Cerebral Amyloid Angiopathy: A Common Cause of Cerebral Hemorrhage

A. Pezzini*1, E. Del Zotto1,2, I. Volonghi1, A. Giossi1, P. Costa1 and A. Padovani1

1Department of Medical and Surgical Sciences, Neurology Clinic, University of Brescia, Brescia, Italy 2Department of Biomedical Sciences and Biotechnology, University of Brescia, Brescia, Italy

Abstract: Amyloid is a term used to describe protein deposits with circumscript physical characteristics: β-sheet configuration, apple green birefringence under polarized light after Congo red staining, fibrillar structure and high insolubility. Cerebral amyloid angiopathy (CAA) defines a clinicopathological phenomenon characterized by amyloid deposition in the walls of leptomeningeal and cortical arteries, arterioles, and, less often capillaries and veins of the central nervous system. CAs are currently classified according to the protein deposited including amyloid β peptide (Aβ), cystatin C (CysC), prion protein (PrPSc), Aβ/ADan, transthyretin (ATR), and gelsolin (AGel). Most often amyloid deposition occurs in sporadic forms. In less common hereditary forms, a mutated variant protein or precursor protein is abnormally metabolized by proteolytic pathways in consequence of specific gene mutations, and accumulates as amyloid. The spectrum of clinical phenotypes associated with CAA-related vasculopathic changes includes both ischemic and hemorrhagic presentations, primary intracerebral hemorrhage (PICH) being probably the most well-recognized. However, in spite of accumulating data and recent progress in understanding the pathogenesis of CAA-related hemorrhage, the exact mechanisms leading to vessel rupture in these cases are yet to be established. This represents, at present, a major limitation to the identification of reliable biomarkers and the development of disease-specific treatment options. The present paper summarizes epidemiologic and clinical aspects of CAA, and highlights the presumed pathomechanisms of amyloid deposition in both sporadic and hereditary forms.

Keywords: Cerebral amyloid angiopathy, intracerebral hemorrhage, β amyloid peptide, hereditary cerebral hemorrhage with amyloidosis.

INTRODUCTION

Definition and Nomenclature

According to the consensus reached at the meeting of the Nomenclature Committee of the International Society of Amyloidosis, held at Woods Hole, MA, 6th and 7th of November, 2006, amyloid is defined as an in vivo deposited material, which can be distinguished from non-amyloid deposits by characteristic fibrillar electron microscopic appearance, typical X-ray diffraction pattern and histological staining reactions, particularly affinity for the dye Congo red with resulting green birefringence. The main constituent of amyloid deposits is the protein fibril. In principle, there is one fibril protein in each type of amyloidosis. A disease associated with (or caused by) amyloid deposits is called ‘amyloidosis’. Systemic amyloidosis is defined as being derived from a plasma protein, while localized amyloids are derived from proteins expressed by cells at the deposition site. Amyloid diseases are categorized according to the type of amyloid-forming protein. As example, the fibril protein Aβ in Alzheimer’s disease (AD) is derived from the Aβ protein precursor (AβPP) [1]. Cerebral amyloid angiopathy (CAA) is the term used to define the deposition of amyloid in the walls of medium- and small-sized leptomeningeal and cortical arteries, arterioles and, less frequently, capillaries and veins of the central nervous system.

HISTORICAL OVERVIEW

In 1907, Alois Alzheimer published his seminal article in which he described the autopsy of a 51-year-old woman with dementia. Among his findings, he saw “...deposit in the cerebral cortex of a peculiar substance which can be recognized without stain and is, in fact, very resistant to staining.” Lacking the techniques of modern molecular biology, Alzheimer and his colleagues were not able to determine the constituents of these deposits [2]. Later research into the nature of such deposits, now known as senile plaques (SPs), showed that they are primarily composed of β-amyloid. Vascular deposition of β-amyloid was not described in the original case reported by Alzheimer.

The first description of histological vascular abnormalities recognizable as vascular β-amyloid deposition was by Gustav Oppenheim in 1909. Oppenheim found foci of necrosis in the brain parenchyma adjacent to hyalinized capillary walls in 6 of 14 autopsied brains of individuals with senile dementia and the pathological changes of Alzheimer disease. He hypothesized that the substance in the capillary walls was the same substance causing the pericapillary necrosis [3]. Oskar Fischer in 1910 also noted the occasional presence of degeneration of the capillary wall. Fischer described 8 stages of “sphaerotrichia cerebri multiplex”, and stage 6 was a “furry” destruction of the vessel wall in association with changes in the perivascular brain parenchyma [4].

In 1938, Scholz published the first article in which the primary focus was the vascular abnormality now recognizable as CAA. He detected this abnormality in the brains of 15 of 104 unselected autopsies, and he suggested it to be a disease of aging. Like Oppenheim and Fischer, he emphasized the deposition of an identical substance in the vessel wall and immediately adjacent perivascular brain parenchyma, and he speculated that the abnormal substance might be amyloid [5]. Later, the disease was named angiopathie dyshorique by Morel. The observation of CAA limited to the vascular media without adjacent parenchymal involvement...
was made in 1954 by Stefanos Pantelakis. He referred to the vascular abnormality as congophilic angiopathy because of the presence of staining with Congo red. When viewed under polarized light, the Congo red-stained vessels exhibited birefringence typical of amyloid. Pantelakis observed many of the pathological features now associated with CAA: (1) preferential involvement of the small arteries and capillaries of the meninges, cerebral cortex, and cerebellar cortex; (2) a topographical distribution favoring the posterior brain regions, most frequently involving the occipital lobes; (3) lack of staining of vessels in the white matter; (4) association with increased age and the presence of dementia; (5) lack of association with hypertension and arteriosclerosis; and (6) lack of association with amyloidosis of the other organs. None of the original descriptions of CAA noted an association with either symptomatic or asymptomatic intracerebral hemorrhage [6].

In 1960, Neumann reported an unusual case of a 45-year-old woman with severe CAA who had 2 symptomatic lobar hemorrhages and multiple small asymptomatic lobar petechial hemorrhages. Neumann speculated that an amyloid-like substance might have weakened the arterial wall but mistakenly concluded that the multiple hemorrhages may have been due to vascular malformations [7]. Over the following 20 years, there appeared multiple case reports and small series that suggested a link between CAA and lobar hemorrhage.

Okazaki et al. published an influential article in 1979 that better defined the relationship of CAA and intracerebral hemorrhage. They identified 23 consecutive cases of moderate to severe CAA from autopsies at Mayo Clinic, Rochester, Minn, over the preceding 10 years. A history of lobar, multiple hemorrhages was very common. Fibrinoid degeneration of the vessel walls with microaneurysm formation was sometimes seen, as well as a double-barreled appearance of the walls of some of the larger leptomeningeal arteries caused by cracking of the arterial media. Okazaki and colleagues concluded that CAA was an underrecognized cause of lobar hemorrhage in the elderly [8].

