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Autosomal dominant adult neuronal ceroid lipofuscinosis

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Chapter 3

AD-ANCL Pathology

Autosomal dominant adult neuronal ceroid-lipofuscinosis:

a novel form of NCL with granular osmiophilic deposits without palmitoyl-protein thioesterase 1 deficiency

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Abstract

We describe the neuropathological and biochemical autopsy findings in three patients with autosomal dominant adult neuronal ceroid lipofuscinosis (ANCL, Parry type; MIM 162350), from a family with six affected individuals in three generations. Throughout the brain of these patients, there was abundant intraneuronal lysosomal storage of autofluorescent lipopigment granules. Striking loss of neurons in the substantia nigra was found. In contrast, little neuronal cell loss occurred in other cerebral areas, despite massive neuronal inclusions. Visceral storage was present in gut, liver, cardiomyocytes, skeletal muscle, and in the skin eccrine glands. The storage material showed highly variable immunoreactivity with antiserum against subunit c of mitochondrial ATP synthase, but uniform strong immunoreactivity for saposin D (sphingolipid activating protein D). Protein electrophoresis of isolated storage material revealed a major protein band of about 14 kDa, recognized in Western blotting by saposin D antiserum (but not subunit c of mitochondrial ATPase (SCMAS) antiserum).

Electron microscopy showed ample intraneuronal granular osmiophilic deposits (GRODs), as occurs in CLN1 and congenital ovine NCL. These forms of NCL are caused by the deficiencies of palmitoyl protein thioesterase 1 and cathepsin D, respectively. However, activities of these enzymes were within normal range in our patients. Thus we propose that a gene distinct from the cathepsin D and CLN1-CLN8 genes is responsible for this autosomal dominant form of ANCL.

Introduction

The neuronal ceroid lipofuscinoses (NCLs or Batten disease) represent a group of progressive neurodegenerative diseases with intraneuronal storage of autofluorescent lipopigment. NCLs have originally been classified in four major categories according to the age of onset: infantile , late-infantile , juvenile and adult forms [10]. A new classification based on phenotypic and genotypic heterogeneity distinguishes 13 variants. Six genes have been cloned (CLN 1,2,3,5,6,8), and mutations in these genes have been identified. Two further genes (CLN4 and 7) have been postulated by exclusion of other genes [11;17]. Inheritance of childhood forms of NCL is autosomal recessive. The NCLs are the most common progressive neurodegenerative disorders in children, with visual loss, epilepsy and cognitive impairment. In adults, the common form of NCL is known as Kufs' disease, which also has autosomal recessive inheritance. In addition, a very rare form of adult NCL with autosomal dominant inheritance (Parry disease; MIM 162350) has been described [16]. Genes associated with adult forms of NCL have not yet been characterized.

In all NCLs, loss of neurons and abundant intraneuronal autofluorescent lysosomal inclusions are seen. Ultrastructural patterns in NCL can be multiform [6]: in CLN3, CLN5 and CLN6 a mix of fingerprint profiles, rectilinear complexes and curvilinear profiles can be found in neurons. In CLN2 curvilinear profiles dominate, while CLN1 (caused by mutations in the palmitoyl protein thioesterase 1 or CLN1 gene) is characterized by granular osmiophilic deposits (GRODs) [19]. In addition to CLN1, GRODs are found in congenital ovine NCL, caused by mutations in the cathepsin D gene[22], as well as in rare undefined types of NCL [3]. In CLN1 and congenital ovine NCL, GRODs are associated with storage of sphingolipid

activator proteins (SAPs, also called saposins) A and D. In most other NCLs the storage material largely consists of the subunit c of mitochondrial ATP-synthase (SCMAS) [7].

Recently, we described the clinical characteristics of a Dutch family with autosomal dominant adult NCL[18]. Here we report on the morphological, histochemical, and biochemical findings in autopsies of two sisters and a brother from this family.

Materials and methods

Patients

Patient 1

From the age of 44 this woman had progressive dementia, epilepsy, myoclonus and parkinsonism. A biopsy of frontal lobe cortex was taken in 1962.

Patient 2 (a daughter of patient 1)

From the age of 46 she had myoclonus, cognitive decline, parkinsonism and epilepsy. She died at the age of 59 years.

Patient 3 (also a daughter of patient 1)

This patient suffered from epilepsy, myoclonus, parkinsonism, depressions and progressive dementia from the age of 42 years, and died at the age of 56.

Chapter 3

Patient 4 (a son of patient 1)

This man had myoclonus from the age of 36 years, followed by cognitive decline, parkinsonism and epilepsy. He died at the age of 56.

Patient 5 (a daughter of patient 2)

This patient had myoclonus and cognitive problems since a pregnancy at the age of 32. Several years before, bilaterally synchronous epileptiform discharges on electroencephalography (EEG) were seen.

Patient 6 (a son of patient 4)

This man had myoclonus of the arms from the age of 25, epilepsy, parkinsonism and EEG features comparable to those of his niece (patient 5).

