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RESEARCH ARTICLE

Modified amyloid variants in pathological subgroups of β-amyloidosis

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Abstract

Objective: Amyloid β (Aβ) depositions in plaques and cerebral amyloid angiopathy (CAA) represent common features of Alzheimer's disease (AD). Sequential deposition of post-translationally modified Aβ in plaques characterizes distinct biochemical stages of Aβ maturation. However, the molecular composition of vascular Aβ deposits in CAA and its relation to plaques remain enigmatic. **Methods**: Vascular and parenchymal deposits were immunohistochemically analyzed for pyroglutaminated and phosphorylated Aβ in the medial temporal and occipital lobe of 24 controls, 27 pathologically-defined preclinical AD, and 20 symptomatic AD cases. **Results**: Sequential deposition of Aβ in CAA resembled Aβ maturation in plaques and enabled the distinction of three biochemical stages of CAA. B-CAA stage 1 was characterized by deposition of Aβ in the absence of pyroglutaminated $Aβ_{N3pE}$ and phosphorylated $Aβ_{pS8}$. B-CAA stage 2 showed additional $Aβ_{N3pE}$ and B-CAA stage 3 additional $Aβ_{pS8}$. Based on the Aβ maturation staging in CAA and plaques, three case groups for Aβ pathology could be distinguished: group 1 with advanced Aβ maturation in CAA; group 2 with equal Aβ maturation in CAA and plaques; group 3 with advanced Aβ maturation in plaques. All symptomatic AD cases presented with end-stage plaque maturation, whereas CAA could exhibit immature Aβ deposits. Notably, Aβ pathology group 1 was associated with arterial hypertension, and group 2 with the development of dementia. **Interpretation**: Balance of Aβ maturation in CAA and plaques defines distinct pathological subgroups of β-amyloidosis. The association of CAA-related Aβ maturation with cognitive decline, the individual contribution of CAA and plaque pathology to the development of dementia within the defined Aβ pathology subgroups, and the subgroup-related association with arterial hypertension should be considered for differential diagnosis and therapeutic intervention.

Introduction

Alzheimer's disease (AD) represents the most common form of dementia, characterized by the accumulation of amyloid β (Aβ) in extracellular plaques and hyperphosphorylated tau in intracellular neurofibrillary tangles (NFTs).[1] The majority of AD patients additionally develop amyloid lesions in the cerebrovasculature.[2] Cerebral amyloid angiopathy (CAA) describes an AD-associated vessel disorder, defined by the deposition of Aβ in leptomeningeal and/or parenchymal arteries, veins, and capillaries.[3] Cerebrovascular Aβ structurally resembles Aβ fibrils in plaques of AD patients.[4] The overall abundance of CAA and plaques in AD[2,5] and the spatio-temporal relation between CAA and plaques in the pathogenesis of AD[6–8] suggest a pathogenic link between both pathologies.

Recently, we revealed a hierarchical sequence for the deposition of different Aβ species in the pathogenesis of AD-related amyloid plaques.[9] Three biochemical stages of Aβ aggregate maturation in plaques (B-Aβ plaque stages) were identified based on the immunohistochemical detection of Aβ and its modified species. B-Aβ plaque stage 1 was defined by the parenchymal deposition of Aβ lacking N-terminal truncated, pyroglutaminated Aβ ($Aβ_{N3pE}$) and phosphorylated Aβ ($Aβ_{pS8}$), whereas Aβ including $Aβ_{N3pE}$ was prevalent in B-Aβ plaque stage 2. B-Aβ plaque stage 3, finally, exhibited $Aβ_{pS8}$ within the parenchymal Aβ aggregates in addition to other forms of Aβ including $Aβ_{N3pE.}$[9]

Post-translational modification of Aβ by N-terminal truncation and pyroglutamination as well as phosphorylation affect the aggregation, stability, and toxicity of Aβ.[10,11] In particular pyroglutamination at glutamate 3 and phosphorylation at serine 8 promote aggregation, thereby enhancing metabolic stability and toxicity of Aβ.[12–16]

Despite the abundance of $Aβ_{N3pE}$ and $Aβ_{pS8}$ in plaques and CAA of AD patients,[9,15,17–20] the molecular composition of CAA lesions and its relation to the progression of AD remain enigmatic. To compare amyloid deposition in CAA and plaques, we analyzed the medial temporal and occipital lobe of control, pathologically-defined preclinical AD (p-preAD), and symptomatic AD cases for the presence of Aβ and its modified forms $Aβ_{N3pE}$ and $Aβ_{pS8}$ in CAA-affected blood vessels.

Materials and Methods

Neuropathology

Brain tissue originated from the brain bank of the "Laboratory of Neuropathology" at the "University of Ulm" (Germany) that collected brain tissue in accordance with German legal regulations. Collection of brain tissue and experimental analyses of this project were approved by the ethics committees of the Universities of Ulm (Germany), Bonn (Germany), and KU Leuven (Belgium) where experiments have been performed (Votes Nos. Bonn: 161/01, 238/04; Ulm: 238/07, 54/08, 57/12; Leuven: S-58102, S-59295).

The brain collection consists of hospital-based autopsy cases that were included into the brain bank at the time point of autopsy. The clinical information, therefore, included only information from files that could be reviewed retrospectively in the respective hospital. Longitudinal data and data from neuropsychological tests were not available.

Morphological analysis of cerebrovascular and parenchymal Aβ lesions was performed in autopsy brains of 24 control, 27 p-preAD, and 20 sporadic AD cases (Table 1). The diagnosis of AD was performed neuropathologically with consideration of clinical information about the cognitive status. Control cases were defined by absence of amyloid plaques including cases with primary age-related tauopathy[21] and occasionally CAA. Non-demented cases with AD pathology, comprising Aβ plaques and NFTs, were designated as p-preAD cases, whereas symptomatic AD cases were characterized by substantial AD pathology and impairment of cognition.[1,22]

Following autopsy, brains were fixed in a 4% aqueous solution of formalin and tissue from both the medial temporal and occipital lobe was embedded in paraffin and cut into sections of 12 μm. Neuropathological diagnosis of AD was performed according to established guidelines of the "National Institute of Aging-Alzheimer's Association" (NIA-AA AD degree)[1] and included (1) the assessment of NFT distribution (Braak-NFT stage)[23,24] on the basis of Gallyas' silver impregnation[24] and/or immunohistochemical staining of abnormal phosphorylated tau (AT8, 1:1000, Pierce Endogen; Rockford, USA),[25] (2) the assignment of neuritic plaque density (Consortium to Establish a Registry for AD (CERAD) score)[26] on the basis of Gallyas' silver impregnation[24] and/or immunohistochemical staining of abnormal phosphorylated tau (AT8, 1:1000, Pierce Endogen; Rockford, USA),[1] and (3) the evaluation of amyloid plaque distribution in the medial temporal lobe (Aβ-MTL phase)[27] on the basis of immunohistochemical staining for $Aβ_{17-24}$ (4G8, 1:5000, Covance; Princeton, USA). Apolipoprotein E (*APOE*) genotypes were determined by restriction isotyping of unfixed brain tissue with HhaI[28] (Table 1).

The severity of CAA-related vessel wall destruction (CAA severity) was graded according to Vonsattel et al.[29]; and the stage of the anatomical expansion of CAA throughout the brain (CAA stage) was rated according to Thal et al.[30] CAA with affection of capillaries was referred to as CAA type 1, and CAA without capillary Aβ deposits was designated as CAA type 2.[31]

Medical examination of control, p-preAD, and AD cases was performed one to four weeks prior to death according to standardized protocols and included the

Table 1. List of control, p-preAD, and AD cases used for analysis of CAA and plaques.

