

Case Report

Senile plaque calcification of the lamina circumvoluta medullaris in Alzheimer's disease

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Vascular calcification is a common phenomenon in the elderly, predominantly appearing in the basal ganglia and in the lamina circumvoluta medullaris of the hippocampus. Calcifications are not an inherent feature of Alzheimer's disease. On the other hand, a rare presenile type of dementia with symmetrical Fahr-type calcifications and numerous neurofibrillary tangles without senile plaques has been described by Kosaka in 1994 and was termed "diffuse neurofibrillary tangles with calcification" (DNTC). We here report a case of Alzheimer's disease with calcifications both in the basal ganglia and in the lamina circumvoluta medullaris of the hippocampus, differing from DNTC by the presence of senile plaques. The calcifications in the hippocampus were not only vascular in nature but also covered amyloid- β - and phosphorylated tau-positive plaque-like structures that were linearly arranged along the dentate fascia in the CA1 sector, an unusual finding of pathogenetic interest.

Key words: Kosaka–Shibayama disease, diffuse neurofibrillary tangles with calcification, Alzheimer's disease, amyloid plaques.

INTRODUCTION

Vascular calcification is a common phenomenon in the elderly, predominantly occurring in the basal ganglia and in the lamina circumvoluta medullaris of the hippocampus.¹ They have to be distinguished from Fahr's disease.² Calcification is not an inherent feature of Alzheimer's disease. On the other hand, a rare presenile type of dementia with symmetrical Fahr-type calcifications, bilateral

temporal lobe atrophy, severe neuronal cell loss, and numerous neurofibrillary tangles without senile plaques was described by Kosaka in 1994 and was termed "diffuse neurofibrillary tangles with calcification" (DNTC).³ It has since been known as Kosaka–Shibayama disease. More recently, this entity has been controversially discussed,^{4,5} although all cases occurring in Japan were thoroughly reviewed,⁶ and similar cases were also reported outside Japan.⁷ We here report a case associated with localized senile plaque calcification, which is not a feature of DNTC.

CLINICAL SUMMARY

The patient was a 73-year-old woman with a medical history of hypertensive cardiac disease, paroxysmal atrial fibrillation and pacemaker insertion, chronic renal insufficiency, diabetes mellitus with polyneuropathy, axial hiatal hernia, and dementia with a course of approximately four years starting with amnesia and disorientation. Two years before death, the patient's general physician ordered psychiatric therapy and treatment with Memantine. Magnetic resonance examination at that time was still unremarkable. However, a neurology consultation one year later made the diagnosis of dementia of Alzheimer type. A sister of the patient also suffered from dementia.

At her last hospitalization, the patient presented with swelling of her face, lips, and tongue, suggestive of angioneurotic edema. Treatment with corticosteroids resulted in a transient improvement only, at the 8th day necessitating intratracheal intubation and high-pressure artificial respiration. This was soon complicated by ventricular fibrillation. Cardiac reanimation was not successful, and the patient died of circulatory failure.

PATHOLOGICAL FINDINGS

General autopsy confirmed cardiac decompensation as the cause of death. The heart weighed 520 g, with bilateral

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Received 29 October 2020; revised 01 April 2021; accepted 13 April 2021.

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hypertrophy and left-sided fibrosis of the ventricular wall. In addition, the lungs showed focally hemorrhagic lesions of bronchopneumonia in the left upper lobe. There was a pronounced struma colloidosa nodosa with colloid nodes of up to 4.5 cm in diameter. Slices of the knee joint showed hypertrophic neosynovial membrane formation and focal chondrocalcinosis. The parathyroid glands were unremarkable, and there were no calcifying lesions in other organs. The formalin-fixed brain weighed 1180 g and was mildly swollen. On coronal slices, there were no appreciable macroscopic changes, apart from some cribriform lesions in the basal ganglia (Fig. 1A).

Case recruitment, autopsy, and data handling were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments as well as with the convention of the Council of Europe on Human Rights and Biomedicine. Informed consent was obtained from the patient's legal representatives. The findings were obtained as part of an extended diagnosis of this patient.

