

Supplementary material (Data, Tables, Figure)

Amyloid precursor protein-fragments-containing inclusions in cardiomyocytes with basophilic degeneration and its association with cerebral amyloid angiopathy and myocardial fibrosis

Lara Maria Krämer¹, Johannes Brettschneider², Jochen K. Lennerz^{1,3}, Daniel Walcher⁴, Lubin Fang⁵, Angela Rosenbohm⁵, Karthikeyan Balakrishnan^{1,6}, Julian Benckendorff¹, Peter Möller¹, Steffen Just⁴, Michael Willem⁷, Albert C. Ludolph⁵, Dietmar Rudolf Thal^{1,8,9}

1 Institute of Pathology, Ulm University, Ulm, Germany

2 RehaClinic Lucerne, Switzerland

3 Department of Pathology, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA

4 Department of Internal Medicine II (Cardiology), Ulm University, Ulm, Germany

5 Department of Neurology, Ulm University, Ulm, Germany

6 Department of Gene Therapy, Ulm University, Ulm, Germany

7 Biomedical Center, Ludwig-Maximilians-Universität Munich, Germany

8 Department of Neuroscience and Leuven Brain Institute, KU-Leuven, Leuven, Belgium

9 Department of Pathology, UZ-Leuven, Leuven, Belgium

Legends:

Supplementary Table 1: List of cases. (Data)

Supplementary Table 2: List of proteins observed for its presence in p62/SQSTM1-positive BD inclusions in cardiomyocytes and the respective antibodies used for this purpose.

Supplementary Fig. 1: Positive controls for immunohistochemistry (**A-D** visualized with avidin-biotin complex and DAB, **E-H** visualized with carbocyanine (Cy) labelled secondary antibodies): **A:** p62/SQSTM1-staining of cytoplasmic dipeptide repeat inclusions in CA4 neurons (arrows) of a case with ALS carrying a C9orf72 hexanucleotide mutation. The lacking nuclear signal serves as intrinsic negative control. **B, C:** Neuritic plaques (arrows) and less strongly the neuropil and the cytoplasm of neurons of AD brain (**B:** temporal neocortex; **C:** hippocampal sector CA1) are marked with the antibodies against the N-terminus of APP (22C11) (**B**) and the A η region of APP (D-epitope antibody) (**C**). The nuclei are not stained and serve as intrinsic negative controls. **D:** The anti-A β ₁₋₁₇ antibody 6E10 clearly stains amyloid plaques (arrows) in AD temporal neocortex whereas the neighboring neuropil remains negative. **E, F:** Dipeptide inclusions in the granule cell layer of the cerebellum of an ALS case carrying the C9orf72 hexanucleotide repeat mutation stained with the p62/SQSTM1 antibody visualized with Carbocyanin-5 (Cy5)-labelled secondary antibodies (**E**) and with the ubiquitin antibody-coupled on biotinylated anti-mouse Fab fragments and labelled with Cy2-conjugated streptavidin (**F**). The inclusions stained with both antibodies in this double labelled section are indicated by arrowheads. Ubiquitin antibodies also mark other lesions that are not detected by the p62/SQSTM1 antibody. **G:** The antibody against the M-epitope of the APP-A η region stains a neuritic plaque with dystrophic neurites (arrowheads) in the parietal cortex of an AD brain (the original Cy 3 color is shown here; the image is not recoded into blue as it is done for triple label immunofluorescence figures!). **H:** Amyloid plaque (arrowhead) in the parietal cortex of an AD brain detected with the polyclonal A β antibody 3552 visualized with Cy3-labelled secondary antibodies (the original color is shown here; the image is not recoded into blue as it is done for triple label immunofluorescence figures!).