No	Age[1]	Gender[2]	Diagnosis[3]	Diabetes mellitus[4]	Hyper-tension[4]	Alcohol abuse[4]	CDR score[5]	Aβ-MTL phase[6]	Braak-NFT stage[7]	CERAD score[8]	NIA-AA AD degree[9]	*APOE* genotype[10]	B-Aβ plaque stage[11]	B-CAA stage
1	60	M	Control	-	+	-	0	0	0	0	not AD	"2/3"	0	0
2	69	F	Control	-	+	-	0	0	1	0	not AD	"3/3"	0	0
3	66	M	Control	-	-	-	0	0	1	0	not AD	"3/3"	0	0
4	71	F	Control	-	+	-	0	0	1	0	not AD	"2/3"	0	0
5	58	F	Control	+	-	-	0	0	0	0	not AD	"3/3"	0	0
6	46	M	Control	-	-	-	0	0	1	0	not AD	"3/3"	0	0
7	45	M	Control	-	-	-	0	0	0	0	not AD	"3/4"	0	0
8	35	M	Control	-	-	-	0	0	0	0	not AD	"3/3"	0	0
9	59	M	Control	-	-	+	-	0	1	0	not AD	"3/4"	0	0
10	57	M	Control	-	-	-	0	0	1	0	not AD	"3/4"	0	0
11	74	M	Control	-	-	-	0	0	1	0	not AD	"3/4"	0	0
12	66	M	Control	-	-	+	0	0	1	0	not AD	"3/3"	0	0
13	61	M	Control	-	+	-	0	0	0	0	not AD	"3/3"	0	0
14	66	M	Control	+	-	-	0	0	1	0	not AD	"2/3"	0	0
15	60	M	Control	-	-	-	0	0	1	0	not AD	"3/3"	0	0
16	69	F	Control	-	+	-	0	0	0	0	not AD	"2/3"	0	0
17	66	F	Control	-	+	+	0	0	0	0	not AD	"3/3"	0	0
18	62	M	Control	-	-	+	0	0	0	0	not AD	"3/3"	0	0
19	72	F	Control	+	+	-	0	0	1	0	not AD	"3/3"	0	0
20	62	M	Control	-	-	-	0	0	0	0	not AD	"3/3"	0	0
21	72	F	p-preAD	+	+	-	0	1	2	0	low	"3/3"	2	0
22	71	M	p-preAD	+	-	-	0	3	2	1	low	"2/4"	3	3
23	68	F	p-preAD	-	-	-	0	2	2	0	low	"2/3"	3	3
24	73	F	p-preAD	-	+	-	0	1	2	0	low	"3/3"	2	0
25	77	F	p-preAD	-	-	-	0	3	2	0	low	"3/4"	3	0
26	78	F	p-preAD	-	-	-	0	3	2	0	low	"3/4"	3	3
27	71	F	p-preAD	-	+	-	0	3	2	1	low	"3/3"	2	3
28	77	F	p-preAD, VD	+	+	-	3	2	3	1	low	"3/3"	2	3
29	73	F	p-preAD	-	-	-	0	1	2	0	low	"2/3"	2	0
30	74	M	p-preAD	+	+	-	0	2	2	0	low	"3/3"	2	0
31	64	M	p-preAD, brain infarction	-	+	-	-	2	1	0	low	"3/3"	2	0
32	74	M	p-preAD	+	+	-	0	4	3	1	intermediate	"3/4"	3	1
33	53	M	p-preAD	-	-	-	0	1	1	0	low	"3/3"	2	0
34	78	F	p-preAD, VD, CBD	-	+	-	3	1	1	0	low	"3/3"	2	0
35	68	F	p-preAD	-	+	-	0	2	1	0	low	"3/3"	2	0

(*Continued*)

Table 1. Continued.

No	Age[1]	Gender[2]	Diagnosis[3]	Diabetes mellitus[4]	Hyper-tension[4]	Alcohol abuse[4]	CDR score[5]	Aβ-MTL phase[6]	Braak-NFT stage[7]	CERAD score[8]	NIA-AA AD degree[9]	*APOE* genotype[10]	B-Aβ plaque stage[11]	B-CAA stage
36	67	F	p-preAD	+	-	-	0	2	2	0	low	"3/3"	2	0
37	82	F	p-preAD, microinfarcts	-	+	-	-	2	1	1	low	"3/3"	2	3
38	87	M	p-preAD	-	+	-	0	3	3	1	intermediate	"3/4"	3	3
39	84	F	p-preAD	-	+	-	0	3	2	0	low	"2/3"	3	0
40	84	F	p-preAD, brain infarction	-	-	-	0	3	3	0	intermediate	"3/3"	3	3
41	88	M	p-preAD, AGD	-	+	-	2	3	2	1	low	"2/3"	3	2
42	83	F	p-preAD	+	-	-	0	3	3	1	intermediate	"2/3"	3	1
43	72	M	p-preAD	+	-	-	0	2	3	0	low	"3/3"	2	0
44	64	M	p-preAD	-	-	-	0	2	1	0	low	"3/3"	2	0
45	63	F	p-preAD, brain infarction	-	-	-	0	4	3	1	intermediate	"3/3"	2	0
46	85	F	p-preAD	+	-	-	0	4	3	1	intermediate	"3/3"	2	0
47	83	M	p-preAD, brain infarction	+	+	-	0	2	3	0	low	"3/3"	1	0
48	79	F	AD	-	-	-	-	3	4	2	intermediate	"3/3"	3	3
49	64	F	AD	-	+	-	-	4	6	3	high	"3/4"	3	2
50	62	F	AD	-	-	-	3	4	6	3	high	"3/4"	3	3
51	84	M	AD	-	-	-	3	4	6	3	high	"3/4"	3	2
52	72	F	AD	-	-	-	1	4	4	2	intermediate	"3/3"	3	2
53	83	M	AD	-	-	-	1	4	4	2	intermediate	"3/4"	3	3
54	78	M	AD	-	-	-	3	4	4	1	intermediate	"3/4"	3	2
55	75	F	AD	-	-	-	0.5	4	3	1	intermediate	"4/4"	3	3
56	84	M	AD, AGD, ALS, VD	-	-	+	3	3	4	2	intermediate	"3/3"	3	3
57	68	F	AD	-	-	-	1	4	6	3	high	"3/3"	3	1
58	82	M	AD	+	+	-	2	3	3	2	intermediate	"3/4"	3	3
59	86	F	AD, AGD	-	-	-	3	4	6	3	high	"3/3"	3	3
60	83	M	AD	-	-	-	3	4	4	2	intermediate	"3/3"	3	3
61	78	F	AD	-	+	-	3	4	5	3	high	"3/4"	3	3
62	89	F	AD	+	-	-	2	4	4	3	intermediate	"3/4"	3	3
63	87	F	AD	-	-	-	3	4	4	1	intermediate	"3/3"	3	2
64	78	M	AD	+	-	-	1	3	4	1	intermediate	"3/3"	3	3
65	89	F	AD	+	-	-	3	4	5	2	high	"3/4"	3	2
66	81	F	AD	+	-	-	3	4	5	1	intermediate	"3/3"	3	2
67	83	M	AD	-	+	-	3	4	5	3	high	"4/4"	3	3
68	68	F	Control, pure CAA	-	+	-	0	0	2	0	not AD	"3/3"	0	3

(*Continued*)

Table 1. Continued.

No	Age[1]	Gender[2]	Diagnosis[3]	Diabetes mellitus[4]	Hyper-tension[4]	Alcohol abuse[4]	CDR score[5]	Aβ-MTL phase[6]	Braak-NFT stage[7]	CERAD score[8]	NIA-AA AD degree[9]	*APOE* genotype[10]	B-Aβ plaque stage[11]	B-CAA stage
69	64	F	Control, pure CAA, LBD	-	+	-	0.5	0	3	0	not AD	-	0	2
70	63	F	Control, pure CAA	-	-	-	-	0	2	0	not AD	-	0	2
71	63	F	Control, pure CAA	+	+	-	1	0	1	0	not AD	-	0	2

[1]age [years].
[2]F = female, M = male.
[3]AD = Alzheimer's disease, AGD = argyrophilic grain disease, ALS = amyotrophic lateral sclerosis, CAA = cerebral amyloid angiopathy, CBD = corticobasal degeneration, LBD = Lewy body disease, p-preAD = pathologically-defined preclinical Alzheimer's disease, VD = vascular dementia.
[4]diabetes mellitus/hypertension/alcohol abuse: present (+), absent (-).
[5]clinical dementia rating (CDR) score.[64]
[6]Aβ - medial temporal lobe (MTL) phase.[27]
[7]Braak - neurofibrillary tangle (NFT) stage.[23,25]
[8]Consortium to Establish a Registry for Alzheimer's disease (CERAD) score.[26]
[9]National Institute on Aging-Alzheimer's Association (NIA-AA) degree of Alzheimer's disease pathology.[1,65]
[10]*APOE* = apolipoprotein E.
[11]B-Aβ plaque stage.[9]

assessment of (1) cognition (including short term and long term memory), (2) speech, writing, and reading, (3) self-dependence and self-care, (4) habit of eating, (5) bladder and bowel continence, and (6) orientation within the hospital setting.[8] Clinical data were used to retrospectively assess clinical dementia rating scores (CDR scores)[32] and for information about arterial hypertension, diabetes mellitus, and alcohol abuse. Cases with CDR scores ≥ 0.5 in conjunction with intermediate to high NIA-AA AD degrees were considered as symptomatic AD cases.[1] Due to missing clinical data, CDR scores could not be obtained for 5 of 24 control, 2 of 27 p-preAD, and 2 of 20 AD cases (Table 1).

