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REVIEW

Brain Pathology

The historical background of hereditary cystatin C amyloid angiopathy: Genealogical, pathological, and clinical manifestations

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Abstract

Hereditary cystatin C amyloid angiopathy (HCCAA) is an Icelandic disease that belongs to a disease class called cerebral amyloid angiopathy, a group of heterogenous diseases presenting with aggregation of amyloid complexes and deposition predominantly in the central nervous system. HCCAA is dominantly inherited, caused by L68Q mutation in the cystatin C gene, leading to aggregation of the cystatin C protein. HCCAA is a very progressive and severe disease, with widespread cerebral and parenchymal cystatin C and collagen IV deposition within the central nervous system (CNS) but also in other organs in the body, for example, in the skin. Most L68Q carriers have clinical symptoms characterized by recurrent hemorrhages and dementia, between the age of 20–30 years. If the carriers survive the first hemorrhage, the frequency and severity of the hemorrhages tend to increase, resulting in death at average of 30 years with mean number of major hemorrhages ranging from 3.2 to 3.9 over a 5-year average life span. The pathogenesis of the disease in carriers is very similar in the CNS and in the skin based on autopsy studies, thus skin biopsies can be used to monitor the progression of the disease by quantifying the cystatin C immunoreactivity. The cystatin C deposition always colocalizes with collagen IV and fibroblasts in the skin are found to be the main cell type responsible for the deposition of both proteins. No therapy is available for this devastating disease.

KEYWORDS

cerebral amyloid angiopathy, collagen IV, cystatin C, hemorrhage, hereditary cystatin C amyloid angiopathy, N-acetylcysteine

1 | THE BACKGROUND STORY

Hereditary cystatin C amyloid angiopathy (HCCAA, MIM #105150; also referred to as hereditary cerebral hemorrhage with amyloidosis-Icelandic type) was first described by a country physician, Arni Arnason [1]. Arnason noticed that many young people (79 individuals documented) living in the western part of Iceland were dying of brain hemorrhage before the age of 40. He also noticed the genetic nature of the disease and that the disorder was characterized by anticipation. Further investigation demonstrated that HCCAA belongs to a group of familial and sporadic diseases called cerebral amyloid angiopathy (CAA) [2]. HCCAA is an ultra-rare hereditary CAA disease that is only found in Iceland [3]. Pathological features of the disease were subsequently reported based on post-mortem brain samples, showing cerebral artery thickening with amyloid deposition in the arterial walls [4]. Later, it was found that the amyloid was derived from cystatin C (formerly known as gamma trace) [5]. Immunohistochemistry study, using cystatin C antibody, showed strong staining in cerebral arteries/arterioles [6] but also to lesser extent in other organs, such as lymph nodes, glands, and skin [7, 8]. This resulted

in HCCAA being classified as a hereditary CAA, the first hereditary CAA disease reported [4]. CAA diseases present with cerebral amyloid and parenchymal deposition in the central nervous system (CNS), where the amyloid can have different protein constituents [9, 10]. CAA is one of the major causes of recurrent intracerebral hemorrhages and progressive dementia, with high annual risk of recurrent strokes [11, 12]. HCCAA is a progressive disease and is known as the most severe form of CAA [3]. HCCAA has been found in several families in Iceland, is inherited in an autosomal dominant way with high penetrance, and is caused by a mutation in the cystatin C gene (*CST3*) [3, 13–16]. *CST3* encodes for a cysteine proteinase inhibitor which is present in all body fluids with the highest concentration in the cerebrospinal fluid (CSF) [17].

All the carriers are heterozygous and have the same founder mutation in the *CST3* gene, a single base substitution, which results in the exchange of leucine for glutamine at amino acid 68 of the protein, hereafter referred to as L68Q [16]. The L68Q mutation makes the protein less stable and more prone to form dimers and high-molecular-weight oligomers/polymers that precipitate [18, 19]. The protein is found in high concentrations in the CSF of healthy individuals, whereas mutation carriers have lower levels of cystatin C or about 1/3 of normal levels [20, 21].

The initial medical diagnosis for living L68Q carriers was a measurement of cystatin C levels in CSF or assessment by Congo red staining on postmortem brain samples [4, 21]. When the cystatin C gene had been cloned and sequenced it became possible to use DNA techniques to make a definite diagnosis for individuals at risk [16, 22].

2 | GENEALOGY

The genealogy of the disease has been well-documented [23]. The ancestors of present-day families were uncovered using "The book of Icelanders" a database set up by deCode genetics containing names and linages of 904,000 Icelanders born since settlement in the year, 874. Whole country census, the earliest from 1703, was used to study family structure and location, and parish church records were used to find dates of birth as well as cause of death if available. Medical records (since 1911) were evaluated for disease diagnosis as were death certificates. Obligate carriers are defined as at-risk persons due to their location in the pedigree. The number of births and deaths each decade since 1800 can be seen in Figure 1. The rate of births was at its zenith until late 1800 but began dropping fast after 1900.

3 | FAMILIES

The disease has been found in several families with origin from the western part of Iceland (Figure 2). Originally

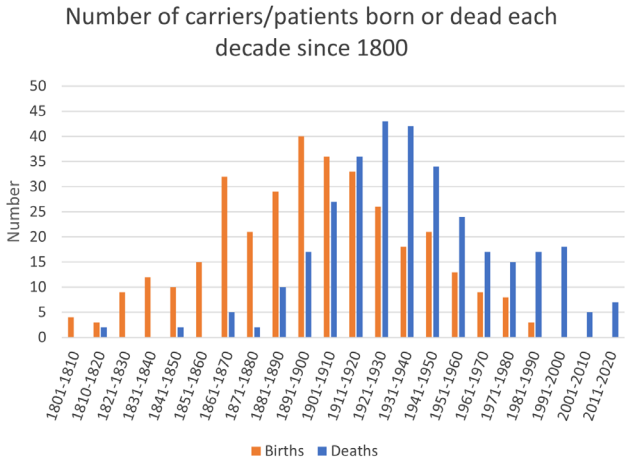

FIGURE 1 Carriers/patients born each decade since 1800. A graph showing the rate of births and deaths for carriers, living carriers are not included. The number of deaths peaked three decades after births.