**SPORADIC AND HEREDITARY FORMS**

CAA mostly occurs in the sporadic form in the elderly, while the rare familial forms occur in younger age. All sporadic forms and most hereditary forms of CAA affecting the human brain are of the Aβ-type (Aβ-CAA, Table 1).

**Sporadic CAA**

Aβ is a normally secreted, ~4 kDa, 40 or 42 amino acids in length, proteolytic product of the 677-770 amino acid, type 1 integral membrane protein, referred to as the AβPP, encoded by a gene on chromosome 21, and it is an internal sequence within the APP beginning 99 amino acids from the carboxyl terminus [9-12]. Generation of Aβ from APP requires two proteolytic events, a proteolytic cleavage at the amino terminus of the Aβ sequence referred to as β-secretase (a membrane-bound aspartyl protease also called β-site APP cleaving enzyme or β-secretase - BACE) [13] and a cleavage at the carboxyl terminus known as γ-secretase (with release of the p3 and the Aβ peptide). In addition, a third proteolytic activity referred to as α-secretase (a member of the A disintegrin and metalloproteinase domain - ADAM - family of metalloproteases), cleaves within the Aβ sequence to release a large secreted derivative thus precluding formation of full-length Aβ and it is therefore usually considered as non-amyloidogenic. The 40-amino-acid-long Aβ (Aβ1–40) is more soluble than the longer Aβ 1–42 and the two molecules differ in the distribution in brain and vessel walls. Aβ1–40 tends to be the major form in the amyloid in artery walls in CAA, whereas Aβ1–42 is more prominent in the plaques in brain tissue [14, 15].

**Epidemiology**

CAA is a common pathology and a fairly common clinical entity in the elderly. As a detectable pathology, cerebrovascular amyloid is present in approximately 10% to 40% of elderly brains and 80% or more in brains with concomitant AD [16]. Even when considering only relatively advanced CAA pathology, it remains a frequent finding. CAA pathology graded as moderate or severe was estimated to be present in 2.3% of 65- to 74-year-olds, 8.0% in 75- to 84-year-olds, and 12.1% in those over 85 years in analysis of brains from Harvard Brain Tissue Resource Center corrected for over-representation of Alzheimer disease referrals [17]. An even higher figure of 21% for the prevalence of CAA graded as severe emerged from other analyses of autopsied individuals aged 85 to 86 [18]. Estimates for the proportion of spontaneous hemorrhages in the elderly that are due to CAA range from 10% to 20% in autopsy series [16] to 34% in clinical series [19].

**Morphology of CAA**

In hematoxylin and eosin-stained sections severe CAA can be recognized by acellular thickening of blood vessel walls. This morphology is, however, non-specific for CAA, since it occurs in a variety of other disorders, including hypertensive angiopathy [20]. In CAA, Aβ is deposited mainly as amyloid-β fibrils in close contact with smooth muscle cells [21-24]. Non-fibrillar, monomeric and oligomeric Aβ was also demonstrated inside smooth muscle cells (SMCs) [21]. Weak and focal immunohistochemical staining of smooth muscle cells could thus be indicative of non-fibrillar Aβ. Depending on the severity of CAA, Aβ deposits have been shown primarily in the abluminal portion of the tunica media, often surrounding smooth muscle cells, and in the adventitia. With increasing severity, Aβ infiltrates all layers of the vessel wall, which shows loss of smooth muscle cells. Finally, the vascular architecture is severely disrupted and “double barrelling”, microaneurysm formation, fibrinoid necrosis, and evidence of perivascular leakage may be seen [25-27]. Intracortical (parenchymal) vessels can show additional spread of Aβ into the surrounding neuropil. Even in very high degrees of CAA-related changes, endothelial cells are well preserved and usually not affected with Aβ depositions. Perivascular hemorrhages are frequent around blood vessels affected with CAA. Several authors reported CAA-associated inflammation/vasculitis [28-30]. Neuropathologically, these cases were characterized by the presence of severe CAA and chronic inflammation within the leptomeninges and in and around the walls of Aβ-laden blood vessels. The perivascular and intramural inflammatory infiltrate consisted of lymphocytes, macrophages, and multinucleated giant cells [29].
Table 1. Amyloid Peptides Causing CAA in Humans

<table>
<thead>
<tr>
<th>Amyloid peptide</th>
<th>Precursor protein</th>
<th>Chromosome</th>
<th>Disease</th>
<th>Mutation</th>
<th>AA substitution</th>
<th>Hemorrhagic stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>APP</td>
<td></td>
<td>Sporadic CAA</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>CAA related to sporadic AD</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>CAA related to familial AD</td>
<td></td>
<td>Associated to PS-1 and PS-2 mutations</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>CAA in Down syndrome</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>HCHWA-Dutch type</td>
<td>G to C (693)</td>
<td>Glu22Gln</td>
<td></td>
<td>Described in two large families from the Netherlands Age at onset: 50 yrs Lobar hemorrhages, focal neurological deficits, dementia, and leukoencephalopathy</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HCHWA-Italian type</td>
<td>G to A (693)</td>
<td>Glu22Lys</td>
<td></td>
<td>Described in 3 Italian families Age at onset: 50 yrs Lobar hemorrhages and dementia</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HCHWA-Flemish type</td>
<td>C to G (692)</td>
<td>Ala21Gly</td>
<td></td>
<td>Described in a Dutch family (discovered in Belgium, therefore called 'Flemish') and a British family Age at onset: 45 yrs Progressive AD-like dementia, in some patients associated with a lobar hemorrhage</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HCHWA-Iowa type</td>
<td>G to A (694)</td>
<td>Asp23Asn</td>
<td></td>
<td>Described in a Iowa family and a Spanish family Age at onset: 50-66 yrs Memory impairment, expressive language disfunction, personality changes, myoclonic jerks, short-stepped gait, no clinically manifest ICH (family from Iowa) Lobar hemorrhages (family from Spain)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HCHWA-Piedmont type</td>
<td>G to C (705)</td>
<td>Leu34Val</td>
<td></td>
<td>Described in one family from the Piedmont region of Italy Age at onset: between 50 and 70 yrs Recurrent lobar hemorrhages, cognitive decline</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HCHWA-Arctic type</td>
<td>A to G (693)</td>
<td>Glu22Gly</td>
<td></td>
<td>Described in one family from northern Sweden Age at onset: about 60 yrs Progressive cognitive decline (no strokes)</td>
<td>-</td>
</tr>
<tr>
<td>ACys</td>
<td>Cystatin C</td>
<td>20</td>
<td>HCHWA-Icelandic type</td>
<td>A to T (68)</td>
<td>Leu68Gln</td>
<td>Described in 9 sub-families in Iceland (one sporadic case in the US); systemic amyloidosis Age at onset: 20 to 30 yrs Recurrent lobar hemorrhages</td>
</tr>
</tbody>
</table>
Histological Diagnosis

