Pathogenic Effects of Cerebral Amyloid Angiopathy Mutations in the Amyloid β-Protein Precursor

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ABSTRACT: Cerebral amyloid β-protein angiopathy (CAA) is a key pathological feature of patients with Alzheimer's disease and certain related disorders. Several mutations have been identified within the Aβ region of the Aβ protein precursor (AβPP) gene that appear to enhance the severity of CAA. A new mutation has been identified within the Aβ region (D23N) of AβPP that is associated with severe CAA in an Iowa kindred. Recently, we showed that E22Q Dutch, D23N Iowa, and E22Q/D23N Dutch/Iowa double-mutant Aβ40 peptides rapidly assemble in solution to form fibrils compared to wild-type Aβ40. Similarly, the E22Q Dutch and D23N Iowa Aβ40 peptides were found to induce robust pathologic responses in cultured human cerebrovascular smooth muscle (HCSM) cells, including elevated levels of cell-associated AβPP, proteolytic breakdown of 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 *in vitro* data suggested that the E22Q Dutch and D23N Iowa substitutions promote fibrillogenesis and the pathogenicity of Aβ towards HCSM cells. Moreover, the presence of both CAA substitutions in the same Aβ peptide further enhances the fibrillogenic and pathogenic properties of Aβ. We also have generated transgenic mouse models to examine the effects of single and double CAA mutations in AβPP *in vivo*. Preliminary analysis of transgenic mouse brains indicates that expression of double-mutant E22Q/D23N Dutch/Iowa AβPP leads to robust deposition of Aβ in a vascular-weighted manner.

KEYWORDS: cerebral amyloid angiopathy (CAA); amyloid β-protein precursor (AβPP); Alzheimer's disease (AD); mutation

INTRODUCTION

Cerebral amyloid angiopathy (CAA) is an age-associated condition that is pathologically characterized by deposition of amyloid in the medial layer of primarily

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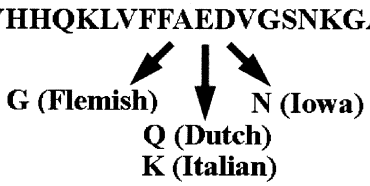

FIGURE 1. Mutations in Aβ associated with familial CAA.

small- and medium-sized arteries and arterioles of the cerebral cortex and leptomeninges.[1–3] This condition is a significant cause of intracerebral hemorrhage and is a key pathologic feature in most patients with Alzheimer's disease (AD) and Down's syndrome.[3–6] In addition to the walls of the cerebral blood vessels, Aβ is also found deposited in plaques within the neuropil of patients with either of these disorders. Aβ is a 39- to 42-amino-acid peptide that has the propensity to self-assemble into insoluble, β-pleated sheet fibrils.[6,7] Aβ is proteolytically derived from a large type-I integral membrane precursor protein, termed the amyloid β-protein precursor (AβPP), which is encoded by a gene located on chromosome 21.[8]

Specific point mutations in the AβPP gene have been identified that fall within the middle of the Aβ region of AβPP, specifically at the adjacent Aβ residues 21 and 22.[8–11] These mutations give rise to mutated forms of the Aβ peptide, which are summarized in FIGURE 1. Interestingly, mutations at these residues appear to associate preferentially with severe CAA and intracerebral hemorrhagic stroke.[8–11] The first CAA mutation in AβPP was identified in patients with Dutch-type hereditary cerebral hemorrhage with amyloidosis.[8,9] This Dutch mutation results in an E22Q substitution in Aβ. Another mutation was found at the same site of the Dutch mutation in an Italian kindred with severe CAA and hemorrhagic stroke.[11] The Italian CAA mutation results in an E22K substitution in Aβ. Both the Dutch and Italian mutations cause extensive early CAA and hemorrhagic stroke with only diffuse Aβ deposits in the neuropil and no neuronal damage.[11–13] Another mutation in Aβ that presents as AD with strong CAA is the A21G Flemish mutation.[10] Recently, a new mutation was identified at a third site within the Aβ region of AβPP that resulted in a D23N substitution.[14] This 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.[14]

