Amyloid β in hereditary cerebral hemorrhage with amyloidosis-Dutch type

Abstract: Hereditary cerebral hemorrhage 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 transporter affinity, and enhanced cell surfaces binding. All these result in amyloid β accumulation in cerebral vessel walls, causing cell death and vessel wall integrity loss, making cerebral vessel walls in hereditary cerebral hemorrhage with amyloidosis-Dutch type more prone to rupture and obstruction, leading to hemorrhages and infarcts. Studying the 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.

Keywords: Alzheimer’s disease, amyloid precursor protein; amyloidosis; cerebral amyloid angiopathy; Dutch mutation.

Introduction

Hereditary cerebral hemorrhage 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 (Levy et al., 1990). HCHWA-D patients suffer from hemorrhagic strokes, infarcts, and vascular dementia (Wattendorff et al., 1995). Life expectancy is reduced: the first stroke occurs between the ages of 40 and 65 and is fatal in two thirds of the patients (Wattendorff et al., 1982; Luyendijk et al., 1986). The patients that survive the first hemorrhage suffer from recurrent strokes (Wattendorff et al., 1982).

HCHWA-D is a rare disease and has only been found in three founder families in the Dutch coastal villages of Katwijk and Scheveningen (Wattendorff et al., 1982; Luyendijk and Bots, 1986). A rough estimate is that likely 400–500 persons are at risk in multigenerational offspring families, but no clear data are available at this moment. An affected family described in Western Australia originates from Katwijk (Panegyres et al., 2005).

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 (Natte et al., 2001). CAA can also be found in at least 80% of Alzheimer’s disease (AD) patients (Yamada, 2012). However, in contrast to AD, the presence of intraneuronal neurofibrillary tangles is low in HCHWA-D and does not correlate with dementia (Natte et al., 2001).
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 (Jarrett et al., 1993). 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) (De Strooper and Annaert, 2000). After processing of APP, Aβ is released into the extracellular space (Haass et al., 1993) where it can form parenchymal plaques or accumulate as vascular deposits in the cerebral vessels causing amyloid angiopathy (Probst et al., 1980). 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 (Levy et al., 1989). The Dutch and Italian are caused by single point mutations at the Aβ region of APP on chromosome 21 (Levy et al., 1990; Bugiani et al., 2010). 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 (Di et al., 2009; Ovchinnikova et al., 2011). 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.

<table>
<thead>
<tr>
<th>Position Aβ</th>
<th>Mutation</th>
<th>Phenotype</th>
<th>Alias</th>
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<tbody>
<tr>
<td>2</td>
<td>Ala673Thr</td>
<td>Not pathogenic</td>
<td>AD</td>
</tr>
<tr>
<td>6</td>
<td>His677Arg</td>
<td>Not pathogenic</td>
<td>AD</td>
</tr>
<tr>
<td>7</td>
<td>Asp678Asn</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>7</td>
<td>Asp678His</td>
<td>Dementia+CAA</td>
<td>AD</td>
</tr>
<tr>
<td>11</td>
<td>Glu682Lys</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>16</td>
<td>Lys687Asp</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>21</td>
<td>Ala692Gly</td>
<td>AD (CAA)</td>
<td>Flemish</td>
</tr>
<tr>
<td>22</td>
<td>Glu693Lys</td>
<td>HCHWA</td>
<td>Italian</td>
</tr>
<tr>
<td>22</td>
<td>Glu693Gln</td>
<td>HCHWA</td>
<td>Dutch</td>
</tr>
<tr>
<td>22</td>
<td>Glu693Gly</td>
<td>AD (CAA)</td>
<td>Arctic</td>
</tr>
<tr>
<td>22</td>
<td>Glu693del*</td>
<td>AD</td>
<td>Japanese</td>
</tr>
<tr>
<td>23</td>
<td>Asp694Asn</td>
<td>AD (CAA)</td>
<td>Iowa</td>
</tr>
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<td>CAA</td>
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<td>Gly708</td>
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<td>AD</td>
</tr>
<tr>
<td>42</td>
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</tr>
<tr>
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</tr>
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<td>Iranian</td>
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<tr>
<td>43</td>
<td>Thr714Ile</td>
<td>AD</td>
<td>Austrian</td>
</tr>
</tbody>
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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 and Frontotemporal Dementia Mutation Database (Cruts et al., 2012) and PubMed: ‘(Lan et al., 2014); ‘(Kaden et al., 2012).
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 (Maat-Schieman et al., 2000).