Supplementary Tab. 1: List of cases (Data)																												
Case number	Age (years)	SEX	AJMTL phase	Brak NfT stage	CERAD score	NIA-AA degree of AD pathology	clinical diagnosis	pathological diagnosis	neuropathological diagnosis	CAA severity	Expansion of atherosclerosis in the cricte of Willis	SVD Stage	Intracerebral hemorrhage	brain infarction	arterial hypertension	diabetes mellitus	Obesity	atrial fibrillation	myocardial infarction	degree of myocardial fibrosis	degree of hypertension-related myocardial fibrosis	BD (p62-positive) inclusion bodies / mm ²	Heart weight	BMI	hyperlipoproteinemia	CDR	GDS	PMI
1	68	m	2	2	0	1	ALS, prostate carcinoma, arterial hypertension	ALS	ALS, SVD, hypoxemia in CA1	2	36,36	2	no	no	yes	no	yes	no	no	2	2	0,2201	365	n.k.	no	0	1	144
2	54	m	0	0	0	0	CLL, stem cell transplantation with GVHD, GBS, arterial hypertension, muscular atrophy	CLL, myocardial hypertrophy, pneumonia	Bergmann cell gliosis, neurogenic muscular atrophy, meningioma anterior cervical root WHD*1	0	0,00	0	no	no	yes	no	no	no	2	2	0,0262	545	22,7	no	0	1	72	
3	60	m	0	0	0	0	HD, kachexia, pneumonia	HD, pneumonia	HD	0	0,00	2	no	no	no	no	n.k.	no	0	0	0	0,0017	340	n.k.	n.k.	n.k.	n.k.	24
4	73	f	0	3	0	0	Carcinoma with unknown primary tumor, kachexia, osteolysis	plasmocytoma	extradural plasmocytoma, neurogenic muscular atrophy	0	45,45	1	no	no	no	no	n.k.	no	2	0	0,0312	280	n.k.	n.k.	n.k.	n.k.	24	
5	76	f	0	2	0	0	Asthma bronchialis, pulmonary emphysema of the lung, ALS, arterial hypertension	pulmonary emphysema, atherosclerosis, ALS	ALS, AGD, neurogenic muscular atrophy	0	50,00	3	no	no	yes	n.k.	n.k.	no	2	2	0,0670	330	n.k.	n.k.	0	1	48	
6	64	f	0	3	0	0	Spinocerebellar syndrome, sepsis-associated encephalopathy, arterial hypertension, colovaginitis, CML?	CML, sepsis-induced multiple organ failure	PD	1	n.d.	0	no	no	yes	no	no	no	2	2	0,1841	280	24,0	no	0,5	1	12	
7	51	m	0	0	0	0	Malignant melanoma, esophageal carcinoma, limbic encephalitis, pneumonia	Status after melanoma surgery, esophageal carcinoma, pneumonia	paraneoplastic limbic encephalitis	0	33,33	0	no	no	no	no	no	no	2	0	0,0083	400	n.k.	no	3	7	72	
8	75	m	1	2	1	1	ALS	ALS	ALS, CAA, SVD	2	45,45	2	no	no	no	n.k.	n.k.	no	0	0	0,0683	385	n.k.	n.k.	0	1	24	
9	62	m	0	1	0	0	alcohol abuse, liver cirrhosis, carcinoma with unknown primary tumor	carcinoma	normal brain	0	8,09	2	no	no	no	no	no	no	2	0	0,0306	410	24,8	no	0	1	12	
10	83	m	0	2	0	0	coronary heart disease, myocardial infarction, chronic renal insufficiency, atrial fibrillation	hypertensive heart disease, biventricular cardiac insufficiency, atherosclerosis, coronary heart disease	SVD, atherosclerosis, multiple old brain infarcts	0	81,82	3	no	yes	yes	no	yes	yes	3	3	0,0153	520	25,3	no	2	7	48	
11	57	m	0	2	0	0	arterial hypertension, ALS	ALS	ALS	0	0,00	2	no	no	yes	no	yes	no	2	2	0,1058	525	30,1	no	0	1	96	
12	56	m	0	0	0	0	OMI	OMI	normal brain	0	36,36	2	no	no	no	no	no	no	0	0	0,0257	350	25,8	no	0	1	48	
13	58	m	0	1	0	0	GBS, status after varicosis surgery, pneumonia	pulmonary embolism	polyradiculitis, toxic myopathy	0	18,18	2	no	no	yes	no	yes	no	0	0	0,0049	530	30,7	no	n.k.	n.k.	48	
14	47	f	0	1	0	0	multiple sclerosis, epileptic seizures, hemiplegia, intracerebral bleeding, obsessive, hyporexia, nikotin abuse	pulmonary embolism, gliomuloseclerosis and renal atherosclerosis	SDH, red brain infarct, atherosclerosis, multiple sclerosis lesions	0	33,33	2	yes	yes	no	yes	yes	no	no	0	0	0,0656	425	n.k.	n.k.	n.k.	n.k.	72
15	61	m	1	2	0	1	ALS, pneumonia	ALS, respiratory failure	ALS	0	0,00	2	no	no	no	no	no	no	2	0	0,1141	340	n.k.	no	0	1	24	
16	53	m	1	1	0	1	ARDS, pneumonia	pneumonia	SVD	0	0,00	2	no	no	no	no	no	no	2	0	0,0812	400	22,0	no	0	1	48	
17	45	m	1	1	0	1	IgA-nephritis and terminal renal insufficiency, pneumonia, atherosclerosis	IgA-nephritis, status after renal transplantation, renal pulmonary embolism, thrombosis	mild SVD, inactivity related muscular atrophy	0	27,27	3	no	no	no	n.k.	n.k.	no	2	0	0,1267	260	n.k.	n.k.	0	1	24	