Tissue material

Tissue material was available from three autopsies (patients 2, 3 and 4). Additional samples, including a brain biopsy sample (patient 1), a rectal biopsy samples (patient 3 and 4) and blood samples (patients 5 and 6) were obtained. All patients were clinically affected.

Tissue from cerebral cortex (frontal, cingulate, temporal), hippocampus, striatum and globus pallidus, thalamus, hypothalamus, pituitary gland, mesencephalon, pons, medulla oblongata, cerebellum and spinal cord was systematically investigated.

Histochemistry and immunohistochemistry

Brain and spinal cord were fixed in formalin for 6 weeks. Paraffin sections were stained for hematoxylin eosin (HE), Klüver-Barrera, periodic acid Schiff (PAS), Nissl, Gomori trichrome, Bodian, Congo red,

Sudan Black B, permanganate-aldehyde fuchsin sequence, and a modified Spielmayer technique as in previous reports [5].

Paraffin slides were also stained by monoclonal antibodies against glial fibrillary acidic protein (GFAP; BioGenex, San Ramon, USA), CD57 (Leu-7, natural killer cells; Neomarkers, Fremont, USA), CD15 (Leu-M1; Becton Dickinson), CD68 (macrophages, Dako, Glostrup, Denmark), tau protein (microtubules; Sigma, St. Louis, USA), HLA-DR-DP-DQ (Dako), ubiquitin (Dako), synaptophysin (BioGenex), neural cell adhesion molecule (Zymed, San Francisco, USA), human neurofilament protein (Dako), neuron specific enolase (BioGenex), smooth muscle actin (BioGenex), human β -amyloid (Dako), Alzheimer precursor protein A4 (Boehringer Mannheim Biochemica) and polyclonal antibodies against desmin (BioGenex). Cathepsin D (Dako, Copenhagen, Denmark) and subunit c of mitochondrial ATP synthase (SCMAS; a generous gift of prof. Eiko Kominami, Jutendo University, Tokyo, Japan) and sphingolipid activator protein D (SAP D, a generous gift of prof. Konrad Sandhoff, University of Bonn, Germany) were detected by polyclonal antibodies as described [5]. LSAB+ detection kit (DAKO) or Vectastain ABC detection kit (Vector Laboratories) were used for immunodetection. Electron microscopy was performed on cerebral cortex, pons, cerebellum, spinal cord, muscle, peripheral nerve and skin, as well as isolated storage material. Standard methods consisted of fixation in glutaraldehyde, post-fixation in osmium tetroxide, embedding in araldite and contrasting with uranyl acetate and lead citrate. A Philips CM10 electron microscope was used.

Enzyme activity assay

Assays of enzyme activity in leukocytes were carried out as described recently, for palmitoyl protein thioesterase (PPT1)[24], tripeptidyl peptidase 1 (TPP1) [28] and cathepsin D[20].

Isolation of the storage material, electrophoresis and Western blotting

Frozen autopsy samples from the frontal, occipital and parietal cortices of patients 2,3 and 4 were pooled and storage material was isolated using CsCl centrifugations as described before[22]. The purity of the isolated storage cytosomes was confirmed by EM, and the protein content of the samples was measured according to Markwell et al.[15]. The storage material was analyzed by 17% SDS-polyacrylamide gels[13] and by Western blotting using antiserum against SAP D, kindly provided by prof. Konrad Sandhoff (University of Bonn, Germany), and against the mitochondrial ATP synthase subunit c, kindly provided by dr. David Palmer (Lincoln University, New Zealand).

Results

Clinical data

Myoclonus in face and arms, epilepsy, parkinsonism, dementia and personality changes were the cardinal clinical features in this family. Moderate visual symptoms and hearing loss occurred. Disease onset varied from 24 to 46 years of age.

For detailed description of clinical characteristics see Nijssen PCG et al. [18].

Macroscopy

The brain of patient 2 weighed 896 g, with severe global cerebral and cerebellar atrophy. The brain had a rubber-like consistency, and the substantia nigra was markedly depigmented. The brain of patient 3 weighed 1012 g, with moderate frontal and cerebellar atrophy. The brain of patient 4 weighed 1144 g, with moderate global atrophy. Heart, intestines, liver, pancreas, kidneys and spine of all 3 patients were normal. Both women had normal female organs.

Light microscopy

Abundant storage of autofluorescent PAS-positive grains was seen in neurons throughout the central nervous system (fig 1A), but not in hypophysis, choroid plexus or dura mater. In some neurons individual grains of ceroid lipofuscin storage material were found; in other neurons more dense aggregates were seen. The nucleus was displaced by the lipopigment to the periphery, and ballooning of the perikaryon was seen. Some neurons, especially Purkinje cells, had a short, funnel-like broadening of proximal cell processes of axons, but probably also of dendrites. Neuronal density in cerebral cortex and basal ganglia was

slightly decreased, but the cytoarchitecture was normal (Prof. H. Braak, Frankfurt, Germany). Neuronal depletion was severe in substantia nigra (fig 1B) (which was depigmented in patient 3) and inferior olive, and moderate in cerebellar nuclei. Slight loss of Purkinje cells was seen in patient 2. The storage granules uniformly stained with permanganate-aldehyde fuchsin sequence, and with Sudan Black B. There was no staining of the storage granules with the modified Spielmayer technique, except for occasional rudimentary deposits. No perikaryal spheroids of any type [5,8] were recorded.