Aβ peptides show different intermediate fibrillization states before plaques, and vascular deposits are formed (Finder and Glockshuber, 2007). The Aβ monomers are amphipathic, with a hydrophobic N-terminal and a hydrophilic 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.

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 (Maat-Schieman et al., 2000). 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 harbor 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 (Maat-Schieman et al., 1996).

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 Aβ40-negative fine diffuse to Aβ40-positive dense plaques (Maat-Schieman et al., 2004). Electron microscopy examination showed that Aβ is non-fibrillar and plasma membrane bound initially, but when the plaques develop, amyloid fibrils accumulate (Maat-Schieman et al., 2000). 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

**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
glial cells, thereby, limiting the neurotoxic soluble form levels of $\alpha$B in HCHWA-D patients’ brains (Maat-Schieman et al., 2004).

$\alpha$B isoforms in HCHWA-D vasculature

While $\alpha$B42 is the main $\alpha$B isoform in parenchymal plaques, $\alpha$B40 is the main component of amyloid deposits in the cerebral vessels of HCHWA-D patients (Ozawa et al., 2002; Nishitsuji et al., 2007). Amino acid sequencing of amyloid that was isolated from leptomeningeal vascular walls showed that both mutated and wild-type $\alpha$B occurs in the vascular deposits of HCHWA-D patients (Prelli et al., 1990). It has been suggested that it is especially the ratio of $\alpha$B40 to $\alpha$B42 that is important for vascular amyloid formation (Herzig et al., 2004). Moreover, an important role for mutated $\alpha$B42 has been proposed. In vascular amyloid of HCHWA-D, wild-type and Dutch-mutated $\alpha$B40 peptides occur in a 1:1 ratio, while only the Dutch mutated $\alpha$B42, and not the wild-type $\alpha$B42, has been detected, suggesting a possible role for Dutch-mutated $\alpha$B42 as a seed for the aggregation of $\alpha$B40 (Nishitsuji et al., 2007). Importantly, all $\alpha$B42 was oxidized at the methionine residue at position 35. The oxidation of Met35 of $\alpha$B42 is known to slow down the rate of fibrillation and aggregation of $\alpha$B42 (Hou et al., 2004). However, the Dutch mutation enables $\alpha$B to fold into different shapes, thereby, creating multiple ways to aggregate (Hou et al., 2004).

In addition to $\alpha$B40 and $\alpha$B42, wild-type $\alpha$B37, wild-type $\alpha$B38, and Dutch-mutated $\alpha$B38 are also present in the vascular amyloid (Nishitsuji et al., 2007). $\alpha$B37 and $\alpha$B38 are less common isoforms of $\alpha$B than $\alpha$B40 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 hemorrhages using a transgenic HCHWA-D mouse model. This indicates that neurons are the main source of Dutch $\alpha$B in the cerebral vessels. Using this model, it was also shown that the Dutch mutation leads to an increased $\alpha$B40:$\alpha$B42 ratio both in parenchymal and cerebrovascular amyloid deposits, and $\alpha$B40 was suggested to be inhibitory for parenchymal $\alpha$B deposition (Herzig et al., 2004). It was discovered in a guinea pig model of the Dutch mutation that $\alpha$B40 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 (Monro et al., 2002). Impaired clearance of $\alpha$B was also shown in a mouse model with the Dutch and Iowa mutation: no detectable plasma $\alpha$B but abundant $\alpha$B deposits were present in cerebral vasculature (Davis et al., 2006). The double mutated $\alpha$B shows a significant lower affinity to the low-density lipoprotein receptor-related protein 1 (LRP1), which is implicated in $\alpha$B clearance, in comparison to wild-type $\alpha$B40 (Deane et al., 2004). Double mutated $\alpha$B also seems to downregulate LRP1 (Deane et al., 2004). However, in this model, it is not clear how each mutation affects the clearance.