Immunohistochemistry

Following deparaffinization, hydration, and blocking, sections of the medial temporal and occipital lobe were incubated for 24 h at room temperature with anti-A$\beta_{17\text{-}24}$ (4G8, 1:5000, Covance; Princeton, USA; formic acid pretreatment), anti-Aβ_{N3pE}[20] (1:100, IBL; Hamburg, Germany; formic acid/microwave pretreatment), or anti-Aβ_{pS8}[12,33] (SA5434/1E4E11, 1:5; formic acid/microwave pretreatment) antibodies. The antibodies used in this study to detect phosphorylated or pyroglutaminated Aβ were raised specifically against synthetic Aβ peptides carrying the phosphorylation or pyroglutamate modification and recognize phosphorylated serine or pyroglutamate residues selectively in the context of the Aβ amino acid sequence. Primary antibodies were detected with biotinylated secondary antibodies and visualized with the ABC method (Vector Laboratories; Burlingame, USA) and 3,3′-diaminobenzidine (DAB; brown color) as chromogen. Sections were counterstained with hematoxylin. Positive and negative controls were performed.

Sensitivity and specificity of phosphorylation-state specific polyclonal (SA5434) and monoclonal (1E4E11) antibodies were examined by preabsorption with synthetic Aβ peptides followed by western immunoblotting and/or immunohistochemistry using brain tissue from transgenic mouse models and human AD cases.[12,17,33] Additional staining with antibodies against A$\beta_{1\text{-}17}$ (6E10; 1:1,250, Covance; Princeton, USA; formic acid pretreatment) was performed on selected sections as described previously.[9]

Analysis of Aβ, Aβ$_{N3pE}$, and Aβ$_{pS8}$ deposition in CAA

Analysis of CAA was conducted in the medial temporal lobe of control, p-preAD, and AD cases stained for Aβ, Aβ_{N3pE}, or Aβ_{pS8} (Table 1). Cases that exhibited Aβ deposits in leptomeningeal and/or parenchymal vessels were considered positive for CAA independent of the severity and extent of CAA pathology. Vascular Aβ deposition was

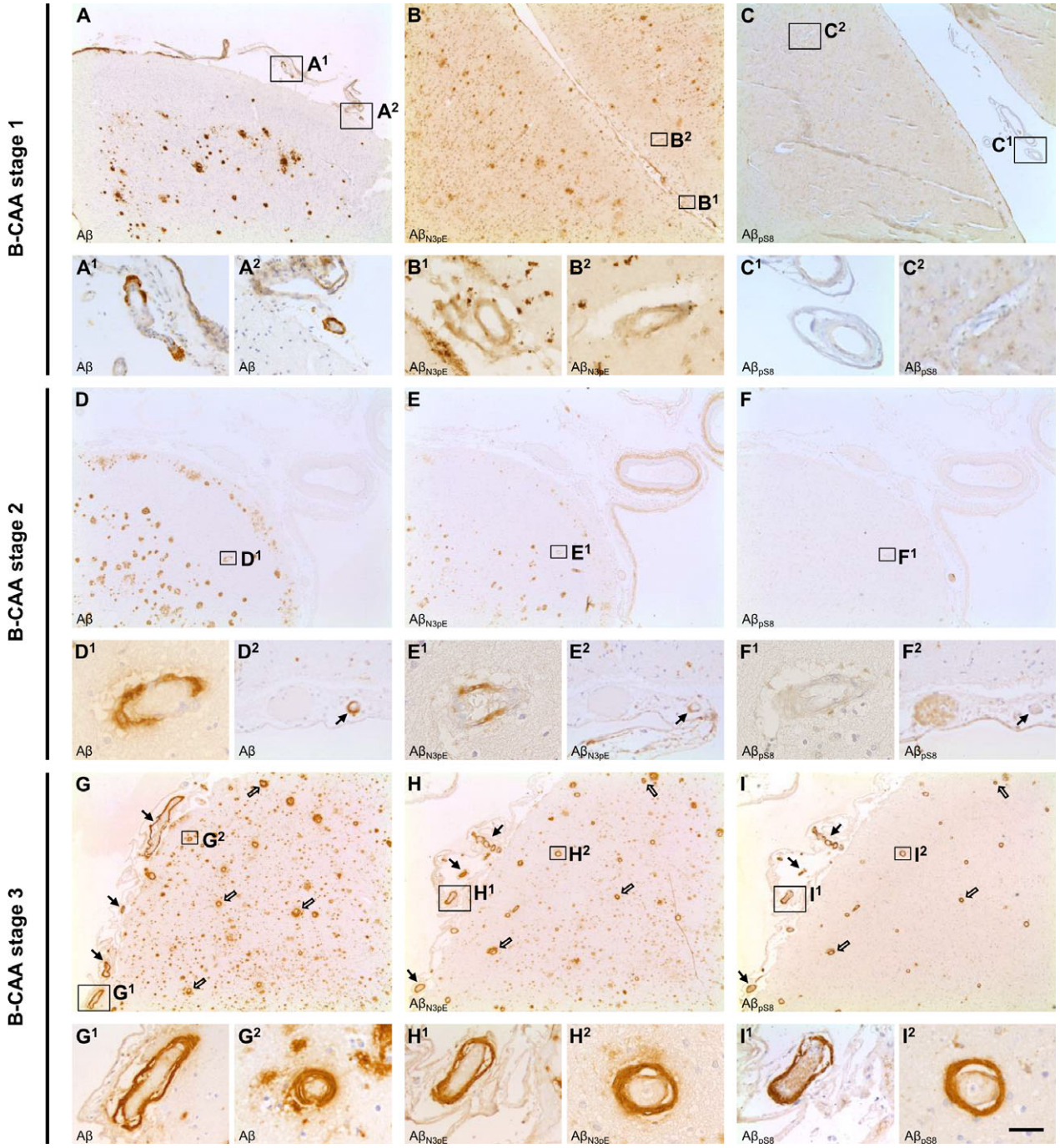

Figure 1. Stages of amyloid maturation in CAA. Detection of Aβ, Aβ_{N3pE}, and Aβ_{pS8} in leptomeningeal (arrow) and parenchymal (open arrow) vessels of AD cases enabled the differentiation of three biochemical stages of amyloid deposition in the pathogenesis of CAA. B-CAA stage 1 (A–C) was characterized by initial deposition of Aβ in the vessel (A) in the absence of Aβ_{N3pE} (B) and Aβ_{pS8} (C) deposition. B-CAA stage 2 (D–F), however, corresponded to the additional deposition of Aβ_{N3pE} (E) whereas the vessels were still devoid of Aβ_{pS8} deposits (F, the intravascularly stained material in one vessel in F and F2 (no arrow) is related to insufficient peroxidase blocking in the erythrocytes and does not correspond to positivity for Aβ_{pS8} as demonstrated in I, I1, and I2). Co-deposition of Aβ (G), Aβ_{N3pE} (H), and Aβ_{pS8} (I) in the vessel could be detected in B-CAA stage 3 (G–I). The figure displays representative images of the temporal cortex of AD cases stained with DAB for Aβ (A, D, G), Aβ_{N3pE} (B, E, H), and Aβ_{pS8} (C, F, I). Scale bar: (A, B, C, D, E, F, G, H, I) 350 μm, (A1, A2, C1, C2, D2, E2, F2, G1, H1, I1) 70 μm, (B1, B2, D1, E1, F1, G2, H2, I2) 35 μm.