Immunohistochemistry

Increased GFAP and CD15 immunoreactivity was found in white matter, due to astrogliosis. HLA-DR-DP-DQ immunoreactivity was increased in striatum and globus pallidus, thalamus, N. ruber, arcuate nuclei, inferior olive and vestibular nuclei, suggesting increased activity of microglia cells. Neurons in cerebellar nuclei and sporadic substantia nigra neurons were labeled by tau antibodies. Diffuse ubiquitin reactivity in white matter, and sporadic ubiquitin labeling of neurons in substantia nigra and the arcuate nuclei was found. In Purkinje cells, the thickening of dendrites had neurofilament immunoreactivity. All neurons throughout the cerebrum stained strongly with SAP D antiserum (Fig 2 A-B), and also the astroglial cells showed positive signal. Further, the spinal cord was filled with SAP D-positive structures, which were, however, irregular in shape and thus represented either disrupted large neurons or, potentially, axonal enlargements filled with storage material. Most neurons displayed no staining for SCMAS, but some were strong or moderately stained.(Fig 2 C-D). Other markers were normal. Visceral (non-neuronal) autofluorescent permanganate aldehyde fuchsin positive storage material was found in cardiocytes, skeletal muscle, liver (hepatocytes and occasional Kupffer cells), smooth muscle of gut,

peripheral nervous system of gut and in skin eccrine glands. Here, histochemical properties were similar to those in neurons.

Electron microscopy

Abundant compact granular osmiophilic deposits (GRODs) were found in neurons throughout the CNS (Fig. 3A). Their size varied from 0.5 to 2.5 μm $\varnothing$. Membrane-bound vacuoles were found in neurons, with polylobulated subunits, filled with GRODs and fat droplets (0.7 μm $\varnothing$) (fig. 3B and C). In spinal cord neurons the prevailing ultrastructure was GRODs, but also membrane covered 'zebra body' inclusions of 1 μm $\varnothing$, containing parallel lamellar profiles, were observed. In glia cells, inclusions up to 4 μm $\varnothing$ were seen filled with numerous dense osmiophilic granules. Sporadic cell processes in cerebral cortex showed membrane-covered groupwise inclusions of fingerprint aggregates (fig. 3D), sometimes associated with lipid droplets of <0.5 μm $\varnothing$. No curvilinear or rectilinear complexes were found.

In muscle, subsarcolemmal lipofuscin grains were found, composed of numerous lipid droplets (size up to 2 μm $\varnothing$), and GRODs. However, few fingerprints were seen in smooth muscle cells of perimysial capillaries and in eccrine sweat glands in the skin, where many GRODs were seen with lipid droplets.

In a rectal biopsy of patient 3 at the age of 45, lipofuscin like inclusions were found in ganglion cells. Further, in a rectal biopsy from patient 4, some intraneuronal inclusions were seen in nerve cell processes in the tunica muscularis.

Analysis of storage material

Storage cytosomes isolated from the pooled autopsy brain tissue of patients 2, 3 and 4 were brown in color and weakly autofluorescent, similar to storage material isolated from other forms of NCLs. Protein electrophoresis revealed a major protein band of about 14 kDa in molecular weight (Fig 4A). In addition, a double band of approximately 40 kDa in molecular weight was detected, while no small molecular weight proteins were seen. In Western blotting the 14 kDa band was recognized by sphingolipid activator protein D antiserum (Fig 4B), while SCMAS was not detected.

Enzyme activities

The activities of PPT1, TPP1, and cathepsin D, (the deficiencies of which are associated with CLN1, CLN2, and congenital ovine NCL respectively), were determined in patients 5 and 6. All activities measured from leukocytes of patients 5 and 6 were normal. PPT1 activities of patients 5 and 6 were 59 nmol/h/mg and 34 nmol/h/mg (reference: 24-100 nmol/h/mg), TPP1 activities were 290 nmol/h/mg and 216 nmol/h/mg (reference: 120-310 nmol/h/mg). Cathepsin D activity in fibroblasts of patient 5 was 3350 pmol/h/mg (reference: 2750-4850 pmol/h/mg).

Discussion

The neuropathological, electron microscopical and biochemical findings in our family unequivocally point to a diagnosis of adult neuronal ceroid lipofuscinosis. The pedigree of this family (fig. 5) indicates an autosomal dominant inheritance pattern. Dominant adult NCL has until recently been described in only two other families[2, 9, 14], and suspected in a few additional isolated cases and small families [1;4]. There was a striking clinical similarity between our family and the Parry family, as discussed before [18]. Recently, a new family with autosomal dominant adult NCL was reported by Josephson et al. [12]. Comparison of the Parry and Josephson families with our family strongly suggests that these patients suffer from a single disease: the light microscopical and ultrastructural findings are virtually identical, and clinical presentation is very similar.