18	0,0301	m	0	0	0	0	hydrops fetalis, pulmonal hypoplasia	hydrops fetalis, vena cava thrombosis	amyoplasia congenita, microinfarcts in the basal ganglia, haemorrhagic signs of haemocoma	0	0,00	0	no	yes	no	n.k.	n.k.	no	0	0	0,0000	16,9	n.k.	n.k.	n.k.	n.k.	24	
19	78	m	4	4	1	2	prostate carcinoma, subcortical vasculare encephalopathy with signs of vascular dementia, meningioma, atrial fibrillation	atherosclerosis, coronary heart disease	AD, VD, meningioma VHD*2	1	100,00	3	no	yes	yes	yes	yes	yes	1	1	0,0000	510	28,7	yes	3	7	48	
20	78	f	1	1	0	1	subcortical vasculare encephalopathy, dementia	circulatory insufficiency, old myocardial infarction, pulmonary emphysema	SVE, corticobasal degeneration, small bleeding near corpus callosum	0	45,45	3	yes	yes	yes	no	yes	yes	2	2	0,0305	290	20,8	yes	3	6	48	
21	56	m	2	2	0	1	celiac disease, pneumonia, cerebellar degeneration and encephalopathy	celiac disease, spleen atrophy, pneumonia	Limbic- and brainstem encephalitis, old red cortical infarct	2	27,27	2	no	yes	no	no	n.k.	n.k.	no	1	0	0,2170	275	n.k.	n.k.	1	6	24
22	43	m	0	1	0	0	Crohn's disease, acute myocardial infarction	sudden cardiac death	normal brain	0	9,09	0	no	no	no	no	n.k.	no	yes	2	0	0,0185	430	n.k.	n.k.	n.k.	120	
23	51	m	0	0	0	0	bladder carcinoma, signs of infection and cognitive deficits	bladder carcinoma, acute pancreatitis, coronary heart disease, pulmonary embolism	brain edema, mild SVD, neurogenic muscular atrophy with accompanied myocathion changes	0	36,36	2	no	no	no	n.k.	n.k.	n.k.	no	2	0	0,0108	390	n.k.	n.k.	n.k.	48	
24	61	f	0	1	0	0	ALS, atrial fibrillation, arterial hypertension, central respiratory failure	ALS, respiratory failure	ALS	0	0,00	1	no	no	yes	no	yes	no	2	2	0,0597	260	n.k.	yes	n.k.	n.k.	48	
25	60	f	4	1	0	1	polyneuropathy, arterial hypertension, hypyepidemia, hypothyreosis	coronary heart disease, pulmonary emphysema	lymphocytic encephalo-myelitis, inactivity-related muscular atrophy	0	54,55	2	no	no	yes	n.k.	n.k.	no	2	2	0,0180	290	n.k.	yes	n.k.	n.k.	96	
26	56	m	0	1	0	0	pulmonary embolism, SDH and SAH, coronary heart disease, alcohol abuse, hepatocephaly, diabetes mellitus	coronary heart disease, liver cirrhosis, left myocardial infarction	SDH, mild SAH, fortobasal hemorrhage, SVD, alcohol-related myopathy of the diaphragma	0	27,27	2	yes	no	no	yes	n.k.	n.k.	no	2	0	0,0039	760	n.k.	n.k.	0	1	72
27	60	m	0	1	0	0	HD, pneumonia	HD	pneumonia, HD	0	36,36	2	no	no	no	no	n.k.	n.k.	no	2	0	0,0319	300	23,9	no	n.k.	n.k.	72
28	59	m	0	0	0	0	sepsis due to pneumokokk with meningitis, status after stroke, status after thrombosis, arterial hypertension, diabetes mellitus	meningitis with focal encephalitis, sepsis due to pneumokokk with multiple organ failure	leptomeningitis with foci of meningencephalitis, old infarct in the left striatum	0	18,18	1	yes	yes	yes	yes	yes	no	no	0	0	0,0158	460	n.k.	yes	0	1	48
29	0,0027	m	0	0	0	0	pulmonary hypoplasia, pulmonary hypertension, mekoniumaspiration, pneumothorax, muscular dystrophy type Becker-Kiener, infection	malformation syndrome, respiratory failure	normal brain	0	0,00	0	no	no	no	n.k.	n.k.	no	0	0	0,0000	8	n.k.	n.k.	n.k.	n.k.	48	
30	69	m	0	1	0	1	ALS, paresis of respiratory muscles	respiratory failure	ALS, cerebellar microbleeds	0	54,55	2	no	no	no	no	no	no	no	2	0	0,0713	340	19,5	yes	n.k.	n.k.	96
31	52	m	0	1	0	0	status after surgery by diverticulitis of the colon, glomerulonephritis, cognitive impairment, pneumonia, gastrointest. bleeding, neurotic syndrome	multiple organ failure	inactivity related muscular atrophy, no morphological correlate for cognitive impairment	0	45,45	2	no	no	no	n.k.	n.k.	no	no	2	0	0,0110	430	n.k.	n.k.	0	1	48
32	52	f	1	1	0	1	AML, status after bone marrow transplantation, COPD by nikotin abuse, vascular malformations at the liver vessels, status after ASD, posterior reversible encephalopathy and intracerebral hemorrhage	AML, status after surgery of an ASD	Fresh ICB, posterior reversible encephalopathy syndrome parieto-occipital, inactivity-related muscular atrophy	0	0,00	2	yes	no	no	no	no	no	no	0	0	0,0000	390	22,3	no	0,5	6	24
