Hereditary Cystatin C Amyloid Angiopathy: Genetic, Clinical, and Pathological Aspects

Palsdottir A[1]; Snorradottir AO[1]; Thorsteinsson L[2]

[1] Institute for Experimental Pathology, Reykjavik, University of Iceland.
[2] The Blood Bank, National University Hospital, Reykjavik, Iceland.

Corresponding author:
Astridur Palsdottir, Institute for Experimental Pathology, University of Iceland, Vesturlandsvegur, 112 Reykjavik, Iceland (E-mail: *astripal@hi.is*)

Hereditary cystatin C amyloid angiopathy (HCCAA) 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 in cystatin C, L68Q, 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, and usually consists of repeated hemorrhages leading to paralysis. Sometimes the initial signs of hemorrhage are dementia and personality changes.

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INTRODUCTION

Hereditary cystatin C amyloid angiopathy (also called hereditary cerebral hemorrhage with amyloidosis-Icelandic type) was first described by a country physician in the northwest of Iceland (7). He noticed that many young people in his region were dying of brain hemorrhage and he also realized the genetic nature of the disease.

Almost 4 decades after the first description of the disease, Congo red staining of tissue sections revealed amyloid deposits in the arteries of the central nervous system (17).

A decade later a protein purified from the amyloid was sequenced and found to be a recently described protein called cystatin C (formerly γ-trace) (10).

Cystatin C is a low molecular weight cystatin of type 2 and is present in all body fluids, predominantly in cerebrospinal and seminal fluids, where it is a major inhibitor of extracellular cysteine proteinases (6). The gene spans 4.5 kb and consists of 3 exons. It is situated on the short arm of chromosome 20, 20p11 (2, 3).

HCCAA is classified as one of the cerebral amyloid angiopathies, CAA (34). The other proteins known to form amyloid in cerebral vessels are: Aβ, prion protein, gelsolin, transthyretin, and the ABri and ADan precursor proteins (34).

FAMILY STUDIES

HCCAA is an autosomal dominant disease which has now been assigned to 212 individuals in 9 sub-families in Iceland, either directly through post-mortem diagnosis, by a study of earlier patient records/death certificates, or by tracing obligatory gene carriers through the families. In most families the mutation is extinct. An example of one sub-family is depicted in Figure 1, showing 6 generations. The disease occurs in every generation and can be traced to a couple that were born in the 18th century.

A study was made of a group of ninety-three HCCAA patients born between 1900 and 1950. The average age of death for this sample population was 30.2 years for women (n = 44) but 32 years for men (n = 49) (Figure 2). The youngest patients died aged 15 years but some lived beyond the age of 50. It is obvious from the pedigree data that the penetrance of the mutation is very high; however, some individuals live a normal lifespan and die of other causes (38).

The patients belong to 9 families, 8 of which originate in the same region (21) around Breidafjordur Bay; one family has also been found in the south of Iceland (Figure 3). The common founder of the 9 sub-families has not yet been identified, although all families share the same L68Q mutation (22, 33). The mutation is found

