SHORT REPORT

Coexistence of CADASIL and Alzheimer's disease

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is caused by point mutations in the Notch3 gene. Presenilins are proteins involved in the cleaving of both Notch and the amyloid precursor protein (APP). In cases of early onset Alzheimer's disease mutations of the presenilin genes (PSEN 1 and PSEN 2) and APP can be found. A 64 year old patient with CADASIL (R169C-mutation) is reported, who, in addition to subcortical infarcts and granular osmiophilic deposits, had numerous senile plaques and neurofibrillary tangles on pathological examination. Mutations in the APP, PSEN1, and PSEN2 genes were not identified. Neuropathological findings of Alzheimer's disease may be found in CADASIL patients.

CADASIL is an autosomal dominant vasculopathy caused by point or splice site mutations in the Notch3 gene coding for the Notch3 transmembrane receptor.¹ The disease is characterised by migraine with aura, recurrent transient ischaemic attacks or strokes, psychiatric disease, and subcortical dementia.² The Notch receptor regulates cellular proliferation, differentiation, and developmental fate switching in a wide variety of tissues.³ Presenilin proteins are pivotal in regulating the proteolytic cleavage of amyloid precursor protein (APP) into amyloid β (A β), and play a similar role in the control of the Notch signalling pathway. Presenilin, or a cofactor associated with presenilin, appears to cleave Notch into an extracellular domain responsible for ligand binding and an intracellular domain that carries the signal transducing capacity.⁴ We report a patient with CADASIL who, in addition to characteristic CADASIL findings, had evidence of Alzheimer's disease on pathological examination.

CASE REPORT

A previously healthy male patient suffered two lacunar brain stem strokes at age 54 and 56 years. After the second stroke the patient's family noted increasing memory loss and apathy. Cardiovascular risk factors were absent. A complete cardiovascular evaluation was normal. The patient's father had died from cerebrovascular disease and his sister and daughter had transient focal neurological symptoms aged 36 and 42, respectively. Visual, brain stem, and somatosensory evoked potentials were normal. A lumbar puncture was done on two occasions and the cerebrospinal fluid showed six oligoclonal bands which were not present in serum. Magnetic resonance imaging (MRI) showed hyperintense T2 spots surrounding the ventricles, in the basal ganglia, and in the pons. No hyperintensities were found in the cerebellum. A cerebral angiogram was normal. Over the following years the patient's gait became severely spastic, micturition disturbances became pronounced, and dementia developed. No formal neuropsychological testing was done during the disease course.

The patient died when aged 64 years. A necropsy examination was done.

Cranial MRI in the patient's daughter and his sister showed white matter hyperintensities, so genetic analysis of exons 3 and 4 of the Notch gene was undertaken.

Pathology

The brain was fixed in 6% formalin. After three weeks of fixation, the following regions were sampled and processed to paraffin: frontal, insular, paramedian parietal and occipital cortex, hippocampus, basal ganglia, mesencephalon, pons, and medulla. A large slice of the cerebral hemisphere was also embedded. Sections were stained with haematoxylin and eosin, Klüver-Barrera, PAS $\pm$ diastase, and Congo red. Immunohistochemistry was performed using β A4, ubiquitin, and tau antibodies. A cortical formalin fixed specimen was rinsed, fixed in glutaraldehyde, and processed for electron microscopy.

The brain weighed 1200 g. On coronal sections, bilateral prominent subcortical infarctions were present. The centrum semiovale was most affected, although the occipital periventricular white matter and the internal capsule and basal ganglia were also involved. Most infarctions were well delineated, with a relatively prominent yellowish border. The temporal lobe, including the hippocampal formation, and the corpus callosum were atrophic. In addition, the lateral and third ventricles were dilated.

On histology, the infarcted areas were surrounded by a palisade of loaded macrophages. Throughout the brain, the medium to small sized arterioles showed a concentric hyaline thickening with the presence of PAS positive granular material, mainly in the media (fig 1). The vessels of the white matter and leptomeninges were most affected.

Another striking feature was the presence of numerous mature senile plaques in the frontal, parietal, and temporal cortex, with some variability in numbers of plaques between the various areas examined. The plaques were β -A4, ubiquitin, and Congo red positive. On the β -A4 stain some vessel walls were also positive. The number of vessels with the granular material clearly outnumbered the vessels with the β -A4 positive material. In the vessels with both deposits, the β -A4 deposits surrounded the granular deposits (fig 1). Some of the senile plaques corresponded to neuritic plaques, as shown by tau staining. This staining also revealed numerous neurofibrillary tangles, mainly in the hippocampus but also in the neocortex (fig 2). According to the criteria of Braak and Braak, the Alzheimer related changes correspond to stage V.⁵

Ultrastructural analysis showed prominent changes in all vessels examined. They consisted of variably sized granular osmiophilic deposits in between the smooth muscle cells of the tunica media, and in between the subendothelial basal lamina material. Convincing deposits of amyloid fibrils were not seen.