33	54	f	0	1	0	0	schizophrenia, SDH, pulmonary embolism?, thabdomyolysis?	pulmonary embolism	chronic parmyelitis with neurogenic muscular atrophy, SDH, capillary telangiectasia at the level of the left cirsulate arsus	0	18,18	1	no	no	no	no	yes	no	no	2	0	0,0051	360	34,6	no	n.k.	n.k.	24
34	46	f	0	1	0	0	ALS	ALS	ALS	0	0,00	2	no	no	no	no	no	no	0	0	0,0064	205	20,0	no	n.k.	n.k.	120	
35	74	f	0	1	0	0	implantation of a hip prothesis, focal epileptic seizures, respiratory failure	atherosclerosis, decompensated left ventricular failure	AGD, SVE	0	80,91	3	no	no	yes	yes	yes	no	no	2	2	0,0213	530	27,3	yes	0	1	24
36	36	m	0	0	0	0	EBV-infection, sepsis, vena porta thrombosis and liver failure, hemochromatosis, hemochromatosis	liver failure with consecutive multiple organ failure	infarct of the adenohypophysis	0	0,00	0	no	no	no	n.k.	n.k.	no	no	2	0	0,0000	430	n.k.	no	0	1	24
37	53	m	0	1	0	0	coronary heart disease, myocardial infarction, hepatopathy	coronary heart disease, myocardial infarction	Null cell microadenoma of the adenohypophysis	0	18,18	0	no	no	no	n.k.	n.k.	yes	2	0	0,0163	500	n.k.	n.k.	0	1	72	
38	63	m	1	1	0	1	stenosis of the aorta, IgA-nephropathy, CMV infection	atherosclerosis, heart failure, pulmonary hypertension, liver cirrhosis, renal failure, aortic valve stenosis, failure of the mitral and tricuspidal valves, edema of the lung	lacunar infarct left thalamus, fresh microinfarct in CA1, SVD	0	n.d.	2	no	yes	no	no	no	no	2	0	0,0703	650	24,0	no	0	1	120	
39	46	m	0	1	0	0	right ventricular failure by tricuspidal valve failure	normal brain	normal brain	0	0,00	0	no	no	no	n.k.	n.k.	no	no	0	0	0,0206	485	n.k.	no	0	1	29
40	64	f	0	1	0	0	malignant melanoma	pulmonary embolism, metastasizing malignant melanoma	multiple metastases of a malignant melanoma, AGD	0	54,55	2	no	no	no	no	no	no	2	0	0,0000	250	28,7	no	0	1	48	
41	49	m	0	0	0	0	mitral valve failure, cerebral and peripheral (lower limb) ischmia, ventricular fibrillation	endocarditis of the mitral valve	multiple lacunar infarcts and microinfarcts, large fresh infarct of the right basal ganglia, SVD	0	100,00	2	no	yes	yes	no	no	no	yes	0	0	0,0229	850	23,7	no	0	1	8
42	75	m	2	2	0	1	atrial fibrillation, ICB, infarct of the thalamus, cystic pancreatic lesions, pneumonia	coronary heart disease, pneumonia, chronic pancreatitis, pancreatic carcinoma, diabetes mellitus, renal failure	multiple brain infarcts (thalamus, midbrain, parietal lobe), old microinfarct in CA1, lymphocytic meningoencephalitis	0	36,36	1	no	yes	yes	yes	n.k.	yes	no	2	2	0,0773	425	n.k.	n.k.	0	1	24
43	72	f	4	4	2	2	dementia, arterial hypertension, obesity, lung carcinoma with pleurocarcinosis, atrial fibrillation	coronary heart disease	AD, metastases of a lung adenocarcinoma, SVD, lacunar infarct of the thalamus	1	45,45	2	no	yes	yes	yes	yes	no	2	2	0,2221	370	n.k.	n.k.	1	6	12	
44	51	m	0	1	0	0	multiple sclerosis, bladder carcinoma, diabetes mellitus, pneumonie	pneumonia	multiple sclerose-Maarburg type/ subtype II	0	33,33	0	no	no	yes	yes	yes	no	no	2	0	0,0544	400	n.k.	yes	3	7	24
45	45	m	0	0	0	0	myocardial infarction, coronary heart disease	myocardial infarction, coronary heart disease	normal brain	0	0,00	1	no	no	no	n.k.	n.k.	yes	2	0	0,0337	410	n.k.	no	0	1	24	
46	73	f	2	1	0	1	Obesity, arterial hypertension, renal cell carcinoma, gastric ulcer, decompensated left ventricular failure	decompensated left ventricular failure by status after renal cell carcinoma	ICBs (basal ganglia, thalamus, brain stem cerebellum), SVD	0	66,67	2	yes	no	yes	yes	n.k.	n.k.	no	2	2	0,1154	725	n.k.	n.k.	0	1	30
47	35	m	0	0	0	0	GIST, endokrine carcinoma of the colon, pancreatic encephalitis, factor V Leiden-mutation	GIST, endokrine carcinoma of the colon, dilatative cardiomyopathy	limbic encephalitis	0	0,00	0	no	no	no	no	no	no	2	0	0,0259	640	27,5	no	0	1	72	
48	85	f	0	2	0	0	arterial hypertension, diabetes mellitus, COPD, chronic renal failure, duodenal perforation, sepsis, pneumonia																					