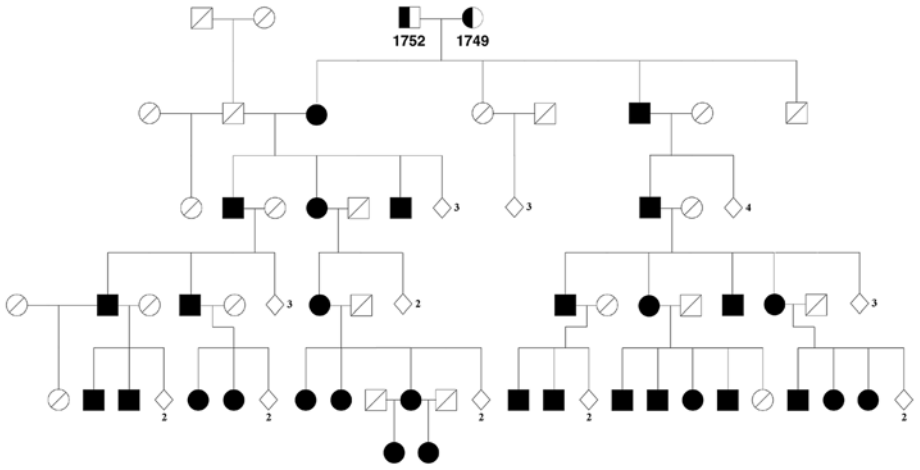

Figure 1. A simplified pedigree showing the HCCAA disease in every generation. Filled signs indicated disease and unfilled signs indicate healthy individuals. Squares signify men and circles women. Triangles indicate several healthy siblings.

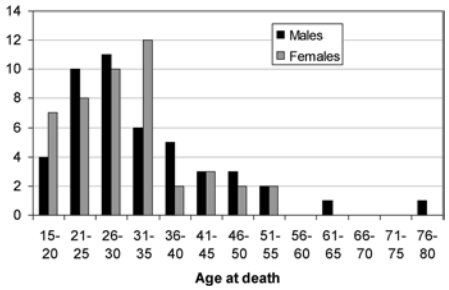

Figure 2. Number of HCCAA patients and their age at death in each 5-year interval is shown.

Figure 3. Map of Iceland showing (hatched) the area around the Bay of Breidafjordur where most of the descendants of the eight original families originated. Also shown is the region in the South of Iceland were one family is found.

