Molecular pathology of hereditary cerebral hemorrhage with amyloidosis-Dutch type

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Chapter 2

Amyloid β in hereditary cerebral hemorrhage with amyloidosis-Dutch type

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Abstract

Hereditary cerebral haemorrhage with amyloidosis–Dutch type is an autosomal dominant hereditary disease caused by a point mutation in the amyloid precursor protein gene on chromosome 21. The mutation causes an amino acid substitution at codon 693 (E22Q), the 'Dutch mutation'. Amyloid β, the product after cleavage of the Amyloid Precursor Protein, is secreted into the extracellular space. The Dutch mutation leads to altered amyloid β cleavage and secretion, enhanced aggregation properties, higher proteolysis resistance, lowered brain efflux transporters affinity and enhanced cell surfaces binding. All this results in amyloid β accumulation in cerebral vessel walls, causing cell death and vessel wall integrity loss, making cerebral vessel walls in hereditary cerebral haemorrhage with amyloidosis–Dutch type more prone to rupture and obstruction, leading to haemorrhages and infarcts. Studying effects of altered amyloid β metabolism due to mutations like the 'Dutch' provides us with a better understanding of amyloid β toxicity, also in other amyloid β diseases like sporadic Cerebral Amyloid Angiopathy and Alzheimer's Disease.

Introduction

Hereditary cerebral haemorrhage with amyloidosis–Dutch type (HCHWA-D) is an autosomal dominant hereditary disease caused by a mutation in the Amyloid Precursor Protein (APP) gene on chromosome 21 (1). HCHWA-D patients suffer from haemorrhagic strokes, infarcts and vascular dementia (2). Life expectancy is reduced: the first stroke occurs between the ages of 40 and 65 and is fatal in two thirds of the patients (3,4). The patients that survive the first haemorrhage suffer from recurrent strokes (4).

HCHWA-D is a rare disease and has only been found in three founder families in the Dutch coastal villages of Katwijk and Scheveningen (3,4). A rough estimate is that likely 400-500 persons are at risk in multi-generational offspring families, but no clear data are available at this moment. An affected family described in Western Australia originates from Katwijk (5).

In HCHWA-D, amyloid beta (Aβ) accumulates in the cerebral vessels (cerebral amyloid angiopathy; CAA). Especially the meningeal arteries and the cerebrocortical arterioles are affected. The amount of CAA, quantified *ex vivo* using computerized morphometry, is strongly associated with the presence of dementia in HCHWA-D, and this is independent of parenchymal plaque density and age (6). CAA can also be found in at least 80% of Alzheimer's disease (AD) patients (7). However, in contrast to AD, the presence of intraneuronal neurofibrillary tangles is low in HCHWA-D and does not correlate with dementia (6).

Aβ results from a cascade of proteolytic cleavages of the APP gene product. The most common Aβ isoforms contain either 40 (Aβ-40) or 42 amino acids (Aβ-42), depending on the site of γ-secretase cleavage. In comparison with Aβ-40, Aβ-42 contains more hydrophobic residues and therefore is more prone to aggregation (8). Alternative cleavage of APP within the Aβ fragment by α-secretase prevents the formation of Aβ and leads to the release of the neuroprotective secreted APP (sAPP) (9). After processing of APP, Aβ is released into the extracellular space (10) where it can form parenchymal plaques or accumulate as vascular deposits in the cerebral vessels causing amyloid angiopathy (11). Brains of HCHWA-D patients show few parenchymal plaques, but multiple vascular Aβ deposits.

In this review, it will be described how the Aβ mutation of HCHWA-D patients modifies Aβ properties regarding aggregation, binding to cerebral vessel wall cells, interplay with extracellular matrix, proteolysis and clearance and how these altered characteristics lead to HCHWA-D pathogenesis.