FIGURE 2 Map of Iceland showing where the HCCAA families lived in 1800. The stripes on the map show where the HCCAA family lived. The oldest known common carriers were a sib pair, a man (born 1684) who moved from the Snæfellsnes peninsula (marked by a star) to the South coast and started a big family, and his sister (born 1670) an ancestor of a big family in the west country.

there were 16 families identified but now they are 14, after some have been connected to ancestors through genealogy connection [24]. Those 14 families include 346 individuals born with the L68Q mutation since 1800 and constitute our database registry. They are only listed if their cause of death is brain hemorrhage in parish records, death certificates confirming the diagnosis, or by DNA diagnosis when available. Included are also obligated mutation carriers, who by their location in the pedigree must have inherited the L68Q mutation. Childless siblings of patients/carriers were omitted if no information was available about their cause of death despite being at risk and dying young. The registry also includes 153 spouses of patients and their age at death. It has not been possible to connect all families to one common ancestor, but the oldest known common ancestor was a man born in 1684 in Snæfellsnes peninsula (marked with

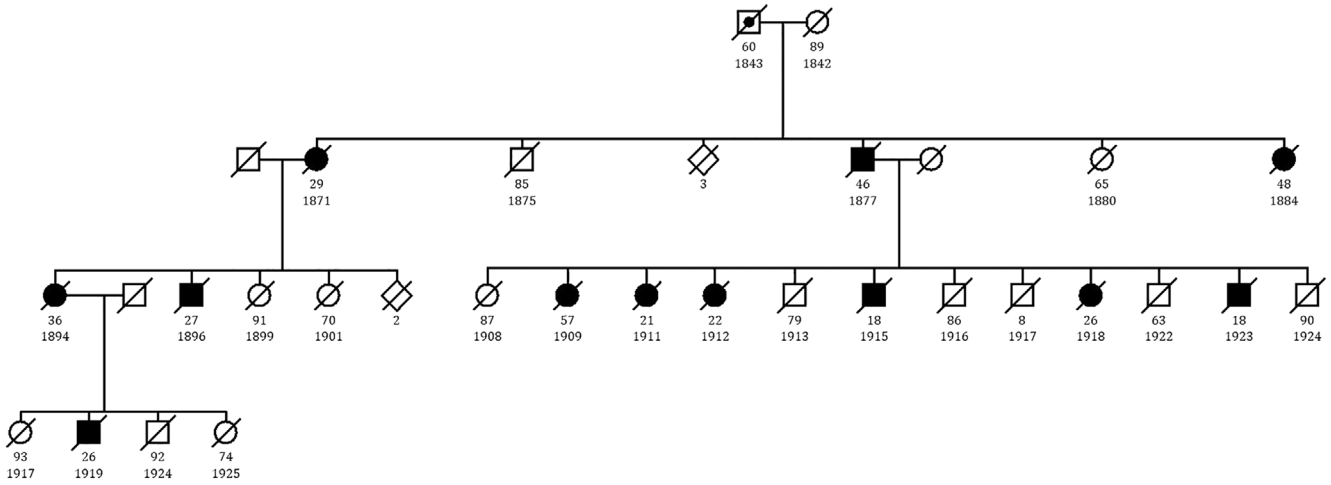

FIGURE 3 Pedigree of a typical HCCAA family. The pedigree shows a typical HCCAA family, women are depicted as circles, men as squares. The year of birth and the age at death are shown under each symbol. Obligate carriers are labeled with a dot and those who got a diagnosis, either in parish records or a death certificate are shown as full symbols. The oldest male is linked to another family.

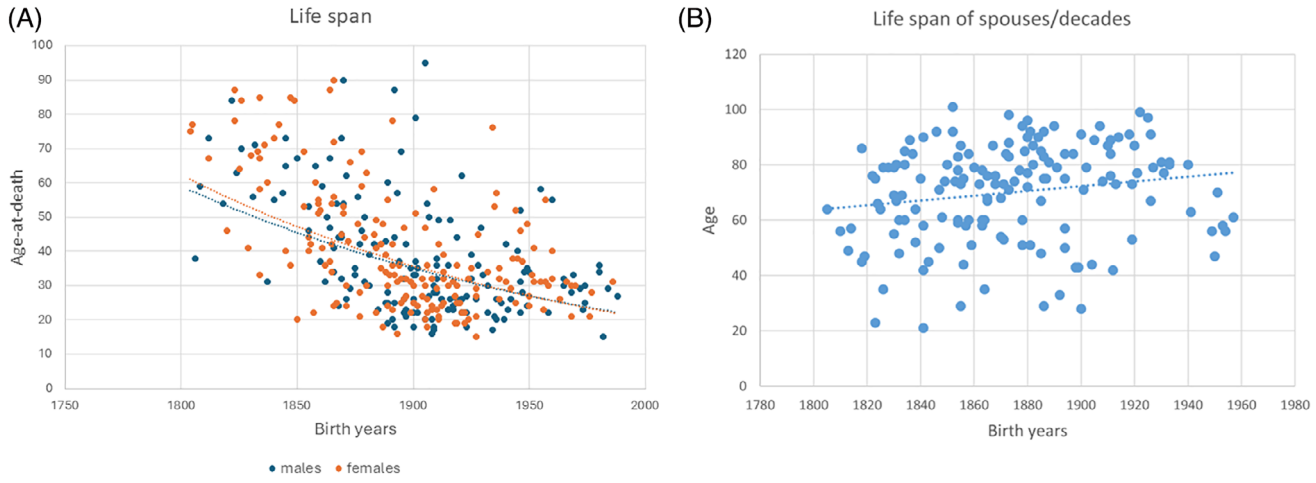

FIGURE 4 Life span of HCCAA carriers and spouses. (A) A graph showing the relationship between the life span and the year of birth of carriers since 1800. The life span fell during the 19th century from about 60 years on average to about age 30 at the end of the century. This was noticeable for both female and male patients. (B) A graph showing the relationship between the life span of the spouses of HCCAA carriers. As shown, the life span of spouses increased slightly after 1800 in contrast to the carriers who's life span dropped significantly after 1800. The dotted lines present the trendlines. Adapted from [23] with more individuals.

