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A Novel *AβPP* Mutation Exclusively Associated with Cerebral Amyloid Angiopathy

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Mutations in *AβPP* cause deposition of Aβ amyloid fibrils in brain parenchyma and cerebral vessels, resulting in Alzheimer's disease (AD) and/or cerebral amyloid angiopathy (CAA). We report a novel mutation (L705V) within the Aβ sequence of *AβPP* in a family with autosomal dominant, recurrent intracerebral hemorrhages. Pathological examination disclosed severe CAA, without parenchymal amyloid plaques or neurofibrillary tangles. This variant highlights the vascular tropism of mutated Aβ, resulting in CAA instead of the pathological hallmarks of AD.

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The cerebrovascular deposition of amyloid fibrils, particularly in leptomeningeal and cortical arteries and arterioles, is a well-recognized cause of intracerebral hemorrhagic stroke, ischemic lesions, and dementia.[1] The amyloid β-peptide (Aβ), released by proteolysis from the amyloid β precursor protein (AβPP), is the amyloid fibril subunit in most forms of cerebral amyloid angiopathy (CAA), such as sporadic, age-related CAA and CAA associated with Alzheimer's disease (AD).[1,2] Aβ-related CAA also includes autosomal dominant disorders (MIM 104760) caused by mutations within the Aβ region of *AβPP*.[2,3] These mutations cluster at codons 692–694, corresponding to residues 21–23 of

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Aβ, and are associated with massive amyloid deposition in vessel walls and variable extent of parenchymal amyloid plaques, leading to a prominent vascular symptomatology, characterized by recurrent intracerebral hemorrhages (ICHs) and death by the sixth or seventh decade of life. Vascular cognitive decline and dementia are often observed in carriers of both the Glu693Gln variant underlying prototypic hereditary CAA-Dutch type[4] and the Glu693Lys mutation,[5] whereas an early-onset dementia compatible with AD has been reported in carriers of either the Flemish Ala692Gly variant[6] or the Arctic Glu693Gly mutant.[7] The Iowa Asp694Asn mutation causes leukoencephalopathy, cortical calcification, and dementia in one kindred[8] and hemorrhagic stroke in one other.[9] We describe a novel Aβ mutation causing severe CAA and fatal ICH, with amyloid deposition selectively affecting vessel walls, without Aβ parenchymal deposits or neurofibrillary tangles.

Materials and Methods

Pathological Examination

Autoptic brain tissue was obtained from the proband and from Subject III:5. Sections of formalin-fixed, paraffin-embedded tissue from frontal, superior temporal, parietal and occipital lobes, gyrus cinguli, cerebellum, hippocampus, basal ganglia, thalamus, hypotalamus, midbrain, and pons were stained with hematoxylin and eosin, Congo red, periodic acid–Schiff, and Bielschowsky stains.

Immunohistochemistry was performed on 6μm sections using anti–Aβ antibodies (clone WO-2 [1:200], selective for residues 4–10, clone G2-10 (1:200) for Aβ40, clone G2-13 (1:200) for Aβ42, ABETA, Germany; clone 4G8 (1:1,000), for residues 17-24; Sigma, St. Louis, MO) and to phosphorylated tau (Serotek Ltd. 3UK, Oxford, UK) and clone AT8 (Pierce, Rockford, IL). Aβ immunostain slides were pretreated twice with citric acid, pH 6.0, for 5 minutes (microwave, 800W), except for 4G8, for which slides were pretreated in 80% formic acid for 10 minutes at room temperature, according to the manufacturer. EnVision+ System (DAKOCytomation, Glostrup, Denmark) was used for antibody detection. Sections were developed with 3,3′-diaminobenzidine and hydrogen peroxide and counterstained with hematoxylin. Positive and negative controls were performed for each antibody.

DNA Analysis

Blood was obtained from four subjects (Fig 1; III:1–III:4) after obtaining written informed consent. DNA was extracted by standard procedures. For Subject III:5, DNA was extracted from paraffin-embedded tissue as described.[10] Exon 17 of *AβPP* was amplified by polymerase chain reaction (PCR) with flanking intronic primers: 5′-ACCTCATCC-AAATGTCCCCTGC-3′ and 5′-TCTCATAGTCTTAAT-TCCCAC-3′. Amplicons were sequenced on a 310 DNA sequencer (Applied Biosystems, Foster City, CA). For restriction analysis a *Tsp*45I site was artificially introduced in the mutated allele by PCR, using a different sense primer (5′-CTAATTGCGTTTATAAATTG-3′) and antisense, mismatched primer 5′-ATGACAACACCGCCCACCgTG-3′ (lowercase indicates mismatched nucleotide). Sequencing of PCR products from one control and one affected subject confirmed the generation of a *Tsp*45I site in the mutated allele only. *ApoE* genotype was assessed according to Cazeneuve et al.[11] for Subjects III:1, III:3, and III:4, but not on paraffin-extracted DNA, because of technical reasons.