Genetics of HCHWA

Three types of HCHWA are known: Dutch, Icelandic and Italian. The Icelandic type is caused by a mutation in the Cystatin C gene (*CST3*) on chromosome 20 (12). The Dutch and Italian are caused by single point mutations at the Aβ region of *APP* on chromosome 21 (1,13). There are more known mutations in the APP gene, inside and outside the Aβ region. The mutations in the Aβ region mainly lead to an AD phenotype, but a mixed pathology (AD and CAA) is also described in patients with the Flemish, Arctic, Iowa, Italian II and II mutations (**Table 1**). The above described mutations are inherited in an autosomal dominant fashion. Recessive pathogenic mutations within the Aβ region of *APP* are also known, like the Japanese mutation (a deletion of glutamine at Aβ's position 22), and the valine substitution for alanine at position 2 (14,15). The point mutation in HCHWA-D, a cytosine for guanine substitution at codon 693 of *APP*, causes an amino acid substitution of glutamine for glutamic acid at position 22 of the Aβ region of APP. The deletion of the glutamine or the substitution of the glutamine for glycine, as present in the Japanese and Arctic types, do not cause the characteristic angiopathy of HCHWA, suggesting that the exact nature of the amino acid substitution is essential for HCHWA pathogenesis. The Dutch (Glu693Gln) and Italian (Glu693Lys) amino acid substitutions lead to a change of charge at Aβ's position 22. These changes in charge specifically enable Aβ40 to bind to the surface of cerebrovascular smooth muscle cells (SMC) and form amyloid fibrils (16). Moreover, the Dutch mutation makes the Aβ peptide more resistant to neprilysin-catalysed proteolysis, probably by interfering with the peptide's backbone spatial fitting into neprilysin's catalytic pocket, thereby increasing Aβ's half-life (17).

The Dutch mutation is located near the α-secretase cleavage site of APP (**Figure 1**), which lies between the lysine at position 16 and the leucine at position 17 of Aβ (9). Patients with HCHWA-D have reduced levels of sAPP in the cerebral spinal fluid (CSF) (18), which may be caused by an altered processing of the precursor protein or alternatively by increased binding of APP to the vessel wall, as described later. Furthermore, the location of the Aβ mutation appears to influence aggregation properties. When studying the Italian, Dutch, Arctic and Iowa mutations in Aβ42 monomers, it was shown that the Italian and Dutch mutation made the Aβ42 monomer aggregate quicker than wild type Aβ42, while the Artic and Iowa mutations made the Aβ42 monomer aggregate slightly slower than wild type Aβ42 (19). This difference in aggregation was attributed to differences in helix propensities in residues 20-23 caused by the mutations: Italian and Dutch mutation increase helix propensity, while Artic and Iowa mutations slightly decrease helix propensity. It is thought that α-helical intermediates play an important role in amyloid oligomerization (19).

Position Aβ	Mutation	Phenotype	Alias
2	Ala673Thr	Not pathogenic	
2	Ala673Val*	AD	
6	His677Arg	Not pathogenic	
7	Asp678Asn	AD	
7	Aso678His[1]	Dementia + CAA	
11	Glu682Lys	AD	
16	Lys687Asp[2]	AD	
21	Ala692Gly	AD (CAA)	Flemish
22	Glu693Lys	HCHWA	Italian
22	Glu693Gln	HCHWA	Dutch
22	Glu693Gly	AD (CAA)	Arctic
22	Glu693del*	AD	Japanese
23	Asp694Asn	AD (CAA)	Iowa
34	Leu705Val	CAA	Italian II
37	Gly708	Not pathogenic	
42	Ala713Thr	AD (CAA)	Italian III
42	Ala713Val	Not pathogenic	
43	Thr714Ala	AD/Epilepsy	Iranian
43	Thr714Ile	AD	Austrian

Table 1. Mutations within the Aβ region of *APP* Aβ= Amyloid β; *= recessive mutation; AD= Alzheimer's disease; CAA= Cerebral Amyloid Angiopathy. The mutations are shown with the affected amino acid, the affected *APP* codon and, if applicable, the amino acid alteration resulting from the mutation. Mutations were found using the Alzheimer Disease & Frontotemporal Dementia Mutation Database (80) and PubMed: [1](81); [2](82).

Cerebral amyloid plaques in HCHWA-D

Aβ deposition in AD is mainly located in the brain parenchyma in the form of plaques. In HCHWA-D, Aβ deposition is mainly found in the cerebral vessel walls, but also some parenchymal Aβ deposition in the form of plaques is present, mainly in the form of 'diffuse' plaques, lacking an amyloid core as in AD (20).

Aβ peptides show different intermediate fibrillization states before plaques and vascular deposits are formed (21). The Aβ monomers are amphiphatic, with a hydrophylic N-terminal and a hydrophobic C-terminal, and are able to adopt different conformations: α-helices, β-sheets or random coils. After arrangement of dimers and trimers, unstable and toxic oligomers are formed. These oligomers contain up to 50 monomers. Subsequently, the oligomers assemble into protofibrils, which are the relatively flexible and rod-shaped precursors of the mature fibrils. Fibrils contain multiple protofibrils and are the main components of amyloid aggregates.