In individuals with and without the Dutch mutation, $\alpha$B40 plasma levels were similar (Bornebroek et al., 2003). On the other hand, the plasma $\alpha$B42 concentration of individuals with the Dutch mutation was significantly lower than in the plasma of their family members that did not carry the mutation (Bornebroek et al., 2003). Of the 22 individuals with the Dutch mutation, seven were still asymptomatic. Plasma concentrations of $\alpha$B40 and $\alpha$B42 in HCHWA-D patients did not correlate with age or severity of the symptoms (Bornebroek et al., 2003), which indicates

### Table 2

<table>
<thead>
<tr>
<th>Clouds</th>
<th>Plaques</th>
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<tr>
<td></td>
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<tr>
<td>Fine diffuse</td>
<td>Dense diffuse</td>
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<tr>
<td>$\alpha$B</td>
<td>$\alpha$B40</td>
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<td></td>
<td>$\alpha$B42</td>
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<td>DN</td>
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<td>APP</td>
<td>-</td>
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<td>Ubiquitin</td>
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<td>Tau</td>
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Adapted from Maat-Schieman et al. (2000). Staining intensity: No staining -, few bundles ±, positive staining/small bundles +, and strong positive staining/clusters ++. $\alpha$B, amyloid $\beta$, DN, degenerating neurites.
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 the 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 the medium of cells with the Dutch mutation, while the Aβ40 concentration was unchanged (Nilsberth et al., 2001).

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 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 (Bornebroek et al., 2003; Herzig et al., 2004).

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 (Nilsberth et al., 2001). 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, hemorrhages are uncommon, whereas parenchymal plaques are abundant in patients with the Flemish- and Arctic-type mutations (Brooks et al., 2004; Basun et al., 2008). 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 peptide 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 (Van Nostrand et al., 1998). 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 (Van Nostrand et al., 2000a). 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 (Van Nostrand et al., 1995), and Aβ fibrils enhance the anticoagulant property of APP (Wagner et al., 2000). As a consequence, an anticoagulant environment is created, leading to an increased chance of hemorrhages. 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 (Van Nostrand et al., 1992).

Moreover, Aβ fibrillation activates an apoptotic pathway in the cerebrovascular smooth muscle cells, leading to cell death (Van Nostrand et al., 2000b). The combination of the cell death and the anticoagulant environment induced by Aβ fibrils in the vessel wall are probably major contributors to the hemorrhages 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 (Jung et al., 2003). MMPs are tissue-remodeling enzymes and turnover basement membranes. Elevated MMP-2 is known to lead to blood brain barrier disruption and causes cerebral hemorrhage; thus, the Dutch Aβ-induced MMP-2 activation and expression probably contributes to loss of vessel wall integrity and consequent hemorrhagic stroke (Jung et al., 2003).

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 (Verbeek et al., 1997). 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 (Corder et al., 1993).

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 (Haan et al., 1994). Furthermore, no association between the ApoE ε4 allele and Aβ plasma levels was found in 22 HCHWA-D patients (Bornebroek et al., 2003). 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 (Verbeek et al., 2000). 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 (Fossati et al., 2012).

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 hemorrhages 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 sulfate proteoglycans (HSPGs) (Hawkes et al., 2011). HSPGs co-localize with the vascular deposits in AD and HCHWA-D (Van Horssen et al., 2001). HSPGs consist of sulfated glycosaminoglycan (GAG) side chains bound to a core protein (Hardingham and Fosang, 1992). Heparin and heparan sulfate are GAGs with side chains showing high Aβ affinity (Snow et al., 1995). The sulfate moieties of the side chains modulate the aggregation (Timmer et al., 2010). Heparin and heparan sulfate both increase the aggregation of Aβ40 with the Dutch mutation, but especially, heparin is a very potent aggregation inducer. Moreover, heparin and heparan sulfate 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 (Timmer et al., 2010). 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 (Van Horssen et al., 2001) 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-localized 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 (Van Horssen et al., 2001). 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 glypicanc-1 are not expressed by vascular cells (Van Horssen et al., 2001). This indicates that implicated HSPGs are not produced by vascular cells but have other sources and travel toward the vascular wall.

