X
123. Bonelli RM, Aschoff A, Niederwieser G, et al. (2002) Cerebrospinal Fluid Tissue Transglutaminase as a Biochemical Marker for Alzheimer's Disease. Neurobiol Dis 11:106–110. doi: 10.1006/nbdi.2002.0535
124. Nemes Z, Fésüs L, Egerházi a, et al. (2001) N(epsilon)(gamma-glutamyl)lysine in cerebrospinal fluid marks Alzheimer type and vascular dementia. Neurobiol Aging 22:403–6.
125. Wang D-S, Uchikado H, Bennett D a, et al. (2008) Cognitive performance correlates with cortical isopeptide immunoreactivity as well as Alzheimer type pathology. J Alzheimers Dis 13:53–66.
126. Zhang W, Johnson BR, Suri DE, et al. (1998) Immunohistochemical demonstration of tissue transglutaminase in amyloid plaques. Acta Neuropathol 96:395–400.
127. Dudek SM, Johnson G V (1993) Transglutaminase catalyzes the formation of sodium dodecyl sulfate-insoluble, Alz-50-reactive polymers of tau. J Neurochem 61:1159–62.
128. Dudek SM, Johnson G V (1994) Transglutaminase facilitates the formation of polymers of the beta-amyloid peptide. Brain Res 651:129–33.
129. Ho GJ, Gregory EJ, Smirnova I V, et al. (1994) Cross-linking of beta-amyloid protein precursor catalyzed by tissue transglutaminase. FEBS Lett 349:151–4.
130. Schmid AW, Condemi E, Tuchscherer G, et al. (2011) Tissue transglutaminase-mediated glutamine deamidation of beta-amyloid peptide increases peptide solubility, whereas enzymatic cross-linking and peptide fragmentation may serve as molecular triggers for rapid peptide aggregation. J Biol Chem 286:12172–88. doi: 10.1074/jbc.M110.176149

131. Wakshlag JJ, Antonyak M a, Boehm JE, et al. (2006) Effects of tissue transglutaminase on beta -amyloid1-42-induced apoptosis. Protein J 25:83–94. doi: 10.1007/s10930-006-0009-1
132. Currò M, Ferlazzo N, Condello S, et al. (2010) Transglutaminase 2 silencing reduced the beta-amyloid-effects on the activation of human THP-1 cells. Amino Acids 39:1427–33. doi: 10.1007/s00726-010-0605-4
133. Borth W, Chang V, Bishop P, Harpel PC (1991) Lipoprotein (a) is a substrate for factor XIIIa and tissue transglutaminase. J Biol Chem 266:18149–53.
134. Cocuzzi E, Piacentini M, Beninati S, Chung SI (1990) Post-translational modification of apolipoprotein B by transglutaminases. Biochem J 265:707–13.