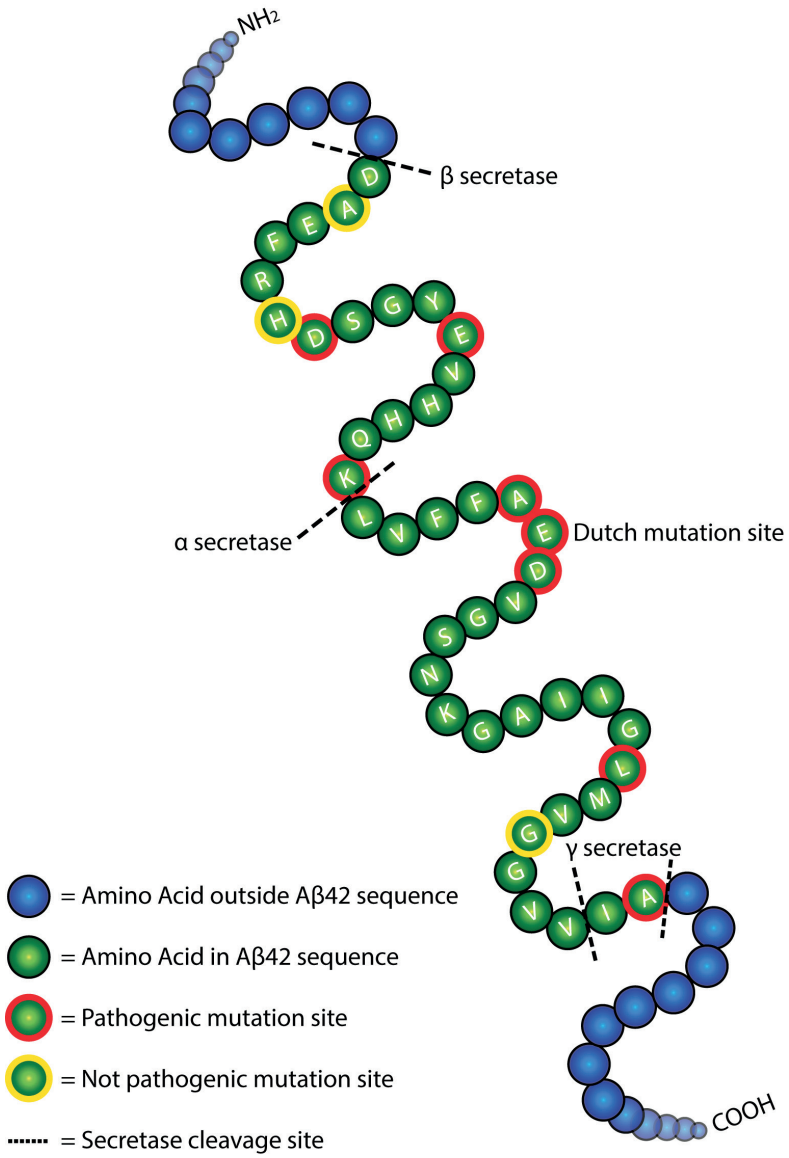

Figure 1: Location of known mutations and secretase cleavage sites in the amyloid beta sequence of the amyloid precursor protein

In the brain parenchyma, there are four different types of parenchymal plaques distinguishable in HCHWA-D: fine diffuse, dense diffuse, coarse and homogeneous. The morphology of these plaques was described by Maat-Schieman and her colleagues (20). Fine diffuse plaques are irregularly shaped, ill-defined, evenly stained, and show finely fibrous Aβ deposits. Dense diffuse plaques are either irregular, ill-defined or rounded and are stained unevenly. Coarse plaques are clusters of small, coarse and strongly staining deposits and homogeneous plaques are well-defined round shaped plaques. While all plaques show Aβ42 staining, Aβ40 staining is present in a small subset of dense diffuse and coarse plaques and in all homogeneous plaques. Only plaques containing Aβ40 harbour degenerating neurites that showed APP and ubiquitin staining. No tau is present in these degenerating neurites. In addition to the plaques, also clouds of Aβ42 were shown to be present throughout the cortex, except around Aβ containing arterioles (**Table 2**). In addition to ubiquitin, other proteins like amyloid-P, cystatin C and ApoE are known to co-aggregate with amyloid deposition in HCHWA-D (22).