The histological diagnosis of amyloidosis is established using special staining for amyloid under light microscopy. Puchtler alkaline Congo-red stain has been the standard method of amyloid staining for a long time [31-33]. The Congo-red stain has undergone several modifications since it was first used by Bennhold in 1922 [33,34]. Since this stain is relatively unstable and has low sensitivity, a control staining with positive specimens is absolutely essential [33]. In contrast, Daylon stain is more sensitive, hence it is more useful for the detection of small amount of amyloid deposition [35,36]. Daylon stain, also named as direct fast scarlet, has recently become more popular. However, a more careful observation is necessary with Daylon stain because it shows a tendency to over-staining. The definite diagnosis of amyloidosis requires the detection of apple-green birefringence under polarized light microscopy. The bundles of rich collagen fibres easily reveal the polarization of white light; therefore, a positive reaction to amyloid stains where the polarization of apple-green light cannot be observed is considered a nonspecific reaction.

Fluorescent microscopy is also useful for the diagnosis of amyloidosis. Thioflavin-S is a sensitive stain for amyloid deposition, and it has been used from long time along with Congo-red staining [37-39]. An intensive green fluorescent reaction is observed; however, over-reaction often disturbs the precise diagnosis. Immunohistochemistry with fluorescent antibodies specific for precursor proteins is a reliable diagnostic complement.

Electron microscopy is another important diagnostic method for amyloidosis. Amyloid fibrils accumulate in dense and sparse patterns. In areas where amyloid fibrils densely aggregate, detection of a single fibril may not be easy [40]. The marginal zone of dense accumulation is better suited for the precise identification of amyloid fibrils. Amyloid fibrils are first laid down in the abluminal aspect of the basal lamina around smooth muscle cells and gradually spread towards the internal elastic lamina of arteries and the endothelium of arterioles. As deposits increase in size the smooth muscle cells may show degenerative features. In capillaries, amyloid fibrils are found within the basal lamina with larger deposits extending into the adjacent neuropil. Immunoelectron microscopy also demonstrates that in the initial phase of Aβ deposition the basal lamina is either unremarkable or shows increased electron density and reticular structures with amyloid fibrils being observed in more advanced lesions.

Topographical Distribution of CAA

In general, the distribution of CAA is characteristically patchy and segmental [26]. In one given histological slide there may be foci showing vessels with varying degrees of amyloid depositions adjacent to foci showing vessels without any amyloid deposition. The patchy distribution of CAA may thus lead to an under-diagnosis of CAA in postmortem examination, as even in severe cases a given histological slide might not contain amyloid-laden blood vessels. It has been shown by many authors that CAA is most frequent in the occipital lobe, followed by either frontal, temporal or parietal lobes, respectively [41-45].

<table>
<thead>
<tr>
<th>Amyloid peptide</th>
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<th>Chromosome</th>
<th>Disease</th>
<th>Mutation</th>
<th>AA substitution</th>
<th>Hemorrhagic stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATTR</td>
<td>Transthyretin</td>
<td>18</td>
<td>Meningovascular amyloidosis</td>
<td>multiple mutations</td>
<td>Polyneuropathy is the main clinical symptom; in a small number of patients ataxia, spasticity and dementia; systemic amyloidosis</td>
<td>in some families</td>
</tr>
<tr>
<td>AGe1</td>
<td>Gelsolin</td>
<td>9</td>
<td>FAF</td>
<td>G to A (654) Asp187Asn</td>
<td>Progressive corneal lattice dystrophy, cranial and peripheral neuropathy, cutaneous amyloidosis; systemic amyloidosis</td>
<td>-</td>
</tr>
<tr>
<td>PrPSc</td>
<td>Prion Protein</td>
<td>20</td>
<td>GSS (Y145Stop)</td>
<td>T to G (145) Y145STOP</td>
<td>Described in one family Progressive cognitive decline</td>
<td>-</td>
</tr>
<tr>
<td>ABri</td>
<td>ABri precursor protein</td>
<td>13</td>
<td>FBD</td>
<td>T to A (266) Stop to Arg</td>
<td>Described in 4 families Age at onset: 45-50 yrs Progressive dementia, cerebellar ataxia, spastic tetraparesis</td>
<td>-</td>
</tr>
<tr>
<td>ADan</td>
<td>ADan precursor protein</td>
<td>13</td>
<td>FDD</td>
<td>10-nucleotide duplication insertion after codon 265</td>
<td>Described in 1 family from Denmark Age at onset: 30 yrs Cataracts, deafness, progressive ataxia, dementia (previously known as &quot;heredopatia ophtalmoto-ento-encephalica&quot;)</td>
<td>-</td>
</tr>
</tbody>
</table>

APP, Aβ precursor protein; AD, Alzheimer’s Disease; PS1, Presenilin 1; PS2, Presenilin 2; HCHWA, hereditary cerebral hemorrhage with amyloidosis; FAF, familial amyloidosis, Finnish type; GSS, Gerstmann-Sträussler-Scheinker syndrome; FBD, familial British dementia; FDD, familial Danish dementia.
Some authors, however, reported the frontal lobe to be the site most frequently involved in CAA [46, 47]. The occipital lobe is not only the site most frequently affected with CAA but also most severely so [41,44]. CAA is rarely seen in the basal ganglia, thalamus, and cerebellum, while both white matter and brainstem are usually spared [48,49]. It is generally assumed that involvement of leptomeningeal arteries represents an early stage in the process of the disease, which is followed by involvement of cortical arteries. Conflicting data have been reported on the involvement of intracortical capillaries and leptomeningeal veins. Veins, however, tend to be affected less frequently than arterial vessels [25].