In the Parry and Josephson families, the EM pattern was dominated by GRODs, and no fingerprints or curvilinear profiles were found. Although some scarce fingerprints were seen in our family (especially in glial cells), ultrastructure of tissue of our patients was also dominated by intraneuronal and glial GRODs. As in the Parry family [2], severe loss of neurons was seen in the substantia nigra, which correlates with the occurrence of parkinsonism in our patients. However, previously reported SPECT studies also indicated striatal D2 receptor loss [18]. Thus, parkinsonism in our family can be attributed to both nigral and striatal degeneration. Neuronal cell loss was strikingly absent in certain areas, like pontine nuclei, despite abundant intraneuronal inclusions. The role of intraneuronal inclusions in the pathophysiology of neurodegenerative diseases has recently been the subject of discussion [26;27]. The relation between neuronal storage and cell loss is unclear in our patients. Regional differences may indicate independence of

neuronal degeneration and the storage process, but it is possible that cell death has occurred as a consequence of inclusion storage. SCMAS is stored uniformly only in CLN2, where it has been suggested to be the substrate of the deficient TPP1 [7]. In CLN 3, 5 and 6 SCMAS storage is generalized, missing only in certain visceral storage sites [7]. In typical CLN1, histochemical SCMAS reactivity is almost absent, found only in some restricted neurons [7]. In all forms of NCLs, the storage material is strongly immunoreactive for SAPs, although SAP A and D are the major storage proteins only in CLN1, congenital ovine NCL and in Schnauzer NCL [21, 22, 23]. In our family, the participation of SCMAS in the storage process was also limited, and restricted to a subpopulation of neurons, while storage of SAPs appeared to be relatively uniform throughout the brain. Thus, immunohistochemically this family represented either a SAP storage disease or a mixture of SAP and SCMAS storage. Accumulation of SAP D was also confirmed by Western blotting from isolated storage cytosomes, which, however, did not contain SCMAS.

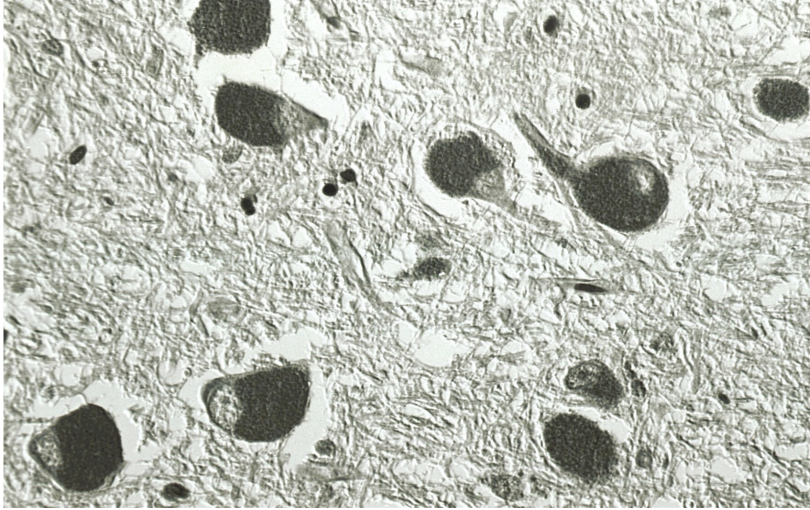
Based on the ultrastructural, immunohistochemical and storage cytosome analyses, the disease of the present family resembles CLN1 and congenital ovine NCL, which are caused by deficiencies of PPT1 and cathepsin D, respectively. Recently, an adult variant of PPT1 deficiency, characterized by GRODs and recessive inheritance, was described in a French family[25]. In the present family, however, the activities of PPT1 and cathepsin D, were normal, excluding them as the cause of the disease. In addition, CLN2 has been excluded as the etiological factor by normal TPP1 enzyme activity. Other NCLs, such as CLN 3, 5, 6, 8 and Kufs' disease have been indirectly excluded by the different clinical presentation, ultrastructure, immunohistochemical and biochemical findings, as well as by the dominant inheritance. In addition to the above-discussed forms of NCL, GRODs have been reported in two

genetically undefined NCLs: the congenital human NCL and adult Schnauzer NCL. The immunohistochemical characteristics of the congenital human (Tyynelä, unpublished data) and the adult Schnauzer NCL resemble those described here [23].

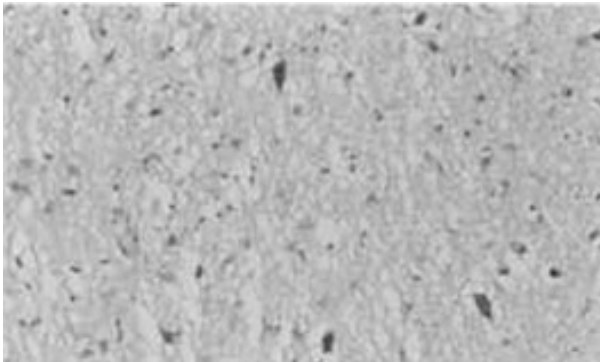
Therefore, it is likely that the gene underlying the presented form of autosomal dominant adult NCL is different from the identified NCL genes as well as from the (undiscovered) Kufs gene. Further genetic and biochemical studies will be needed to define this novel genetic locus.