Supplementary Tab. 2: Proteins observed for its presence in BD inclusions in cardiomyocytes

	Cardiomyocyte inclusion bodies	Antibodies and staining protocol
p62/SQSTM1	+	Mouse; Clone 3/p62 LCK ligand, BD Transduction Laboratories, Mountain View, CA, USA, 1:500
Ubiquitin	+	Polyclonal rabbit; Ubiquitin, DAKO, Glostrup, Denmark, 1/100
Ubiquilin	+	Polyclonal rabbit; Anti-UBQLN, Acris Antibodies, San Diego, CA, USA, 1/100, microwave pretreatment
APP-N-terminus (22C11)	+	Mouse; Clone, 22C11, Millipore, 1/75, microwave pretreatment
APP-N-terminus (9023)	(+)	Polyclonal rabbit: 9023, ThermoScientific, Fremont, USA, 1/100, microwave pretreatment
APP-D-Epitope (D-epitope = DALMPSLT)	-	Polyclonal rabbit; 9478D, 9480D, (18), 1/50, microwave pretreatment
APP-M-Epitope (M-epitope = MISEPRISYG)	-	Polyclonal rabbit; 9475M, 9476M, (18), 1/50, microwave pretreatment
A β ₁₋₁₇ (6E10)	-	Mouse; Clone 6E10, Covance, Dedham, USA, 1/1000, formic acid pretreatment
A β ₁₇₋₂₄ (4G8)	-	Mouse; Clone 4G8, Covance, Dedham, USA, 1/5000, formic acid pretreatment
A β (3552)	-	Polyclonal rabbit; 3552, (44), 1/1000, formic acid pretreatment
APLP2	-	Polyclonal rabbit: ThermoScientific, Rockford, USA, 1/200, microwave pretreatment
Smooth muscle actin,	-	Mouse; Clone 1A4, DAKO, Glostrup, Denmark, 1/200, microwave pretreatment
Myosin (fast twitch)	-	Mouse; Clone MY-32, Bio-Genex, The Hague, Netherlands, 1/50, microwave pretreatment
abnormal phosphorylated τ -protein	-	Mouse; Clone AT-8, Thermo-Scientific – Pierce Biotechnology, Rockford, IL, USA, 1/1,000
pTDP43 (phosphorylated transactive DNA binding protein)	-	Polyclonal rabbit; pS409/410-2, Cosmo Bio Co., Ltd, Tokyo, Japan, 1/10000, microwave pretreatment
TPD43	-	Mouse; Clone 2E2-D3, Novus Biologicals, Littleton, CO, USA, 1:2000, formic acid and microwave pretreatment
α -synuclein	-	Mouse; Clone KM51, Leica Biosystems – Novocastra, Newcastle, UK, 1/40, formic acid pretreatment
CD-56	-	Mouse; Clone BC56C04, Biocare, Concord, CA, USA, 1/50, microwave pretreatment
Desmin	-	Mouse; Clone D33, Linaris, Dossenheim, Germany, 1/100
Filamin-C	-	Polyclonal rabbit; anti-FLNC, HPA006135, Sigma-Aldrich Chemie Gmbh, Munich, Germany