a star in Figure 2) who moved to the south coast of Iceland where two large families can be traced to him. Many families in the western part of Iceland can be traced to his siblings and cousins. The disease has died out in 9 families but is still found in 5 families today. A typical pedigree of a HCCAA family is shown in Figure 3. As is expected in an autosomal dominantly inherited disease approximately half of adult siblings inherited the L68Q mutation.

4 | THE AGE OF THE L68Q MUTATION

deCode genetics used microsatellite analysis of 36 L68Q carriers to determine the age of the mutation. It was estimated that it occurred 18 generations ago, placing it in around year 1550 [23]. It can be estimated that the mutation spread to several regions in the west and south of Iceland for 3 centuries as it had no negative consequences for carriers until the middle of the 19th century.

5 | A SHARP DROP IN LIFE SPAN IN THE MIDDLE OF THE 19TH CENTURY

When all carriers and patients are pooled ($n = 346$) and life span studied with respect to year of birth a trend of shorter life span over time is observed: Beginning with those born around 1840 their life span became shorter and shorter with each decade [23] (Figure 4a). This

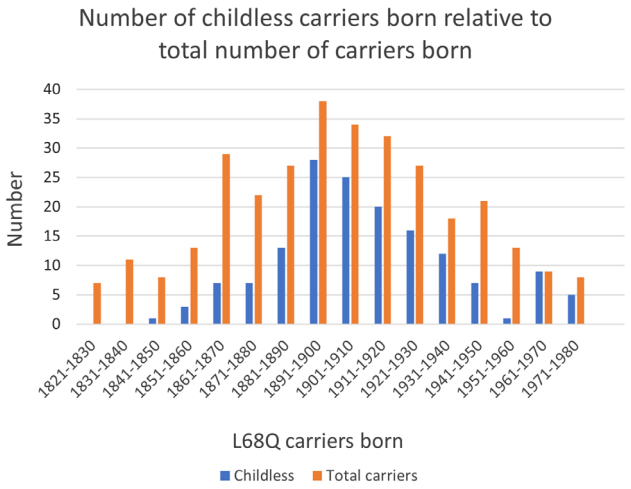

FIGURE 5 Number of childless carriers born. A graph showing that the number of childless carriers born increased relative to the total number of carriers born. The number of childless individuals increased as the penetrance of the cystatin C L68Q mutation increased during the 19th century. The first signs of disease or death often occurred too early for marriage or childbirth.

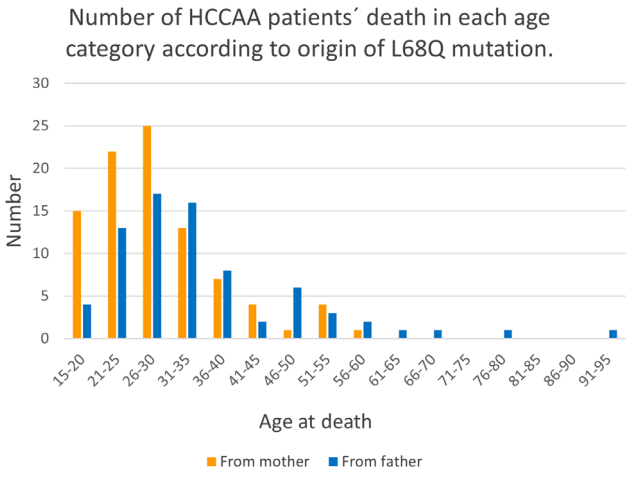

FIGURE 6 Maternal or paternal inheritance. A graph shows that carriers who got the L68Q gene from their mother had a shorter life span than those who inherited it from their father.

happened fast and sometimes parents and offspring died in the same decade. By 1900 the average life span was down to 30 years on average and has remained so since. The life span of the spouses of carriers/patients was used as control as they shared the same diet and housing. In the middle of the 19th century, the life span was the same for carrier/patients and spouses but that of spouses ($n = 153$) increased slightly during the same time (Figure 4b). As the penetrance of the mutation increased during the 19th century, the number of childless carriers increased, and clinical symptoms of disease or death often occurred too early for marriage or having children (Figure 5).

In the 5 extended families that exist today, a small subset (2%–4%) of DNA-typed carriers are known to get symptoms later and die later than the typical carriers who die as young adults. These five families are the only families that still have living carriers/patients. This was first described in one family in 1996 [25]. This effect could be the result of another modifying gene or potential environmental factor as the effect is not stable. Sometimes longer-living carriers have short-lived offspring and vice versa. Interestingly there is at least one known case of a longer-living patient dying not of brain hemorrhage but of heart failure due to amyloid in the heart. Approximately 2%–4% of obligate carriers have longevity.

6 | PARENT-OF-ORIGIN EFFECT ON LIFE SPAN

Looking at the origin of the mutated gene, that is, depending on whether it was inherited from the mother or father it became evident during the 19th century that those who got the gene from their mother had a shorter life span than those who inherited it from their father. The difference grew during the 19th century and around 1900 life span for those with the gene from their mother was 9.4 years shorter than those who got it from their fathers [23]. The origin of this difference is unknown, but because they occurred at the same time as the environmental effect they might be related. After 1900 the difference in life span is more pronounced with patients inheriting the mutation from their mother dying younger compared to those who got the mutation from their father (Figure 6).