In the initial stages of HCHWA-D, Aβ deposition in the form of clouds and fine diffuse plaques are present. With age, clouds disappear and plaque density increases from Aβ 40 negative fine diffuse to Aβ40 positive dense plaques (23). Electron microscopy examination showed that Aβ is non-fibrillar and plasma membrane bound initially, but when the plaques develop, amyloid fibrils accumulate (20). This development can be visualized with Congo red staining that shows increased fluorescent activity *ex vivo*. Non-fibrillar Aβ is assumed to be cleared by glial cells, thereby limiting the neurotoxic soluble form levels of Aβ in HCHWA-D patients' brains (23).

		Clouds	Plaques			
			Fine diffuse	Dense diffuse	Coarse	Homogeneous
Aβ						
	Aβ40	-	-	- to ++	- to ++	++
	Aβ42	+	+	++	++	++
DN						
	APP	-	-	- to +	- to +	±
	Ubiquitin	-	-	- to +	- to +	±
	Tau	-	-	-	-	-

Table 2. Staining intensities of parenchymal clouds and plaques Adapted from (19). Staining intensity: No staining -, few bundles ±, positive staining/small bundles + and strong positive staining/clusters ++. Aβ = Amyloid beta, DN = Degenerating Neurites.

Aβ isoforms in HCHWA-D vasculature

While Aβ42 is the main Aβ isoform in parenchymal plaques, Aβ40 is the main component of amyloid deposits in the cerebral vessels of HCHWA-D patients (24,25). Amino acid sequencing of amyloid that was isolated from leptomeningeal vascular walls showed that both mutated and wild type Aβ occurs in the vascular deposits of HCHWA-D patients (26). It has been suggested that it is especially the ratio of Aβ40 to Aβ42 that is important for vascular amyloid formation (27). Moreover, an important role for mutated Aβ42 has been proposed. In vascular amyloid of HCHWA-D, wild type and Dutch mutated Aβ40 peptides occur in a 1:1 ratio, while only the Dutch mutated Aβ42 and not the wild type Aβ42 has been detected, suggesting a possible role for Dutch mutated Aβ42 as a seed for the aggregation of Aβ40 (24). Importantly, all Aβ42 was oxidized at the methionine residue at position 35. The oxidation of Met35 of Aβ42 is known to slow down the rate of fibrillation and aggregation of Aβ42 (28). However, the Dutch mutation enables Aβ to fold into different shapes, thereby creating multiple ways to aggregate (28). In addition to Aβ40 and Aβ42, wild type Aβ37, wild type Aβ38 and Dutch mutated Aβ38 are also present in the vascular amyloid (24). Aβ37 and Aβ38 are less common isoforms of Aβ than Aβ40 and differ at the C-terminus.

It was shown that neuronal expression of APP with the Dutch mutation was sufficient to induce HCHWA pathology, i.e. CAA, smooth muscle cell degeneration and haemorrhages using a transgenic HCHWA-D mouse model. This indicates that neurons are the main source of Dutch Aβ in the cerebral vessels. Using this model it was also shown that the Dutch mutation leads to an increased Aβ40: Aβ42 ratio both in parenchymal and cerebrovascular amyloid deposits and Aβ40 was suggested to be inhibitory for parenchymal Aβ deposition (27). It was discovered in a guinea pig model of the Dutch mutation that Aβ40 accumulates around the blood vessels and in the brain due to a reduced clearance from the cerebrospinal fluid and impaired transport over the blood brain barrier, because of the lower affinity for central nervous system efflux transporters (29). Impaired clearance of Aβ was also shown in a mouse model with the Dutch and Iowa mutation: no detectable plasma Aβ but abundant Aβ deposits were present in cerebral vasculature (30). The double mutated Aβ shows a significant lower affinity to the low-density lipoprotein receptor-related protein 1 (LRP1), which is implicated in Aβ clearance, in comparison to wild type Aβ40 (31). Double mutated Aβ also seems to down-regulate LRP1 (31). However, in this model, it is not clear how each mutation affects the clearance.

In individuals with and without the Dutch mutation, Aβ40 plasma levels were similar (32). On the other hand, the plasma Aβ42 concentration of individuals with the Dutch mutation was significantly lower than in the

plasma of their family members that did not carry the mutation (32). Of the 22 individuals with the Dutch mutation, 7 were still asymptomatic. Plasma concentrations of Aβ40 and Aβ42 in HCHWA-D patients did not correlate with age or severity of the symptoms (32), which indicates that plasma Aβ does not play a major role in the pathology. It is important to note that the detection method used in this study was only appropriate for soluble Aβ. Because Dutch mutated Aβ42 aggregates more readily than wild type Aβ42, the detected decline of Aβ42 in plasma of individuals with the Dutch mutation could also be due to the lack of detection of aggregated Aβ42. However, reduced Aβ42 levels as a consequence of the Dutch mutation were confirmed by *in vitro* experiments that showed a decreased Aβ42 concentration in medium of cells with the Dutch mutation, while the Aβ40 concentration was unchanged (33).