EFFECTS OF CAA MUTATIONS IN Aβ: *IN VITRO* STUDIES

It is noteworthy that, like the Dutch E22Q and Italian E22K CAA mutations at position 22 of Aβ, the Iowa D23N CAA mutation results in a loss of charge at the

adjacent position 23 of Aβ. We previously reported that charge-altering mutations at position 22 of Aβ, as found in the Dutch and Italian disorders, enhance the *in vitro* pathogenic properties of Aβ towards cultured human cerebrovascular smooth muscle (HCSM) cells.[15] Thus, we recently characterized the effects of this new Iowa CAA mutation on the amyloidogenic processing of AβPP and on the fibrillogenic and pathogenic properties of Aβ.[16] We found that, similar to the Dutch CAA mutation, the Iowa CAA mutation had no effect of the processing of AβPP and production of Aβ peptide in transfected cells. We next tested the effect of the D23N Iowa mutation on the fibrillar assembly of Aβ40. To do this, we performed a quantitative Congo red binding/precipitation assay to measure the rate Aβ40 fibril assembly. Similar to E22Q Dutch Aβ40, D23N Iowa Aβ40 rapidly assembled in fibrils compared to wild-type Aβ40. It is noteworthy that a double E22Q/D23N Dutch/Iowa mutant Aβ40 also rapidly assembled into fibrils. Fibril assembly of the Dutch Aβ40, Iowa Aβ40, and double-mutant Dutch/Iowa Aβ40 was confirmed by transmission electron microscopy. These studies showed that, similar to Dutch CAA mutant Aβ, Iowa CAA mutant Aβ and double-mutant Dutch/Iowa Aβ possess potent fibrillogenic properties.

Although the Dutch and Iowa CAA mutant Aβ peptides possess potent fibrillogenic properties *in vitro*, this alone cannot explain their selectivity for causing primarily robust cerebrovascular Aβ deposition and pathology with little parenchymal pathology *in vivo*. This suggests that perhaps the CAA mutant Aβ peptides have targeted pathogenic effects towards the cerebral vasculature. In this regard, we previously reported that pathogenic forms of Aβ strongly bind and assemble into fibrils on the surface of cultured HCSM cells.[17,18] Using quantitative immunofluorescent labeling, we found that Dutch, Iowa, and double-mutant Dutch/Iowa Aβ40 were potently bound to the surface of HCSM cells, whereas wild-type Aβ40 was not. Similarly, the same CAA mutant Aβ peptides were assembled into fibrils on the HCSM cell surface as determined by quantitative thioflavin T fluorescent labeling.[16]

There is a loss of vascular smooth muscle cell α-actin in CAA-affected vessels *in vivo*.[1,19,20] Similarly, we previously demonstrated that proteolytic breakdown of vascular smooth muscle cell α-actin occurs in cultured HCSM cells as part of an apoptotic response to pathogenic forms of Aβ.[21] We examined this pathologic response in HCSM cells treated with the different CAA mutant forms of Aβ. Dutch mutant Aβ40 and Iowa mutant Aβ40 caused a similar marked loss in vascular smooth muscle cell α-actin. However, double-mutant Dutch/Iowa Aβ40 produced a strikingly more robust loss in vascular smooth muscle cell α-actin.[16]

A similar pattern was observed when HCSM cell death was measured in response to treatment with the different CAA mutant Aβ peptides. Dutch mutant Aβ40 and Iowa mutant Aβ40 each caused an approximately 40% loss in HCSM cell viability, whereas double-mutant Dutch/Iowa Aβ40 was more cytotoxic, causing >80% loss in HCSM cell viability.

Together, these *in vitro* studies show that E22Q Dutch mutant Aβ and D23N Iowa mutant Aβ possess similarly enhanced fibrillogenic and pathogenic properties towards HCSM cells, consistent with the effect of these mutations in causing familial CAA.[8,9,11,14] The presence of both the Dutch and Iowa CAA substitutions together in Aβ enhances the cerebrovascular pathogenic properties of the peptide further. This suggests that loss of one (−) charge at either position 22 or 23 leads to strong CAA and that loss of both (−) charges would further increase the severity of CAA.