7 | POSSIBLE ENVIRONMENTAL EFFECT ON LIFE SPAN

The fact that the changes in life span occurred all over the country points to common environmental factors taking place all over the country at the same time, that is, second half of the 19th century. The most parsimonious explanation is the change in diet which occurred at the same time.

For centuries the diet of Icelanders had been based on fishing and dairy products from cows and sheep. Barley could not be grown after 1500 due to colder climate and the only fruit available was wild berries. Lack of salt meant that food had to be preserved in acid whey in barrels. Around 1840 grain and sugar imports gradually grew, with annual amount of imports being recorded [26]. The diet shifted from one very low in carbohydrates to a more "European-style" diet with grain and sugar import [27].

8 | HCCAA IN USA AND CANADA

The parish church records list Icelanders who emigrated to USA or Canada, in the last decades of the 19th

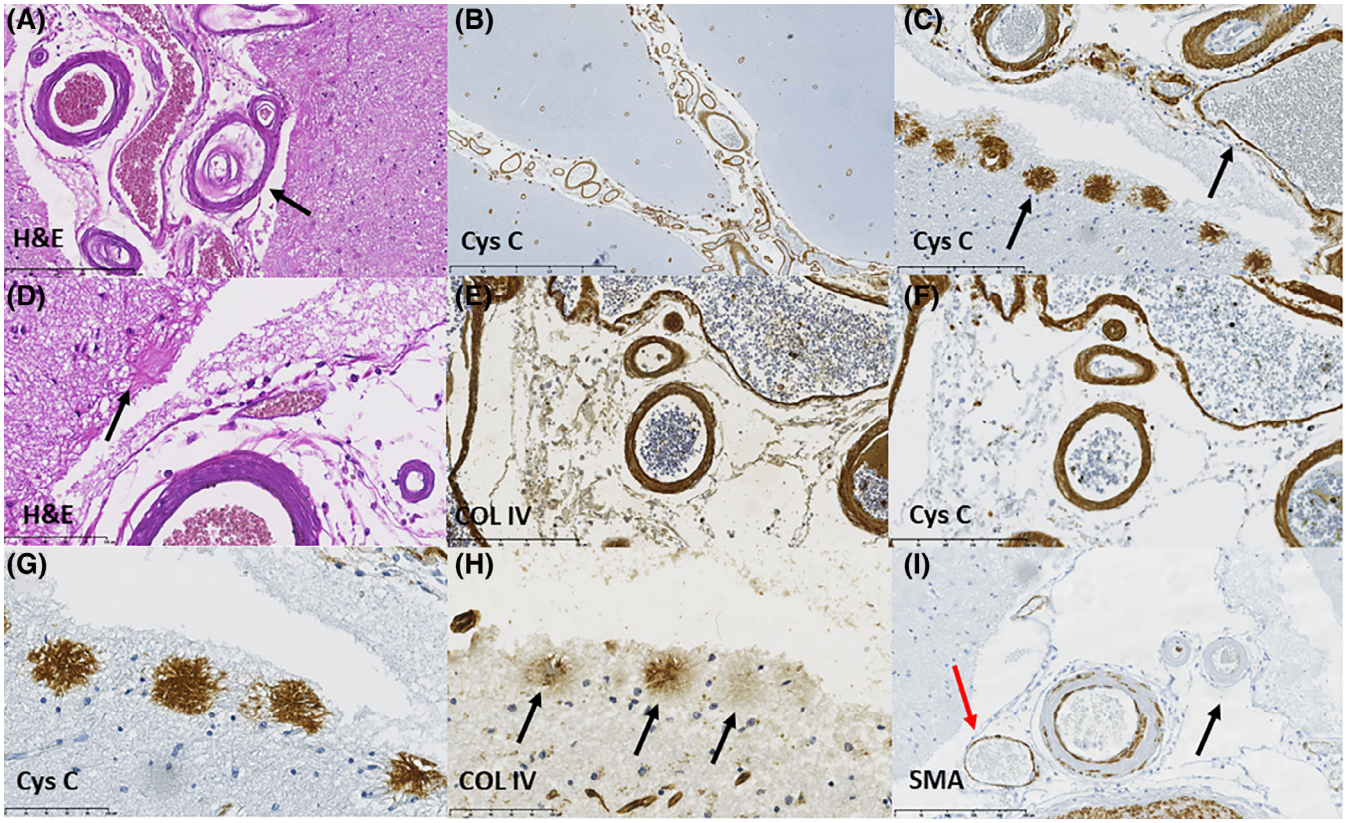

FIGURE 7 Brain pathology in HCCAA patients. (A)–(I) Post-mortem brain samples showing leptomeningeal vessels and the surface of a sulcus in the cerebrum. (A) H&E staining shows thickened and acellular arterial vessel walls, smaller arteries/arterioles are more affected, and affected vessel with double barrel lumen (arrow). (B) Severe cystatin C deposition (cystatin C amyloid complexes) in all layers of the vessel wall in almost all vessels in leptomeningeal space and in the cerebral cortex, with deposition most prominently in medium and small-sized arteries and arterioles. Some arterioles with completely occluded lumen. (C) Cystatin C immunostaining showing focal parenchymal cystatin C deposits/plaques (amyloid cystatin C complexes) at the surface of a sulcus of the cerebrum (arrow pointing to one plaque). Deposition is also evident in vein (arrow). (D) Arrow pointing to plaque in the surface of a sulcus in the cerebrum in H&E staining. (E) Collagen IV immunostaining in vessels, dense accumulation of collagen IV in both arteries/arterioles and veins/venules, in all layers of the vessel walls. (F) Cystatin C deposition in same vessels as seen in (E) shows very similar distribution as collagen IV accumulation. (G), (H) Same focal deposits/plaques in adjacent sections stained with cystatin C (G) and collagen IV (H), arrows point to collagen IV staining in same focal deposits as seen in cystatin C staining. (I) Affected arteries/arterioles with scarce SMA staining and arterioles with no SMA cells left (arrow), red arrow pointing to venule with mostly intact SMA layer. Scale bar: 250 μm on all figures.