**Grading of CAA**

Two grading systems are commonly used in routine neuropathology. Olichney et al. [27] proposed the scale: 0, no Aβ-positive blood vessels; 1, scattered Aβ positivity in either leptomeningeal or intracortical blood vessels; 2, strong, circumferential Aβ positivity in either some leptomeningeal or intracortical blood vessels; 3, widespread, strong, circumferential Aβ positivity in leptomeningeal and intracortical blood vessels; 4, same as 3 with additional dyshoric changes. This system has a rather quantitative approach, whereas Vonsattel et al. [50] graded CAA with respect to the severity of pathological changes in a given blood vessel: mild, amyloid is restricted to the tunica media without significant destruction of smooth muscle cells; moderate, the tunica media is replaced by amyloid and is thicker than normal; severe, extensive amyloid deposition with focal wall fragmentation or even double barrelling of the vessel wall, microaneurysm formation, fibrinoid necrosis, and leakage of blood through the blood vessel wall. Despite the practical value of these two grading systems, they have some limitations. The system described by Olichney et al. [27] links leptomeningeal and intracortical involvement, and does not allow scoring cases with strong positivity in intracortical vessels but without (strong) positivity in leptomeningeal vessels. The system by Vonsattel et al. [50], on the other hand, does not distinguish between leptomeningeal and intracortical affection. Because of these limitations and despite the practical value of these two grading systems, to date, standardized neuropathological criteria for rating CAA are not available.

**Clinical Features**

Amyloid deposition in cerebral blood vessels can have several clinical consequences (Table 2).

**Table 2. Recognised Clinical Manifestations of CAA**

| Primary intracerebral haemorrhage (PICH) | Dementia |
| Cerebral infarcts | Transient ischaemic attacks |
| Seizures | Subarachnoid haemorrhage |
| CAA-related central nervous system vasculitis |

First, it can remain asymptomatic, as suggested by the neuropathological observation that during “normal” ageing approximately 50 per cent of individuals over 80 years of age have CAA. Second, it can weaken the vessel wall, causing rupture and intracerebral hemorrhage (ICH). Finally, it can obliterate the vessel lumen, leading to ischemia (cerebral infarction, “incomplete infarction”, and leukoencephalopathy). Focal neurological deficits, disturbances of consciousness, stepwise dementia, and death can occur as a consequence of these vascular mechanisms.

Although all these clinical manifestations are noteworthy, the most well-recognised manifestation of CAA is PICH. CAA-related hemorrhages (CAAH) account for 5-20% of all spontaneous (non-traumatic) cerebral hemmorhages in elderly subjects, though ICH were found only in 5.4% of autopsy-confirmed CAA. CAAH tends to be lobar (due to the involvement of superficial cortical and leptomeningeal vessels) and recurrent or multiple simultaneous (widespread nature of the angioopathy). Hypertension is less commonly associated with lobar hemorrhages [51]. There is no pathognomonic clinical feature of CAAH. Headache, focal neurological deficit, seizures and altered level of consciousness occur depending on the size and location of hemorrhage, although headache and seizures are more common in lobar than in deep hemorrhages. PICH due to CAA can be small and asymptomatic. An important manifestation of CAA is PICH caused by therapy with anticoagulants or thrombolytic agents [52].

The clinical differentiation of CAA-related versus non-CAA-related symptomatology may be very difficult and unreliable, as there is significant overlap in diseases that result in acute neurologic deficits, transient ischemic attack (TIA)-like symptoms, and dementia. The diagnosis of CAA can only be made with certainty after histologic investigation of affected brain tissue, obtained at autopsy or brain-biopsy. In practice it is very often found unexpectedly at post-mortem investigation. Non-invasive diagnostic criteria have been developed in the mid-1990s as a tool to both improve and standardize the diagnosis of CAA during life and have been recently validated in a clinicopathological study [53]. CAA is considered “probable with supporting pathology” when, in combination with appropriate clinical data, pathologic tissue from a biopsy performed at the time of hematoma evacuation reveals amyloid angiopathy. CAA is considered “probable” if there is an appropriate clinical history as well as imaging findings of multiple cortical-subcortical hematomas in a patient 55 years or older, with no other clinical or radiologic cause of hemorrhage. Finally, clinical data suggesting CAA and the imaging finding of a single cortical-subcortical hematoma in a patient older than 55 years, without other causes of hemorrhage, leads to a diagnosis of “possible” CAA (Table 3).

**Pathogenesis**

Aβ-CAA occurs as a sporadic disorder in the elderly and is present in virtually all patients with AD or Down syndrome [25,41,54]. Therefore, AD and CAA share a hallmark neuropathologic finding: deposition of the Aβ peptide in the central nervous system. It is therefore surprising that the clinical occurrence and manifestations of AD and CAA appear largely distinct. Although the two diseases can frequently coexist, the majority of patients diagnosed with CAAH do not have pre-existing symptoms of AD [55-57]. Similarly, only a minority of AD subjects demonstrate hem-
orrhages suggestive of advanced CAA [58]. These observations raise important questions on what factors predispose Aβ to deposit primarily in vessels rather than in brain parenchyma. Three main hypotheses on the origin of vascular amyloid have been proposed: a neuronal, a blood or systemic, or a vessel wall origin.

The neuronal origin hypothesis suggests that neurons are the source of vascular amyloid and is supported by the observations that neurons are the main source of APP and Aβ in brain [59-61]. This hypothesis is strongly supported by the transgenic mouse work.

The systemic hypothesis proposes that Aβ might be transported from blood to the walls of cerebral vessels (and brain) since in humans Aβ is present in the circulation [62,63]. A receptor-mediated transport of Aβ across the blood brain barrier (BBB) into the vasculature/brain has in fact been demonstrated and is mediated to some extent through RAGE (receptor for advanced glycation end-products) [64]. However, a major argument against a hematogenous origin of Aβ is provided by transgenic mice that constitutively overproduce the APP C-terminal fragment and have been shown to generate Aβ [66-68]. Furthermore, cerebrovascular SMCs isolated and cultured from mouse brains secrete Aβ [69]. Although Aβ production by vascular cells seems not to be a prerequisite for Aβ-CAA, Aβ from SMCs may contribute to its formation. However, the experimental proof, ie, a transgenic mouse model of Aβ-CAA in which human APP expression is confined to the vessel wall, is still lacking.

Aβ-CAA as a Result of Impaired Aβ Clearance

Elevated Aβ steady-state levels, either caused by an up-regulation of Aβ anabolism or a down-regulation of catabolism, promote Aβ accumulation [70,71]. The brain is equipped with different mechanisms to clear and keep Aβ at physiological, non-amyloidogenic concentrations [72-74]. Understanding the mechanism of these clearing pathways allows insights into pathomechanisms of Aβ-CAA.