EFFECTS OF CAA MUTATIONS IN Aβ: *IN VIVO* STUDIES

Two similar transgenic mouse lines that specifically overexpress Swedish mutant AβPP in neurons under control of the Thy1 promoter were shown to develop senile plaques and, as a secondary and later event, CAA.[22,23] In older mice of these particular transgenic lines, evidence has been presented of smooth muscle cell degeneration and small cerebral hemorrhages.[24] However, other transgenic mouse lines that overexpress AβPP in neurons only develop senile plaques with much less evidence of cerebrovascular Aβ deposition.[25,26] These differences may result from the type of human AβPP transgene, the promoter used, and/or mouse background. Nevertheless, use of the Thy1 promoter and the Swedish mutant form of AβPP in the former two transgenic lines resulted in abundant production of wild-type Aβ and secondary deposition of CAA. Employing a similar approach, we have used the Thy1 promoter to express high levels of different forms of human AβPP in neurons. We used three variations of human AβPP-770 cDNA. First, we used a Swedish mutant AβPP-770 cDNA. The Swedish two-point mutations result in the double K670N/M671L substitutions immediately upstream of the β-secretase cleavage site in AβPP and are associated with the Swedish form of familial AD.[27] These were incorporated since studies have shown that the presence of the Swedish double mutation in AβPP causes much higher levels of amyloidogenic processing and results in 8- to 10-fold higher levels of wild-type Aβ peptide.[28,29] This transgene will allow us to evaluate the pathogenic effects of wild-type Aβ in this model. Second, we used human AβPP-770 containing the Swedish double mutation in conjunction with the Dutch CAA point mutation. The Dutch mutation results in the E22Q substitution of Aβ.[8,9] This mutation was chosen since the resulting mutant form of Aβ causes early and severe CAA,[12,13] our target phenotype. Therefore, including both the Swedish and Dutch mutations in the AβPP-770 transgene should result in high levels of Aβ containing the Dutch E22Q substitution, which is highly pathogenic for the cerebral vasculature and development of CAA. Third, we used human AβPP-770 containing the Swedish double mutation in conjunction with both the Dutch- and Iowa-type CAA point mutations. Including the Swedish, Dutch, and Iowa mutations in the same AβPP-770 transgene will result in high levels of Aβ containing the adjacent Dutch E22Q and Iowa D23N substitutions. Our recent studies showed that double Dutch/Iowa mutant Aβ is even more pathogenic for cerebrovascular cells than either single mutation alone. We expect these latter transgenic mice to develop earlier and more severe pathology. The use of these three human AβPP transgenes will allow a direct comparison of the pathogenic effects of wild-type, Dutch, and double Dutch/Iowa Aβ peptide in an otherwise identical *in vivo* paradigm.

We have performed some preliminary experiments for immunostaining for Aβ in fresh frozen sections prepared from 5-month-old transgenic and wild-type mice. For this, we used an HRP conjugate of a monoclonal antibody to Aβ (designated 66.1) that we recently developed in our lab. As shown in FIGURE 2, the two human AβPP Swedish/Dutch/Iowa (SwDI) lines B and F (lower panels) showed a dramatic number of Aβ deposits in cortex. Widespread deposition was observed in the cortex and hippocampal regions. In comparison, few deposits were observed in the similarly aged human AβPP Swedish/Dutch (SwD) line B (upper right) or no deposits in the similarly aged human AβPP Swedish (Sw) (upper middle), even though comparable

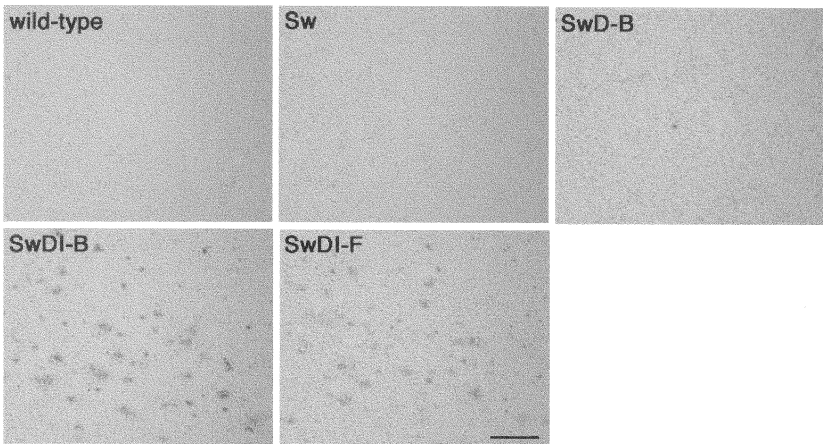

FIGURE 2. Immunostaining for Aβ in 5-month-old transgenic mouse brains. Frozen brain sections were cut at 10 μm, immunostained with a monoclonal antibody to Aβ, and developed with diaminobenzidine. Scale bar: 200 μm.