century. Emigrants were recorded in a book called Vesturfaraskrá (New World emigrants' lists) [28]. At least 4 at-risk individuals left Iceland and sailed to USA/Canada during those years. Their obituaries in the local Icelandic newspapers printed in America mention diseases like stroke and "illness of the brain" but the HCCAA disease does not seem to have spread there as their descendants died young. The search for more at-risk emigrants is ongoing.

9 | PATHOLOGICAL FEATURES

Extensive research on post-mortem brain samples was performed by Snorradottir et al. with an emphasis on structural changes within the arterial wall of affected vessels and within the brain parenchyma [29, 30]. These studies revealed that at the stage of autopsy, the severity of CAA due to cystatin C amyloid complex deposition in all patients examined is always classified as severe (Figure 7A–C), using criteria defined by Vonsattel et al. [31]. Amyloid deposition is found in all vessel types, but small/medium-sized arteries and arterioles are most affected. This severity of the deposition in post-mortem brain samples is irrespective of age or brain area, for example, leptomeninges, cerebrum, thalamus, midbrain, cerebellum, and basal ganglia. The topography and progression of the CAA in HCCAA is Stage 3, defined by criteria established by Thal et al. [32, 33]. The criteria divide CAA into three stages based on CNS distribution. In stage 1, CAA is present in leptomeningeal or parenchymal vessels of neocortical areas, in stage 2 in allocortical, midbrain, and cerebellar vessels, and Stage 3, in subcortical nuclei such as the thalamus, the basal ganglia, the lower brainstem or the white matter. As CAA is found in all brain areas in HCCAA post-mortem brains, the CAA in HCCAA is Stage 3. Focal cystatin C deposits and perivascular deposits can be found in about 1/3 of