Microscopic brain samples were taken from 10 regions from both the hemispheres, and sections from formalin-fixed, paraffin-embedded blocks were stained with hematoxylin and eosin (HE), Masson's trichrome, Congo red, periodic acid-Schiff (PAS), and von Kossa.⁸

For immunohistochemistry, paraffin-embedded sections were deparaffinized in xylene and hydrated in a series of graded alcohols until finally water is used. The required antigen retrieval for ionized calcium-binding adaptor molecule 1 (Iba1) was carried out after autoclaving tissue slides for 15 min at 121°C in 10 mM Tris-HCl pH 8.0. The sections

were incubated for 72 h at 4°C with the primary antibodies (see Table 1), followed by biotinylated species-specific secondary antibodies and ExtrAvidin-peroxidase (Cat. No. #E2886; Sigma-Aldrich; 1:1000). 3,3'-Diaminobenzidine was the chromogen, and hematoxylin, the counterstain.

Consistent with the macroscopic appearance, the basal ganglia showed a rarefaction of the perivascular tissue, resulting in a status cribrosus predominantly in the globus pallidus on light microscopy. Here, the blood vessels showed rather a massive vascular calcification of the Fahr type as the underlying change (Fig. 1B). No other calcifications were present, except for small concretions in the choroid plexus and for calcified lesions in the hippocampus *vide infra*. Additional minor changes included a mild to moderate rarification of Purkinje cells with increased number of Bergmann glia to some extent, most likely caused by past hypoxic episodes.

In accordance with the clinical history of dementia, the major microscopic findings were neurodegenerative in nature. Both histological, histochemical, and immunohistochemical examinations revealed widespread changes, allowing the diagnosis of Morbus Alzheimer. Senile plaques were found in the cerebral neocortex, hippocampus, and amygdala (Fig. 1C). Their density was graded as Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score B, and the grade of amyloid- β deposition was Thal A β phase 3.^{11,12} Mild amyloid angiopathy, affecting occasional small leptomenigeal and superficial cortical vessels, was observed. The immunohistochemical distribution of neurofibrillary tangles was Braak stage IV (Fig. 1D).¹³ Globoid tangles were found in occasional neurons in the nucleus basalis

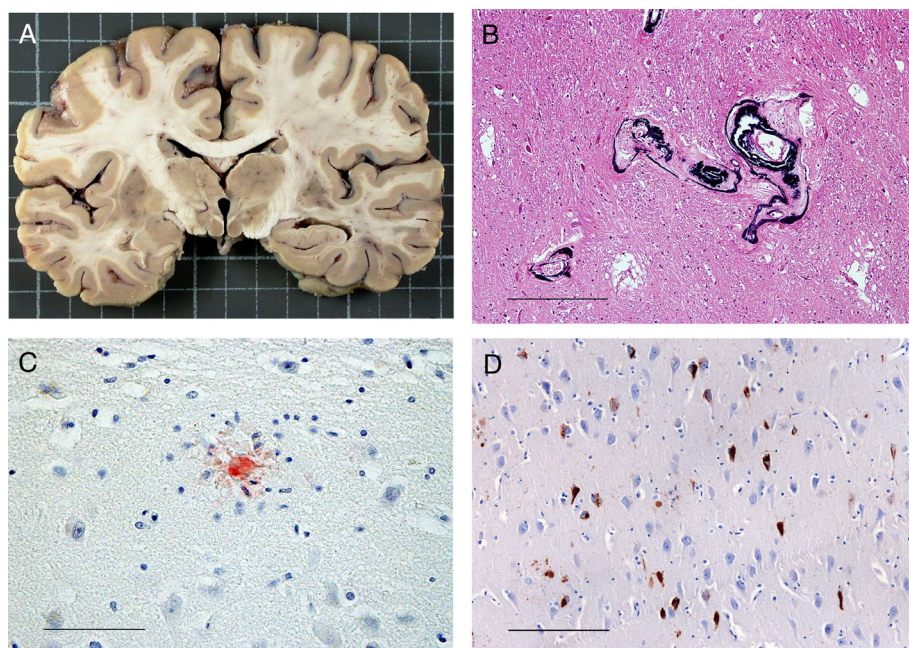


Fig 1 Macroscopic (A) and microscopic (B-D) findings of the brain. (A) A coronal slice at midlevel exhibits some cribriform lesions in the basal ganglia (square size: 1 cm). (B) Fahr-type vascular calcifications are observed in the globus pallidus on HE staining. (C) A classic senile plaque identified on Congo red staining is found in the hippocampal CA4 sector. (D) Neurofibrillary tangles identified by phosphorylated tau immunohistochemistry with the antibody 10F8 are seen in the hippocampal CA1 sector. Scale bars: 10 μ m (C), 40 μ m (B, D).