Results

Clinical Data

We studied a three-generation Italian family with autosomal dominant, recurrent hemorrhagic stroke in the fifth to eighth decades of life. The proband (see Fig 1, III:1), a 63-year-old man, was referred for weakness and paresthesias on his left hand and confusional state. A computed tomography (CT) scan showed a small right parietal ICH, appearing as a subarachnoid hemorrhage (Fig 2, top). Old hemorrhagic foci were shown on gradient-echo magnetic resonance imaging, with diffuse white matter hyperintensities on T2-weighted images (see Fig 2, bottom). Two months after this stroke, he died from two additional lobar hemorrhages and an autopsy was performed. A first-degree cousin (see Fig 1, III:5), presented at age 50 years with motor impairment and paresthesias on her right arm. A CT scan showed a single left frontoparietal hemorrhage, involving the cortical and subcortical white matter with evidence of severe deep white matter degeneration. Seven years later, she had a right frontal hemorrhage. She died at age 58 years from a large right frontal and temporoparietal ICH and an autopsy was performed. Her sister (III:3) was referred at age 72 years for confusional state without headache or motor symptoms. A CT scan showed a large, left temporal hemorrhage involving the deep white matter. Their brother (see Fig 1, III:4) presented with a left frontal hemorrhage at age 70 years. Three poroencephalic cavities affecting the right frontal and occipital lobes were observed on CT. Subsequently, three lobar ICHs led to global cognitive decline on a multifactual basis. He is the only affected subject who presented with cognitive impairment. Another proband's cousin (III:2) is well at age 74 years. The proband's father (II:3) and two paternal siblings died in their 70s from stroke.

Pathological Examination

Microscopic examination under polarized light, after Congo red staining, showed apple-green birefringent amyloid deposits in small- and medium-sized arteries of the leptomeninges and cerebral and cerebellar cortex. Features of severe CAA were observed, such as the "vessel-within-vessel" configuration and hemorrhages originating from affected vessels (Fig 3). Evidence of secondary microvascular degeneration, as hyalinization of small arteries (see Fig 3), microaneurysm and focal lymphocytic infiltration of vessel walls, with two mi-