Figures

Fig 1 Light microscopy of paraffin embedded tissue sections from patients with adult NCL.



1A Periodic-acid Schiff (PAS) staining of pontine nuclei, showing intraneuronal storage of PAS-positive material, with ballooning of axon hillocks.



1B In substantia nigra, severe loss of neurons was seen. (Bodian stain).

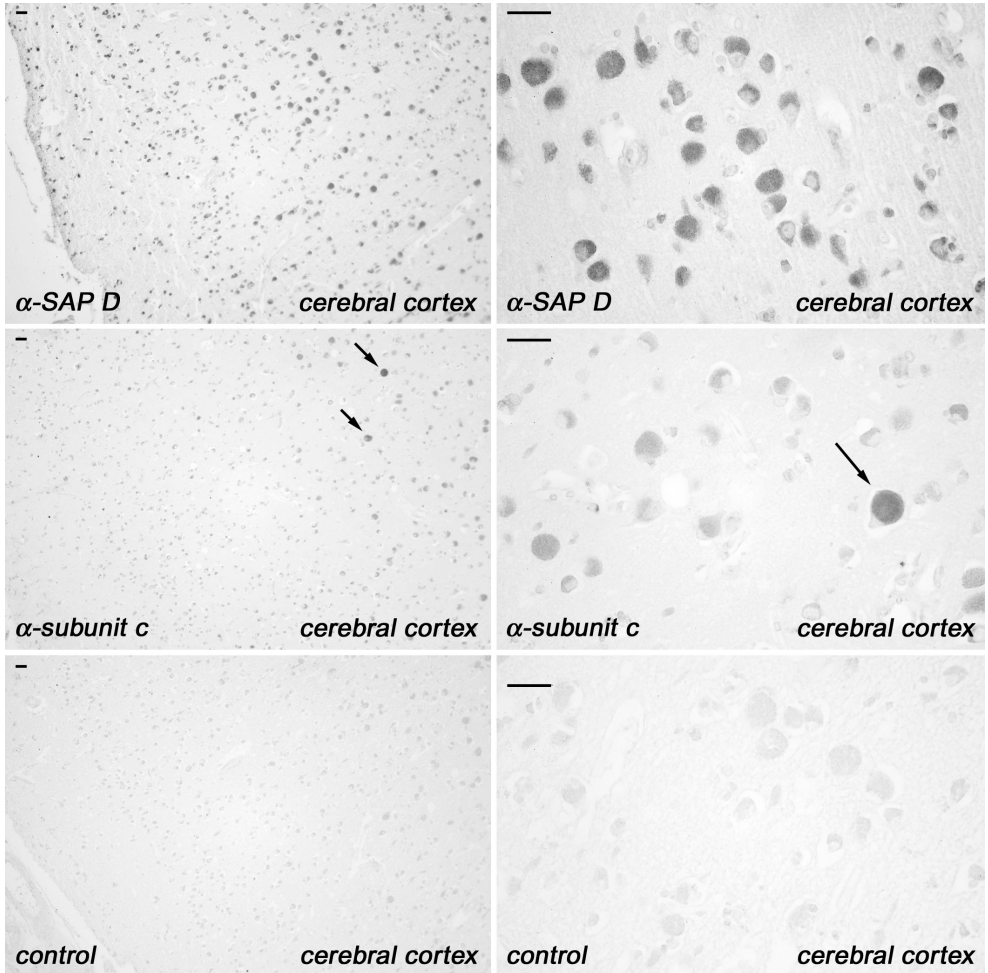


Fig 2 Immunohistochemical staining of paraffin embedded tissue sections from patients with ANCL. Bars, 20 μ meter.

A (top left) Sphingolipid activator protein D (SAP D) antiserum strongly stained the neurons throughout the cerebral cortex. Also the glial cells close to the surface of the cortex show positive staining for SAP D.

B (top right) At higher magnification, the neurons showed pronounced accumulation of SAP D-positive storage material.