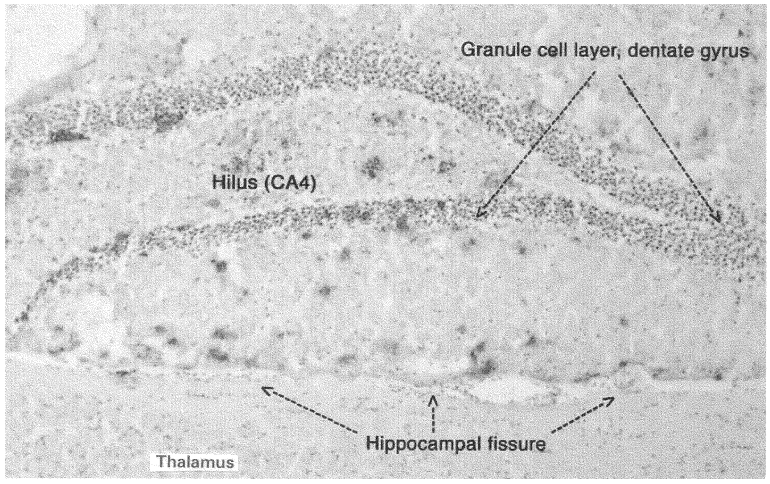

FIGURE 3. Immunostaining for Aβ in the hippocampal region of a 5-month-old Thy1-AβPP SwDI transgenic mouse. Frozen brain sections were cut at 20 μm, immunostained with a monoclonal antibody to Aβ, and developed with diaminobenzidine. Aβ deposits were observed preferentially in vascular-rich regions, including the hippocampal fissure and interface between the granule cells of the dentate gyrus and the hilus.

amounts of human AβPP are expressed in all of the transgenic mice (data not shown). As expected, no Aβ immunostaining was detected in the wild-type mice (upper left). Similar immunostaining results were obtained with the commonly used anti-Aβ monoclonal 6E10 (data not shown).

Upon viewing the hippocampal region of the Thy1-AβPP SwDI transgenic mice, it was observed that there is a relatively "nonrandom" distribution of Aβ deposition that favors perivascular regions between adjacent brain structures, that is, where blood vessels tend to run through the brain. As shown in FIGURE 3, this nonrandom or "vascular-weighted" spatial distribution is observed in the region of the ventral hippocampal fissures and the interface between the granule cells of the dentate gyrus and the hilus (CA4). Further rigorous pathological analysis of our different transgenic mouse lines is in progress.

SUMMARY

In conclusion, our recent data show that single and double charge-altering mutants of Aβ associated with familial CAA exhibit an increasing enhancement of pathogenic properties towards cultured cerebrovascular cells. These findings provide the rationale for our continuing efforts to study the effect of one or two charge-altering CAA mutations in AβPP on the preferential development and severity of CAA in transgenic mice. We also present data showing that we have successfully generated multiple transgenic mouse lines expressing robust levels of human Swedish AβPP, human Swedish/Dutch CAA mutant AβPP, or human Swedish/Dutch/Iowa CAA mutant AβPP. Furthermore, we have found that, in relatively young (5-month-old) Thy1-human Swedish/Dutch/Iowa CAA mutant transgenic mice, there is robust deposition of Aβ in the cortex and hippocampal regions. The Aβ deposited in these particular transgenic mice appears to be vascular-weighted. Although these preliminary findings are very exciting, future work needs to be done to biochemically and pathologically characterize the CAA-related pathology in our different transgenic mouse paradigms that will provide further insight into the pathogenic mechanisms that underlie the development of CAA.

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REFERENCES

1. VINTERS, H.V. 1987. Cerebral amyloid angiopathy: a critical review. Stroke **18:** 311–324.
2. IWAMOTO, N., T. ISHIHARA, H. ITO *et al*. 1993. Morphological evaluation of amyloid-laden arteries in leptomeninges, cortices, and subcortices in cerebral amyloid angiopathy with subcortical hemorrhage. Acta Neuropathol. **86:** 418–421.
3. CORIA, F. & I. RUBIO. 1996. Cerebral amyloid angiopathies. Neuropathol. Appl. Neurobiol. **22:** 216–227.
4. GLENNER, G.G., J.H. HENRY & S. FUJIHARA. 1981. Congophilic angiopathy in the pathogenesis of Alzheimer's degeneration. Ann. Pathol. **1:** 120–129.