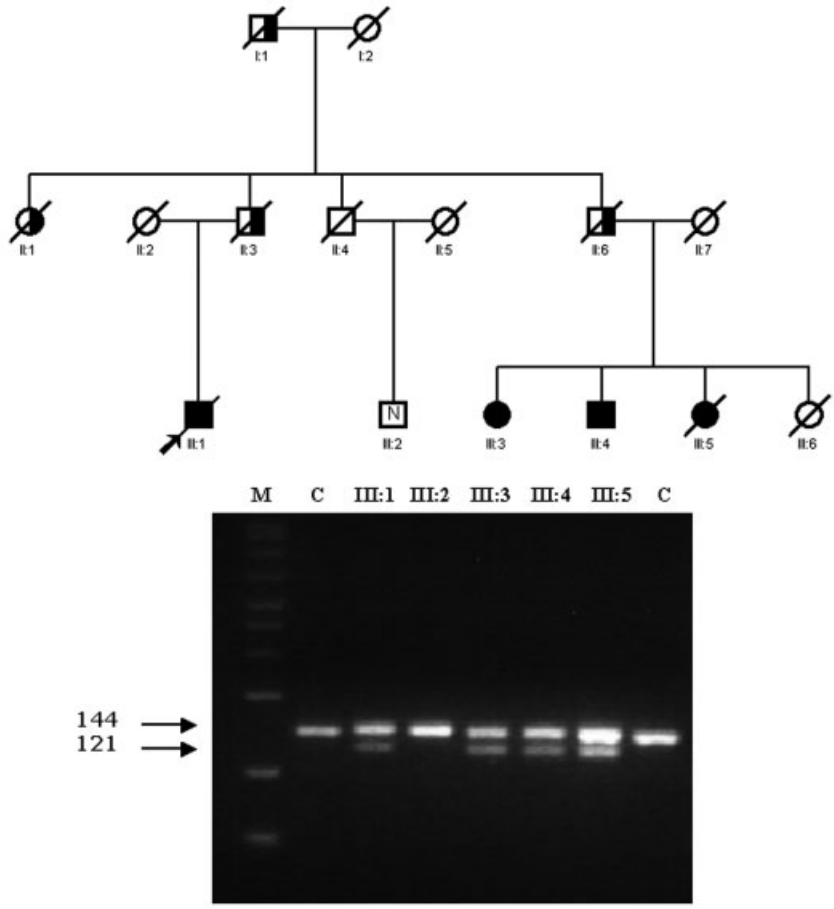

Fig 1. Pedigree of the Italian family with cerebral amyloid angiopathy (CAA). The proband is indicated by an arrow. Subjects clinically affected and carriers of the mutation are indicated by solid symbols. Subjects who died of stroke and are presumed to have been affected are indicated by partially filled symbols. Dead subjects are indicated by a diagonal line through the symbols. Open symbols indicate asymptomatic subjects not tested. N indicates tested subjects, no mutation found. A Tsp*45I restriction endonuclease assay was used to detect the L705V mutation after polymerase chain reaction amplification with a mismatched oligonucleotide. An additional 121bp fragment is observed in the proband (III:1) and in his affected cousins (III:3, III:4, and III:5) compared with one healthy relative (III:2) and two unrelated controls (C). The small cleavage fragment of 23bp is not shown. M = 50–2,000bp ladder (BioRad, Hercules, CA).*

crothrombi, was present. Amyloid specifically immunostained with anti–Aβ antibodies, demonstrating the presence of both Aβ40 and Aβ42 species (see Fig 3). Numerous capillaries showed Aβ deposits. CAA was present in the proband in all blocks analyzed (one from each frontal, parietal, temporal, and occipital lobes and one from cerebellum). In each block, Aβ accumulation was observed in the large majority of meningeal vessels, whereas cortical vessels were variably affected, ranging from at least one to two positive small- and medium-sized arteries in the majority of 100× fields in frontal cortex up to six in sections from the occipital and cerebellar areas. Vessel wall thickening was limited, with a few stenotic vessels observed in occipital cortical regions. Neither diffuse nor neuritic amyloid plaques were found in the brain parenchyma. 4G8 antibody confirmed the absence of Aβ plaques and did not show amorphous Aβ aggregates (see Fig 3). Neither dystrophic neurites nor neurofibrillary tangles were observed in all sections analyzed.

Genetic Analysis

Sequencing of exon 17 of *AβPP* showed a G to C transversion in the first nucleotide of codon 705 in the proband and his three affected cousins. This substitution results in a valine for leucine replacement at residue 34 of Aβ. Patients were heterozygous for this mutation, as confirmed by restriction analysis (see Fig 1). The mutation was absent in one unaffected relative and in 100 controls. *ApoE* genotyping of the proband and two affected cousins (III:3 and III:4) showed that they are homozygous for ε3 allele.

