Autosomal dominant arteriopathic leuko-encephalopathy and Alzheimer's disease*

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A 49-year-old man suffered from progressive dementia and seizures leading to death after 2 years. CT scans showed severe cortical-subcortical atrophy and hypodensity of the white matter. His father had died at about the same age with similar clinical signs. Two sisters and one brother were also affected. Neuropathological study revealed predominant involvement of the cerebral white matter with myelin loss, gliosis and type I lacunes. The small arteries and arterioles of the white matter and basal ganglia, and, to a lesser extent those of the subarachnoidal space, displayed fibrosis and replacement of the media by an eosinophilic, PAS positive, Congo Red negative, granular substance. Electron microscopy showed swollen myocytes surrounded by collagen, elastin and a compact electron-dense material. Immunofluorescence using antibodies against IgA, IgG,

IgM, C1q and C3 stained the abnormal media weakly. In the cortex, there were diffuse senile plaques and neurofibrillary tangles. Immunohistochemistry demonstrated $\beta/A4$ positive material in cortical senile plaques but not in arterial walls. Adventitial macrophages were, however, immunoreactive for gamma-trace. Systemic arterioles were normal. The vascular changes and leuko-encephalopathy are comparable to those described in 'Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy' (CADASIL). Similar vascular changes were also observed in non-familial cases. An association with Alzheimer changes in the cortex has not been described previously. The relationship between both diseases and the role of each in the causation of the dementia is unclear.

Keywords: Alzheimer's disease, arteriopathy, dementia, hereditary disease, leukoencephalopathy, stroke

Introduction

A new hereditary syndrome 'Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy' (CADASIL) [23] has been described in seven unrelated families in Europe [5, 14, 17, 18, 19, 21, 22]. This condition is characterized by recurrent subcortical ischaemic strokes that may lead to dementia, with severe involvement of the white matter on neuro-imaging studies. Neuropathological study has been performed in a few cases with variable findings [1, 5, 19–21].

We report pathological findings in a new case from an Italian family: the patient also showed cortical changes characteristic of Alzheimer's disease.

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The patient, a 49-year-old man, a Canadian immigrant from Italy. had suffered from meningitis aged 12 years, and from tuberculosis of the lung and the knee joint when 20 years old. In 1982, at the age of 40, he complained of memory disturbance. One year later, he was admitted to Hôpital Notre Dame, in Montreal, for dementia with signs suggestive of frontal or subcortical involvement including intellectual slowing, with difficulty in concentration, decreased insight, and behavioural disturbances. EEG showed diffuse subcortical slow wave activity. CT scan revealed cortical atrophy, mild ventricular dilatation and bilateral periventricular hypodensity. CSF was normal. A stereotactic brain biopsy of the right inferior frontal gyrus was performed in August 1983. There were no changes suggesting Alzheimer's disease or Pick's disease. The subcortical white matter was considered unremarkable. However, recent review of the slides demonstrated three small arteries in the white matter displaying granular degeneration of the media.

The patient's status worsened rapidly and progressively without any stroke-like episode. Aetiological investigations of the leukoencephalopathy including biochemistry (urinary oligosaccharides, hexosaminidases, arylsulfatases, β -galactosidase and cerebrosidase). auditory and visual evoked potentials, angiographies (aortography, carotid angiogram) and C3 and C4 assays, RA test, ANA, protein electrophoresis on serum and CSF were normal.

In 1984, he was hospitalized in a permanent care centre. He had dysarthria, marked gait disturbance and was finally confined to bed with severe dementia, marked spasticity and incontinence.

In April 1991, he was in a vegetative state with decortication and generalized seizures. He was re-admitted to Hôpital Notre Dame following a grandmal episode. CT scan showed severe cortico-subcortical atrophy with widening of the sulci and ventricular dilatation. There was also marked leukoencephalopathy with diffuse hypodensity and lacunes in the deep cerebral white matter (Figure 1). CSF was normal. Biochemical investigations for leukodystrophies ruled out metachromatic leukodystrophy, Krabbe's disease or adrenoleukodystrophy. Biopsies of nerve, muscle and skin were remarkable: in particular, they did not show any vascular abnormality. Mitochondrial disease was ruled out.