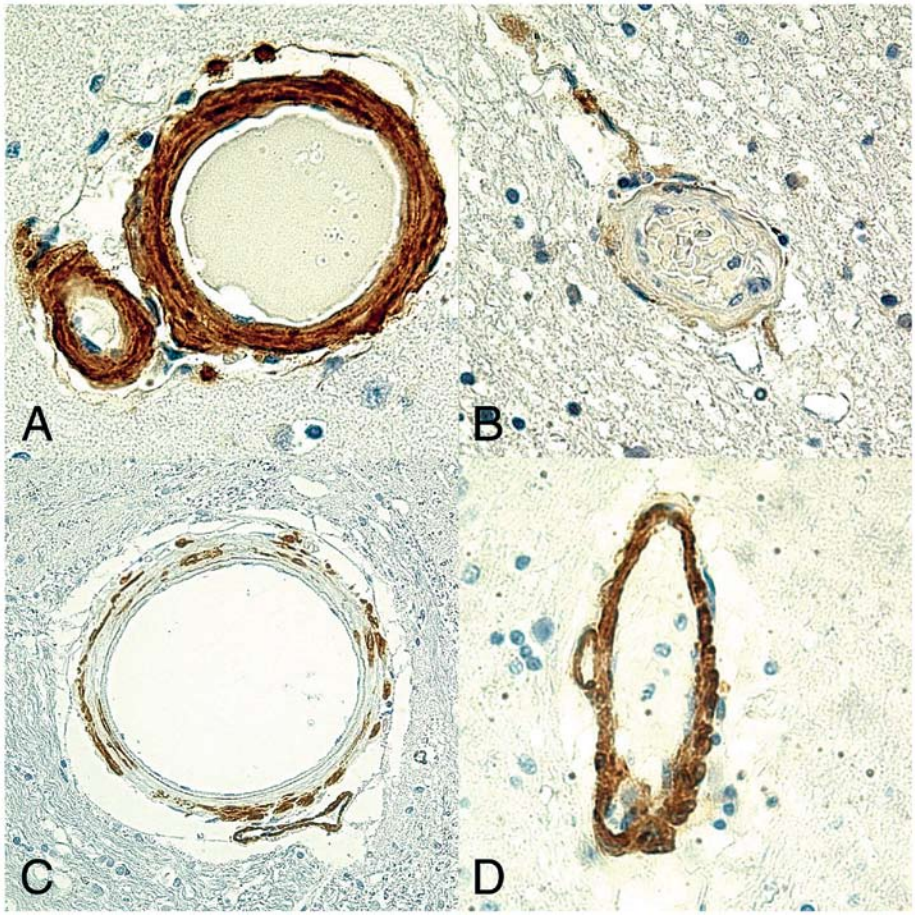

Figure 4. An immunohistochemistry study of brain tissues of patients that died of HCCAA. Antibodies: Cystatin C polyclonal antibody (DAKO; 1:500) and monoclonal anti smooth muscle α-actin (DAKO; 1:50). **A.** Immunostaining using cystatin C antibody, HCCAA patient. Intense staining of tunica media of arterioles (brown). **B.** Immunostaining using cystatin C antibody, control sample. Weak staining of adventia but no staining in tunica media of an arteriole. **C.** Immunostaining using smooth muscle α-actin antibody, HCCAA patient. Few scattered smooth muscle cells in media, which is mostly composed of amyloid. **D.** Immunostaining using smooth muscle α-actin antibody, control sample showing strong staining of media. Magnification: ×40.

exclusively in Iceland with one exception in a sporadic case found in the United States (15). A systematic search for the L68Q mutation in young patients with brain hemorrhage has not yielded any cases outside Iceland (29, 30).

CLINICAL FEATURES

The main clinical feature of HCCAA is stroke in a previously healthy young adult (17, 26, 40). A typical presentation is the sudden appearance of symptoms of intracerebral hemorrhage which are appropriate to the location of the hematoma. Massive sensorimotor hemiparesis with or without aphasia or symptoms of neglect are the most common initial symptoms. Usually there is no precipitating event, but on occasion the symptoms appear to be initiated by exercise. The great majority of patients present with the typical symptoms but atypical presentation, including primary mental symptoms, such as progressive dementia or psychiatric symptoms, is rarely seen. This atypical presentation is more common in the few patients who present in the fifth decade or later, but such cases have been very rare (38).

The diagnosis is based on the typical symptoms occurring in a person in the late-teens, 20s or early-30s. In general, there is a family history of the disease and the risk of the disease is usually known to the individual as typically a parent, one grandparent and usually uncles and aunts have died of the disease. However this is not always the case and sometimes there is only the history of a parent dying young.

Commonly the patient will survive the first hemorrhage and go on to have further strokes; if he or she survives long enough multi-infarct dementia will emerge. The symptoms of HCCAA are not distinguished from intracerebral hemorrhage due to other causes and it is only the young age of the patient and presence of a family history that provide clues to the diagnosis.

GENETICS/DIAGNOSIS

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 (16). A quantitative test formed the basis of the initial diagnosis of pre-symptomatic family members. Once the cystatin C gene was cloned and characterized (1), it became possible to use molecular techniques to accurately diagnose the disease by detecting the L68Q disease mutation, which causes the loss of an AluI restriction site, first by Southern blotting (33) and later by PCR (4). 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 L68Q mutation results from a substitution of a

thymidine (T) for an adenine (A) base in exon 2 of the cystatin C gene (4, 24).

The mutation, which is situated in the hydrophobic core of the protein (5, 13) makes the L68Q variant less stable and more prone to dimerization and aggregation (12). It has been shown to form dimers through 3-dimensional domain swapping resulting in the loss of inhibitory activity (20). The L68Q gene carriers have normal cystatin C values (wild-type and mutated) in their plasma, suggesting that abnormal breakdown of the mutant protein is responsible for the low values in the cerebrospinal fluid (16). The low levels of protease inhibitors in the CSF (compared to blood) could possibly contribute to the breakdown of mutated cystatin C (A. Grubb, personal communication). Cystatin C dimers are found in the plasma of HCCAA patients but only the monomer is found in the blood of healthy controls (9). No antibody exists which can differentiate between the normal and the mutant form of cystatin C.

The advent of the genetic test, genetic councelling, and better information about the genetic nature of the disease has resulted in fewer births of gene carriers since 1988. It is estimated that very few gene carriers are now left in 3 sub-families.