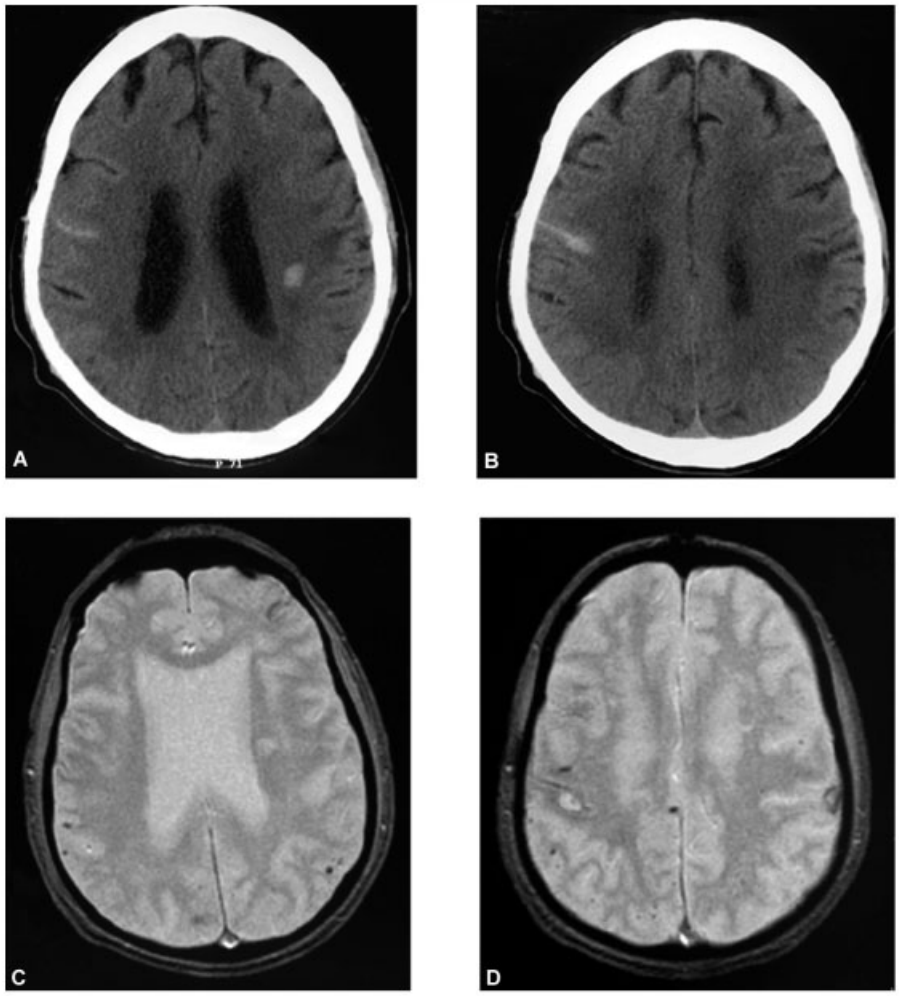

Fig 2. Computerized tomography (CT) scans (A, B) of the proband showed the presence of a small, right parietal intraparenchymal hemorrhage associated with a subarachnoid hemorrhagic effusion. An additional small intraparenchymal hemorrhagic lesion was observed in the left corona radiata. T2-weighted magnetic resonance imaging scans (C, D) similarly disclosed the presence of the same intraparenchymal hemorrhages, together with multiple cortical hypointensities particularly in the parietal lobes, corresponding to old hemorrhagic foci.

Discussion

A key issue in the pathogenesis of cerebral amyloidoses is the definition of in vivo mechanisms that underlie the parenchymal versus vascular deposition of amyloid fibrils in brain,[12] resulting in either AD or CAA or both. Substitutions at residue 21–23 of Aβ are preferentially associated with massive accumulation of amyloid in cerebral blood vessels. Several studies on the effects on these mutations on APP processing[7] and on the fibrillogenic and cytotoxic properties of mutant Aβ peptides[5,7] have attempted to explain the molecular basis of this "vascular-weighted" distribution of amyloid.[13]

We report a novel Aβ mutation, Leu34Val, exclusively associated with CAA. A causative role is strongly supported by its complete segregation with the disease and by specific staining of amyloid with anti–Aβ antibodies. This variant displays unique neuropathological features, represented by the selective localization of amyloid into the vessel walls. Although the overall CAA load seems limited, cracking of vessel walls with "vessel-within-vessel" configuration and other features of secondary microvascular degeneration[14] support the prominent vascular symptomatology observed in all patients. A major difference with the pathology of other known Aβ variants is the absence of parenchymal lesions, including either diffuse and neuritic amyloid plaques or amorphous Aβ aggregates. Similarly, neither dystrophic neurites nor neurofibrillary tangles were observed in all sections. The contribution of *apoE* genotype to this vascular phenotype seems unlikely, because three affected members do not carry the ε2 allele that has been connected with severe CAA.[15]

