Pathogenic Effects of D23N Iowa Mutant Amyloid β-Protein*

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Cerebral amyloid β-protein angiopathy (CAA) is a key pathological feature of patients with Alzheimer’s disease and certain related disorders. In these conditions the CAA is characterized by the deposition of Aβ within the cerebral vessel wall and, in severe cases, hemorrhagic stroke. Several mutations have been identified within the Aβ region of the Aβ protein precursor (AβPP) gene that appear to enhance the severity of CAA. We recently described a new mutation within the Aβ region (D23N) of AβPP that is associated with severe CAA in an Iowa kindred (Grabowski, T. J., Cho, H. S., Vonsattel, J. P. G., Rebeck, G. W., and Greenberg, S. M. (2001) Ann. Neurol. 49, 697–705). In the present study, we investigated the effect of this new D23N mutation on the processing of AβPP and the pathogenic properties of Aβ. Neither the D23N Iowa mutation nor the E22Q Dutch mutation affected the amyloidogenic processing of AβPP expressed in H4 cells. The A21G Flemish mutation, in contrast, resulted in a 2.3-fold increase in secreted Aβ peptide. We also tested synthetic wild-type and mutant Aβ40 peptides for fibrillogenesis and toxicity toward cultured human cerebrovascular smooth muscle (HCSM) cells. The E22Q Dutch, D23N Iowa, and E22Q,D23N Dutch/Iowa double mutant Aβ40 peptides rapidly assembled in solution to form fibrils, whereas wild-type and A21G Flemish Aβ40 peptides exhibited little fibril formation. Similarly, the E22Q Dutch and D23N Iowa Aβ40 peptides were found to induce robust pathologic responses in cultured HCSM cells, including elevated levels of cell-associated AβPP, proteolytic breakdown of smooth muscle cell α-actin, and cell death. Double mutant E22Q,D23N Dutch/Iowa Aβ40 was more potent than either single mutant form of Aβ in causing pathologic responses in HCSM cells. These data suggest that the different CAA mutations in AβPP may exert their pathogenic effects through different mechanisms. Whereas the A21G Flemish mutation appears to enhance Aβ production, the E22Q Dutch and D23N Iowa mutations enhance fibrillogenesis and the pathogenicity of Aβ toward HCSM cells.

Cerebral amyloid angiopathy (CAA) is a common pathology found at increased frequency in patients with Alzheimer’s disease (AD) and related disorders such as Down’s syndrome and hereditary cerebral hemorrhage with amyloidosis of the Dutch type (1–7). Aβ is a 39–43-amino acid peptide that is proteolytically derived from its larger parent transmembrane molecule amyloid-β precursor protein (AβPP) (8). Although AβPP exhibits a variety of biological activities, its role as the parent molecule of the Aβ peptide has drawn the most interest (9). In this regard, full-length AβPP can undergo proteolytic cleavage by β- and γ-secretases to liberate the Aβ peptide. Alternatively, full-length AβPP can be proteolytically processed by an enzyme termed α-secretase at position 16 of the Aβ domain, resulting in a non-amyloidogenic membrane-spanning carboxyl-terminal fragment and truncated secretory forms of AβPP that are released into the extracellular environment.

Mutations in the AβPP gene have been linked to several familial diseases. Substitutions at residues flanking the Aβ region of AβPP give rise to early-onset AD, apparently through effects on the processing of AβPP and production of Aβ (8). Other mutations have been identified that fall within the Aβ region of AβPP, specifically at the adjacent Aβ residues 21 and 22 (10–14). Interestingly, mutations at these residues appear to associate preferentially with CAA (10–14). Extensive CAA can associate with several clinical syndromes, including intracerebral hemorrhagic stroke (2, 15) and dementia with white matter destruction (16, 17).

We recently identified a mutation at a third site within the Aβ region of AβPP that resulted in substitution of asparagine for aspartic acid at Aβ position 23 (AβPP D694N) (18). The mutation was carried by an Iowa family with a three-generation history of autosomal dominant dementia with onset in the sixth or seventh decade and, in two patients studied radiographically, extensive white matter abnormalities and posterior cortical calcifications. Neuropathological examination of the proband revealed severe CAA with numerous small cortical hemorrhages and both cortical and subcortical infarctions. Abundant neurofibrillary tangles and dystrophic neurites were also present, a possible result of either a direct toxic effect of the mutant amyloid peptide or hypoxia due to the severe vascular narrowing. Aβ plaques were relatively sparse and generally of diffuse morphology.