Drainage of Aβ with Interstitial Fluid Along Perivascular Spaces

It has been suggested that Aβ drains retrograde to blood flow with interstitial fluid (ISF) along perivascular spaces of parenchymal and leptomeningeal vessels to cervical lymph nodes [73,75-77]. This reverse perivascular transport may be driven by pulsations of the blood vessel wall [78]. Several observations in transgenic mice are in line with this drainage hypothesis and stress its importance for Aβ-CAA. For instance, APP-Dutch mice produce Aβ exclusively in neurons; however, Aβ is deposited solely within vessel walls, ie, distant to the site of production. This observation suggests that Aβ is transported with ISF before it aggregates at the vasculature [79]. Further support for extracellular ISF-mediated transport of Aβ has been provided by in vivo microdialysis [80] and neural grafting experiments [81]. The site of initial cerebrovascular Aβ accumulation is consistent with the putative ISF drainage pathways. Initially, Aβ deposits are seen within basement membranes of arteries that are located at the abluminal side of SMCs [49]. This has been shown to occur

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Table 3. Boston Criteria for Diagnosis of CAA-Related Hemorrhage [53]

<table>
<thead>
<tr>
<th>1. Definite CAA</th>
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<tbody>
<tr>
<td>Full post-mortem examination demonstrating:</td>
<td></td>
</tr>
<tr>
<td>• Lobar, cortical, or corticosubcortical hemorrhage</td>
<td></td>
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<tr>
<td>• Severe CAA with vasculopathy [40]</td>
<td></td>
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<tr>
<td>• Absence of other diagnostic lesion</td>
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</table>

<table>
<thead>
<tr>
<th>2. Probable CAA with supporting pathology</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical data and pathologic tissue (evacuated hematoma or cortical biopsy) demonstrating:</td>
<td></td>
</tr>
<tr>
<td>• Lobar, cortical, or corticosubcortical hemorrhage</td>
<td></td>
</tr>
<tr>
<td>• Some degree of CAA in specimen</td>
<td></td>
</tr>
<tr>
<td>• Absence of other diagnostic lesion</td>
<td></td>
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</tbody>
</table>

Probable CAA

| Clinical data and MRI or CT demonstrating: | |
| • Multiple hemorrhages restricted to lobar, cortical, or corticosubcortical regions (cerebellar hemorrhage allowed) | |
| • Age ≥ 55 years | |
| • Absence of other cause of hemorrhage* | |

4. Possible CAA

| Clinical data and MRI or CT demonstrating: | |
| • Single lobar, cortical, or corticosubcortical hemorrhage | |
| • Age ≥ 55 years | |
| • Absence of other cause of hemorrhage* | |

*Other cause of intracerebral hemorrhage: excessive warfarin (INR > 3.0); antecedent head trauma or ischemic stroke; CNS tumor, vascular malformation, or vasculitis; and blood dyscrasia or coagulopathy. (INR > 3.0 or other non-specific laboratory abnormalities permitted for diagnosis of possible CAA).
in both transgenic mice and humans [79,82]. Changes of the vessel wall properties might impair ISF drainage and therefore promote Aβ aggregation and Aβ-CAA formation.

**Elimination of Aβ by Direct Transport into the Circulation**

In addition to perivascular drainage, Aβ is also cleared actively across the blood-brain barrier to the blood [83-85]. This translocation is mediated by the low-density-lipoprotein receptor-related protein-1 (LRP-1) [85,86] or by IgG-assisted clearance by the neonatal Fc receptor [87]. LRP-1 also interacts with many proteins that are involved in amyloidosis, ie, apolipoprotein E (ApoE) and Aβ, as well as BACE1, and presenilin (PS) 1 [88-90]. Several observations highlight the importance of LRP-1 in the development of Aβ-CAA. First, it has been suggested that the polymorphism in LRP-1 is associated with Aβ-CAA formation [91]. Second, LRP-1, although abundant in brain microvessels (including capillaries, arterioles, and small venules) in young mice and humans, is downregulated in older animals and AD brain [85]. Third, a deletion of the mesenchyme homeobox 2 (MEOX2) gene results in both reduced LRP-1 levels and an impaired Aβ efflux from the mouse brain [92]. Thus, an age-related reduction in LRP-1-mediated Aβ translocation into blood could lead to elevated Aβ levels in the perivascular space and therefore favour the development of Aβ-CAA.

**Degradation by Aβ-Cleaving Enzymes**

Aβ has been shown to be degraded within the brain by proteolytic cleavage. Neprilysin, located primarily in synapses and axons of neurons, is a major Aβ-cleaving enzyme in the brain. Impaired cleavage of Aβ could lead to its accumulation [74,93], a hypothesis that is supported by many observations. Intracerebral infusion of an inhibitor of neprilysin, for instance, induced amyloidosis in rats [57] and neprilysin-deficient mice reveal a region specific increase of Aβ levels that correlates with the susceptibility of the AD brain to amyloid deposition [94]. Interestingly, genetic polymorphisms in the regulatory region of neprilysin have been suggested to be associated with a higher risk for developing Aβ-CAA [95]. Moreover, Aβ with the Dutch, Flemish, Arctic or Italian mutations is more resistant to neprilysin-independent proteolysis compared to Aβ wild-type (wt) [96]. In addition to cleavage by neprilysin, Aβ has also been shown to be degraded in the brain by insulin-degrading enzyme (IDE) [97] and endothelin-converting enzyme-1 (ECE-1) [98].

**Mutant forms of Aβ have Different Propensities to Aggregate**

The majority of the mutated forms of Aβ occurring in familial Aβ-CAA are not only less efficiently cleaved and cleaved, but also show different fibrillogenic properties compared to Aβ wild type. Moreover and relevant for the in vivo situation, mixing Aβ40 wild type with mutated Aβ40 has been shown to enhance aggregation [99]. These mostly in vitro observations suggest that mutated Aβs involved in familial Aβ-CAA show enhanced aggregation properties that are even more pronounced when mutated and wild type Aβ is mixed. It is obvious that such enhanced aggregation properties of mutated Aβ will generally lead to reduced clearance via the above described clearance mechanisms.