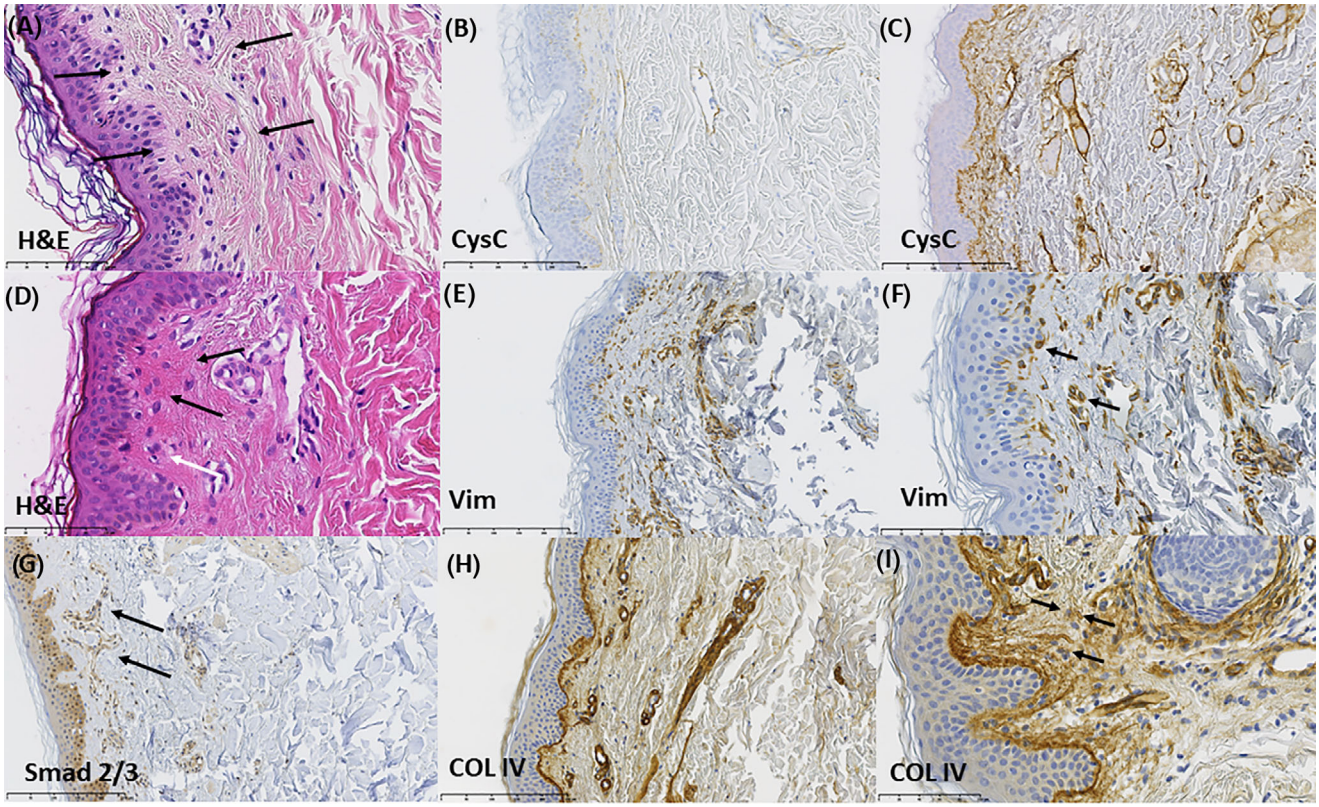

FIGURE 8 Skin pathology in HCCAA patients. (A) H&E staining in skin biopsy from a carrier shows normal skin structure, apart from increased density of cells in the upper dermis, right beneath the epidermis (arrows). (B) Cystatin C immunostaining in asymptomatic carrier with relativity weak staining in the basement membrane between epidermis and dermis and in basement membranes in vessels in the dermis. (C) Extensive cystatin C deposition (reflective of cystatin C amyloid complexes) in a symptomatic carrier, most evident in the basement membrane between epidermis and dermis and in basement membrane vessels in the dermis. (D) Higher magnification of H&E staining showing eosinophilic material right beneath epidermis and increased density of cells (arrows). (E) Increased density of vimentin-positive cells in the same area as seen in (D), that is, in the upper dermis. (F) A higher magnification of vimentin-positive cells, these cells have the activated appearance of fibroblasts (arrows point to two examples). (G) SMAD 2/3 positivity in activated fibroblasts in the upper dermis and in vessels (arrows). (H) Collagen IV immunostaining with intense positivity in basement membranes, especially between epidermis and dermis. (I) Collagen IV immunostaining in higher magnification shows cells (which are also vimentin-positive) with collagen IV staining (arrows). Scale bar: 250 μm on all figures.

patients and in all brain areas mentioned above, these parenchymal deposits show strong cystatin C immunoreactivity (Figure 7C) and are visible by H&E staining (Figure 7D). The presence of focal deposits and the stage of the CAA underlies the severity of the disease state in young patients compared to other CAA diseases.

In addition to deposition of cystatin C in vessel walls, there are extensive changes in the walls of affected vessels characterized by degeneration of the smooth muscle cells (SMA), endothelia, fragmentation of the elastic layer, and extensive accumulation of extracellular matrix proteins, collagen IV, laminin and aggrecan [29, 30]. The distribution of the basement membrane protein collagen IV within the arterial wall (Figure 7E) is very similar to cystatin C (Figure 7F). Focal deposits are also immunoreactive to collagen IV (Figure 7G, H). The distribution of cystatin C and collagen IV immunoreactivity in affected vessels and in the parenchyma is such that cystatin C deposition is always accompanied with collagen IV accumulation. Cystatin C amyloid complex deposition begins in the basement membrane around SMA cells in the media of the vessel wall. As the amyloid and collagen IV deposition progresses in the vessels, degeneration of SMA becomes more evident, and in more affected arteries/arterioles, complete loss of SMA is seen (Figure 7I). The post-mortem brain samples show the end-stage pathology, but skin samples from carriers can show intermediate events in the pathogenesis of the disease, as very similar pathogenesis is seen in the skin and in the CNS [34].

H&E staining of patient's skin biopsies shows normal skin structure except from increased number of cells right beneath epidermis (Figure 8A, arrows) and in some carriers, there is mild inflammation. The cystatin C deposition in carriers begins initially in the basement membrane between epidermis and dermis. As the disease progresses, cystatin C amyloid complexes are found in basement membranes around vessels, hair follicles, and sweat/fat glands. Symptomatic carriers have significantly higher levels of cystatin C immunoreactivity in their skin than