Table 1 List of antibodies used in immunohistochemistry

Antibody-targeted antigen	clone/Cat. No.	Manufacturer	dilution
Amyloid- β 17–24	4G8 (#800701)	Biologend	1:500
Pyroglutamated amyloid- β Abeta-pE3	#218003	Synaptic Systems	1:500
Pyroglutamated amyloid- β Abeta-pE3	D5N5H (#1497)	Cell Signaling Technology	1:400
Phosphorylated tau, pS202	10F8	Roboscreen GmbH Leipzig	1:100
Phosphorylated tau, pS202/pT205	AT8	Thermo Fisher Scientific	1:100
Neurofilament protein	2F11	Thermo Fisher Scientific	1:600
Aggregated α -synuclein	5G4 (#847-0102004001)	Roboscreen GmbH Leipzig	1:300
Ubiquitin	P4D1 (# sc-8017 HRP)	Santa Cruz Biotechnology	1:50
Iba1	E4O4W	Cell Signaling Technology	1:200
Biotinylated donkey anti-mouse IgG	# 711-065-150	Jackson ImmunoResearch	1:500
Biotinylated donkey anti-rabbit IgG	# 711-065-152	Jackson ImmunoResearch	1:500

Meynert and locus coeruleus. In addition to the tangles, there were Hirano bodies and/or Simchowicz bodies of granulovacuolar degeneration in some pyramidal cells in all sectors of the Ammon's horn (data not shown).

The most conspicuous finding of the Ammon's horn was seen in the lamina circumvoluta medullaris, displaying two kinds of calcification. Vascular calcification of very fine and occasional coarser granules lining the wall of numerous radial capillaries and some small blood vessels was observed between the dentate fascia and the pyramidal cell layer. More calcifications close to the dentate fascia and lined around its lateral knee were irregular or rounded, up to plaque-sized nets of strong hematoxyphilia corresponding to the nearby vascular calcifications. The outer and incipient ones were composed of very fine lines and streaks, the central ones with coarser streaks and dots at their crossings (Fig. 2A, B). The underlying rounded and plaque-sized tissue structures were negatively stained with Congo red but immunoreactive for amyloid- β indicating diffuse plaques (Fig. 2C). In contrast to senile plaques with crystalline amyloid elsewhere, they were negative for N-terminally truncated, pyroglutamated amyloid- β (pE3 amyloid- β). The senile plaque nature of the tissue underlying the nets of calcification was further confirmed by decoration of several dystrophic neurites with phosphorylated tau (Fig. 2D). The coarser calcified nets contained some neurofilament protein-positive swollen axons, proving an incipient tissue reaction (Fig. 2E). On consecutive sections, all strongly hematoxyphilic, respectively, calcified structures, both the calcified vessels and the calcified nets, were positively stained with PAS (Fig. 2F). The calcific nature of the hematoxyphilic nets was proven by their positivity on von Kossa staining just as the nearby vascular calcifications (Fig. 2G). A microglial reaction was not observed here, in contrast to the classical plaques elsewhere (Fig. 2H). Other neuropathological hallmarks, such as Lewy bodies, agyrophilic grains, and undefined protein aggregates, were ruled out by probing hippocampal and temporal tissue sections with monoclonal antibodies

against aggregated α -synuclein (clone 5G4) and ubiquitin (clone P4D1) (data not shown).