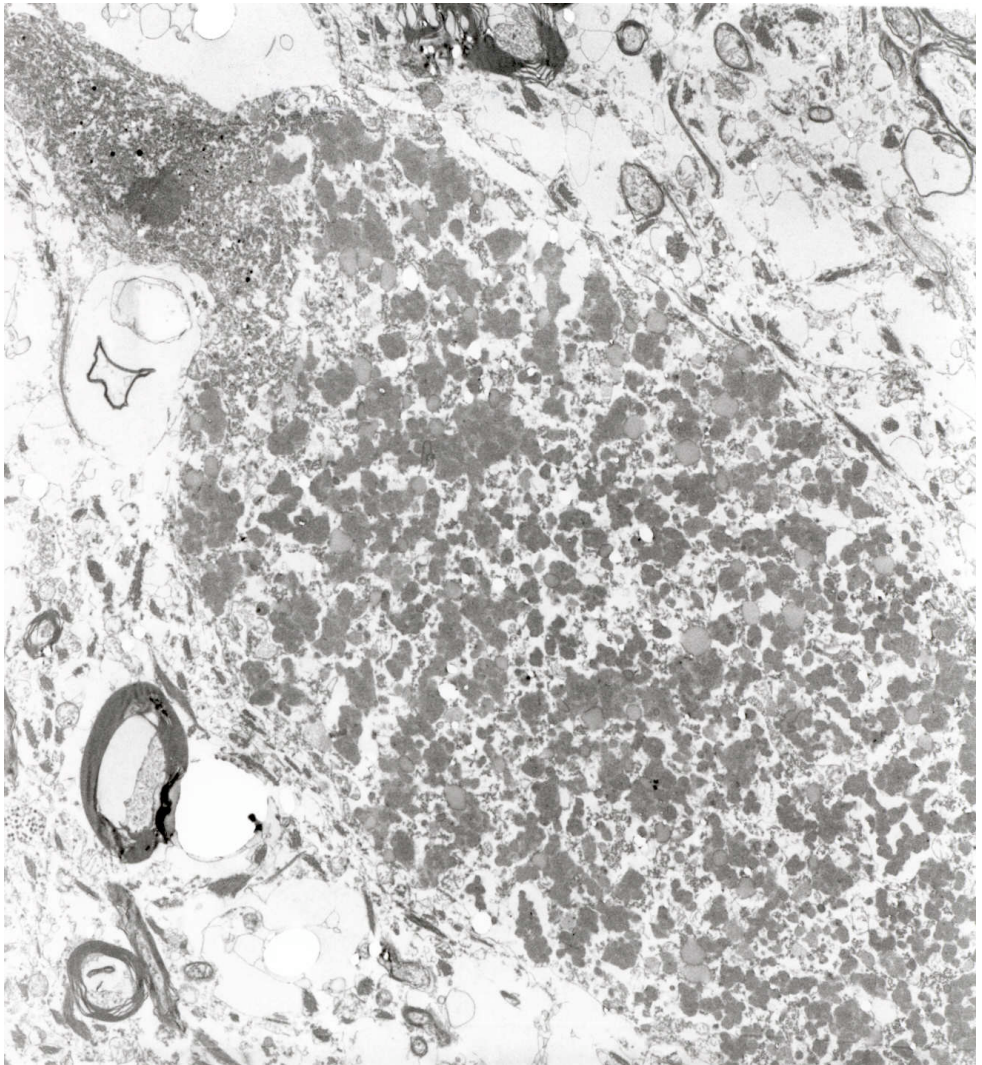
Chapter 3

C (middle left) In contrast, antiserum against subunit c of the mitochondrial ATP synthase (SCMAS) stained selected neurons in the cerebral cortex (arrows).

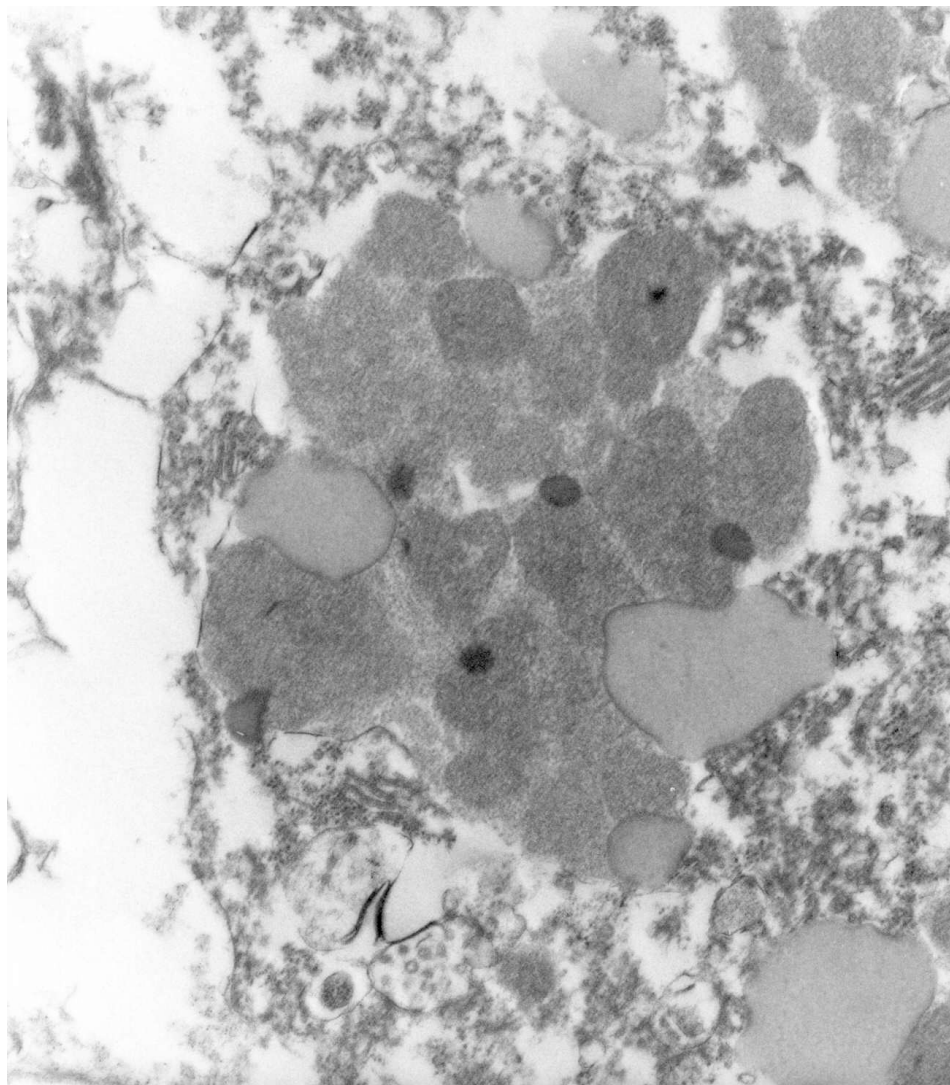
D (middle right) At higher magnification, one neuron in the deeper cortical layers shows strong positive staining for SCMAS whereas the neighbouring neurons are negative or mildly stained.