Decreased Aβ42 levels in plasma of HCHWA-D patients and in the cell model with the Dutch mutation suggest that the ratio of Aβ40:Aβ42 is elevated in HCHWA-D as compared to healthy individuals. The importance of relatively lower Aβ42 concentrations in the pathophysiology of HCHWA-D was shown in animal studies. When increasing the Aβ42 expression in transgenic HCHWA-D mice by crossing them with Aβ42 overexpressing mice, amyloid deposits were redistributed from the cerebral vessels to the parenchyma (27,32).

The nature of the mutation within Aβ was shown to be crucial in the Aβ40:Aβ42 ratio in cell models of the Flemish and Arctic mutations, where an increase in Aβ42 was present (33). Because the locations of the Dutch, Flemish and Arctic mutations are comparable (Table 1), it is not the actual mutation location but the substitution to glycine that probably affects the Aβ40:Aβ42 ratio. However, the mechanism behind this altered Aβ40:Aβ42 ratio is still unknown. Interestingly, haemorrhages are uncommon, whereas parenchymal plaques are abundant in patients with the Flemish and Arctic type mutations (34,35). This supports the role of Aβ42 in amyloid accumulation localization, as suggested in animal studies.

Aβ fibril assembly at cell surfaces

Assembly of Aβ fibrils to cell surfaces is believed to be crucial in the loss of vessel wall integrity in HCHWA-D. The assembly of Aβ fibrils has been intensively studied. Both wild type and Dutch mutated Aβ40 did not substantially assemble into fibril sheets in solution of 25 μM Aβ40, which is the Aβ peptides concentration shown to evoke pathological responses in cerebrovascular smooth muscle cells. However, at the same concentration, but in the presence of cultured cerebrovascular smooth muscle cells, Dutch mutated Aβ40 did assemble in fibrils (36). This was not the case for wild type Aβ. So Dutch mutated Aβ40 fibril formation is facilitated in the vicinity of smooth muscle cells.

After Aβ fibrillation, sAPP is able to bind to the Aβ fibrils at the smooth muscle cell surface (37). The binding of APP leads to the presence of the Kunitz-type protease inhibitor (KPI) domain, which is part of most of the APP isoforms. The KPI domain inhibits coagulant factors XIa and IXa (38), and Aβ fibrils enhance the anticoagulant property of APP (39). As a consequence, an anticoagulant environment is created, leading to an increased chance of haemorrhages. The binding of sAPP prevents its efflux from the brain, and could thus explain the reduced levels of sAPP in the CSF in HCHWA-D patients (40).

Moreover, Aβ fibrillation activates an apoptotic pathway in the cerebrovascular smooth muscle cells, leading to cell death (37). The combination of the cell death and the anticoagulant environment induced by Aβ fibrils in the vessel wall are probably major contributors to the haemorrhages in HCHWA-D patients. Also, Dutch Aβ induces increased expression and activation of matrix metalloproteinase 2 (MMP-2) in smooth muscle cells and this is believed to contribute to the Dutch Aβ-induced cell death (41). MMPs are tissue remodelling enzymes and turnover basement membranes. Elevated MMP-2 is known to lead to blood brain barrier disruption and causes cerebral haemorrhage, thus the Dutch Aβ-induced MMP-2 activation and expression probably contributes to loss of vessel wall integrity and consequent haemorrhagic stroke (41).

In addition to smooth muscle cells, pericytes are also prone to surface Dutch type Aβ fibril formation. The pericytes are even more vulnerable to the Aβ-induced degeneration compared to the smooth muscle cells (42). Pericyte degeneration was shown to be dependent on Apolipoprotein E (ApoE) genotype. ApoE is known to be the major risk factor for AD, and carrying one or two ε4 alleles is associated with a dose-dependent increase in AD risk (43).