Table 2. Partial Spearman's rank correlations (control for age/gender).

	Correlation coefficient	*P*-value
B-CAA stage		
CAA stage	**0.910**	**<0.001**
CAA severity	**0.911**	**<0.001**
Aβ plaque load	0.163 (0.007)*	0.264 (0.967)*
$A\beta_{N3pE}$ plaque load	0.216 (0.055)*	0.137 (0.761)*
$A\beta_{pS8}$ plaque load	**0.469** (-0.044)*	**0.001** (0.807)*
CTRL/p-preAD/AD	**0.442**	**<0.001**
CDR score	**0.535**$^{+}$	**<0.001**$^{+}$
NIA-AA AD degree	**0.403**	**0.001**
Aβ-MTL phase	**0.394**	**0.001**
Braak-NFT stage	**0.509**	**<0.001**
CERAD score	**0.599**	**<0.001**
B-Aβ plaque stage	**0.500**	**<0.001**
APOE ε4 allele		
B-CAA stage	**0.357**	**0.003**
CAA severity	**0.372**	**0.002**
B-Aβ plaque stage	**0.379**	**0.002**
Aβ-MTL phase	**0.387**	**0.001**

Aβ = amyloid β, AD = Alzheimer's disease, *APOE* = apolipoprotein E, B-CAA stage = biochemical CAA stage, B-Aβ plaque stage = biochemical Aβ plaque stage, CAA = cerebral amyloid angiopathy, CDR = clinical dementia rating, CERAD = Consortium to Establish a Registry for Alzheimer's disease, CTRL = control, MTL = medial temporal lobe, NFT = neurofibrillary tangle, NIA-AA = National Institute on Aging-Alzheimer's Association, p-preAD = pathologically-defined preclinical Alzheimer's disease; Aβ/$A\beta_{N3pE}$/$A\beta_{pS8}$ plaque load[9] [* numbers display the correlation coefficients and *P*-values when restricting the correlation analysis to cases with Aβ pathology, whilst numbers in brackets correspond to the correlation coefficients and P-values when restricting the correlation analysis to cases with CAA pathology (B-CAA stage ≥1)], Aβ-MTL phase[27], B-Aβ plaque stage[9], Braak-NFT stage[25], CAA severity[29], CAA stage[30], CDR score[32] [+ correlation analysis of CDR scores was restricted to cases without VD, CBD, LBD, and/or AGB], CERAD score[26], NIA-AA AD degree[1,65]. Values in bold represent statistically significant results.

determined independently for Aβ, $A\beta_{N3pE}$, and $A\beta_{pS8}$ for each case. Cases that did not exhibit vascular Aβ, $A\beta_{N3pE}$, and $A\beta_{pS8}$ deposition in the medial temporal lobe were additionally analyzed for CAA in the occipital lobe.

Quantification of Aβ, $A\beta_{N3pE}$, and $A\beta_{pS8}$ plaque loads

Aβ, $A\beta_{N3pE}$, and $A\beta_{pS8}$ plaque loads were quantified in the temporal cortex (Brodmann area 36) of control, p-preAD, and AD cases (Table 1) stained with anti-$A\beta_{17-24}$ (Aβ plaque load), anti-$A\beta_{N3pE}$ ($A\beta_{N3pE}$ plaque load), or anti-$A\beta_{pS8}$ ($A\beta_{pS8}$ plaque load) antibodies as previously published.[9] ImageJ 1.46 (National Institutes of Health; Bethesda, USA) was used to delineate the temporal cortex and, similarly, to delineate the plaques at morphological identification. The area covered by the plaques was calculated and related to the area of the temporal cortex to assess the plaque load.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 22 (IBM; Chicago, USA). Partial Spearman's rank correlation (control for age/gender) was used to evaluate the association amongst CAA- and AD-related parameters. Multinomial logistic regression (control for age/gender) was applied to compare Aβ pathology groups for their association with CAA- and AD-related parameters, vascular risk factors, and alcohol abuse. Linear regression (control for age/gender) was used to determine the effect of B-CAA and B-Aβ plaque stages on NIA-AA AD degree, CDR score, vascular risk factors, and alcohol abuse.

B-Aβ plaque stages and amyloid plaque loads used for statistical analysis were obtained from a previous publication in which the present cases were analyzed for the biochemical composition of Aβ aggregates in plaques.[9]

Results

Molecular differentiation of amyloid deposition in CAA

To analyze amyloid composition of CAA, brains of control (including cases with CAA in the absence of plaques), p-preAD, and AD cases were stained with antibodies

Table 3. Distribution of cases within different B-Aβ plaque and B-CAA stages.

	B-CAA stage 0	B-CAA stage 1	B-CAA stage 2	B-CAA stage 3	Number
B-Aβ plaque stage 0	**20** *(28.2%)*	**0** *(0.0%)*	**3** *(4.2%)*	**1** *(1.4%)*	**24**
B-Aβ plaque stage 1	**1** *(1.4%)*	**0** *(0.0%)*	**0** *(0.0%)*	**0** *(0.0%)*	**1**
B-Aβ plaque stage 2	**13** *(18.3%)*	**0** *(0.0%)*	**0** *(0.0%)*	**3** *(4.2%)*	**16**
B-Aβ plaque stage 3	**2** *(2.8%)*	**3** *(4.2%)*	**8** *(11.2%)*	**17** *(23.9%)*	**30**
Number	**36**	**3**	**11**	**21**	**71**

Absolute (number of cases in bold) and relative (percentage of cases in italics) frequency of B-Aβ plaque and B-CAA stages. Assignment of cases with CAA and/or plaque pathology to the Aβ pathology groups is color-coded [light gray boxes = group 1 (CAA-predominant Aβ maturation); black boxes = group 2 (equal maturation of Aβ in CAA and plaques); dark gray box = group 3 (plaque-predominant Aβ maturation)] whereas control cases without CAA and AD pathology were indicated in white.

Table 4. Multinomial logistic regression models (control for age/gender).

	Aβ pathology group 1 vs Aβ pathology group 2			Aβ pathology group 1 vs Aβ pathology group 3			Aβ pathology group 2 vs Aβ pathology group 3		
	P-value	odds ratio	95% confidence interval	*P*-value	odds ratio	95% confidence interval	*P*-value	odds ratio	95% confidence interval
CAA severity	0.583	0.520	0.050–5.376	**0.016**	**0.060**	**0.006–0.597**	**0.001**	**0.031**	**0.004–0.240**
CAA stage	0.535	0.543	0.079–3.734	**0.014**	**0.072**	**0.009–0.588**	**0.002**	**0.133**	**0.037–0.481**
CAA type 1	0.452	2.812	0.190–41.587	0.689	1.729	0.119–25.179	**0.035**	**4.861**	**1.115–21.183**
CAA type 2	0.249	4.385	0.355–54.137	**0.040**	**12.106**	**1.123–130.467**	0.162	2.761	0.665–11.452
APOE ε4 allele	0.998	2.678×10^{-9}	-	0.998	1.176×10^{-8}	-	**0.021**	**0.228**	**0.064–0.804**
Arterial hypertension	**0.010**	**0.028**	**0.002–0.426**	**0.042**	**0.080**	**0.007–0.918**	0.160	2.898	0.657–12.788
Diabetes mellitus	0.282	0.272	0.025–2.914	0.726	0.687	0.084–5.606	0.207	2.530	0.597–10.713
Alcohol abuse	1.000	1.398	-	1.000	1.172 x 10^{-8}	-	0.998	2.279×10^{-8}	-
Aβ-MTL phase	**0.008**	**5.279**	**1.530–18.210**	**0.042**	**2.867**	**1.039–7.907**	0.134	1.841	0.829–4.089
Braak-NFT stage	**0.037**	**3.008**	**1.069–8.466**	0.137	2.095	0.790–5.555	0.131	1.436	0.897–2.298
CERAD score	**0.034**	**3.322**	**1.092–10.103**	0.433	1.517	0.535–4.296	**0.021**	**2.190**	**1.128–4.253**
Aβ plaque load	**0.048**	**1.328**	**1.002–1.759**	0.124	1.222	0.947–1.577	0.303	1.087	0.928–1.273
$Aβ_{N3pE}$ plaque load	**0.009**	**2.613**	**1.271–5.374**	0.057	1.816	0.982–3.357	0.106	1.439	0.926–2.237
$Aβ_{pS8}$ plaque load	**0.000**	**2.514×10^{77}**	**1.034×10^{77}–6.112×10^{77}**	-	-	-	0.099	2.110	0.868–5.128
CDR score	**0.000**	**3.733×10^{10}**	**2.096×10^{10}–6.650×10^{10}**	0.997	6.324×10^{-13}	-	0.389	1.289	0.724–2.296