PATHOLOGICAL ASPECTS OF HCCAA

Macroscopic pathology. Postmortem examination of the brain from patients shows in most cases multiple lesions of different ages and sizes, apparently hemorrhagic in nature, located in both the cerebral cortex and white matter of all lobes of the brain as well as in the basal ganglia region (26). Organs outside the central nervous system do not show any macroscopic pathologic changes (31, 32, 39).

Histopathology. Microscopic examination shows a widespread hyalinization of the walls of arteries and arterioles throughout the brain and spinal cord, which concentrically narrows the vessels, in some cases to the point of complete occlusion. Vessels of the optic nerve are also affected. The affected vessels frequently show splitting of their media and aneuyrism formation, and perivascular fibrosis is often found. In some cases parenchymal vessels have increased perivascular space, sometimes containing hemosiderin loaded macrophages.

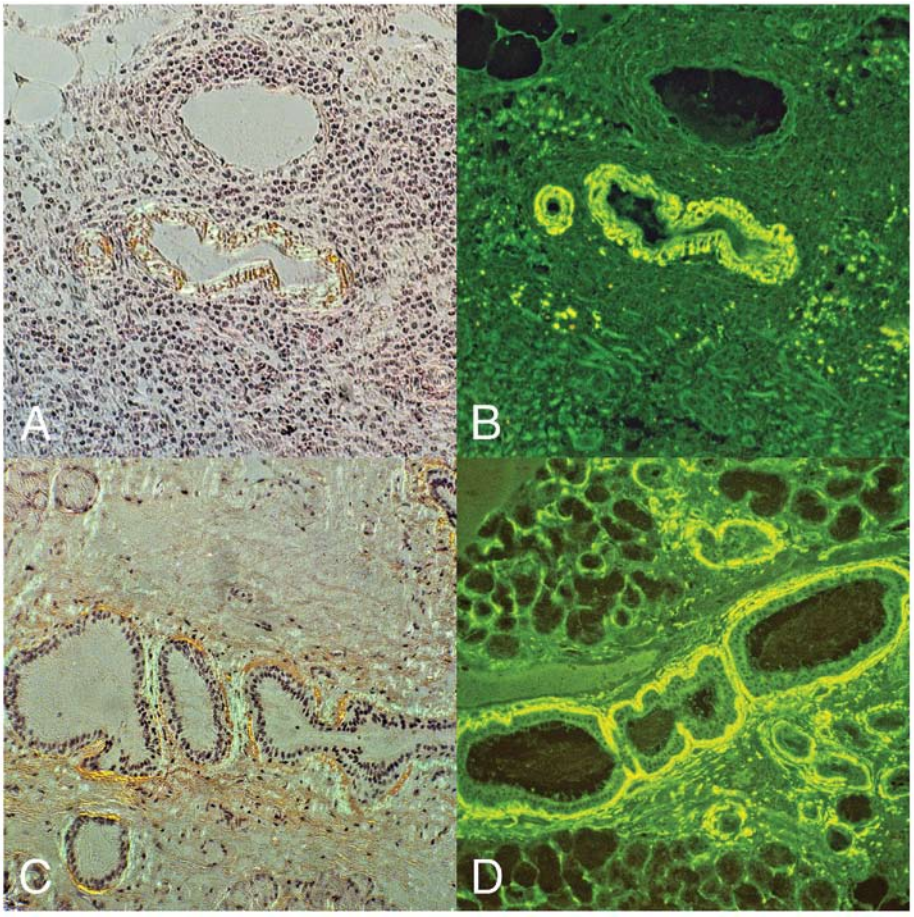

Figure 5. An immunohistochemistry study and Congo red staining of peripheral tissues of patients that died of HCCAA. Antibodies: Cystatin C polyclonal antibody (DAKO; 1:500) **A.** Paratracheal lymph node. Staining with Congo red. Greenish birefringence of Congophilic amyloid deposits is seen in the arterial wall, using polarized light. **B.** Cystatin C immunostaining of the Congophilic layer in the same section. **C.** Submandibular salivary gland. Staining with Congo red. Greenish birefringence of Congophilic amyloid deposits are seen in the interlobular connective tissue, using polarized light. **D.** Submandibular salivary gland. Cystatin C immunostaining of the interlobular layer. Magnification: ×40.