However, in a study of 36 carriers of the Dutch mutation and 10 related controls, the ApoE ε4 genotype did not influence the age of onset of HCHWA-D, the occurrence of dementia, number of strokes nor the age at death (44). Furthermore, no association between the ApoE ε4 allele and Aβ plasma levels was found in 22 HCHWA-D patients (32). In contrast with the clinical findings, cultures of human brain pericytes with an ε4/ε4 genotype showed more Dutch Aβ-induced cell death than cultures with other ApoE genotypes (45). It is not clear what causes this inconsistency between clinical and *in vitro* studies.

In endothelial cells *in vitro*, Aβ protofibrils and fibrils induce apoptosis, and these effects are significantly stronger for Dutch mutated Aβ than wild type Aβ (46). Thus Aβ fibril formation in the vessel wall leads to an anticoagulant environment and the degeneration of three different cell types in the cerebral vessel walls, leading to CAA. This CAA leads to the haemorrhages in HCHWA-D.

The role of extracellular matrix components in cerebral amyloid angiopathy

As discussed above, reduction of Aβ clearance through the vessel wall plays a role in Aβ accumulation in the vessel wall. A major characteristic of cerebral vessels is the blood brain barrier, which prevents certain molecules to pass through the vessel into the brain and *vice versa*. Extracellular matrix (ECM) properties in the vessel wall are important for this perivascular filter by forming and maintaining basement membranes. The basement membranes are important for regulating cell growth, differentiation and migration and consist of laminins, nidogens, collagen and heparan sulphate proteoglycans (HSPGs) (47). HSPGs co-localize with the vascular deposits in AD and HCHWA-D (48). HSPGs consist of sulphated glycosaminoglycan (GAG) side chains bound to a core protein (49). Heparin and heparan sulphate are GAGs with side chains showing high Aβ affinity (50). The sulphate moieties of the side chains modulate the aggregation (51). Heparin and heparan sulphate both increase the aggregation of Aβ40 with the Dutch mutation, but especially heparin is a very potent aggregation inducer. Moreover, heparin and heparan sulphate both inhibit the cytotoxicity of cerebrovascular cells that is induced by Dutch mutated Aβ40, probably because increased aggregation prevents interactions of toxic monomeric, oligomeric or prefibrillar species of Dutch mutated Aβ40 (51). So HSPGs are modulators of Aβ aggregation and inhibitors of Dutch mutated Aβ40 cytotoxicity.

There are differences in HSPG subtype expression between AD and HCHWA-D (48) that suggest a different role for these HSPG subtypes in the different disorders. Immunohistochemical examination of AD and HCHWA-D *post mortem* brain tissue showed that the HSPG subtype agrin specifically co-localised with the vascular Aβ40 deposits in HCHWA-D, a co-localization that is less frequent in AD. In contrast, another HSPG subtype, syndecan-2 is only present in vascular deposits in AD, but not in HCHWA-D (48). These results suggest that vascular deposits in AD and HCHWA-D arise *via* different mechanisms.

Interestingly, HSPG subtypes that are usually associated with vascular basement membranes were not found in CAA, while CAA associated HSPGs syndecan-2 and glypican-1 are not expressed by vascular cells (48). This indicates that implicated HSPGs are not produced by vascular cells but have other sources and travel towards the vascular wall.

A protein that co-localizes with ECM proteins in CAA is tissue transglutaminase (tTG) (52). tTG is an enzyme involved in posttranslational modifications of proteins, like covalently cross-linked proteins (53). It plays an important role in the remodelling of the ECM after tissue injury and cell stress (54). It is known that tTG mediates Aβ40 dimerization through covalent intermolecular cross-linking and thereby seeding aggregation

(55). In early stage CAA, tTG is increased in affected vessel walls and co-localizes with Aβ deposition. This tTG could originate from endothelial cells or smooth muscle cells around which the Aβ accumulates. In later stages, co-localization is absent and tTG encloses the Aβ deposition in an abluminal and a luminal halo as shown in **Figure 2** (56). The tTG in the abluminal halo is assumed to be produced by fibroblasts in leptomeningeal vessels or astrocytes in parenchymal vessels, while tTG in the luminal halo is produced by endothelial cells in all vessel types. Moreover, ECM components fibronectin and laminin colocalize with the tTG in the halos (56). The tTGs cross-link fibronectin and laminin, and thereby stabilize the CAA. In conclusion, tTG might play an important role in the formation of vascular deposits in CAA patient.