Figure 1. Last CT performed one month before death. Marked cortico-subcortical atrophy with widening of the sulci and ventricular dilatation. There is also marked diffuse hypodensity in the deep cerebral white matter.

The patient died one month later in May 1991, 9 years after the onset of neurological signs.

The family pedigree (Figure 2) was difficult to obtain and is largely incomplete. The Canadian wife of the patient was only partially aware of the patient's family history. The family had lived in Northern Italy. The mother of the patient had died aged 68 years in a permanent care centre, from a dementing disease clinically diagnosed as Alzheimer's disease. The father of the patient had died aged 48 years in a military hospital from a disease clinically very similar to that of the propositus but had not undergone any radiological or pathological examination. They had six children. One boy had died in early childhood. The eldest man died around 60 years of age. His cause of death is unknown. Two sisters and one brother are still alive. The brother aged 67 and one sister aged 57 are clinically normal. The other sister is 61: she has manifested several episodes of transient paralysis. Each of them has three children all clinically normal but none of the patient's nephews has had children. CT scans have been performed on all the Italian relatives of the patient. They were positive in the three siblings but negative in the children. The patient's son and the daughter are



Figure 2. Family pedigree. The patient is number 6. Squares, male subjects. Circles, female subjects. Diamonds, subjects whose sex is unknown. Oblique slash, deceased. Empty symbols, subject normal clinically. Black symbol, CADASIL. Striped symbol. Alzheimer's disease. Bordered symbols, subjects radiologically examined.

clinically normal but have not yet been radiologically examined.

Pathological examination

Post-mortem examination showed aspiration bronchopneumonia. The heart weighed 380 g. Although slight fibrous hyperplasia of the intima was frequent in visceral arteries, they did not show changes similar to those found in the central nervous system (cf. infra).

Gross examination of the brain (1170 g) and spinal cord was performed following fixation in 10% buffered formalin. Coronal sections of the cerebral hemispheres, sections of the brain stem and cerebellum perpendicular to their axis and horizontal sections of the spinal cord were examined. There was mild cortico-subcortical atrophy with widening of the sulci and ventricular dilatation. There was also bilateral, roughly symmetrical, yellowish discolouration of the deep white matter of the cerebral hemispheres, more prominent in the frontal areas. These areas resembled ill-defined infarcts. Small lacunes measuring 1 mm or less in diameter were present in the deep hemispheric white matter. Somewhat larger lacunes were seen in the basal ganglia. The brain stem, cerebellum and spinal cord were macroscopically normal.

Microscopical examination

Blocks from many regions of the cerebral hemispheres, basal ganglia, brain stem, cerebellum, spinal cord, optic nerves, peripheral nerves, and psoas muscle were embedded in paraffin. Large slices of cerebral hemisphere were also embedded in paraffin. Sections were stained with haematoxylin and eosin. Wolcke stain for myelin, Klüver and Barrera method. Bielschowsky silver stain, Bodian silver impregnation combined with Luxol-fast-blue, Masson's trichrome, periodic acid Schiff (PAS). Congo Red, and orcein for elastin.

Immunostaining was performed on paraffin sections by an indirect immunoalkaline phosphatase (APAAP) method using polyclonal antibodies raised against $\beta/A4$ amyloid protein (Dako), Tau protein (courtesy of André Dalacourte) and fibrinogen (Dako). All sections were counterstained with haematoxylin. For amyloid, immunostaining was also performed by an avidin biotin peroxidase technique, utilizing formic acid for pretreatment of tissue sections to enhance amyloid immunoreactivity and immunolabelling as previously described [25, 26], using polyclonal antibodies raised in rabbits to a 28 amino-acid peptide fragment representing a portion of the Alzheimer A4 or β peptide [25, 26] and a 18 amino-acid peptide fragment representing aminoacids 20 to 37 (inclusive) of the reported gamma-tracelike protein found in the microvessels of patients with hereditary cerebral haemorrhage with amyloidosis-Icelandic [27]. Immunofluorescence was performed on formalin fixed embedded material using antibodies to IgA, IgG, IgM, C1q and C3.

Small specimens of formalin-fixed tissue were rinsed in cacodylate buffer, fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in epoxy resin and stained with uranyl acetate and lead citrate for electron microscopy.