Aβ = amyloid β, *APOE* = apolipoprotein E, CAA = cerebral amyloid angiopathy, CDR = clinical dementia rating, CERAD = Consortium to Establish a Registry for Alzheimer's disease, MTL = medial temporal lobe, NFT = neurofibrillary tangle; $Aβ/Aβ_{N3pE}/Aβ_{pS8}$ plaque load,[9] Aβ-MTL phase,[27] Braak-NFT stage,[25] CAA severity,[29] CAA stage,[30] CAA type,[31] CDR score,[32] CERAD score.[26] Bold values represent statistically significant results.

against non-modified epitopes of Aβ ($Aβ_{17-24}$, $Aβ_{1-17}$) or the post-translationally modified species $Aβ_{N3pE}$ and $Aβ_{pS8}$. Deposition of Aβ, including $Aβ_{N3pE}$ and $Aβ_{pS8}$, was detected in arteries, veins, and/or capillaries of the leptomeninges and/or the parenchyma (Fig. 1). Of the 35 cases with CAA detected through anti-$Aβ_{17-24}$ staining, 32 cases (91.4%) also showed vascular $Aβ_{N3pE}$, whereas vascular $Aβ_{pS8}$ was limited to 21 cases with CAA (60%). Thereby, $Aβ_{pS8}$ was exclusively detected in cases also exhibiting $Aβ_{N3pE}$. Triple label immunofluorescence revealed that non-modified (6E10-positive) Aβ, $Aβ_{N3pE}$, and $Aβ_{pS8}$ could colocalize in the same vessels (Fig. S1). Moreover, all cases in which CAA could be detected with antibodies against $Aβ_{17-24}$ also showed a positive staining for $Aβ_{1-17}$.

Our findings indicate the sequential deposition of distinct post-translationally modified Aβ species in CAA analogous to the biochemical stages of Aβ deposition in amyloid plaques (B-Aβ plaque stages),[9] defining three biochemical (immunohistochemical) stages of CAA (B-CAA stages). B-CAA stage 1 was characterized by deposition of Aβ in the absence of $Aβ_{N3pE}$ and $Aβ_{pS8}$; B-CAA stage 2 was defined by the additional deposition of $Aβ_{N3pE}$; and B-CAA stage 3 corresponded to the deposition of Aβ including $Aβ_{N3pE}$ and $Aβ_{pS8}$ (Fig. 1). In the combined cohort of control, p-preAD, and AD cases, three of 35 cases with CAA (8.6%) presented with B-CAA stage 1, whereas B-CAA stage 2 was prevalent in 11 of 35 cases with CAA (31.4%). Twenty one of 35 cases with CAA (60%) exhibited B-CAA stage 3 (Table 1).

The B-CAA stages highly correlated with the overall anatomical expansion of CAA as represented by the CAA stage[30] and the severity of CAA-related vessel wall damage according to the Vonsattel grading[29] ($P < 0.001$, Table 2).

Heterogeneous amyloid deposition in CAA and plaques

Analysis of Aβ deposition in CAA and plaques revealed heterogeneity between B-Aβ plaque and B-CAA stages. On the one hand, B-CAA stage 1 and 2 could be detected in cases with B-Aβ plaque stage 3. Two cases with B-Aβ plaque stage 3 even presented without CAA. On the other hand, cases with B-CAA stage 3 could exhibit initial stages of amyloid deposition in plaques (B-Aβ plaque stage 2) or no amyloid plaques at all (Table 1). The distribution of the distinct B-Aβ plaque stages within the different B-CAA stages of control, p-preAD, and AD cases indeed supported the heterogeneous deposition of modified Aβ in CAA and plaques (Table 3). Accordingly, analysis of control, p-preAD, and AD cases with Aβ pathology revealed no significant correlation between the B-CAA stages and the $Aβ/Aβ_{N3pE}$ plaque load ($P \geq 0.137$, Table 2). However, the B-CAA stages weakly correlated with the $Aβ_{pS8}$ plaque load ($P = 0.001$, Table 2). The

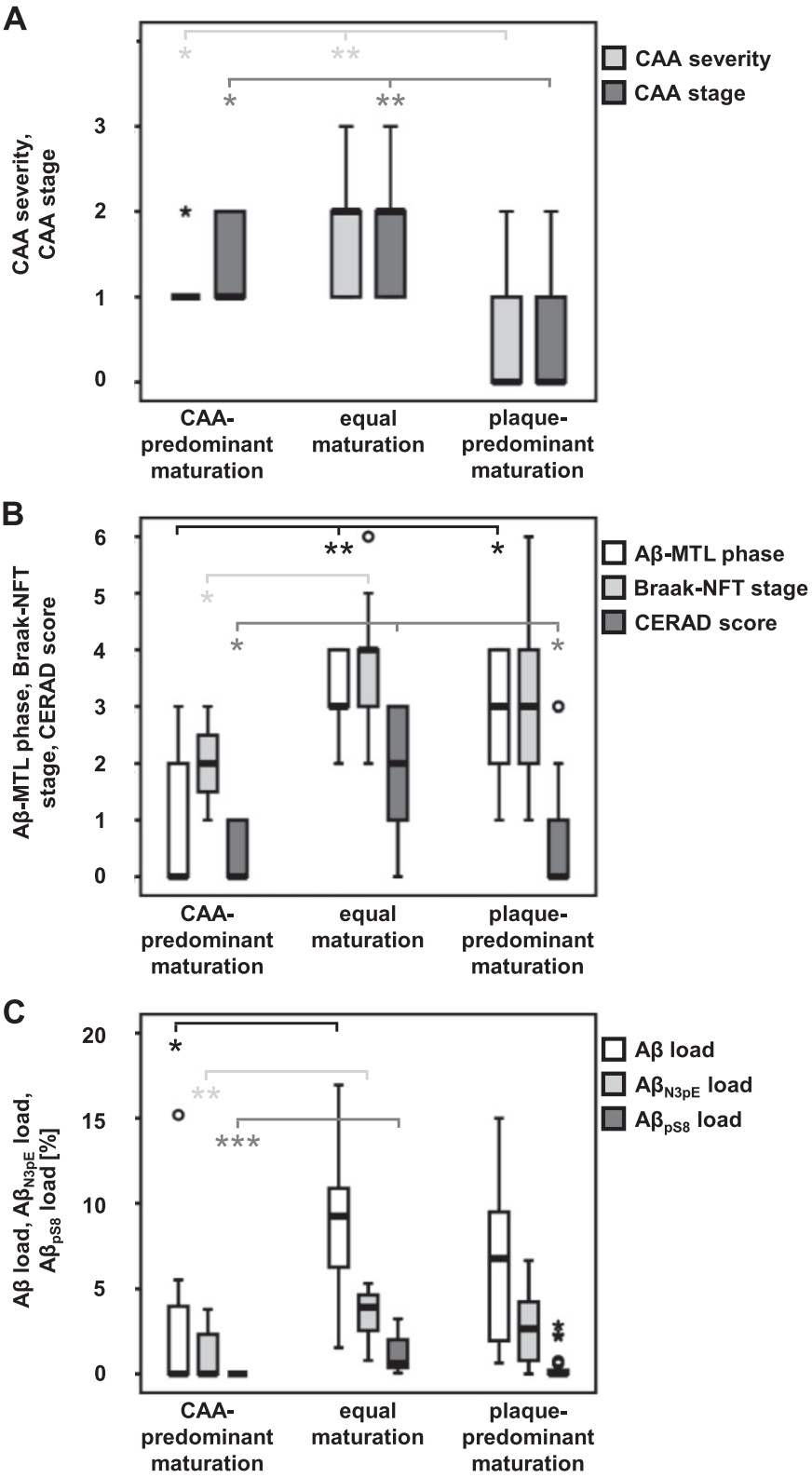

Figure 2. Neuropathologic associations of Aβ pathology groups. Relation of case groups for Aβ pathology to CAA stage (of CAA distribution)[30] and CAA severity[29] (A), to Aβ-MTL phases,[27] Braak-NFT stages,[23] and CERAD scores[26] (B), and to Aβ, Aβ_{N3pE}, and Aβ_{pS8} plaque loads (C).