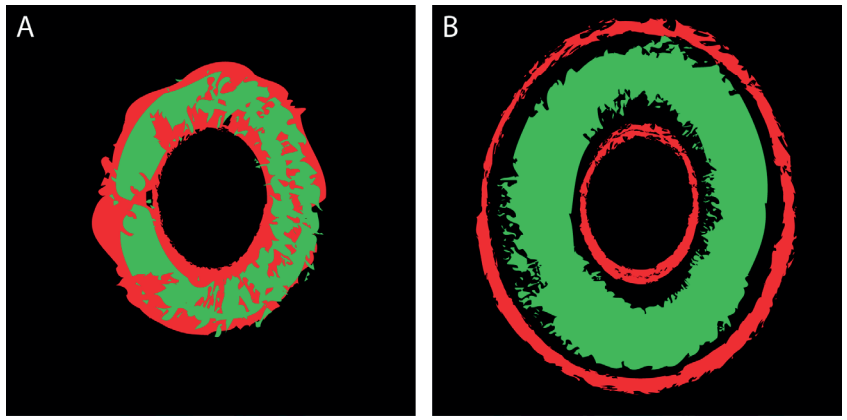

Figure 2 Schematic representation of tissue transglutaminase (tTG) and amyloid-β (Aβ) localisation in cerebral amyloid angiopathy in the neocortex of HCHWA-D patients. Aβ is shown in green, tTG in red. A. Early stage CAA: Aβ and tTG co-localize. B. Late stage CAA: Aβ and tTG do not co-localize anymore. Two halos of tTG are present: one luminal and one abluminal.

More recently, another important ECM modulator, lysyl oxidase (LOX) has been implicated in HCHWA-D and AD. LOX converts primary amines in peptide chains into aldehydes which interact to form cross-links between proteins. LOX is best known for its cross-linking of elastins and collagens in basement membranes and the ECM to maintain structural integrity (57), but HSPGs are also substrates of LOX (58). LOX is believed to play a role after tissue injury and is secreted by cells that are attracted to the brain injury sites (59). Elevated cross-linking of ECM by LOX increases permeability of the basement membrane, and thus destabilizes the vessels. LOX is present

within reactive astrocytes associated with parenchymal plaques in AD and HCHWA-D and LOX immunoreactivity is significantly increased in CAA affected vessels (58).

Potential therapies for HCHWA-D

Over the past few years, extensive research has been conducted on potential therapies for AD with the main focus on preventing formation and deposition of Aβ and tau, or increasing their clearance. Strategies reducing Aβ formation would also be interesting for HCHWA-D. Recent research has shown promising results in reducing Aβ production using RNA interference. RNA interference is a technique that down regulates gene expression by inducing degradation of targeted mRNA. Allele specific APP down regulation using short interfering RNA improved behaviour in an Alzheimer mouse model carrying the Swedish mutation (60). Using the same model, central and peripheral administration of an antisense oligonucleotide targeting APP, reduced formation of Aβ and improved the AD phenotype (61). However, APP has multiple morphoregulatory functions, like regulation of neurite outgrowth and complete knock-down of APP expression could lead to major side effects (62). Also the formation of the toxic Aβ peptides from APP could be prevented by increasing α-secretase activity or inhibiting the β- or γ-secretase activity. Epigallocatechin-gallate (EGCG), a compound that is also found in green tea, upregulates α-secretase and thereby promotes non-amyloidogenic processing of APP (63). Bryostatin 1 promotes α-secretase processing of APP by activating protein kinase C (64) and is currently in phase II clinical trials (Blanchette Rockefeller Neurosciences Institute).

Six small molecule BACE inhibitors are now tested in phase I trials (AZD3293, CTS-21166, E2609, PF-05297909 and TAK-070) and one (MK-8931) in phase II/III (65). Inhibiting γ secretase activity is not the best option, since γ secretase is involved in other pathways, like the Notch pathway (66). However, a "Notch-sparing γ secretase modulator" called Avagacestat has been tested in phase II, but led to worsening cognitive function, just like the phase III γ secretase inhibitor Semagacestat (67). Two other γ secretase targeting compounds (CHF-5074 and NIC5-15) are tested in phase II, but no results have been announced at this moment (67).

Another therapeutic agent that has been investigated for AD and could be interesting for HCHWA-D is *Scyllo*-inositol, an inhibitor of Aβ aggregation that demonstrated a decrease in CAA in an AD mice model (TgCRND8) after prophylactic administration (68). But clinical efficiency outcomes in a phase two clinical trial of AD patients using 250 mg *Scyllo*-inositol were not significantly different from placebo and higher dose studies were discontinued due to increased infections and mortalities (69).