E (bottom left) and F (bottom right) In control experiments (where primary antiserum was omitted or replaced by pre-immune serum) there was no staining.

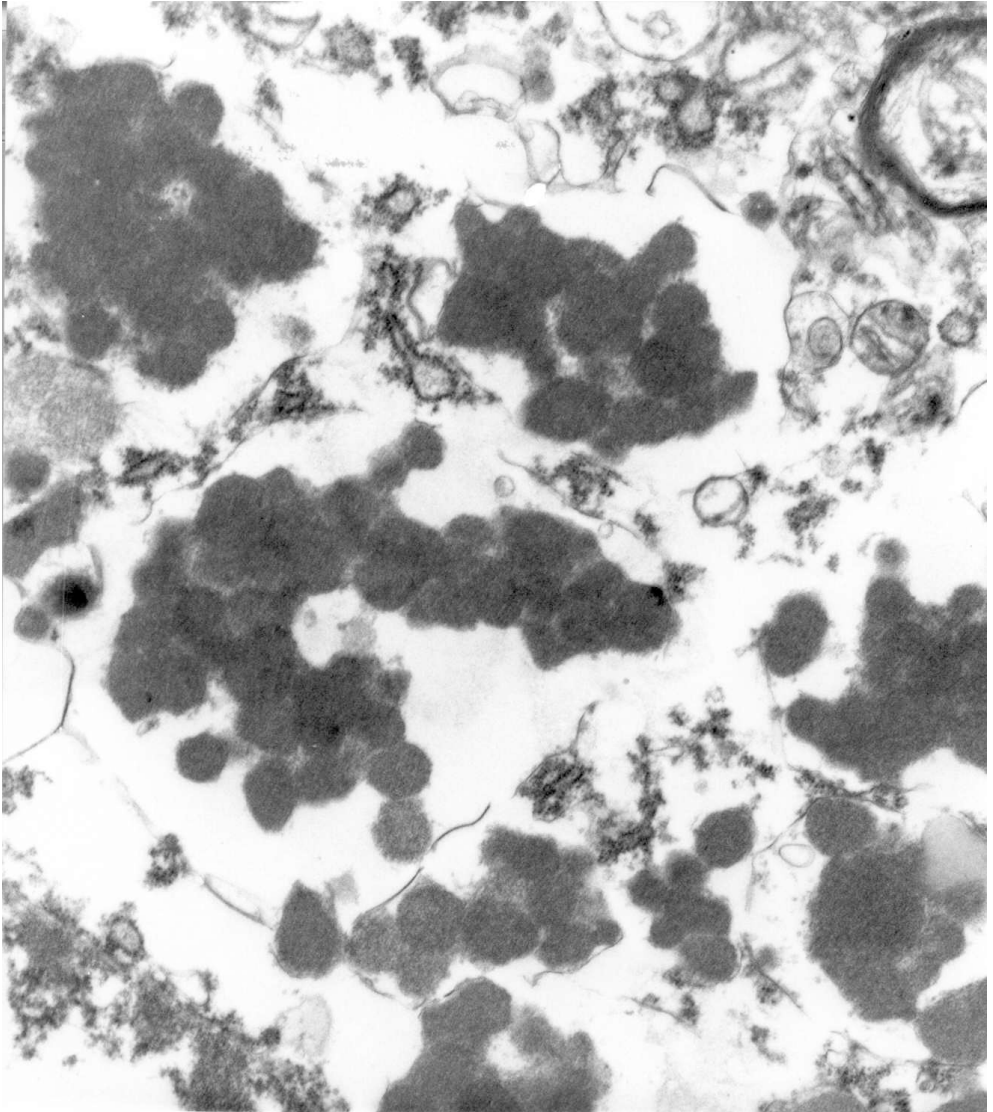
Fig. 3 Electron micrographs of autopsy brain material from patients with adult NCL.