Table 5. Prevalence of arterial hypertension within Aβ pathology groups.

	Aβ pathology group 1	Aβ pathology group 2	Aβ pathology group 3
Arterial hypertension	**6** *(85.7%)*	**4** *(23.5%)*	**11** *(40.7%)*
No arterial hypertension	**1** *(14.3%)*	**13** *(76.5%)*	**16** *(59.3%)*

Absolute (number of cases in bold) and relative (percentage of cases in italics) frequency of (no) arterial hypertension within Aβ pathology groups. Of the 20 control cases without Aβ pathology that could not be assigned to either of the Aβ pathology groups, seven cases (35%) exhibited arterial hypertension, whilst arterial hypertension was not observed in 13 cases (65%).

Table 6. Linear regression models (control for age/gender).

Model		B-CAA stage		B-Aβ plaque stage	
		β-coefficient	*P-value*	*β-coefficient*	*P-value*
1	NIA-AA AD degree	0.087	0.248	**0.823**	**<0.001**
2	CDR score	**0.401**	**0.005**	0.216	0.173
3	Arterial hypertension	−0.105	0.319	-	-
4		-	-	**−0.205**	**0.022**
5	Diabetes mellitus	−0.133	0.213	-	-
6		-	-	−0.053	0.569
7	Alcohol abuse	−0.068	0.522	-	-
8		-	-	−0.169	0.064

Aβ = amyloid β, AD = Alzheimer's disease, CAA = cerebral amyloid angiopathy, CDR score = clinical dementia rating score,[32] B-CAA stage = biochemical CAA stage, B-Aβ plaque stage = biochemical Aβ plaque stage,[9] NIA-AA AD degree = National Institute on Aging-Alzheimer's Association Alzheimer's disease degree[1,65]; model 1 - model 2: dependent variables: NIA-AA AD degree, CDR score; independent variables: B-CAA stage, B-Aβ plaque stage; confounding variables: age, gender; model 3 - model 8: dependent variables: B-CAA stage, B-Aβ plaque stage; independent variables: arterial hypertension, diabetes mellitus, alcohol abuse; confounding variables: age, gender; – = variable is not included in the model. Values in bold represent statistically significant results.

dissociation of the B-CAA stages from the plaque load became particularly obvious when restricting the correlation analysis to cases with CAA pathology (≥ B-CAA stage 1) ($P \geq 0.761$, Table 2).

Based on the distribution of the B-Aβ plaque and B-CAA stages, cases with Aβ pathology could be subclassified into three groups of Aβ aggregate maturation: group 1 corresponded to cases with biochemically more advanced maturation of CAA pathology (CAA-predominant group: B-CAA stage > B-Aβ plaque stage; this group included CAA cases without plaque pathology); group 2 comprised cases with equal biochemical maturation of Aβ aggregates in CAA and plaques (equal maturation group: B-CAA stage = B-Aβ plaque stage; this group contained only cases with end-stage Aβ pathology); and group 3 referred to cases with biochemically more advanced maturation of plaque pathology (plaque-predominant group: B-CAA stage < B-Aβ plaque stage; this group included 16 cases without CAA). Seven of 51 cases with Aβ pathology (13.7%) were assigned to group 1 whereas group 3 comprised 27 of 51 cases with Aβ pathology (53.0%). Notably, 17 of 51 cases (33.3%) exhibited equal maturation of Aβ in CAA and plaques, thus being classified into group 2 (Table 3).

Association of Aβ pathology groups with CAA- and AD-related pathology

Comparison of the CAA-predominant (group 1), plaque-predominant (group 3), and equally maturating (group 2) cases by multinomial logistic regression (controlled for age and gender) revealed no association with the CAA stage and severity when comparing groups 1 and 2 ($P \geq 0.535$, Table 4). However, group 3 exhibited lower levels of CAA severity and expansion throughout the brain than the other two groups ($P \leq 0.016$, Table 4; Fig. 2A).

Notably, cases with capillary CAA (CAA type 1) more likely belonged to group 2 than to group 3 compared to cases without capillary Aβ deposits (CAA type 2) or without CAA ($P = 0.035$, Table 4). No significant difference became obvious between groups 1 and 2, or groups 1 and 3 ($P \geq 0.452$, Table 4). In contrast, the presence of CAA type 2 significantly increased the probability of a case for belonging to group 1 compared to group 3 ($P = 0.040$, Table 4) that could not be observed for group 2 ($P \geq 0.162$, Table 4). The *APOE* ε4 allele frequency was higher in group 2 compared to group 3 ($P = 0.021$, Table 4). Furthermore, group 1 ($P \leq 0.042$, Table 4), but not groups 2 and 3 ($P = 0.160$, Table 4), was associated with arterial hypertension. The differential prevalence of arterial hypertension within the Aβ pathology groups indeed supported the association with CAA-related Aβ maturation (Table 5). None of the groups showed an association with diabetes mellitus or alcohol abuse ($P \geq 0.207$, Table 4).

Cases in groups 2 or 3 presented with higher Aβ-MTL phases compared to group 1 ($P \leq 0.042$, Table 4). However, no significant difference was detected between groups 2 and 3 ($P = 0.134$, Table 4). Higher Braak-NFT stages were observed in group 2 (equal maturation) compared to group 1 with CAA-predominant Aβ pathology ($P = 0.037$, Table 4) whereas no significant difference became obvious between groups 1 and 3, or groups 2 and 3 ($P \geq 0.131$, Table 4). Likewise, CERAD scores for neuritic plaque pathology were higher in group 2 than in groups 1 and 3 (plaque-predominant maturation) ($P \leq 0.034$, Table 4). Groups 1 and 3 did not differ significantly ($P = 0.433$, Table 4; Fig. 2B).

Notably, cases of groups 1 and 2 showed significant differences in the Aβ, $A\beta_{N3pE}$, and $A\beta_{pS8}$ plaque load ($P \leq 0.048$, Table 4). Comparison of group 3 with groups