Previous studies demonstrated that Aβ mutants are

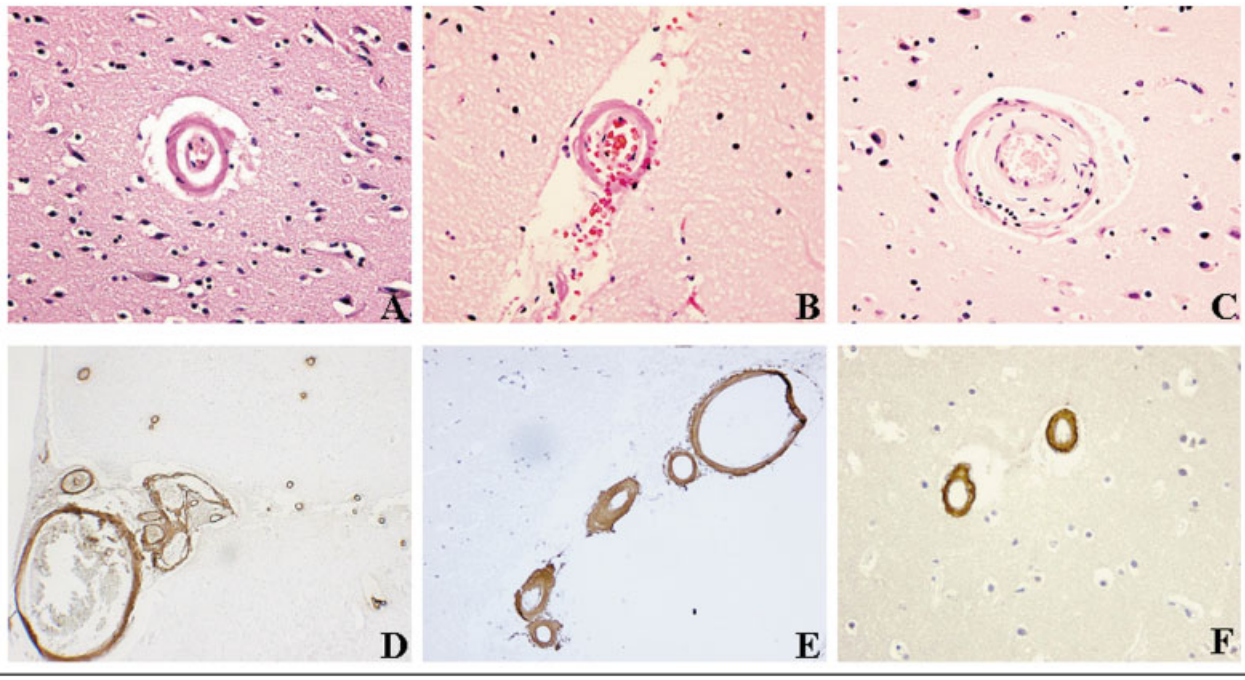

Fig 3. Neuropathological findings in proband. Features of severe amyloid angiopathy were observed after staining with hematoxylin and eosin, including "vessel-within-vessel" changes (A; ×400), presence of hemorrhage originating from an affected cortical vessel characterized by partial splitting of the wall (B; ×400), and artery hyalinization (C; ×400). Staining with anti–Aβ 4G8 antibody (D; ×100) demonstrates the presence of Aβ amyloid deposits in leptomeningeal and cortical vessel walls. No parenchymal deposits were observed around vessels. Vascular amyloid deposits are stained with anti–Aβ40 (E; original magnification, ×200) and anti–Aβ42 (F; ×200) antibodies.

associated with enhanced protofibril formation[7] and/or changes in fibrillogenic properties.[12] This aggregation propensity is correlated with peptide hydrophobicity and charge neutralization,[16] and various equations were proposed to predict the relative propensity of a peptide to aggregate in physiologic solutions. One algorithm[17] correlates well with experimental evidence of the aggregation kinetics of Aβ isoforms and predicts the higher aggregation propensity of Aβ42 compared with Aβ40. According to this algorithm (http://tango.embl.de) AβL34V would have an approximate 20% increase in the β aggregation parameter in the adjoining sequence, compared with the wild type. The effect would be mainly caused by the peculiar hydrophobic contribution for aggregation of valine in position 34, which could favor more then leucine the β sheet structure.

Experimental studies are required to validate this bioinformatic prediction and possibly shed light on the molecular basis of the exclusive targeting of this Aβ peptide to cerebral vessels.

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A CTLA4^{high} Genotype Is Associated with Myasthenia Gravis in Thymoma Patients

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Myasthenia gravis (MG) in thymoma patients depends critically on intratumorous generation and export of mature autoreactive CD4$^+$ T cells. Why non-MG thymomas fail to produce CD4$^+$ T cells is unknown. We studied three single-nucleotide polymorphisms of the cytotoxic T-lymphocyte–associated antigen 4(*CTLA4*) gene in thymoma patients, nonthymoma early-onset MG patients, and control subjects. Surprisingly, the CTLA4^{high} genotype +49A/A, which is protective against several autoimmune diseases, exerted a prominent predisposing effect to paraneoplastic MG in thymoma patients. The unusual disease association with a CTLA4^{high} genotype implies a unique pathogenesis of paraneoplastic MG, with high CTLA4 levels possibly supporting the nontolerogenic selection of CD4$^+$ T cells in MG-associated thymomas.

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The cytotoxic T-lymphocyte–associated antigen 4 (*CTLA4*) gene has been a primary candidate for genetic susceptibility to autoimmune diseases, including type 1 diabetes,[1,2] Graves' disease,[1,3] autoimmune hypothyroidism,[1,3] multiple sclerosis,[4] and systemic lupus erythematosus (SLE).[5] CTLA4 is a critical negative regulator of T-cell activation, which competitively interferes with the binding of CD28 to B7-1 and B7-2 on antigen-presenting cells.[6] Several polymorphisms of the *CTLA4* gene, including the only coding single-

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