Genetic analysis

Genomic DNA was extracted from white blood cells using a traditional differential salt precipitation. Exons 3 and 4 of the Notch3 gene^{1 6} were amplified by polymerase chain reaction and sequenced on an ALF DNA sequencer (Amersham Pharmacia Biotech) by solid phase dideoxy sequencing (the primer sequences are available on request).

Both daughter and sister of the patient were heterozygous for a C to T mutation on position 583 of exon 4, which leads to a substitution from arginine to cysteine on position 169 (Arg169Cys or R169C) of the Notch gene product.

Figure 1 (A) PAS positive granular deposits in the tunica media of a vessel as seen in CADASIL. (B) Same vessel surrounded by β -A4 positive deposits typical of Alzheimer's disease.

Figure 2 Tau positive neurofibrillary tangle (arrowhead) and neuritic plaque (arrow) in the neocortex as found in Alzheimer's disease.

Direct sequencing of the exons 3 to 12 of the PSEN1 and PSEN2 genes, and of exons 16 and 17 of the APP gene was done in the patient's sister and daughter. No mutations were identified.

DISCUSSION

In this patient, histopathological study of the brain showed two types of lesion. The combination of PAS positive granular osmiophilic material in the wall of medium sized to small vessels with subcortical infarcts is characteristic of CADASIL.⁷ In addition, the presence of hippocampal and neocortical amyloid plaques (some of which corresponded to neuritic plaques), neurofibrillary tangles, and β -A4 deposits in vessel

walls imply the presence of Alzheimer's disease. Interestingly, in the vessels with both granular and β -A4 deposits, the β -A4 always surrounded the inner granular "CADASIL" deposits, suggesting that the disease processes are related.

The frequency of Alzheimer's disease in the general population between age 50 and 65 years is around 1% in large epidemiological studies.⁸ Subclinical pathological features of Alzheimer's disease are relatively frequent in patients in this age group, but are usually absent in the neocortex. The presence of numerous senile plaques and neurofibrillary tangles in the neocortex in this patient argues against preclinical Alzheimer's disease and suggests that established Alzheimer's disease contributed to his dementia.⁹ However, as detailed serial neuropsychological testing was not done, more direct proof is lacking.

Most of the published pathological examinations in CADASIL patients did not reveal Alzheimer's disease pathology, and CADASIL features are absent in classical Alzheimer's disease. We found only one recent report in which both diseases coexisted, and this at a young age. That report described a 51 year old patient with progressive dementia, seizures, and leucoencephalopathy in whom necropsy showed a non-amyloid, non-atherosclerotic vasculopathy and granular osmiophilic material characteristic of CADASIL, combined with cortical neurofibrillary tangles and senile plaques. Genetic analysis was not available.¹⁰

Our findings suggest that there might be a subgroup of CADASIL patients who, in addition to classical CADASIL findings, have Alzheimer's disease pathology. Alzheimer's disease may not only contribute to the cognitive impairment in these patients, but also share similar molecular mechanisms with CADASIL, as suggested by the role of presenilins in the processing of both APP and Notch. Whether Alzheimer's disease leads to accelerated CADASIL pathology, or conversely whether CADASIL leads to accelerated Alzheimer's disease in these patients, is at present unknown and should be the object of further study. Presenilins or a cofactor associated with presenilin cleave both the amyloid precursor protein and the notch protein. The cleaved notch protein then exerts its actions by inducing or inhibiting presently unknown downstream genes.⁴ In CADASIL the cleaved ectodomain of the protein accumulates in the cell and this excess protein might lead to a toxic cascade that induces amyloid deposition.¹¹ Dysfunctional notch signaling could also induce or inhibit genes that are important in the pathogenesis of Alzheimer's disease or in presenilin function. These hypotheses, however, remain entirely speculative.

Researchers who study the dementia associated with CADASIL as the expression of a pure white matter behavioural syndrome should bear in mind that the presence of unsuspected Alzheimer's disease might confuse the clinical and the behavioural syndrome.¹²

Our findings also imply that pathological examination of patients with CADASIL remains necessary even though the diagnosis of CADASIL can now be made with skin biopsy, MRI, and genetic testing.^{6 13}

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