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Capillary CAA and perivascular Aβ-deposition: Two distinct features of Alzheimer's disease pathology

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ABSTRACT

Cerebral amyloid angiopathy (CAA) is frequently seen in Alzheimer's disease (AD) cases and represents one of its histopathological hallmarks. CAA is characterized by amyloid β-protein (Aβ) deposits within vessel walls. In addition to arteries and veins capillaries can also be affected. Aβ deposition into the capillary wall is, thereby, known as capillary CAA (capCAA) and strongly associated with the apolipoprotein E *APOE*ε4 allele as a risk factor. Aβ deposits along the pericapillary glia limitans are described as pericapillary Aβ (pericapAβ: synonymous with pericapillary CAA in other studies). Here, we studied the relationship between pericapAβ and capCAA in 58 human autopsy cases. Although pericapAβ and capCAA were more frequently found in AD cases compared to controls and although they exhibited a correlation to one another, detailed analysis revealed that there is a significant number of cases with pericapAβ lacking capCAA and vice versa. Moreover, single capillaries show either both pathologies or pericapAβ or capCAA only. There was no local association between these pathologies when analyzing multiple capillaries in each given case. Moreover, pericapAβ predominantly exhibited $Aβ_{42}$ whereas capCAA contained both $Aβ_{42}$ and $Aβ_{40}$. These differences as well as differences in the related astroglial reaction indicate that pericapAβ and capCAA are not directly linked. PericapAβ appears to represent initial Aβ accumulation along the glia limitans that is involved in the perivascular drainage of apoE and Aβ regardless of the *APOE* genotype whereas capCAA could be explained by a limited transendothelial clearance of apoE4–Aβ complexes compared to apoE2/3–Aβ complexes.

1. Introduction

Alzheimer's disease (AD) is histopathologically characterized by the deposition of the amyloid β-protein (Aβ) and by the generation of neurofibrillary tangles (NFTs) [1,2]. In addition to parenchymal Aβ-plaques Aβ is also deposited in the vessel wall referred to as cerebral amyloid angiopathy (CAA) [3–6]. Vascular Aβ-deposition has also been demonstrated in transgenic mice that express APP driven by a neuron-specific promoter indicating that neuron-derived Aβ is the subject of deposition in the vessel wall [7]. Drainage along perivascular channels and basement membranes was identified as an important clearance mechanism for neuron-derived Aβ [8,9]. Alterations of the perivascular clearance of Aβ are related to vessel wall changes due to small vessel disease [9,10] resulting in an alteration of the blood-brain barrier (BBB) at least in the precapillary segment [11,12].

Apolipoprotein E (apoE) is involved in physiological perivascular clearance of the extracellular fluid [13] and its *APOE* ε4 allele has been reported to be a major risk factor for sporadic AD [14,15]. Moreover, Aβ-deposition in the walls of capillaries (capCAA) is strongly associated with the *APOE* ε4 allele constituting the distinction of two types of CAA: CAA-type 1 representing capCAA with or without CAA in arteries and/ or veins whereas CAA-type 2 refers to CAA cases without capCAA [16]. By contrast, parenchymal Aβ-deposits clustered along the glia limitans around capillary walls are predominately composed of $Aβ_{42}$ and increase in frequency with the progression of AD [17,18] whereas capCAA is detected in only 51% of AD cases [19]. Due to the anatomical relationship of these pericapillary Aβ-deposits they were also named capCAA previously [17] but here will be referred to as pericapillary Aβ deposits (pericapAβ). PericapAβ represents parenchymal Aβ-deposits around capillaries regardless whether the capillaries contain Aβ-deposits within their wall [17]. This pericapAβ is different from the dyshoric plaques that are, by definition attached to capillary Aβ-deposits [16,20]. It is not clear whether capCAA and pericapAβ are biologically linked or whether they represent two different features of AD pathology. However, capillaries as well as astrocytes

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constituting the glia limitans represent important parts of the BBB. It is, therefore, tempting to assume a link between both, capCAA and pericapAβ, and alterations of the BBB integrity including glia limitans function.

To address these questions we studied 58 human autopsy cases with and without capCAA and pericapAβ. Our results show that capCAA and pericapAβ are not directly linked but that both pathologies point to alterations in Aβ-clearance from brain.

2. Materials and methods

2.1. Neuropathology and human sample characterization

Our cohort consists of 58 non-selected autopsy cases from the Pathological Institute of the Otto Wagner Hospital (Vienna, Austria) and the Municipal hospital of Offenbach am Main (Germany). All autopsies and tissue retrieval were performed in accordance to the Austrian and German laws. This study was approved by the local ethical committee. The patient age ranged between 62 and 104 years (mean 84.7 years, ±8.45 years; 33 female, 25 male). This sample included 23 AD cases and 35 non-AD controls (Table 1). Non-AD controls included non-demented cases with and without Aβ-deposition and NFTs, cases with argyrophilic grain disease, vascular lesions, Parkinson's disease and NFT-predominant dementia (Table 1). Demented as well as non-demented patients had been examined 1–4 weeks prior to death according to standardized protocols utilized for routine clinical and neurological examinations of patients upon admission to hospital. These protocols included the assessment of cognitive function and the ability to perform activities associated with daily life: care for and dress oneself, eating habits, bladder and bowel continence, speech patterns, reading and writing skills, short-term and long-term memory, and orientation within the hospital setting. These data were used to determine whether individuals clinically fulfilled the DSM-IV criteria for dementia [21]. AD was diagnosed when dementia was observed and when the degree of AD-related pathology indicated a high likelihood for AD according to standardized criteria [22]. In the event that there were no further changes in a demented brain than AD pathology with an intermediate likelihood for the disease these cases were also classified as AD. The likelihood for AD was assessed strictly following the recommended criteria [22]. A given likelihood was diagnosed only in the event that both CERAD and Braak NFT-stage met the criteria with the exception that cases with Braak NFT-stages V and VI and sparse neuritic plaques (i.e. CERAD-score 1) were supposed to have an intermediate likelihood for AD.