APOE GENOTYPING

ApoE genotyping was performed using the protocol of Zivelin *et al.*⁹ For extraction of DNA from formalin-fixed brain tissue, we combined proteinase K digestion with prior tissue heat treatment as recommended.¹⁰ In brief, 20 mg of cerebellar tissue was chopped and washed two times in 1 mL phosphate-buffered saline, heated for 15 min at 95°C in 1 mL 10 mM tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.5), followed by overnight digestion with 200 μ g of proteinase K in 400 μ L digestion buffer at 55°C. Reaction was stopped by adding five-volume PB reagent, and each aliquot was loaded onto a spin column of a QIAquick PCR Purification Kit (Quiagen, Dusseldorf, Germany) with 30 μ L DNA elution. *ApoE* was amplified using a OneTaq (New England Biolabs, Ipswich, MA, USA) with 5% dimethyl sulfoxide (DMSO) under the condition of primer annealing for 45 sec at 65°C over 40 cycles. Afl III/ Hae II digestion was proceeded in primer extension mix supplemented with 0.1 vol cutsmart buffer (New England Biolabs) for 2 h. The amplified 218 bp product was digested into 145, 50, and 23 bp fragments yielding the genotype $\epsilon 3/\epsilon 3$.⁹

DISCUSSION

According to a recent study, there is a great variation in the distribution of senile plaques in the CA1 sector of the hippocampus among the cases, but plaques are absent in the stratum lacunosum-moleculare.¹⁴ The plaque-like diffuse amyloid- β deposition with phosphorylated tau-immunoreactive neurites in this location is, therefore, an unusual finding. Even more unusual and, to our knowledge not described so far, are the calcific deposits of these plaques. They do not fall in the spectrum of DNTC calcifications. The case presented here, with both calcified and non-calcified plaques, cannot be designated as DNTC because