In the present study, we investigated the pathogenic mechanism of the Iowa AβPP mutation. Studies of position 22 mutations suggest that they may generate an Aβ peptide with properties of altered fibrillogenesis (19–21) and increased toxicity to cerebrovascular cells (21–26). The alternative possibility is that the Iowa mutation may affect the production rather than the fibrillogenesis of Aβ.
Fig. 1. AβPP mutations associated with familial CAA. A, mutations at positions 3 of Aβ have been found in familial cases of CAA: Aβ21 (Flemish), Aβ22 (Dutch), and Aβ23 (Iowa). B, Aβ in the brain of an individual with the AβPP Iowa mutation. Total Aβ immunoreactivity was detected in the temporal lobe of a 68-year-old man with the AβPP D694N mutation, using 7-μm paraffin-embedded sections pretreated with 99% formic acid and stained for Aβ (antibody 6E10) as described (12). Severe deposition of Aβ in the walls of cerebral blood vessels but very little parenchymal Aβ was observed. Scale bar = 200 μm.

than the biochemical properties of Aβ, a mechanism that has been suggested for the Flemish glycine for alanine substitution at position 21 (27, 28). Our data indicate that the Iowa mutations result in increased toxicity of Aβ to cerebrovascular cells in culture.

**EXPERIMENTAL PROCEDURES**

**Materials—** Aβ peptides were synthesized by solid-phase Fmoc (9-fluorenlymethoxycarbonyl) amino acid chemistry, purified by reverse phase high performance liquid chromatography, and structurally characterized as previously described (29). The lyophilized Aβ peptides were first resuspended in sterile distilled water to a concentration of 250 μM. Before addition to HCSM cells, the peptides were diluted to a final concentration of 25 μM in serum-free culture media. The secondary structures of the resuspended peptides were determined by circular dichroism spectroscopy as previously described (30). The anti-Aβ antibody was visualized by ECL detection system (Amersham Pharmacia Biotech). Only faint immunoreactivity was observed in cells transfected with vector alone. Aβ-specific bands from transfected cells were visualized by film using a Bio-Rad GS-700 densitometer. Relative levels of immunoreactivities were compared by analysis of variance (Statview, Abacus Concepts).

**Aβ40 Sandwich ELISA—** Aβ40 levels in conditioned media were measured by ELISA using Aβ antibodies 2G3 for capture and biotinylated 3D6 for detection (33). 2G3 was developed against Aβ 33–40 and does not cross-react with Aβ42; 3D6 recognizes Aβ1–5 and detects only Aβ with the amino-terminal aspartic acid (33). 96-Well plates (Costar, NY) were coated overnight at room temperature with 2G3 (1 μg/ml) and blocked in 0.25% human serum albumin for 1 h at room temperature. Then 100 μl of Aβ40 standards and samples of conditioned media (in triplicate) were applied for 1 h and washed three times with 0.05% Tween 20 in Tris-buffered saline. Then 100 μl of biotinylated 3D6 (0.2 μg/ml) was applied for 1 h and washed three times with 0.05% Tween 20 in Tris-buffered saline. Each sample was washed with Tris-buffered saline, and then reacted with Aβ42/43. The reaction was developed with the colorimetric substrate Slow TMB substrate (Pierce). The absorbance was read at 450 nm.

**Assembled Aβ Congo Red Binding/Precipitation Assay—** Aβ samples were initially resuspended in distilled water to a concentration of 200 μM and then immediately diluted to a final concentration of 100 μM in 100 mM Tris-HCl (pH 7.5). Triplicate samples of each peptide were continuously incubated at 37 °C on a rocking platform. At the designated time points, 100-μl aliquots of each sample were collected and incubated with 2 μl of a sterile filtered Congo red solution (1.5 mg/ml in distilled water) for 1 h at room temperature in the dark. Then the samples were centrifuged at 14,000 × g for 10 min. The resulting supernatants were placed in a 96-well microtiter plate, and the absorbance was read at 492 nm. The extent of Aβ peptide assembly was reflected in the loss of absorbance compared with buffer and Congo red-only controls.