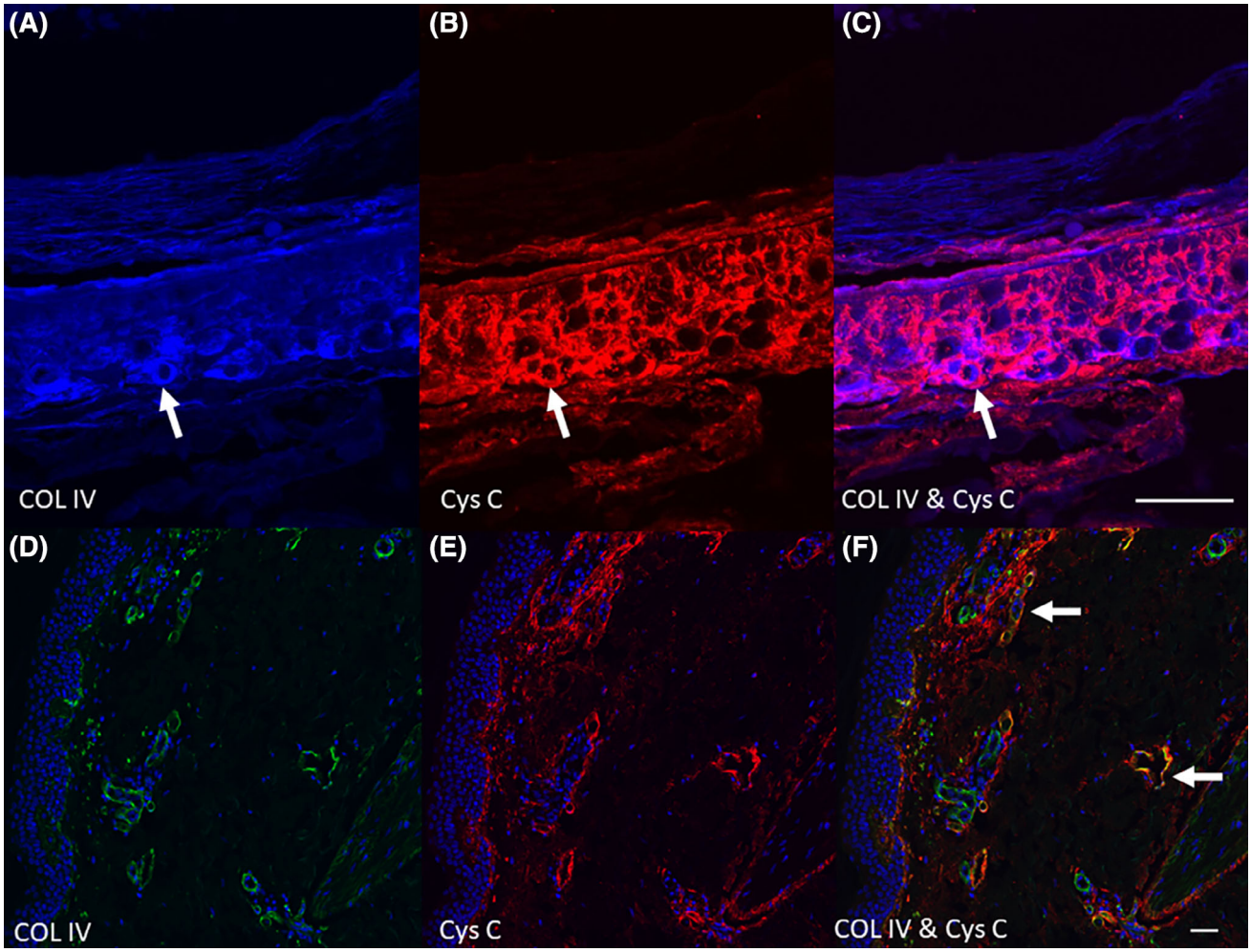

FIGURE 9 Confocal immunofluorescence staining in cerebral vessel and in skin biopsy from patient/carrier. (A)–(C) Immunofluorescence staining in the vessel wall of cerebral artery, showing especially the media of the wall, where initial cystatin C deposition (amyloid cystatin C complexes) occurs. The immunoreactivity for collagen IV and cystatin C reveals a close association between both proteins. The arrows point to a basement membrane around one smooth muscle cell with collagen IV and cystatin C positivity. A spatial overlap (purple color) of both fluorescent labels is noticeable in (C). (D)–(F) Immunofluorescence staining in skin biopsy, revealing close association between collagen IV and cystatin C with spatial overlap of both fluorescent labels (yellow color) in (F). Scale bar: 50 μm on all figures.

asymptomatic carriers (Figure 8B, C), thus the progression of disease in the CNS can be monitored by quantifying cystatin C immunoreactivity in skin biopsies. The proliferation of cells seen in the upper dermis is most prominent within the same areas cystatin C complexes are found in greatest quantity (Figure 8D), and are immunoreactive to vimentin, which is marker for, for example, fibroblasts (Figure 8E). The vimentin immunoreactive fibroblasts have activated appearance, enlarged cell bodies, and processes (Figure 8F), are Smad 2/3 positive (Figure 8G) but not-SMA positive (data not shown), suggesting that they are not myofibroblasts but rather proto-myofibroblasts [35]. Phosphorylation of Smad2/3 is a well-documented downstream event in the TGFβ signaling pathway, TGFβ initiates its pro-fibrotic action on fibroblasts. Activated fibroblasts produce ECM proteins, such as collagen IV and fibronectin [36, 37]. Collagen IV accumulation is evident in the basement membrane between epidermis and dermis and basement membranes in vessels, hair follicles, and sweat/fat glands in carriers (Figure 8H). Strong collagen IV positivity is also seen in fibroblasts in the upper dermis (Figure 8I). Collagen IV immunoreactivity is elevated in both asymptomatic and symptomatic carrier, suggesting that collagen IV accumulation is a primary event in the etiology of the disease. The accumulation of collagen IV in cerebral vessels/parenchyma and the skin is in very close association with cystatin C deposition, with spatial overlap of both proteins (Figure 9). Basement membrane changes due to collagen IV are early and important events in HCCAA pathogenesis that could facilitate cystatin C deposition and aggregation. HCCAA is therefore both amyloid and fibrotic disease, both take part in weakening the arterial wall with SMC degeneration, which can lead to micro and macro hemorrhage/s and infarcts [29, 30, 34].

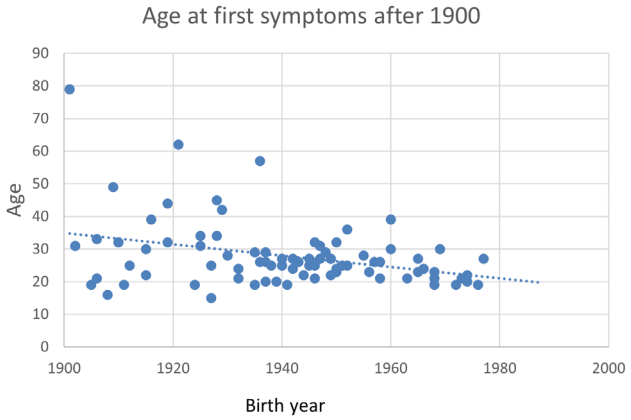

FIGURE 10 Age at first symptoms. The graph shows age at first symptoms in L68Q carries born after the year 1900. Age at first symptoms range from 16 to 79-years-old with only three carriers over 50 years. Most carriers have the first symptoms between 20 and 30-years-old with mean age at death 30-years-old.