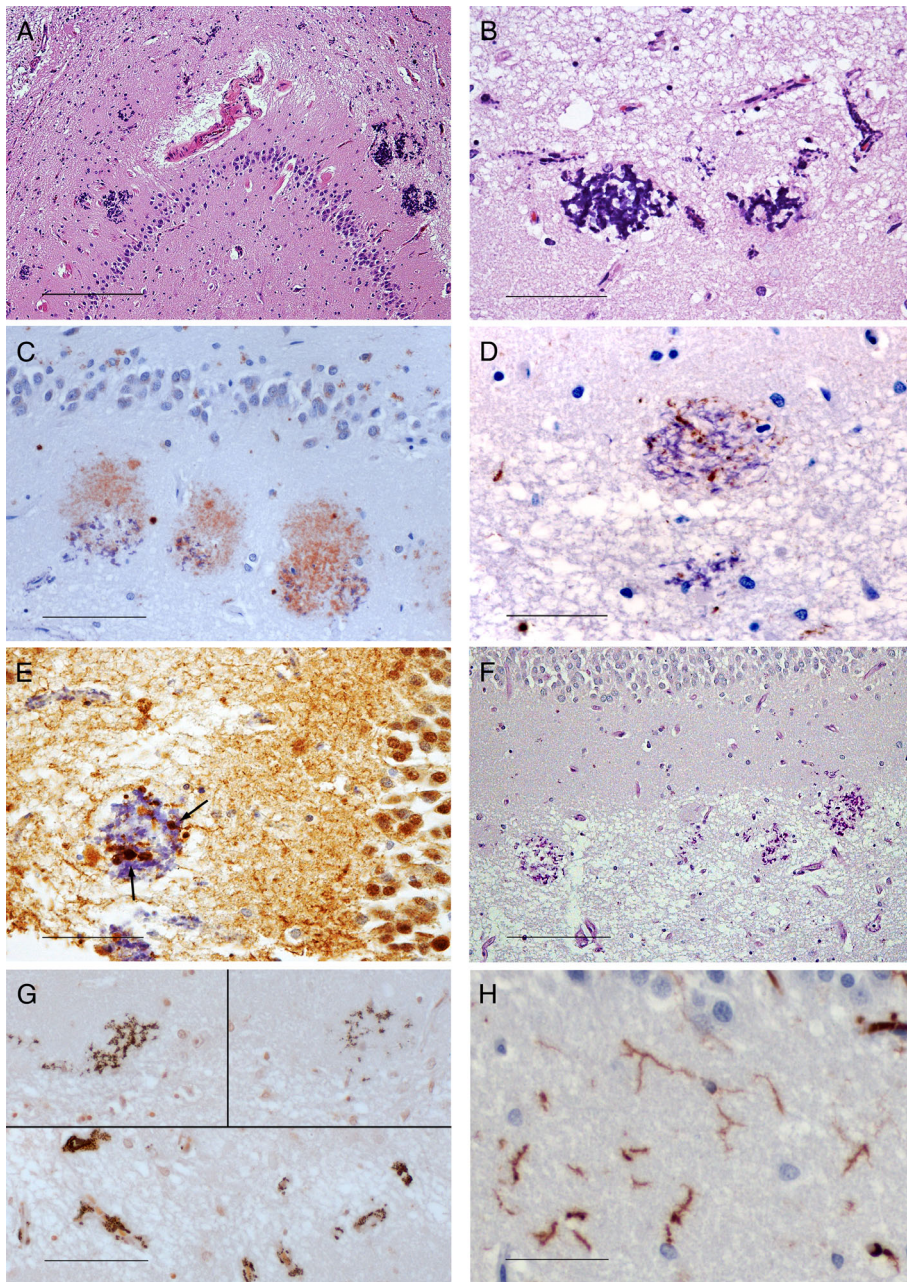


Fig 2 Microscopic findings of calcified plaques in the lamina circumvoluta medullaris of the Ammon's horn. (A) Calcified plaques are aligned along the dentate fascia, and calcified small vessels are also observed on HE staining. (B) Calcified capillaries and plaques are found in the vicinity on HE staining. (C) Amyloid- β immunohistochemistry with the antibody 4G8 reveals the spatial concordance of calcification with calcified diffuse senile plaques; however, some immunoreactive areas are not associated with calcification. (D) Phosphorylated tau immunohistochemistry with the antibody AT8 decorates dystrophic neurites belonging to senile plaque nature. (E) Neurofilament protein immunohistochemistry reveals plaque-associated axonal spheroids (arrows) as a reactive tissue change. (F) Calcified nets positively stained with PAS are analogous to their hematoxylinophilicity. (G) von Kossa staining identified calcified blood vessels (lower panel) and calcified plaque-like structures (upper panels). (H) Iba1 immunohistochemistry reveals microglial cells not enriched in calcifying deposits. Scale bars: 10 μ m (B-E, G, H), 20 μ m (F), 40 μ m (A).

plaques, by definition, are absent in the latter. The plaque calcification can, furthermore, not be associated with the knee joint chondrocalcinosis in this case because brain calcification may occur in hemochromatosis but not in chondrocalcinosis.¹⁵ The most probable explanation of the observed calcific deposits is that they are concomitant with the vascular calcification of this region. In a correlative, quantitative study of the human hippocampus in non-Alzheimer subjects, high density areas, as detected on computed tomography, were histologically always validated as calcification of precapillaries, capillaries, and arteries of the molecular and granular layer of the dentate gyrus.¹⁶

The calcific nature of the deposits is obvious already by their structures and strong hematoxyphilicity, and the von Kossa positivity is regarded as histological proof. In an earlier investigation on cerebral vascular calcification, PAS positivity has also been described.¹⁷ A recent animal experimental study has shown that PAS positivity of certain neurons may even precede their calcification.¹⁸ It is, therefore, not surprising that in our study the hematoxyphilic structures, including the plaque-associated nets, were all PAS-positive.

Although vascular calcification of the lamina circumvoluta medullaris is not an unusual finding in the elderly, senile plaque calcification not been described in Alzheimer's disease. A previous study on autopsy cases

suggested that vascular fibrosis and calcification could delete Alzheimer's disease pathology, arresting plaque formation and leading to a loss of plaques in the molecular layer of the dentate gyrus.¹⁹ However, in a recent study on a mouse model of human tauopathy, hippocampal calcifications and neuronal osteopontin deposits have been colocalized with phosphorylated tau.²⁰ It would be of interest to extend such experimental studies to models of human amyloid- β deposition.

ACKNOWLEDGMENTS

We thank Mrs. Hella Oy for excellent technical assistance and Dr. Ingolf Lachmann Roboscreen for providing the tau and synuclein antibodies 10F8 and 5G4. We acknowledge the support of Jens Stieler for graphical design.

Open access funding enabled and organized by Projekt DEAL.

DISCLOSURE

The authors declare no conflict of interest for this article.

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