Histopathological study revealed two types of lesions.

1 An arteriopathic leukoencephalopathy which was characterized by severe myelin pallor of the deep white matter sparing the U fibres and numerous type 1 lacunes [16] in the white matter and basal ganglia (Figure 3). There was severe myelin loss with areas of malacia, incomplete preservation of axons and marked astrocytic gliosis. The leukoencephalopathy was apparently caused by a severe non-arteriosclerotic, nonamyloid, angiopathy. The small arteries and arterioles penetrating the white matter and basal ganglia and, to a lesser extent, those of the subarachnoid space displayed two different pathological patterns which could be associated in the same vessel and probably corresponded to different stages of the disease. The most conspicuous change was a concentric thickening of the arterial wall mainly due to an eosinophilic granular deposit in the media which, in some areas, surrounded swollen myocytes (Figure 4). This deposit was PAS positive and stained red by Masson's trichrome. It was not stained by methods specific for amyloid. It was only weakly stained by orcein, probably because of remaining, fragmented elastic fibres separated by the deposits. Immunofluorescence using antibodies to IgA, IgG, IgM, C1q and C3 weakly stained the abnormal media.



Figure 3. Coronal section of the left cerebral hemisphere through the lateral geniculate body demonstrating arteriopathic leukoencephalopathy. There is diffuse myelin pallor of the deep white matter sparing the U-fibres and type 1 lacunes in the lateral part of corpus callosum, internal capsule and basal ganglia. Paraffin embedding. Wolcke stain for myelin.

Immunocytochemistry for fibrinogen did not stain the granular deposit. The secondary type of change included splitting of elastic lamina and myofibroblastic proliferation of the intima, resulting in marked arteriolar stenosis. It probably represented a later stage of the vascular disease.

Similar although less severe arteriolar changes were found in the optic nerves, cerebellum, brain stem and spinal cord. Secondary Wallerian degeneration of the corticospinal tracts was present in the brain stem and spinal cord. The large cervical and cerebral arteries did not show significant changes.



Figure 4. Non-amyloid, non-arteriosclerotic arteriopathy in arterioles of the deep white matter, **a**, and leptomeninges, **b**. Note concentric thickening of the arterial wall with intimal hyperplasia, **a**, eosinophilic granular deposit in the media around swollen myocytes, and fibrosis of the adventitia. H & E. $\times 250$.

Electron microscopy showed fragmentation of elastin. Myocytes were frequently swollen and were surrounded by collagen and a compact electron dense granular material (Figure 5). This material was frequently adjacent to myocytes. It was also occasionally related to endothelial cells.

2 Cortical changes characteristics of Alzheimer's disease were numerous. These were obvious on silver stains but were more easily seen by immunostaining for the protein $\beta/A4$ amyloid and Tau protein. Senile plaques and tangles were diffuse throughout the entire neocortex but more numerous in the temporal regions, particularly in Ammon's horn, hippocampus, and amygdala. They were not found in basal ganglia, brain stem and cerebellum. There was no associated amyloid angiopathy. Gamma trace was demonstrated only in the adventitia of vessels, in some pericytes and perivascular macrophages.

Discussion

The clinical data and family history in the present case are consistent with the diagnosis of CADASIL. The progressive course of the disease, in the absence of any recurrent attack of focal brain dysfunction. is atypical. However, such a progressive course was found by Tournier-Lasserve *et al.* [22] in 20% of their cases; in addition, one sister of our patient, whose CT scan showed a leukoencephalopathy, had several episodes of transient paralysis. On the other hand, patients with radiological evidence for leukoencephalopathy may remain asymptomatic in the early stages of the disease. It is possible that in our patient, the onset of dementia occurred earlier due to associated Alzheimer's disease.