**Transmission Electron Microscopy—** Aliquots of the Aβ peptide samples used in Congo red binding experiments described above were

**Fig. 2. Processing of AβPP mutant AβPP751.** H4 neuroglioma cells were transfected with vectors expressing wild-type or CAA mutant AβPP751; cell extracts and conditioned media were analyzed for full-length AβPP and AβPP fragments generated by wild-type (W), Flemish (F), Dutch (D), and Iowa (I) mutants. A, representative immunoblots of full-length AβPP, secreted AβPPα (AβPPα), and C83. B, relative amounts of full-length AβPP and fragments compared among the wild-type AβPP (defined as 1.0, dotted line). Aβ40 in conditioned media was analyzed by ELISA. Data represent the mean ± S.D. of four independent experiments. Production of Aβ from Flemish AβPP was elevated 2.3-fold (p < 0.0001).
Temperature. The HCSM cells were rinsed with PBS three times, and antibody (Amersham Pharmacia Biotech) diluted 1:200 for 2 hours with blocking solution (Research Genetics, Huntsville, AL) for 30 min. The cells were then incubated in serum-free medium overnight before treatment. The cells were then incubated in serum-free medium for 6 days in the absence or presence of 25 μM freshly solubilized Aβ peptides. The cells were then rinsed with PBS three times and fixed with 2% paraformaldehyde in PBS for 20 min at room temperature. The HCSM cells were rinsed with PBS after fixation, stained with 0.1% thioflavin T for 10 min at room temperature, and rinsed with 80% ethanol three times. 250 μl of PBS was added to each well, and thioflavin T fluorescence was measured at excitation wavelength 440 nm and emission wavelength 485 nm using a Cytofluor II fluorescence plate reader. Each time point was performed in triplicate, and five fields were scanned for each well.

Immunoblotting Analyses of HCSM Cellular Proteins—Near-confluent cultures of HCSM cells were incubated with or without 25 μM freshly solubilized Aβ peptides for 6 days. After incubation, the HCSM cells were rinsed with PBS three times, and the cells were solubilized in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, and protease inhibitor mixture (Roche Molecular Biochemicals). The cell lysates were centrifuged at 14,000 × g for 10 min to remove insoluble material. Protein concentrations were determined by the method of Bradford (35). Cell lysates were stored at −70 °C until analysis. Aliquots of the cell lysate samples were electrophoresed in non-reducing SDS, 10% polyacrylamide gels, and the proteins were electroblotted onto Hybond nitrocellulose membranes (Amersham Pharmacia Biotech). Unoccupied sites on the membranes were blocked with 5% nonfat milk overnight in PBS with 0.05% Tween 20. The membranes were probed with mAb P2 1:1000, the peroxidase activity on the membranes was detected using an enhanced chemiluminescence system and analyzed using a Fluor-S Multi-Analyst (Bio-Rad) with the manufacturer’s Multi-Analyst software.

RESULTS

Iowa Mutation at Position 23 in Aβ Is Associated with Familial CAA—A newly described mutation at AβPP position 694, causing an aspartate to asparagine substitution, is associated with familial CAA in a kindred in Iowa (12). This mutation is at position 23 of the Aβ peptide, immediately downstream of two other AβPP residues with mutations associated with familial CAA, residue 21, A to G substitution (Flemish mutation), and residue 22, E to Q (Dutch). E to K (Italian), and E to G (Arctic) substitutions (Fig. 1A). Immunostaining for Aβ in a brain tissue sample from an individual with the Iowa mutation reveals extensive CAA (Fig. 1B).

Effects of CAA Mutations on AβPP Processing and Aβ Production—We first determined whether the AβPP Iowa mutation altered the processing of AβPP in human H4 neuroglioma cells. These cells are derived from the human central nervous system and have been used to study the effects of AβPP mutations on AβPP processing (28, 32). The production of full-length AβPP, secreted AβPPa and C83 (the amino- and carboxyl-terminal products of α-secretase cleavage of AβPP) were measured by quantitative immunoblotting, whereas Aβ (produced from β- and γ-secretase cleavage of AβPP) was measured by ELISA.
separate experiments. Triplicate wells read in five different fields per well from two to three
was subtracted from each sample. Data represent the mean
In each case the background fluorescence from untreated HCSM cells
multi-well plate reader as described under
B quantitatively measured by immunofluorescence labeling using mAb6E10 (A)
or fluorescent thioflavin T binding (B) using a Cytofluor II fluorescence
multi-well plate reader as described under “Experimental Procedures.” In
each case the background fluorescence from untreated HCSM cells
was subtracted from each sample. Data represent the mean ± S.D. of
triplicate wells read in five different fields per well from two to three
separate experiments. Lanes 1, wild-type Aβ40; lanes 2, Flemish mutant Aβ40; lanes 3, Dutch mutant Aβ40; lanes 4, Iowa mutant Aβ40; lanes 5, double Dutch/Iowa mutant Aβ40. a.u., absorbance units.