10 | CLINICAL SYMPTOMS AND LOCATION OF HEMORRHAGES

Clinical symptoms in HCCAA patients are similar to other CAA diseases, except patients are typically younger and symptoms are more severe. The first symptom often happens unexpectedly in previously healthy normotensive young HCCAA carriers, as they have sudden appearance of stroke symptoms. Some patients have a history of headaches, resembling migraine, and dementia symptoms prior to hemorrhage [4, 9, 38–40]. The patient will often survive the first hemorrhage but continue to have recurrent hemorrhages of varying frequency and severity. The hemorrhages can lead to sensorimotor hemiparesis with or without aphasia, symptoms of neglect, and progressive dementia. Many patients throughout the years have been admitted to psychiatric hospital due to personality changes with gradual deterioration [4, 25, 38, 40]. In most cases, strokes become more frequent and more severe as the disease progresses [38, 40]. Jensson et al. [40] surveyed on 127 patients and showed that age of death was most common between 20 and 40 years and only very few lived past 50 years. Thus, some patients die immediately or within days or weeks after their first hemorrhage, and some live for a few years but are crippled by recurrent hemorrhages, resulting in severe dementia, personality changes, and/or paralysis. Looking at medical history of carriers born after 1900, the age of onset of symptoms ranges from 16 to 79 years, with only 2%–4% of subjects surviving beyond 50 years. Most carriers have their first hemorrhage between the ages of 20–40 years (Figure 10). The mean number of alarming symptomatic macro hemorrhages before death is 3.9 if patient lives through the first hemorrhage and the duration of symptoms or life span after first hemorrhage is at average 5 years. This is in accordance with studies from 1989 [38, 39], where medical history of 19 patients were examined, and found that mean age at first hemorrhage was 25.2 years and mean number of hemorrhages over 5-year period was 3.2. However, while frequency of major bleeds ranges between 3.2 and 3.9 on average in these studies over 5-year timeframe, it should be noted that multiple smaller hemorrhages have been observed at autopsy that were subclinical in presentation.

Hemorrhages are observed in most brain areas, for example, cerebrum, cerebellum, basal ganglia, thalamus, and ventricles. The most severe hemorrhages are found in the basal ganglia, midbrain, and thalamus, often extending to the ventricles [4, 6, 30, 40]. Patients often have micro hemorrhages (seen only in post-mortem brain samples) and microinfarcts that do not cause alarming clinical symptoms, but rather contribute to the gradual deterioration and dementia [30]. The wide distribution of CAA and related hemorrhages is rarely seen in other CAA diseases except in severe cases. Cognitive decline in AD and Aβ-CAA is more progressive in patients with moderate to severe CAA but can also be independent of CAA and closely associated with parenchymal changes, such as microinfarcts and other lesions [2, 41, 42].

11 | THERAPEUTIC INTERVENTIONS

The monomer of cystatin C does not precipitate in the cerebral vasculature or other organs. The L68Q mutation makes the mutated cystatin C protein less structurally stable and more prone to dimerization and aggregation compared to wild-type cystatin C which is stable as a monomer [18, 19]. The mutated protein forms amyloid through stepwise process with formation of dimers, and oligomers of different sizes ultimately aggregating into cystatin C amyloid complexes. L68Q carriers have cystatin C dimers in the CSF and blood plasma and as mentioned low levels of cystatin C in their CSF [16, 20, 43, 44]. Basement membrane alterations consisting of collagen IV accumulation due to activation of fibroblasts could lead to impaired perivascular drainage of cystatin C in the CSF and entrapment of dimeric cystatin C in the thickened cerebral basement membrane. This scenario could explain low levels of cystatin C in CSF, that is, the dimers could be more prone to aggregate into higher molecular weight amyloid complexes with resulting tissue deposition. Pathologies of Aβ in Aβ-CAA and AD are both considered to be driven by failure of perivascular drainage [45, 46]. As mentioned, fibroblasts are activated by Smad2/3/TGFβ in the skin of carriers, studies on fibroblasts show that cystatin C can directly prevent binding of TGFβ to its receptor and hinder TGFβ signaling cascade [47]. Low levels of cystatin C in carriers CSF could result in increased TGFβ signaling. Increased TGFβ and Smad3 decrease intracellular levels of glutathione in fibroblasts, which can lead to increased production of ECM proteins, e.g. collagen IV [48–51]. Therapeutic interventions for the pathogenesis of the

disease could therefore be to hinder amyloid/oligomer/dimer formation and hinder activation of fibroblasts by TGFβ. In 2021, March et al. [52] published a pilot study demonstrating that N-acetylcysteine (NAC) breaks cystatin C oligomers into monomers in cellular models by interrupting disulfide bonds. NAC, a precursor of glutathione, elevates intracellular glutathione levels, modulates oxidative stress in cells, and has anti-fibrotic effects on fibroblasts [53, 54]. The changes in life span of carriers and diet occurred at same time. Earlier, the diet was more ketogenic, and after mid-19th century the diet contained for example more glucose and grain [24]. Ketogenic diet upregulates glutathione synthesis, whereas high levels of glucose impair it [55, 56].

All at-risk family members who are being referred for genetic testing or pursuing genetic testing on their own, are offered genetic counseling prior to testing as well as after the test results are available. They are counseled about the mode of inheritance, disease presentation, progression, and life expectancy. The fact that the quantity of cystatin C amyloid complex deposition in skin is associated with the progression of the disease in the CNS shows that skin biopsies can be used to assess disease progression and could, therefore, be of use in the evaluation of therapeutic interventions. In the pilot study, treatment with NAC in six subjects showed reduced cystatin C complex deposition in skin biopsies, with 50%–90% reduction detected following 9 months of NAC therapy [52]. These results led to full phase IIa clinical trial in 17 L68Q carriers and the outcome from that study further supported the beneficial effects of NAC on biomarker responses in HCCAA patients. Importantly, they also suggested that NAC reduced both frequency and severity of cerebrovascular events including both hemorrhagic and ischemic stroke. While these results are promising, NAC doesn't cross the blood-brain barrier nearly as well as other derivatives for this class of drugs, such as NAC amide (NACA) which is lipophilic and shows over tenfold greater efficacy over NAC in cellular models and reaches multi-fold higher concentration in the brain and intracellularly where the actionability of the drug is most relevant for prevention of protein aggregation [57–59]. Thus, NAC, and more importantly NACA, present encouraging treatment options for HCCAA patients and potentially also for other CAAs as well as AD disease.

AUTHOR CONTRIBUTIONS

Asbjorg Osk Snorradottir, Hakon Hakonarson, Astridur Palsdottir: Writing and editing the manuscript. All authors approved the submitted version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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