Neuropathology showed an arteriopathic leukoencephalopathy associated with a non-arteriosclerotic,



Figure 5. Electron microscopy of the media of small arteries in the white matter showing, **a**, swollen myocyte surrounded by compact electron dense material which in **b** appears granular at higher magnification. **a**, \times 12 000. **b**, \times 60 000.

non-amyloid, angiopathy [6, 12]. Neuropathological study has been performed only in a few cases regarded as CADASIL. The pathological findings in our case, closely resemble those described by Davous and Fallet-Bianco [5] and Baudrimont et al. [1]. In contrast, the arteriolar changes in our case are quite different from those described and illustrated by Sourander and Walinder [19, 20] which also involved the visceral arteries and consisted of fibrous thickening of the arterioles and capillaries with abundant collagen deposition but no deposit of granular material. In the report of Stevens et al. [21], the lesions are not illustrated but the authors do not describe either arteriopathic leukoencephalopathy or granular deposits in the media. Another possible CADA-SIL case was described by Van Bogaert [4] in two sisters whose father was possibly affected. The leukoencephalopathy resembled Binswanger's disease but the arteriolar changes were not precisely described. Japanese cases with subcortical vascular encephalopathy in normotensive young adults have also been reported [10, 28, 29]. However the arteriolar changes were arteriosclerotic and the disease was either familial with recessive transmission, or sporadic. On the other hand, arteriolar changes comparable to those in our case, were also observed in cases with no obvious family history by Estes et al. [7], Faust et al. [8] and Berthier et al. [3]. Although the description of the vascular changes is not characteristic in this latter report, the slides were reviewed by one of us (M.B.) who confirmed similar changes. Genetic linkage analysis recently performed in two CADASIL families [14, 22] has assigned the disease to chromosome 19q12 [23]. In one case from one of these families, neuropathological examination showed changes similar to those noted in our patient [1]. Further genetic studies are necessary to determine whether all the familial cases included in the definition of CADASIL, even those with differing pathology, as well as the sporadic cases with similar clinico-pathological features, correspond to a single disease entity.

The pathogenetic mechanism of the arteriolar changes is unknown. Davous and Fallet-Bianco [5] noticed that specific methods for elastic fibres strongly stained the granular deposits and proposed that the disease may be due to degeneration of elastic fibres. Electron microscopy was not performed in that case. Our ultrastructural findings do not support this hypothesis. The vascular deposits were usually found at a distance from the fragmented elastin. In contrast, they were closely related to myocytes and occasionally to endothelial cells. The granular electrondense material is more reminiscent of the immune deposits of systemic lupus erythematosus or cryoglobulinaemia. The possibility of an immunopathological process could be in keeping with the description, in some pathological cases, of perivascular inflammatory infiltrates, with the observation of steroid responsive peripheral blood lymphocyte abnormalities [2] in two patients of the family reported by Sourander and Walinder [19, 20] and with the occurrence of dysglobulinaemia in one case of the series of Tournier-Laserve et al. [22] whose condition improved following plasmapheresis. However, the absence of deposits in the visceral arteries is puzzling. In our case, immunofluorescence was performed under suboptimal conditions, on paraffin embedded sections, and the specificity of this finding remains questionable [11]. This deposit could represent a secondary non-specific finding following a primary arteriolar lesion of other origin. The finding of gamma trace-like immunoreactivity around some vessels is intriguing in view of the presence of this molecule in the adventitia of patients with severe cerebral amyloid angiopathy who experience cerebral haemorrhages [24. 27, 30]. In the latter group, gamma trace immunoreactivity has been found in both adventitial macrophages/ pericytes and, astrocytes, suggesting a role for gamma trace in exacerbation of 'weakening' or degeneration of the vessel wall; a mechanism that may also be operative in CADASIL patients.

Cortical changes characteristic of Alzheimer's disease have never been described in CADASIL cases to our knowledge. In our patient, these changes were numerous and diffuse in the whole cerebral cortex, and could not be regarded as insignificant in a 49-year-old man. Although biopsy findings suggest that they occurred after the vascular changes, they are highly likely to have contributed to the dementia. The patient's mother had died at age 68 from a dementing disease clinically diagnosed as Alzheimer's disease. Unfortunately no information could be obtained concerning her parents. The possibility of a random association between an autosomal dominant arteriopathic leukoencephalopathy transmitted by his father and Alzheimer's disease possibly transmitted by his mother cannot be excluded as a familial form of Alzheimer's disease was reported in a large kindred of Italian origin [9]. It is also of interest that linkage studies have shown that late onset Alzheimer's disease maps to chromosome 19 [15].

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