Fig. 5. Quantitative cell surface measurements of CAA mutant Aβ40 peptides on cultured HCSM cells. Near-confluent cultures of HCSM cells were incubated for 6 days in the absence or presence of the different CAA mutant Aβ40 peptides (25 μM each). The HCSM cells were extensively washed, then fixed, and cell-surface Aβ was quantitatively measured by immunofluorescence labeling using mAbE10 (A) or fluorescent thioflavin T binding (B) using a Cytofluor II fluorescence multi-well plate reader as described under “Experimental Procedures.” In each case the background fluorescence from untreated HCSM cells was subtracted from each sample. Data represent the mean ± S.D. of triplicate wells read in five different fields per well from two to three separate experiments. Lanes 1, wild-type Aβ40; lanes 2, Flemish mutant Aβ40; lanes 3, Dutch mutant Aβ40; lanes 4, Iowa mutant Aβ40; lanes 5, double Dutch/Iowa mutant Aβ40. a.u., absorbance units.

Effects of CAA Mutations on in Vitro Aβ Fibril Assembly—Since the newly described Iowa mutation in AβPP did not affect AβPP amyloidogenic processing, we next evaluated its affect on Aβ fibril assembly. In these experiments we used a quantitative Congo red binding/precipitation assay to determine the in vitro assembly of wild-type and CAA-mutant Aβ40 peptides. We focused our studies on Aβ40 peptides because of their abundance in CAA and our previous in vitro investigations on their cerebrovascular cytotoxic effects of Aβ40 (22–24, 30, 36). The wild-type and Flemish Aβ40 peptides exhibited little assembly over the 48-h time period, with <15% precipitation of Congo red (Fig. 3). The Dutch and Iowa mutant Aβ40 peptides, in contrast, rapidly assembled in solution and precipitated nearly all of the Congo red by 6 h (Fig. 3). The E22Q Dutch and D23N Iowa mutation reside at adjacent sites within AβPP levels.

Fig. 6. Effects of CAA mutant Aβ40 peptides on HCSM cell-associated AβPP levels. Near-confluent cultures of HCSM cells were incubated for 6 days in the absence or presence of the different CAA mutant Aβ40 peptides (25 μM each). After 6 days the culture medium was removed, and the cells were rinsed, solubilized, subjected to SDS-polyacrylamide gel electrophoresis, and subsequently analyzed by quantitative immunoblotting using mAbP2–1 as described under “Experimental Procedures.” A, representative immunoblot; B, summary of the quantitative data. The data are presented as -fold increase in cell-associated AβPP compared with untreated HCSM cells. The data presented are the mean ± S.D. of six to nine separate determinations. Lanes 1, no Aβ control; lanes 2, wild-type Aβ40; lanes 3, Flemish mutant Aβ40; lanes 4, Dutch mutant Aβ40; lanes 5, Iowa mutant Aβ40; lanes 6, double Dutch/Iowa mutant Aβ40.

(Fig. 2). The Flemish, Dutch, and Iowa mutations did not cause a significant change in amounts of full-length AβPP, secreted AβPPα, or C83 (Fig. 2B). No significant differences in the levels of AβPP fragments were observed after correcting for slight differences in total AβPP expression. Because we observed a high degree of Aβ40 immunoreactivity in both vessel and parenchymal Aβ deposits in the brain of the patient with the Iowa mutation (12), we hypothesized that increased Aβ40 production might be associated with this mutation. Using the same transiently transfected cells, we found the Flemish mutation led to a significant 2.3-fold increase in the amount of secreted Aβ40 in the conditioned culture medium, confirming earlier reports (27, 28). In contrast, the Dutch and Iowa mutations did not affect Aβ40 secretion (Fig. 2B).