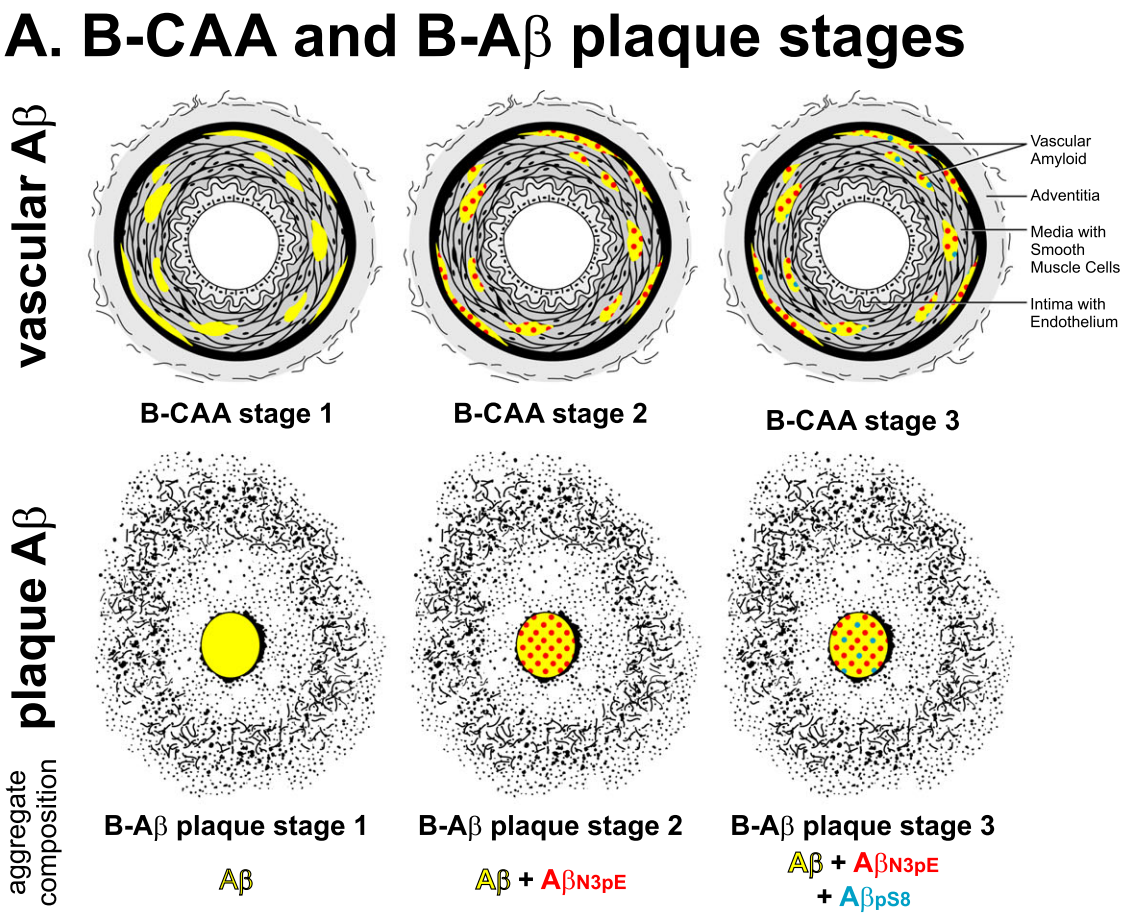

Figure 3. Amyloid maturation within A*β* pathology groups. Schematic representation of the biochemical (immunohistochemical) stages of CAA- (B-CAA stage) and plaque- (B-A*β* plaque stage) related A*β* maturation (A) and their balance in distinct A*β* pathology groups (B).

1 or 2, however, revealed no association with the Aβ, $A\beta_{N3pE}$, or $A\beta_{pS8}$ plaque load ($P \geq 0.057$, Fig. 2C).

Since six cases presented with vascular dementia (VD), corticobasal degeneration (CBD), Lewy body disease (LBD), and/or argyrophilic grain disease (AGD) additional to AD pathology that might contribute to cognitive decline, multinomial logistic regression of CDR scores was restricted to cases with "pure" CAA and/or AD pathology, thereby preventing the distortion of statistics through the contribution of these co-morbidities to dementia. Notably, group 2 exhibited higher CDR scores compared to group 1 ($P < 0.001$, Table 4). No significant difference was detected between groups 1 and 3, or groups 2 and 3 ($P \geq 0.389$, Table 4).

Association of B-CAA stages with AD-related pathology, risk factors, and clinical progression

CAA was prevalent in 4 of 24 control cases (16.7%), 11 of 27 p-preAD cases (40.7%), and 20 of 20 AD cases (100%). All B-CAA stages could be detected in p-preAD and AD cases, whereby two of 11 p-preAD cases with CAA (18.2%) and one of 20 AD cases with CAA (5%) exhibited B-CAA stage 1. B-CAA stage 2 was prevalent in one of 11 p-preAD (9.1%) and seven of 20 AD (35%) cases with CAA. B-CAA stage 3, however, became obvious in eight of 11 p-preAD cases with CAA (72.7%) but only in 12 of 20 AD cases with CAA (60%). In this context, it is important to re-note that 16 of 27 p-preAD cases (59.3%) did not exhibit CAA. Three of four control cases with CAA (75%) exhibited B-CAA stage 2, whereas B-CAA stage 3 was prevalent in one of four control cases with CAA (25%) (Table 1).

Partial Spearman's rank correlation (controlled for age and gender) revealed a moderate correlation between the B-CAA stages and (1) the progression of AD pathogenesis (control, p-preAD, AD) or (2) the degree of dementia, provided by the CDR score ($P < 0.001$, Table 2). Correlation of the B-CAA stages with the CDR score was restricted to cases without VD, CBD, LBD, and/or AGD to avoid bias caused by these co-morbidities. Furthermore, the anatomical expansion of amyloid plaques (Aβ-MTL phases) as well as the B-Aβ plaque stages, Braak-NFT stages, CERAD scores, and NIA-AA AD degrees showed a weak to moderate correlation with the B-CAA stages ($P \leq 0.001$, Table 2). Interestingly, the B-CAA stages also correlated with the *APOE* ε4 allele frequency similar to the CAA severity, the B-Aβ plaque stages, and the Aβ-MTL phases ($P \leq 0.003$, Table 2). Linear regression (controlled for age and gender) furthermore revealed a negative association of arterial hypertension with the B-Aβ plaque stages ($P = 0.022$, Table 6) but not with the B-CAA stages ($P = 0.319$, Table 6). Diabetes mellitus and alcohol abuse did not affect the B-CAA or B-Aβ plaque stages ($P \geq 0.064$, Table 6).

To clarify the impact of plaque- and CAA-related Aβ maturation on the development of AD according to the NIA-AA AD criteria and the degree of dementia as described by the CDR score, we calculated two linear regression models (controlled for age and gender) including both B-CAA and B-Aβ plaque stages. Plaque maturation (B-Aβ plaque stages; $P < 0.001$, Table 6) but not CAA maturation (B-CAA stages; $P = 0.248$, Table 6) correlated with the progression of AD (NIA-AA AD degree). Accordingly, all symptomatic AD cases exhibited B-Aβ plaque stage 3 but only 60% of symptomatic AD cases presented with B-CAA stage 3. Additional linear regression (restricted to cases without VD, CBD, LBD, and/or AGD) indicated that CAA maturation significantly contributed to the degree of cognitive decline (CDR score) ($P = 0.005$, Table 6). As expected from the finding that all cases with symptomatic AD exhibited B-Aβ plaque stage 3, no additional impact of plaque-related Aβ maturation on cognitive decline was detected ($P = 0.173$, Table 6).

Discussion

Biochemical stages of CAA-related Aβ maturation (B-CAA stages)

The combined detection of different Aβ variants revealed a hierarchical sequence of Aβ deposition in CAA that could be differentiated into three distinct stages (Fig. 3A): B-CAA stage 1 corresponded to the deposition of Aβ not modified by pyroglutamination and/or phosphorylation; B-CAA stage 2 was characterized by the additional deposition of $A\beta_{N3pE}$; and B-CAA stage 3, finally, included $A\beta_{pS8}$. This sequential deposition of Aβ in CAA corresponds to the previously observed hierarchical sequence for the deposition of modified Aβ in plaques,[9] suggesting that vascular and parenchymal Aβ deposition represent two aspects of a common biochemical process of Aβ maturation. A common sequence of Aβ deposition in CAA and plaques is indeed supported by the correlation of the B-CAA stages with the B-Aβ plaque stages and with the anatomical expansion of amyloid plaques and CAA-affected blood vessels throughout the brain. Although studies with transgenic mouse models expressing the amyloid precursor protein in neurons[34] and on the drainage of parenchymal Aβ[35] suggest a neuronal origin of Aβ in CAA, it remains to be determined whether modified Aβ species detected in the vasculature originate from the parenchyma or whether modification can occur within

vessels. However, despite the common sequence of Aβ deposition in CAA and plaques, the segregation of CAA and plaque pathology became particularly obvious through the case-by-case analysis of B-CAA and B-Aβ plaque stages and the absent correlation of the B-CAA stages with amyloid plaque load.