An important feature of HCHWA-D is assembly of toxic Aβ fibrils at cell surfaces of cerebrovascular cells. The antioxidant catalase, which binds and degrades Aβ, was shown to inhibit this Aβ fibril-induced cell death in human brain pericytes (70).

The heat shock protein HspB8 could also inhibit Aβ40 accumulation at the cell surface and this reduced accumulation resulted in reduced death of cerebrovascular cells (71). This made HspB8 an interesting candidate for HCHWA-D therapy. However, more research on heat shock proteins showed that these proteins induce interleukin-6 secretion in HCHWA-D, eventually leading to an inflammatory response (72).

The endogenous bile acid Tauroursodeoxycholic acid (TUDCA) is another agent that shows therapeutic potential by preventing Aβ accumulation. Administration of TUDCA reduced amyloid deposition and prevented the defects in spatial, recognition and contextual memory in APP/PS1 mice (73) and was shown to prevent Dutch mutated Aβ-induced apoptosis of cultured cerebral endothelial cells (74).

In HCHWA-D there is a detrimental MMP-2 activation. Using MMP inhibitors, this activation can be diminished and thereby smooth muscle cell viability can be increased (41), which could lead to a lower incidence of cerebral haemorrhages.

As discussed above, ECM components play a major role in CAA. ECM modulators are therefore promising therapeutic targets for HCHWA-D. However, tTG is not a suitable target, since interfering with tTG could lead to destabilization of the vascular Aβ deposits and consequently enhance the chance for vessel wall rupture and haemorrhages. In contrast, lowering LOX activity could be an interesting therapeutic possibility, since elevated LOX activity in CAA leads to increased permeability of the basement membrane.

Immunotherapy directly targets the toxic Aβ peptides. Several vaccines have been developed for the treatment of AD, and these vaccines were promising in pre-clinical animal models. However, these vaccines did not lead to clinical improvement in several trials. This must probably be explained by the fact that in these trials participants already showed a (severe) clinical phenotype, whereas the pathogenic mechanism must already have been active for years. It is likely that individuals with 'pre-clinical' AD may benefit more from these vaccines. However, it is still a challenge to identify pre-clinical AD. This is not the case for pre-clinical HCHWA-D, because the majority of individuals with the Dutch mutation will develop symptoms of HCHWA-D.

It should be noted that because aggregated Aβ is hard to dissolve, it is better to target Aβ in the soluble state. In addition, dissolving the vascular deposits could also lead to disruption of the vessel wall, increasing the chance of haemorrhages. The clearance of soluble Aβ could be stimulated by the widely used drugs caffeine and rifampicin, since these drugs both upregulate the blood brain barrier transporter P-glycoprotein and rifampicin also upregulates LRP1 in wildtype mice (75). Since the proteolytic degradation of soluble Aβ is stimulated by ApoE, Cramer and colleagues hypothesized that enhancement of ApoE expression with the retinoid X receptor agonist bextarotene could promote Aβ clearance and microglial phagocytosis. They showed that administration of bextarotene led to a decrease in soluble and insoluble Aβ40 and Aβ42 levels, a decrease in cortical and hippocampal plaque burden and improved cognitive function of APP/PS1 mice (76). However, although the decrease in soluble Aβ was replicated (77,78), the decrease in plaque burden could not be replicated (77–80). Moreover, it is still unknown if this treatment would have an effect on CAA.

Conclusion

The Dutch mutation at position 22 of Aβ leads to multiple altered Aβ characteristics: charge alteration of the Aβ peptide leading to enhanced binding to cell surfaces and consequent Aβ accumulation, resistance to proteolysis and lowering of the affinity to brain efflux transporters.

The Dutch mutated Aβ is mainly produced in neurons, but forms fibrils at surfaces of cells in the vessel walls, where ECM modulators create an aggregation-promoting environment. The Aβ and sAPP in the vascular deposits promote cell degeneration and create an anticoagulant environment, which can eventually lead to haemorrhages.

Moreover, the elevated Aβ40: Aβ42 ratio in HCHWA-D suggests an inhibitory role for Aβ40 in parenchymal aggregation, but there is also an important role for Aβ42 as a seed for aggregation of Aβ40 in the cerebral blood vessels. Studies into HSPG subtypes suggest that vascular deposits in AD and HCHWA-D arise *via* different mechanisms.

Studying HCHWAs and their mutations provides us with a better understanding of the effects of Aβ and the differences among Aβ isoforms, which not only gives more insight in HCHWA pathogenesis, but also in other amyloidosis diseases, like sporadic CAA or AD.

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