References:

- (18) Willem, M. et al. η -Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature* (2015).
- (44) Page, R. M. et al. Beta-amyloid precursor protein mutants respond to gamma-secretase modulators. *J Biol Chem* 285, 17798-17810 (2010).

Supplementary Fig. 1: Positive controls for immunohistochemistry (**A-D** visualized with avidin-biotin complex and DAB, **E-H** visualized with carbocyanine (Cy) labelled secondary antibodies): **A:** p62/SQSTM1-staining of cytoplasmic dipeptide repeat inclusions in CA4 neurons (arrows) of a case with ALS carrying a C9Orf72 hexanucleotide mutation. The lacking nuclear signal serves as intrinsic negative control. **B, C:** Neuritic plaques (arrows) and less strongly the neuropil and the cytoplasm of neurons of AD brain (**B:** temporal neocortex; **C:** hippocampal sector CA1) are marked with the antibodies against the N-terminus of APP (22C11) (**B**) and the A η region of APP (D-epitope antibody) (**C**). The nuclei are not stained and serve as intrinsic negative controls. **D:** The anti-A β_{1-17} antibody 6E10 clearly stains amyloid plaques (arrows) in AD temporal neocortex whereas the neighboring neuropil remains negative. **E, F:** Dipeptide inclusions in the granule cell layer of the cerebellum of an ALS case carrying the C9Orf72 hexanucleotide el disease; SVE = subcortical vascular encephalopathy; VD = vascular dementia; **E:** Cy5-labelled secondary antibodies (E and with the ubiquitin antibody-coupled on biotinylated anti-mouse Fab fragments and labelled with Cy2-conjugated streptavidin (**F**). The inclusions stained with both antibodies in this double labelled section are indicated by arrowheads. Ubiquitin antibodies also mark other lesions that are not detected by the p62/SQSTM1 antibody. **G:** The antibody against the M-epitope of the APP-A η region stains a neuritic plaque with dystrophic neurites (arrowheads) in the parietal cortex of an AD brain (the original Cy 3 color is shown here; the image is not recoded into blue as it is done for triple label immunofluorescence figures!). **H** Amyloid plaque (arrowhead) in the parietal cortex of an AD brain detected with the polyclonal A β antibody 3552 visualized with Cy3-labelled secondary antibodies (the original color is shown here; the image is not recoded into blue as it is done for triple label immunofluorescence figures!).