**The Aβ40:42 Ratio Determines Vascular Versus Parenchymal Amyloid Formation**

Many studies have revealed that Aβ42 is much more fibrillogenic than the more soluble Aβ40 [100,101]. Amyloid formation is thought to be a nucleation-dependent phenomenon with nucleation being the rate-determining step of amyloidogenesis. Aβ42 can accelerate the nucleation of Aβ40 [101] and, just as interesting, Aβ40 has been shown to inhibit the fibril formation of Aβ42 [102]. The formation of large aggregates however depends on factors that not only affect nucleation but also elongation. Mixing both Aβ40 and Aβ42 at different concentrations can either enhance or retard nucleation and elongation. This shows that Aβ species in specific compositions have different aggregation propensities [103,104]. In vivo, the ratio of Aβ40:42 seems to be important in determining whether Aβ is deposited in brain parenchyma or within walls of the cerebrovasculature. Overexpression of human wild-type APP also causes an increase in both Aβ40 and Aβ42 and results in parenchymal and vascular amyloid in APP wild type transgenic mice [79]. This is similar to the parenchymal and vascular amyloid observed in early-onset familial AD caused by a duplication of the APP locus and to amyloidosis in Down syndrome [105,106]. Thus, increasing the production and secretion of total Aβ, ie, Aβ40 and Aβ42, leads to an increase in both, parenchymal and vascular amyloid. Consistently, overexpression of human BACE should also result in an acceleration of both parenchymal and vascular amyloidosis. The “Indiana” and “London” mutations at position 717 of APP near the γ-secretase cleavage site do not alter total Aβ production, but specifically elevate Aβ42 levels and therefore decrease the ratio of Aβ40:42 [107,108]. In PDAPP and APP/Ld mice which harbor this mutation vascular amyloid is less pronounced and increased Aβ42 production seems to provoke predominantly parenchymal Aβ deposits [109]. This finding is in line with observations made in patients carrying the APP717 mutation that show mainly parenchymal amyloid with only minor vascular deposition [110]. Thus, increasing the more insoluble and fibrillogenic Aβ42, seems to mediate primarily amyloid deposition in the brain parenchyma. In contrast a high Aβ40:42 ratio appears to promote Aβ-CAA. This is consistent with the finding that Aβ40 is the major Aβ species of vascular amyloid in humans and mouse models and demonstrates that the more soluble and less fibrillogenic Aβ40 is needed for the development of significant vascular amyloid. According to these findings, the presence of Aβ40 seems to be an important player in the formation of severe Aβ-CAA; however, several lines of evidence indicate that Aβ40 may not be sufficient for cerebrovascular amyloid formation. The finding that Aβ42 seems to be initially deposited within vessels walls in human brain [111] suggests that Aβ42 acts as a seed not only for parenchymal but also for vascular amyloid formation. Experimental evidence supporting the view that the Aβ40:42 ratio of neuron-derived Aβ determines amyloid formation in different brain compartments, ie, parenchymal vs vasculature, has been provided by various crosses of transgenic mice. APP mutations causing familial Aβ-amyloidosis affect the production and steady-state levels of Aβ40 or Aβ42. While elevation of both Aβ40 or Aβ42 (with no significant change in Aβ40:42 ratio) results in a general increase in amyloidosis, a decrease in the
Aβ40:42 ratio due to elevated Aβ42 favours the formation of parenchymal over vascular amyloid. Conversely, an elevated Aβ40:42 ratio seems to result in vascular amyloid formation in the absence of significant parenchymal amyloid plaques, most likely due to a lack of parenchymal Aβ42 seeding.

**Differences in Pathogenesis of Senile Plaques and CAA**

Although Aβ is the primary component of both SPs and CAA there are a number of observations that support the assumption of different causative mechanisms of either pathological lesion. As stated above, CAA in AD and Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type (HCHWA-D) is mainly composed of both Aβ1-40 and Aβ1-42 [112], but in advanced stages Aβ1-40 becomes the predominant form [113-119]. The Aβ content of CAA differs from that of diffuse plaques, where mainly Aβ1-42/43 [120] is found, but shows more similarity to classic SPs, that contain both Aβ1-40 and Aβ1-42/43. Moreover, high tissue content of soluble Aβ1-40 was not correlated to abundance of SPs but was associated with CAA [121]. Several proteins, such as heparan sulphate proteoglycans (HSPGs), are intimately associated with SPs and NFTs, but also with CAA. HSPGs may affect amyloid formation, probably through the action of the highly sulfated, negatively charged glycosaminoglycan side chains [122]. Different species of HSPGs have been characterized, including agrin, perlecan, syndecan and glypicanc, the expression of which differs in SP and CAA [123]. Syndecan-1 and-3 are found in SPs, but not in CAA, and, whereas agrin is abundantly expressed in SPs, it is only present in a minority of Aβ-laden vessels. Glypicanc-1 is predominantly expressed in CAA. These results suggest that specific HSPG species may be involved in the pathogenesis of CAA and that the pathogenesis of SPs and CAA differs with regard to involvement of HSPGs. Since many years it has been known that inflammatory reactions are associated with SPs. Verbeek *et al.* showed that involvement of inflammatory components in CAA is restricted to activation of the complement system, which results in the presence of complement factors, such as C1q, C3c and the membrane attack complex, in CAA [124]. Other inflammatory proteins, that are present in SPs, such as α-antichymotrypsin, α2-macroglobulin and intercellular adhesion molecule-1, were absent or detectable only in minute amounts. These data suggest that an incomplete inflammatory response occurs in CAA as compared to SPs, which was confirmed by the absence of activated cells of the monocyte/macrophage lineage around CAA compared to unaffected vessels. As mentioned above, vasculitis may be occasionally observed in association with CAA. Aβ is toxic towards both neurons and cerebrovascular cells. However, several differences in the response of cultured cerebrovascular cells and neurons to Aβ have been described [125]. First, especially ‘pre-aggregated’ Aβ-peptides are toxic for cultured neurons [126-128], whereas only soluble, non-aggregated Aβ-peptides are toxic for human brain pericytes (HBPs) [129] and SMCs [130]. Second, degeneration of cultured neurons can be induced by different Aβ peptides (Aβ25-35, Aβ1-40, Aβ1-42 etc.), whereas only Aβ1-42 (and not Aβ1-40) caused degeneration of HBPs [129] and SMCs [131]. Moreover, HCHWA-D Aβ1-40 is more toxic towards cultured cerebrovascular cells than wild-type Aβ1-40, whereas this relation was not observed for cultured neurons [132]. Finally, it has been suggested that Aβ neurotoxicity involves oxidative stress. For example, oxidative markers, such as increased levels of superoxide dismutase [133,134] and lipid peroxidation [135,136] were present in AD brains, and various reports demonstrated that Aβ toxicity in neurons *in vitro* is characterized by formation of hydrogen peroxide and lipid peroxides [137,138]. Furthermore, Aβ neurotoxicity can be inhibited by various free radical scavengers or anti-oxidants [137,139-142]. In contrast, however, Aβ-induced cell death in HBPs and SMCs does not seem to be associated with oxidative stress [143]. Neither free radicals nor oxidatively modified proteins could be detected in human SMCs exposed to pathogenic Aβ [144], Aβ did not induce superoxide radicals in rat SMCs [145], and catalase did not abrogate toxicity of Aβ towards rat vascular SMCs [146].