Cross-sectional autopsy studies cannot prove the sequential deposition of Aβ and its modified forms. However, the following arguments strongly support a sequential process: (1) none of the cases showed deposition of $A\beta_{pS8}$ in the absence of $A\beta_{N3pE}$, (2) the B-CAA stages correlate with the sequential expansion of plaques throughout the MTL[27] which correlates with increased amyloid PET-tracer retention,[36–38] and (3) the sequential occurrence of Aβ and its modified forms in line with the B-Aβ plaque stages within the human brain has been confirmed in a mouse model for AD.[39] Thus, there is at least indirect evidence that the B-CAA stages indeed represent a sequential process of Aβ, $A\beta_{N3pE}$, and $A\beta_{pS8}$ deposition.

Case groups for plaque- and CAA-related Aβ maturation (Aβ pathology groups)

Despite the correlation of the B-CAA stages with the B-Aβ plaque stages and the Aβ-MTL phases, significant variations between the B-CAA and B-Aβ plaque stages existed within individual cases. The specific composition of vascular and parenchymal Aβ deposits indicated three distinct case groups for Aβ pathology (Fig. 3B, Table 3): group 1 was defined by predominant Aβ maturation in CAA (B-CAA stage > B-Aβ plaque stage); group 2 included cases with equal Aβ maturation in CAA and plaques (B-CAA stage = B-Aβ plaque stage); and group 3 was characterized by predominant Aβ maturation in plaques (B-CAA stage < B-Aβ plaque stage), including cases without CAA. Interestingly, these case groups showed additional neuropathological associations. Cases with CAA-predominant pathology showed less advanced Aβ-MTL phases for the anatomical expansion of plaque pathology, whereas cases with plaque-predominant pathology exhibited less widespread and severe CAA. Previous neuropathological studies revealed that CAA cases could differ in their relation between CAA severity and AD-related plaque pathology.[3,7,40–42] Thus, our finding of differences in the balance of Aβ maturation between CAA and plaques might provide an explanation at the molecular level for the well-known variation in CAA in relation to plaque pathology.[3,7,40–42]

The association of the CAA-predominant pathology group with arterial hypertension furthermore indicates that this condition could affect the leading site of Aβ maturation and, thereby, might act as additional risk factor for Aβ seeding and maturation. This is indeed supported by the development of CAA in a rat model for hypertension.[43] Thus, arterial hypertension should be taken into account for therapeutic intervention because it might modify the pathological picture of AD to the CAA-predominant pattern.

The length of Aβ could also play an important role in the balance between vascular and parenchymal Aβ deposition. $A\beta_{40}$ predominantly occurs within vascular Aβ deposits whereas $A\beta_{42}$ predominates in plaques.[44] Likewise, mouse models producing mainly $A\beta_{40}$ develop a CAA-predominant Aβ pathology and mice exhibiting augmented amounts of $A\beta_{42}$ show negligible CAA pathology but abundant plaques.[45] The identification of B-CAA stages with the same Aβ maturation sequence as plaques suggests that post-translational modifications could occur in both $A\beta_{40}$- and $A\beta_{42}$-predominant aggregates in blood vessels and parenchymal plaques. However, since vascular deposits in the human brain contain both $A\beta_{40}$ and $A\beta_{42}$ even in early stages of pathogenesis,[31,44] it might require transgenic animal models to specifically address the question whether the ratio of $A\beta_{40}$ and $A\beta_{42}$ influences the site of Aβ aggregate maturation.

Furthermore, the site of leading Aβ maturation might attract further proteins to accumulate. This interpretation corresponds to the finding that seeds of Aβ aggregates could induce further Aβ deposition[46–48] and argues in favor of the view that the presence of local seeds determines the aggregation pattern. However, it will be important to investigate whether the apparent maturation of Aβ in individual deposits results from addition of modified species to existing Aβ aggregates or whether already aggregated Aβ within deposits undergoes post-translational modification. Previously, we demonstrated $A\beta_{N3pE}$ and $A\beta_{pS8}$ within non-detergent extracts of human and APP transgenic mouse brains,[9,12,17] indicative for the presence of these Aβ variants in monomeric or soluble oligomeric form. Whether already aggregated and deposited Aβ is amenable to pyroglutamination and/or phosphorylation is not known. Own preliminary studies suggest that Aβ aggregates can be phosphorylated at serine 8 *in vitro* but further work would be required to proof that this could occur *in vivo*. It has also been shown that plaques and CAA in human and transgenic mouse brains contain N-terminally truncated Aβ species[19,49–53] that would also be detected by the anti-$A\beta_{17\text{-}24}$ antibody used in this study. Since the cerebrovascular deposits were detected with antibodies raised against $A\beta_{17\text{-}24}$ and $A\beta_{1\text{-}17}$, it is quite likely that these deposits contain non-truncated Aβ. Only some early plaque types, such as fleecy amyloid, appeared to contain N-terminal truncated Aβ with a non-identified N-terminus as reported previously.[54,55] The simultaneous presence of truncated forms of Aβ other than $A\beta_{N3pE}$ and $A\beta_{pS8}$ in B-CAA stage 1 cerebrovascular Aβ deposits, however, could not

be excluded. Additional to the aggregation-promoting effect, phosphorylation, and pyroglutamination also affect the proteolytic degradation of Aβ monomers and the stability of Aβ aggregates.[13,56–58] Thus, pyroglutamination could potentially favor further phosphorylation and, thereby, increase the stability of Aβ aggregates, and exaggerate Aβ aggregation.

Contribution of CAA-related Aβ maturation to cognitive decline

The B-CAA stages significantly correlated with the CDR score representing cognitive decline. However, only cases with equal maturation of Aβ in CAA and plaques showed an association with cognitive decline, suggesting that Aβ maturation in CAA represents only one of numerous factors that contribute to dementia. The mere presence of end-stage CAA maturation (that was observed both in CAA-predominant and equal maturation cases) seems to be insufficient to determine the development of dementia on its own. Rather end-stage plaque maturation has to be present. Accordingly, all symptomatic AD cases presented with end-stage plaque maturation. In contrast, only 60% of symptomatic AD cases exhibited full CAA maturation, supporting the importance of plaque maturation for the development of dementia. However, a multiple linear regression model revealed only the selective impact of CAA maturation on the CDR score, indicating that the impact of plaques on the development of dementia might only partially be ascribed to Aβ maturation but that instead the high level of other AD neuropathologic changes that correlate with end-stage plaque maturation, such as NFT pathology,[59] might contribute to the course of dementia in AD.

Molecular characterization of amyloid pathology, based on the composition of CAA and plaques, might not only help to identify pathological subgroups of β-amyloidosis but also to understand the effect of therapeutic interventions. Aβ immunization, for example, could reduce the amyloid load within plaques but simultaneously exacerbate CAA pathology and CAA-related hemorrhages.[60,61] The association of CAA-predominant Aβ maturation with arterial hypertension argues for a higher risk for therapy-related bleedings in these patients as both CAA and arterial hypertension represent risk factors for intracerebral hemorrhages.[62, 63]

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Author Contribution

Conception and design of study: JW, DRT, JG; acquisition and analysis of data: (i) neuropathology: DRT, (ii) production and characterization of non-commercial antibodies: SK, JG, JW, (iii) immunohistochemistry: JG, DRT, ARU, SK, JW, (iv) *APOE* genotyping: EG, JG, (v) clinical assessment: CAFVA, (vi) statistical analysis: JG, DRT; draft of figures and manuscript: JG, DRT, JW.

Conflicts of Interest

DRT received consultancies from Covance Laboratories (UK) and GE-Healthcare (UK), a speaker honorarium from GE-Healthcare (UK), and collaborated with Novartis Pharma Basel (Switzerland), Probiodrug (Germany) and Janssen Pharmaceutical Companies (Belgium). CAFVA received honoraria from serving on the scientific advisory board of Nutricia GmbH and Hongkong University Research council, received funding for travel and speaker honoraria from Nutricia GmbH, Novartis Pharma GmbH, Lilly Deutschland GmbH, Desitin Arzneimittel GmbH, Biogen, and Dr. Willmar Schwabe GmbH & Co. KG, and collaborated with Roche Diagnostics GmbH, Biologische Heilmittel Heel GmbH, and ViaMed GmbH. All other authors declare no conflicts of interest with the content of the publication.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1: Vascular colocalization of modified Aβ.