Effects of CAA Mutations on in Vitro Aβ Fibril Assembly—Since the newly described Iowa mutation in AβPP did not affect AβPP amyloidogenic processing, we next evaluated its affect on Aβ fibril assembly. In these experiments we used a quantitative Congo red binding/precipitation assay to determine the in vitro assembly of wild-type and CAA-mutant Aβ40 peptides. We focused our studies on Aβ40 peptides because of their abundance in CAA and our previous in vitro investigations on their cerebrovascular cytotoxic effects of Aβ40 (22–24, 30, 36). The wild-type and Flemish Aβ40 peptides exhibited little assembly over the 48-h time period, with <15% precipitation of Congo red (Fig. 3). The Dutch and Iowa mutant Aβ40 peptides, in contrast, rapidly assembled in solution and precipitated nearly all of the Congo red by 6 h (Fig. 3). The E22Q Dutch and D23N Iowa mutation reside at adjacent sites within AβPP levels.
formed transmission electron microscopic analysis of the different Aβ peptide preparations. Consistent with the above findings, Dutch, Iowa, and Dutch/Iowa Aβ40 showed abundant characteristic fibrils (Fig. 4), whereas similar structures were rare or absent in preparations of wild-type or Flemish Aβ40 (data not shown). Together, these studies demonstrate that Iowa mutant Aβ40, like Dutch mutant Aβ40, exhibits an increased propensity to assemble into β-sheet containing fibrils. Furthermore, this property appears to be more enhanced in double-mutant Dutch/Iowa Aβ40.

**Effects of CAA Mutations on HCSM Cell Surface Aβ Binding and Fibril Assembly**—Previously, we showed that forms of Aβ that are pathogenic for HCSM cells exhibit robust binding and fibril assembly on the surface of these cells (24, 30, 36). We therefore examined the ability of the CAA-mutant Aβ40 peptides to bind to HSCM cells by performing quantitative immunoblotting using mAb to vascular smooth muscle cell α-actin as described under “Experimental Procedures”. A, representative immunoblot; B, summary of the quantitative data. The data are presented as % decrease in HCSM cell α-actin compared with untreated HCSM cells. The data presented are the mean ± S.D. of three to six separate determinations. Lanes 1, no Aβ control; lanes 2, wild-type Aβ40; lanes 3, Flemish mutant Aβ40; lanes 4, Dutch mutant Aβ40; lanes 5, Iowa mutant Aβ40; lanes 6, double Dutch/Iowa mutant Aβ40.

**Effects of CAA Mutations on HCSM Cell α-Actin Degradation**—The effects of double mutant Dutch/Iowa Aβ40 on degradation of vascular smooth muscle cell α-actin were quantified by immunoblotting with mAbs to each respective protein as described under “Experimental Procedures.” Incubation with Dutch/Iowa (lanes 4), Iowa (lanes 5), or double mutant Dutch/Iowa (lanes 6) Aβ40 caused a sharp increase in cell-associated AβPP (Fig. 6) and decrease in vascular smooth muscle cell α-actin (Fig. 7) compared with untreated HCSM cells (lanes 1). The effects of double mutant Dutch/Iowa Aβ40 on degradation of vascular smooth muscle cell α-actin were noticeably more robust than either single mutant form of Aβ (Fig. 7). In contrast, wild-type (lanes 2) or Flemish (lanes 3) Aβ40 had no effect on the cell-associated AβPP levels or vascular smooth muscle cell α-actin levels in HCSM cells. In parallel experiments, the extent of HCSM cell death was determined as described under “Experimental Procedures.” Similar to the above results, Dutch and Iowa CAA mutant Aβ40 peptides induced HCSM cell death, whereas wild-type and Flemish Aβ40 peptides did not (Fig. 8). Again, the double mutant Dutch/Iowa Aβ40 was found to be much more toxic to the HCSM cells than either single mutant form of Aβ.
CAAs are a significant age-related risk factor for hemorrhagic stroke and a common pathological feature of AD and certain related disorders. This condition is characterized by deposition of amyloid in the medial and adventitial layer of primarily small and medium-sized arteries and arterioles of the cerebral cortex and leptomeninges (1–7). Cerebrovascular Aβ deposition is intimately associated with smooth muscle cells in the vessel wall (2, 38–40). It has been suggested that the subsequent degeneration of the smooth muscle cells is involved with loss of vessel wall integrity (38, 39, 41). CAA and intracerebral hemorrhage are the primary pathologies of a growing number of hereditary disorders that result from distinct point mutations within the Aβ domain of AβPP. These include the A21G Flemish mutation (12), E22Q Dutch mutation (10, 11), and E22K Italian mutation (14). A recent new addition to this list is the D23N Iowa mutation (18). The Iowa CAA disorder is characterized by progressive dementia with pathological findings of extensive cerebrovascular Aβ deposition (often severely narrowing or occluding the vessel lumen), small foci of hemorrhage and ischemic infarction, widespread neurofibrillary tangles, and relatively sparse senile plaques. It is unclear how the D23N Iowa mutation promotes cerebrovascular Aβ deposition. In the present study we investigated the effects of the D23N Iowa mutation on AβPP processing and Aβ fibrillogenic and pathogenic properties toward HCSM cells.