**Genetic Risk Factors of Aβ-CAA**

The ApoE ε4 allele is a known genetic risk factor for developing both AD and Aβ-CAA [147-150]. In contrast, ApoE ε2 seems to confer a protective effect on AD, but increases the risk of hemorrhage in Aβ-CAA patients [151,152]. ApoE binds to LRP-1 (74), and is regulated in the central nervous system by the low density lipoprotein receptor (LDLR) [153]. ApoE interacts with soluble and aggregated Aβ *in vitro* [154,155] and *in vivo* [156,157]. Since murine ApoE colocalizes with both parenchymal and vascular Aβ deposits it is likely to be involved in parenchymal and vascular amyloidosis. In fact, ApoE facilitates, but is not required, for Aβ fibril formation since mice on an ApoE-null background still develop parenchymal Aβ deposits; however, most of these deposits are diffuse in nature. These observations demonstrate that ApoE is essential for vascular amyloid formation and that the effect of ApoE in combination with the ratio of Aβ40:42 or Aβ40 levels may facilitate Aβ-CAA [158]. Further studies tested the role of human ApoE alleles on the formation of parenchymal and vascular amyloid. The presence of the ε4 allele led to substantial Aβ CAA with only few parenchymal amyloid deposits. The ε3 allele, however, resulted in almost no vascular and parenchymal amyloidosis. In young mice, an increased ratio of Aβ40:42 was observed in brain extracellular pools and a lower Aβ40:42 ratio in cerebrospinal fluid (CSF), suggesting that ApoE ε4 causes altered clearance and transport of Aβ within different brain compartments. These findings highlight again the importance of a high Aβ40:42 ratio for the formation of vascular amyl-oid.

**Hereditary forms of Aβ-CAA**

**Dutch Mutation**

The Dutch form of familial CAA, HCHWA-Dutch type was the first to be characterized and remains by far the best described. HCHWA-Dutch type is a rare autosomal dominant disorder caused by a mutation at codon 693 of the APP gene, resulting in the substitution of a glutamine for a glutamic acid at position 22 of the Aβ sequence (AβE22Q) [159,160]. Excessive Aβ deposition was found in arterioles and arteries in the leptomeninges and in the cerebral and cerebellar cortex of affected patients. Moreover, different types of diffuse SPs were present, the distribution of which is age-related, but neuritic plaques or NFT were rarely observed [160,161]. Almost all affected patients develop in-
tracerebral hemorrhages and infarcts, often leading to early death [162]. Dementia due to CAA is a common finding in HCHWA-D patients over 40 years old, which seems to be independent of plaque and NFT pathology. The disease afflicts members of 2 large families that originate from the coastal village of Katwijk in The Netherlands. A common founder is likely but has not been identified in records tracing to the beginning of the 17th century. Information is currently available on about 200 patients either alive (approximately 50) or deceased; more than 400 others are at 50% risk for inheriting the disease. Although some of the patients have moved to other parts of The Netherlands or other countries (including the United States, Germany, South Africa, and Australia), the majority lives in Katwijk or the neighboring coastal village of Scheveningen.

**Italian Mutation**

HCHWA-Italian type is caused by an APP mutation at codon 693, substituting a glutamic acid for a lysine at residue 22 of Aβ (AβE22K). HCHWA-Italian type is associated with stroke, cognitive decline, and in some cases seizures. Aβ deposits were found in meningo-cortical blood vessels and in cerebral parenchyma. The extensive vascular Aβ deposits appeared amorphous rather than fibrillar. Abnormal tau was absent, except in the hippocampus [163].

**Iowa Mutation**

The Iowa mutation in codon 694 within the Aβ region of APP resulted in substitution of an asparagine for aspartic acid (D694N) at Aβ position 23 [164]. Neuropathologically these patients are characterized by severe CAA with numerous small cortical hemorrhages and both cortical and subcortical infarcts. Abundant NFTs and dystrophic neurites were also present. Aβ plaques were relatively sparse and generally of diffuse morphology. Although small hemorrhages could be identified by magnetic resonance imaging (MRI) and postmortem examination in the Iowa pedigree, there were no reported episodes of clinically manifest ICH, a notable contrast to carriers of the Dutch mutation. A second family from Spain carrying the identical mutation demonstrated symptomatic ICH in 3 of 4 affected members [165], however, indicating that in some settings this mutation can produce major cerebral hemorrhages.

**Flemish Mutation**

Persons carrying the Flemish APP mutation, a mutation in codon 692 resulting in a substitution of an alanine by a glycine at residue 21 of the Aβ sequence present not only with severe CAA and, in most cases, cerebral hemorrhage, but also with AD with large core plaques, which enclose a vessel or are associated with vascular walls. The dementia seen in patients carrying the Flemish mutation was compatible with AD both clinically and neuropathologically. The mutation has been reported in 2 families, a 4-generation Dutch family [166], with 17 affected members identified from family informants or medical records, and a British family [167], with 5 affected members.

**Piedmont Mutation**

This is the APP mutation most recently identified in association with CAA. Again it lies within the Aβ region of APP, but away from the previously identified position 21 to 23 cluster of mutations. This G→C transversion in APP codon 705 leads to a valine for leucine substitution at Aβ residue 34. Four members of a 3-generation family from the Piedmont region of Italy had lobar hemorrhages [168].

**Arctic Mutation**

The Arctic APP (APParc) mutation identified in a family in northern Sweden spanning 4 generations was found to cause early-onset AD in a group of Swedish relatives with prominent vascular symptomatology. The mutation at codon 693 of APP/residue 22 of Aβ determines an A→G transition resulting in substitution of glycine for glutamine (AβE22G). MRI examination showed small infarcts and/or ischemic lesions, similar to what is seen in vascular dementia, without obvious neurological symptoms [169].

**Biological Considerations**

Although some alterations in APP processing have been associated with these mutations (particularly the Flemish mutation) [170-176], their primary effects appear to be on...
the biochemical and cell biological properties of the peptide itself. Isolated Dutch and Italian Aβ peptides have been reported to form β-sheet conformations, aggregate, and generate fibrils more readily than wild-type Aβ [177,178] and to be toxic to cultured human leptomeningeal SMCs [179,180]. The presence of the E22Q mutation might not only cause quantitative differences in the kinetics of Aβ fibrillogensis and aggregation [181] but could also influence the assembly of the wild-type Aβ peptides by providing Dutch fibril nuclei from which the wild-type or mixed fibrils could elongate [182,183]. Also, it has been demonstrated that a short peptide partially homologous to the central hydrophobic region of Aβ (residues 17–21), but containing amino acids that prevent the adoption of a β-sheet structure (i.e. proline), binds Aβ and inhibits amyloid fibril formation in vitro, suggesting an important role of this region for the fibrillogenesis [184].

The E22Q peptide has an increased propensity to self-aggregate and form amyloid-like fibrils when compared with the wild-type and E22K variants. Fibrillogensis with both wild-type and E22K progresses at a lower rate, perhaps reflecting the initial unordered conformation and the stability of the peptides in solution. Furthermore, Miravalle et al. observed that the Aβ40 E22Q peptide produces apoptosis in cultured human brain microvascular endothelial cells (HCEC) at a low concentration (25 μM), while the wild-type Aβ40 and the mutant E22K do not have an apoptotic effect even at concentrations as high as 50 μM. This suggests that intermediate conformers and/or the final fibrils, rather than the soluble peptide, are involved in the cytotoxic mechanisms [179].