The brains were fixed in a 4% aqueous formaldehyde solution for at least three weeks and underwent neuropathological screening. Blocks from the medial temporal lobe (MTL) were excised at the levels of the anterior limit of the dentate gyrus, and/or the level of the lateral geniculate body [23] as well as from the occipital cortex (Brodmann areas 17, 18, and 19). All tissue blocks were embedded in paraffin. The paraffin blocks were sectioned at 10 μm.

Neurofibrillary changes were detected using the Gallyas silver-staining method as well as anti-τ-immunohistochemistry (AT8, Innogenetics, Belgium, 1/1000) [24–27]. Neuritic plaques were diagnosed either in Gallyas- or Bielschowsky-stained sections. The presence of amyloid deposits was assessed using anti-Aβ immunohistochemistry (4G8, Covance, Emeryville, CA, USA [28], 1/5000, formic acid pretreatment).

Diagnosis of the stages in the development of neurofibrillary changes (Braak NFT-stage) and the semiquantitative assessment of neuritic plaques (CERAD-score) were performed in accordance with published and recommended criteria [22,26,29,30]. For staging of Aβ-pathology, we used a previously published protocol for four phases of β-amyloidosis in the MTL [31]. This hierarchically-based procedure facilitates study of the topographic distribution pattern of Aβ-deposition in additional brain regions [31,32]: *Phase 1* represents Aβ-deposition that is restricted to the temporal neocortex. *Phase 2* is characterized by the presence of additional Aβ-plaques in the entorhinal cortex and/or in the subiculum-CA1 region. *Phase 3* is marked by the presence of Aβ-plaques in the outer zone of the molecular layer of the fascia dentata, subpial band-like amyloid, and/or presubicular "lake-like" amyloid. The existence of further Aβ-plaques in CA4 and/or the pre-α layer of the entorhinal cortex characterizes *phase 4* of Aβ-deposition in the MTL.

CAA was diagnosed whenever vascular Aβ-deposition was observed and the severity of CAA was graded according to Vonsattel [33]. For subclassification of CAA types, we noted whether capillary Aβ-deposits were present. In the event of capillary Aβ-deposition, cases were classified as CAA-type 1 (capCAA), whereas those lacking capillary Aβ but having Aβ-deposits in arteries or veins were referred to as CAA-type 2 [16]. In addition, we assessed the extent of pericapAβ that was defined by linear Aβ-deposits along the glia limitans frequently associated with adjacent Aβ-deposits in the surrounding neuropil. The glia limitans constitutes the border between the perivascular space (i.e. Virchow–Robin space) and the brain parenchyma [34], and is, thus, easily identified by its morphology.

2.2. Immunohistochemistry

In each case, abnormal phosphorylated τ-protein was visualized with a monoclonal antibody (AT-8) and Aβ in plaques, vessels and pericapillary deposits using an antibody raised against $Aβ_{17–24}$ (4G8). In five representative cases, ApoE was detected using a monoclonal antibody (Covance: D6E10 [13], 1/500, 24 h at 22 °C, microwave and formic acid pretreatment). The primary antibodies were detected either with a biotinylated secondary antibody and the ABC complex (Vectastain: Vector Laboratories, Burlingame, CA, USA), and this reaction was subsequently visualized with 3,3-diaminobencidine (DAB) or with an alkaline phosphatase labeled secondary antibody visualized with Permanent Red® (Dako, Glostrup, Denmark). Immunostained paraffin sections were counterstained with hematoxylin. Positive and negative controls were included.

Double immunolabeling was performed to demonstrate the spatial relationship between apoE, glial fibrillary acidic protein (GFAP) expression, and vascular Aβ-deposition. GFAP was visualized either with a polyclonal rabbit IgG antibody (1/1000, DAKO, 24 h at 22 °C) or with a monoclonal antibody (1/20, G-A-5, Boehringer-Mannheim, 24 h at 22 °C), and Aβ either with anti-$Aβ_{17–24}$ (4G8), anti-$Aβ_{42}$ (1/200, MBC42, 24 h at 22 °C [35]) or with a polyclonal antibody directed against $Aβ_{N1D}$ (1/100, [36], 24 h at 22 °C, microwave and formic acid pretreatment). Antibodies directed against the N-terminus of Aβ have been shown to stain all vascular Aβ-deposits just as C-terminus specific anti-Aβ-antibodies [16,37]. One primary antibody was detected with a carbocyanine 2-labeled secondary antibody specifically directed against either mouse or rabbit IgG (Dianova, Hamburg, Germany), whereas the second primary antibody was detected using a carbocyanine 3-labeled secondary antibody specifically directed against either mouse or rabbit IgG (Dianova, Hamburg, Germany). All tissue sections were viewed with a Leica DMLB fluorescence microscope. Digital photographs were obtained with a Leica DC 500 camera and were edited for publication layout with the assistance of a CorelPhotopaint® software, release 12.0.

2.3. Morphological analysis

To clarify whether capCAA and pericapAβ depend on one another we assessed whether the cases exhibited capCAA and/or pericapAβ.

To determine the local relationship between pericapAβ and capCAA we restricted our analysis to the 27 cases with capCAA.