Thrombi, both fresh and organized, are also found. Veins and capillaries are either minimally affected or unaffected (31, 39). Biochemical characterization of the Congophilic material has shown that the amyloid deposits are largely composed of cystatin C protein (10, 14, 24).

Amyloid in the central nervous system. The cystatin C staining is strongest in the central nervous system (Figure 4A, B). It can be seen in the walls of practically all small arteries and arterioles in the cerebrum, optic nerve, basal ganglia, hippocampus, and leptomeninges (26, 39).

In some cases the leptomeninges are strongly stained with cystatin C, mainly in the spinal pia and in the arachnoid granulations of the superior sagittal sinus. Extensive cystatin C deposits have also been seen in the interstitial tissue of the basal ganglia and hippocampus, mainly in a perivascular location (32, 39). Leptomeningeal and cortical vessels are also associated with an increase or activation of monocyte/macrophage lineage cells (41).

The deposition of cystatin C amyloid in the vascular walls is associated with a loss of smooth muscle cells in the media of the vessel wall, as demonstrated by the disappearance of α-smooth muscle cell actin immunoreactivity (Figure 4C, D). A difference is seen between the large elastic arteries and the muscular arteries, the latter being more severely affected. This loss of smooth muscle cells is in agreement with an earlier study of HCCAA (43) and has also been described in Aβ-CAA in Alzheimer disease (23). Amyloid laden cerebral vessels in patients with Aβ-CAA were shown to have less smooth muscle and to rupture more easily in direct correlation to their cystatin C immunoreactivity (19).

So far limited pathologic studies have been performed on brain vessels in HCCAA. The amyloid P component has been found in the brain vessels of a HCCAA patient (11, 26, 35). In the brain vessels of patients with Aβ-CAA of Alzheimer disease, both sporadic and hereditary, cystatin C can be detected as a secondary amyloid by immunostaining (18, 25, 42). DNA analysis of one case with sporadic CAA showed that the cystatin C gene was wild-type (27). However, Aβ amyloid immunoreactivity has not been found deposited in the cystatin C amyloid in HCCAA (18) and neither senile plaques nor neurofibrillary tangles of the Alzheimer disease type have been seen in brains of HCCAA patients.

Amyloid in other organs. Cystatin C amyloid deposits have been localized immunohistochemically to tissues ouside the central nervous system, eg, in skin (8) and in arteries of lymph nodes (Figure 5A, B). The central arteries of the splenic corpuscles and its venous sinuses show the same reaction. Interlobular connective tissue of the submandibular salivary gland shows a moderate reactivity when stained with anticystatin C (26, 39) (Figure 5C, D). However the arteries were practically negative. Strong and clear congophilic substance has also been demonstrated in sinusidal vessels of the zona fasiculata of the adrenal cortex.

Other related diseases. Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) has some clinical features which are similar to HCCAA in that both diseases cause brain hemorrhage due to amyloid deposition in brain arteries or arterioles (28, 34). Both diseases are inherited in an autosomal dominant manner. However in HCHWA-D, the amyloid protein is mutated Aβ protein, while in HCCAA it is mutated cystatin C (28). Also in HCHWA-D the age of onset is somewhat higher than in HCCAA (28).

It is not known why the cystatin C amyloid is deposited in the brain vessels of HCCAA patients and how this leads to brain hemorrhage. It is possible that the transforming growth factor β-1 (TGF-β1) plays a role, as cystatin C and TGF-β1 interact in 2 ways. TGF-β1 can increase the expression of cystatin C (12, 36, 37) and recently cystatin C was shown to bind to the TGF-β1 receptor, TGFRII, thus inhibiting the interaction of TGF-β1 with its receptor (36). Dysregulation of TGF-β1 signalling due to lowered cystatin C levels in the cerebrospinal fluid of gene carriers could possibly contribute to the disease.

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