5. HIRAI, S. & K. OKAMOTO. 1993. Amyloid beta/A4 peptide associated with Alzheimer's disease and cerebral amyloid angiopathy. Intern. Med. **32:** 923–925.
6. PRELLI, F., E. CASTANO, G.G. GLENNER *et al*. 1988. Differences between vascular and plaque core amyloid in Alzheimer's disease. J. Neurochem. **51:** 648–651.
7. SELKOE, D.J. 1996. Amyloid β-protein and the genetics of Alzheimer's disease. J. Biol. Chem. **271:** 18295–18298.
8. LEVY, E., M.D. CARMAN, I.J. FERNANDEZ-MADRID *et al*. 1990. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science **248:** 1124–1126.
9. VAN BROECKHOVEN, C., J. HAAN, E. BAKKER *et al*. 1990. Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). Science **248:** 1120–1122.
10. HENDRIKS, L., C.M. VAN DUIJN, P. CRAS *et al*. 1992. Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. Nat. Genet. **1:** 218–221.
11. TAGLIAVINI, F., G. ROSSI, A. PADOVANI *et al*. 1999. A new βPP mutation related to hereditary cerebral haemorrhage. Alzheimer's Rep. **2:** S28.
12. LUYENDIJK, W., G.T.A.M. BOTS, M. VEGTER–VAN DER VLIS *et al*. 1988. Hereditary cerebral hemorrhage caused by cortical amyloid angiopathy. J. Neurol. Sci. **85:** 267–280.
13. WATTENDORFF, A.R., B. FRANGIONE, W. LUYENDIJK *et al*. 1995. Hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D): clinicopathological studies. J. Neurol. Neurosurg. Psychiatry **59:** 699–705.
14. GRABOWSKI, T.J., H.S. CHO, J.P.G. VONSATTEL *et al*. 2001. Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. Ann. Neurol. **49:** 697–705.
15. MELCHOR, J.P., L. MCVOY & W.E. VAN NOSTRAND. 2000. Charge alterations of E22 in the amyloid β-protein enhance its pathogenic properties. J. Neurochem. **74:** 2209–2212.
16. VAN NOSTRAND, W.E., J.P. MELCHOR, H.S. CHO *et al*. 2001. Pathogenic effects of D23N "Iowa" amyloid β-protein. J. Biol. Chem. **276:** 32860–32866.
17. VAN NOSTRAND, W.E., J. MELCHOR & L. RUFFINI. 1997. Pathologic cell surface amyloid β-protein fibril assembly in cultured human cerebrovascular smooth muscle cells. J. Neurochem. **69:** 216–223.
18. MELCHOR, J.P. & W.E. VAN NOSTRAND. 2000. Fibrillar amyloid β-protein mediates the pathologic accumulation of its secreted precursor in human cerebrovascular smooth muscle cells. J. Biol. Chem. **275:** 9782–9791.
19. VINTERS, H.V., D.L. SECOR, S.L. READ *et al*. 1994. Microvasculature in brain biopsy specimens from patients with Alzheimer's disease: an immunohistochemical and ultrastructural study. Ultrastruct. Pathol. **18:** 333–348.
20. KAWAI, M., R.N. KALARIA, P. CRAS *et al*. 1993. Degeneration of vascular muscle cells in cerebral amyloid angiopathy of Alzheimer disease. Brain Res. **623:** 142–146.
21. DAVIS, J., D.H. CRIBBS, C.W. COTMAN *et al*. 1999. Pathogenic amyloid β-protein induces apoptosis in cultured human cerebrovascular smooth muscle cells. Amyloid **6:** 157–164.
22. CALHOUN, M.E., P. BURGERMEISTER, A.L. PHINNEY *et al*. 1999. Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid. Proc. Natl. Acad. Sci. U.S.A. **96:** 14088–14093.
23. VAN DORPE, J., L. SMEIJERS, I. DEWACHTER *et al*. 2000. Prominent cerebral amyloid angiopathy in transgenic mice overexpressing the London mutant of human APP in neurons. Am. J. Pathol. **157:** 1283–1298.
24. WINKLER, D.T., L. BONDOLFI *et al*. 2001. Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy. J. Neurosci. **21:** 1619–1627.
25. GAMES, D., D. ADAMS, R. ALESSANDRINI *et al*. 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature **373:** 523–527.
26. HSIAO, K., P. CHAPMAN *et al*. 1996. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. Science **274:** 99–102.
27. MULLAN, M., F. CRAWFORD, K. AXELMAN *et al*. 1992. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β-amyloid. Nat. Genet. **1:** 345–347.

28. CITRON, M., T. OLTERSDORF, C. HAASS *et al*. 1992. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature **360:** 672–674.
29. GOLDE, T.E., X.D. CAI, M. SHOJI *et al*. 1993. Production of Aβ from normal amyloid β-protein precursor (βAPP) and the mutated βAPPs linked to familial Alzheimer's disease. Ann. N.Y. Acad. Sci. **695:** 103–108.