Previous studies have reported that different Aβ CAA mutations can differentially affect amyloidogenic processing of AβPP. To investigate the possible effects of the D23N Iowa CAA mutation on AβPP amyloidogenic processing, we used a cell transfection paradigm to express different CAA-mutant forms of AβPP. We found that the A21G Flemish mutation increased the production of Aβ peptide, whereas the E22Q Dutch mutation did not (Fig. 2), consistent with previous reports (27, 28). Similar to the Dutch mutation, the D23N Iowa mutation did not increase Aβ production in transfected cells (Fig. 2). This suggests that the CAA observed in patients with the Iowa mutation is unlikely to result from overproduction of Aβ peptide.

The above results suggested that the D23N Iowa mutation instead might cause CAA by altering the properties of the Aβ peptide. In this regard, previous studies have demonstrated that E22Q Dutch mutant Aβ peptides exhibit enhanced rates of fibril assembly compared with wild-type Aβ peptides (19–21). Since the D23N Iowa mutation results in the loss of a negative charge similar to the adjacent E22Q Dutch mutation, we speculated that Iowa mutant Aβ may also exhibit enhanced rates of fibrillogenesis. Indeed we found that D23N Iowa mutant Aβ40 showed enhanced assembly that was nearly identical to E22Q Dutch mutant Aβ40 (Fig. 3). Double-mutant Dutch/Iowa Aβ40 also assembled rapidly, further implicating the importance of the loss of charge of Glu-22 and Asp-23 in augmenting the fibrillogenic properties of Aβ.

We previously demonstrated that E22Q Dutch mutant Aβ40 robustly binds and assemblies into fibrils on the surfaces of cultured HCSM cells, a cell type intimately associated with the pathology of CAA (24, 30, 36). Since D23N Iowa mutant Aβ40 possesses potent fibrillogenic properties like E22Q Dutch mutant Aβ40, it was not surprising that Iowa mutant and double Dutch/Iowa mutant Aβ40 peptides also accumulated and assembled into fibrils on the HCSM cell surface (Fig. 5). Other studies of the Aβ peptide have suggested that the D23N substitution may alter the kinetics of β-sheet formation. The finding that Flemish Aβ40, like wild-type Aβ40, does not readily assemble on HCSM cells further suggests that Flemish Aβ elicits a CAA phenotype by a distinct mechanism.

We have also previously shown that cell surface fibril assembly of pathogenic Aβ is required for inducing downstream pathologic responses in HCSM cells, including cell surface accumulation of secreted AβPP, degradation of vascular smooth muscle cell α-actin, and ultimately an apoptotic cell death (24, 30, 36, 37). The accumulation of secreted AβPP is mediated by its high affinity binding to the Aβ fibrils that assemble on the HCSM cell surface and coincides with the induction of smooth muscle cell α-actin degradation and cell death (36). Following the same pattern for HCSM cell surface Aβ fibril assembly observed in Fig. 5, the Dutch, Iowa, and double Dutch/Iowa mutant Aβ40 peptides potently induced each of these pathologic responses, whereas the wild-type and Flemish mutant Aβ40 peptides did not. It is noteworthy that double Dutch/Iowa mutant Aβ40 was noticeably more robust than either single mutant form of Aβ40 in both the degradation of smooth muscle cell α-actin and loss in cell viability (Figs. 7 and 8, respectively). These findings underscore the importance of cell surface Aβ fibril assembly as the key event in the induction of pathologic responses in HCSM cells. It should be emphasized that Aβ toxicity to HCSM cells may not be the only pathogenic effect of these peptides in familial CAA. Other mechanisms, such as effects on endothelial cell function or vessel narrowing, may also underlie the cause of dementia in these individuals.

Although CAA is commonly observed in patients with AD, the reason why specific mutations within Aβ manifest primarily as CAA remains unclear. The exception is the Flemish mutation, which presents as a mixed AD/strong CAA phenotype (12). On the other hand, patients with either the Dutch, Italian, or newly described Iowa mutations present severe cerebrovascular Aβ deposition with lesser extents of AD neuropathology. Brains from these patients demonstrate numerous diffuse, early stage Aβ deposits in the parenchyma but few of the mature, dense-cored senile plaques characteristic of AD (5, 6, 14, 18, 21). This observation suggests that this particular group of CAA mutations may alter Aβ in a manner that specifically enhances pathogenic interactions with the cerebral vasculature.

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