As to the Arctic substitution, it specifically increases the rate of protofibril formation [185,186]. Aβ 42 and Aβ40 plasma levels were shown to be significantly decreased in carriers of this mutation. Low levels of Aβ were also observed in the youngest mutation carriers 20–30 years before the expected onset of the disease, suggesting a long period of biochemical abnormality before clinical onset.

All the 3 mutations at codon 693 (Arctic, Dutch and Italian) led to decreased Aβ42 concentrations in conditioned media, whereas increased levels of both Aβ42 and Aβ40 in media were found for the Flemish APP692 mutation.

Cell culture systems have found Dutch, Italian, Iowa, and Dutch-Lowa double mutant Aβ to have increased tendency to form fibrils on cell surfaces and trigger toxic responses [187,188,175,189]. Interestingly, Dutch Aβ42 may be too fibrillogenic to promote CAA, causing the peptide to form deposits before reaching the surface of vascular cells [190,191]. Finally, studies of Aβ degradation and clearance have suggested that Dutch, Italian, Arctic, Flemish, and Dutch-Iowa Aβ may have decreased susceptibility to proteolytic breakdown or transport out of the central nervous system relative to wild-type Aβ [192-194].

**Other Hereditary Aβ-CAs**

Several other mutations are known in the APP gene (see Table 1), which cause early-onset AD but are not associated with excessive CAA. These mutations are encompass substitutions of residues flanking the Aβ region of APP and apparently act through altered processing of APP and altered production of Aβ.

**NON-Aβ FORMS OF CAA**

Apart from Aβ-CAA several other forms of CAA are known that are formed by the deposition of the following proteins: amyloid-British protein (ABri) in familial British dementia (FBD), amyloid-Danish protein (ADan) in familial Danish dementia (FDD), cystatin C in HCHWA-Icelandic type, gelsolin in familial amyloidosis Finnish type (FAF), prion protein (PrP) in Gerstmann-Sträussler-Scheinker (GSS) syndrome and TTR in meningovascular amyloidosis (see Table 1). Although involvement of leptomeningeal or cerebral vessels has been described in all these syndromes PICH rarely dominates the clinical picture, with the remarkable exception of HCHWA-Icelandic type.

**Hereditary Cystatin C Amyloid Angiopathy**

Hereditary cystatin C amyloid angiopathy (HCCAA) (also called HCHWA-Icelandic type) is a rare, fatal amyloid disease in young people in Iceland caused by a mutation in Cystatin C, which is an inhibitor of several cysteine proteinases, such as cathepsins S, B, and K. The same mutation at codon 68 of Cystatin C gene at chromosome 20 resulting in a glutamine for a leucine in the cystatin C gene (168Q), has been found in all patients examined so far pointing to a common founder. Most of the families can be traced to a region in the northwest of Iceland, around Breidafjordur bay. Mutated Cystatin C forms amyloid, predominantly in brain arteries and arterioles, but also to a lesser degree in tissues outside the central nervous system such as skin, lymph nodes, testis, spleen, submandibular salivary glands, and adrenal cortex. The amyloid deposition in the vessel walls causes thickening of the walls leading to occlusion or rupture and resulting in brain hemorrhage. Although the amyloid can be detected outside the brain, the clinical manifestation is restricted to the brain [170].

The patients/gene carriers have reduced levels of cystatin C (wild-type and mutated) in their cerebrospinal fluid compared to healthy individuals. The total value is about one third of the normal value of cystatin C [195]. The genetic tests show that all the patients investigated so far harbour the same mutation and furthermore all the gene carriers tested so far have turned out to be heterozygous for the mutation. The mutation, which is situated in the hydrophobic core of the protein [196,197] determines changes in the biologic properties of the protein, making the L68Q variant less stable and more prone to dimerization and aggregation [198]. Dimmers and aggregates of the Leu68Gln variant recombinant protein are formed at normal body temperature, nearly 25°C lower than that needed for wild-type cystatin C dimerization [199]. Furthermore, while dimerization of wild-type occurs in compartments with high concentrations of the protein, the Leu68Gln variant dimerizes at lower concentrations. Actually, while cystatin C dimers and monomers are found in the plasma of HCCAA patients, only the monomer is found in the blood of healthy controls [200]. The dimerization process influences the equilibrium between the proteolytic and the inhibitory activities, and is considered, therefore, relevant for the physiological regulation of cysteine protease activity and for amyloid formation.

Based on these observations it was suggested that the effect of the Leu68Gln substitution on cystatin C is similar to
that caused by amino acid replacements in other amyloid forming proteins: Glu22Gln variant of Aβ deposited in cerebral vasculature of patients with HCHWA, Dutch type (HCHWA-D) [201,202], immunoglobulin light-chain associated with light-chain amyloidosis [203], transtatatin variants found in familial amyloid polyneuropathy [204], and amyloid formed in lysoyme amyloidosis [205]. The first step of this mechanism is a change in conformation followed by the opening of new hydrophobic surfaces, finally leading to the fibrillogenic step. The variant protein differs from the wild-type protein by an increased amount of hydrophobic components on the protein surface [206]. The exposure of hydrophobic fragments to a polar environment is an unfavorable thermodynamic state that often results in protein oligomerization and aggregation. This could be the driving force of amyloid formation.

Overall, hereditary forms of CAA are generally more severe than sporadic forms of CAA as might be expected from a fully penetrant dominant mutation, with earlier age of onset, severe clinical course, and earlier age of neurologic devastation or death. Both forms of disease tend to produce lobar hemorrhages large and small, subcortical white matter lesions, and cognitive impairment that may be at least partly independent of AD pathology. A notable difference is that ApoE genotype may have less effect on the course of hereditary than sporadic CAA, possibly reflecting the overriding role of the Aβ mutation in determining clinical course.

CONCLUSIONS

Apart from the hereditary forms which should be considered rare conditions affecting young adults, CAA is a common disease in elderly individuals and its incidence and severity increase with age. The most important clinical implication of CAA is its role as a frequent cause for non-traumatic, non-hypertensive, cerebral hemorrhage. Recently, several useful animal and in vitro models for CAA have been developed which have provided more detailed insight into the pathogenesis of CAA. However, in spite of this progress, more research and refined models are necessary to further study mechanisms, significance and impact of Aβ-CAA for developing effective therapies.

REFERENCES

Alzheimer disease.


Cerebral Amyloid Angiopathy-Related Hemorrhage