A protein that co-localizes with ECM proteins in CAA is tissue transglutaminase (tTG) (De Jager et al., 2013). tTG is an enzyme involved in posttranslational modifications of proteins, like covalently cross-linked proteins (Lorand and Graham, 2003). It plays an important role in the remodeling of the ECM after tissue injury and cell stress (Ientile et al., 2007). It is known that tTG mediates Aβ40 dimerization through covalent intermolecular cross-linking and thereby seeding aggregation (Schmid et al., 2011). In early stage CAA, tTG is increased in affected vessel walls and colocalizes with Aβ deposition. This tTG could originate from endothelial cells or smooth muscle cells around which the Aβ accumulates. In later stages, colocalization is absent, and tTG encloses the Aβ deposition in an abluminal and a luminal halo as shown in Figure 2 (De Jager et al., 2013). 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 (De Jager et al., 2013). 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 patients.

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 (Kagan and Li, 2003), but HSPGs are also substrates of LOX (Wilhelmus et al., 2013). LOX is believed to play a role after tissue injury and
Figure 2  Schematic representation of tissue transglutaminase (tTG) and amyloid-β (Aβ) localization 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. Figure adapted from De Jager et al., 2013.

is secreted by cells that are attracted to the brain injury sites (Gilad et al., 2001). 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 (Wilhelmus et al., 2013).

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 downregulates gene expression by inducing degradation of targeted mRNA. Allele-specific APP downregulation using short interfering RNA improved behavior in an Alzheimer mouse model carrying the Swedish mutation (Rodriguez-Lebron et al., 2009). Using the same model, central and peripheral administration of an antisense oligonucleotide targeting APP, reduced formation of Aβ, and improved the AD phenotype (Farr et al., 2014). However, APP has multiple morphoregulatory functions, like regulation of neurite outgrowth, and complete knockdown of APP expression could lead to major side effects (Gralle and Ferreira, 2007). 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 (Smith et al., 2010). Bryostatin 1 promotes α-secretase processing of APP by activating protein kinase C (Yi et al., 2012) 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 (Yan and Vassar, 2014). Inhibiting γ-secretase activity is not the best option, as γ-secretase is involved in other pathways, like the Notch pathway (Sato et al., 2012). 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 (Mikulca et al., 2014). 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 (Mikulca et al., 2014).

Another therapeutic agent that has been investigated for AD and could be interesting for HCHWA-D is Scylla-inositol, an inhibitor of Aβ aggregation that demonstrated a decrease in CAA in an AD mice model (TgCRND8) after prophylactic administration (McLaurin et al., 2006). 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 (Salloway et al., 2011).

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 (Rensink et al., 2002).

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 (Wilhelmus et al., 2006). 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 (Wilhelmus et al., 2009).

The endogenous bile acid tauroursodeoxycholic acid (TUDCA) is another agent that shows therapeutic potential by preventing Aβ aggregation. Administration of TUDCA reduced amyloid deposition and prevented the defects in spatial, recognition, and contextual memory in APP/PS1 mice (Lo et al., 2013) and was shown to prevent Dutch-mutated Aβ-induced apoptosis of cultured cerebral endothelial cells (Viana et al., 2009).

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 (Jung et al., 2003), which could lead to a lower incidence of cerebral hemorrhages.

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, as interfering with tTG could lead to destabilization of the vascular Aβ deposits and consequently enhance the chance for vessel wall rupture and hemorrhages. In contrast, lowering LOX activity could be an interesting therapeutic possibility, as 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 preclinical 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 ‘preclinical’ AD may benefit more from these vaccines. However, it is still a challenge to identify preclinical AD. This is not the case for preclinical 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 hemorrhages. The clearance of soluble Aβ could be stimulated by the widely used drugs caffeine and rifampicin, as these drugs both upregulate the blood brain barrier transporter P-glycoprotein, and rifampicin also upregulates LRPI in wild-type mice (Qosa et al., 2012). As 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 bexarotene could promote Aβ clearance and microglial phagocytosis. They showed that administration of bexarotene 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 (Cramer et al., 2012). However, although the decrease in soluble Aβ was replicated (Fitz et al., 2013; Veeraraghavalu et al., 2013), the decrease in plaque burden could not be replicated (Fitz et al., 2013; Price et al., 2013; Tesser et al., 2013; Veeraraghavalu et al., 2013). 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 hemorrhages.

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.

References


J.A. Kamp et al.: Amyloid β characteristics in HCHWA-D