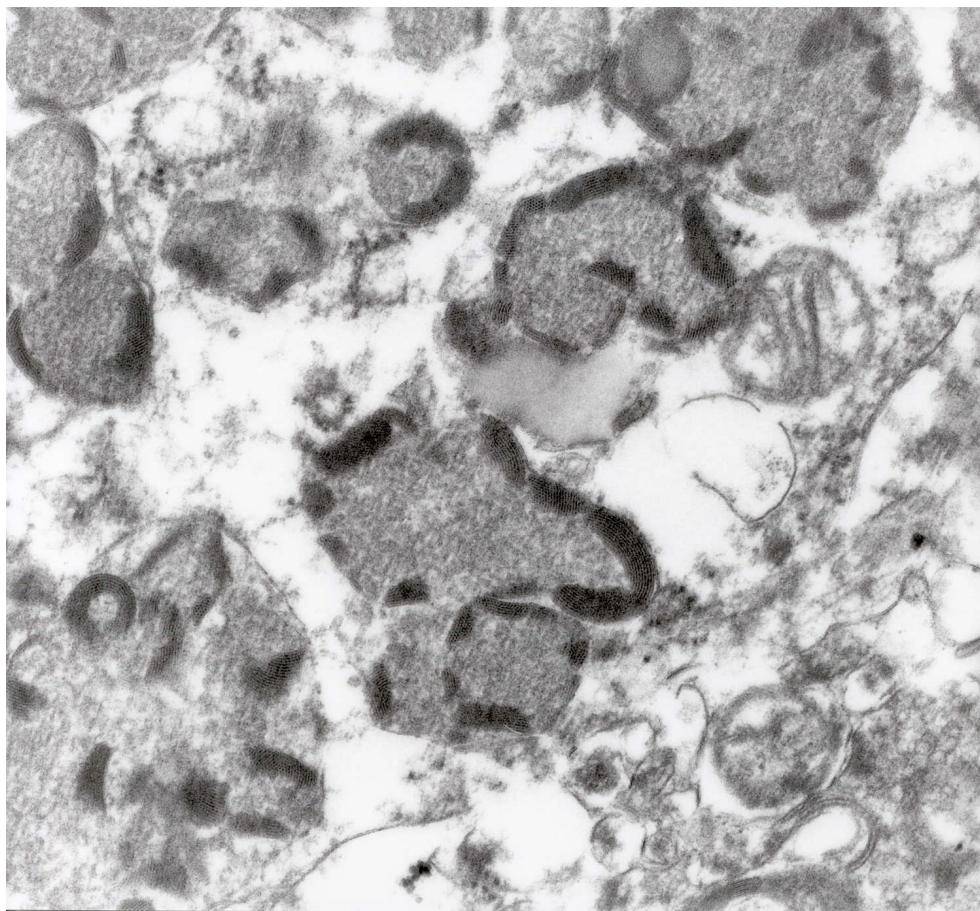
3A Patient 3, cerebral cortex. Electron micrograph of a ballooned neuronal perikaryon showing massive storage of electron dense granular inclusions with lipid droplets. Magnification x 6075.



3B Patient 2. Neuronal storage in a cerebellar Purkinje cell: polylobulated inclusion revealing numerous globular GROD and lipid droplets. Magnification x 40500



3C Patient 2. Neuronal storage in cerebral cortex: polyglobular subunits filled with GROD in clear vacuoles. Magnification x 31500.



3D Patient 2. Electronmicrograph of a neuronal process containing abundant membrane-bounded granular storage, electrodense fingerprints and lipid droplets. Magnification x 78750

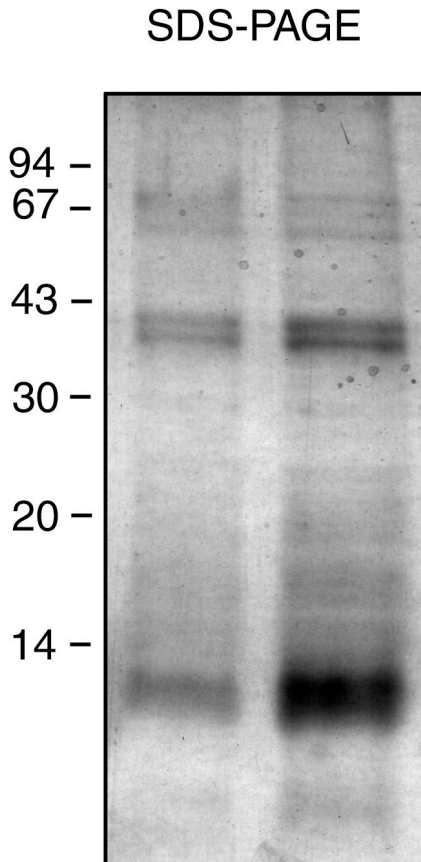


Fig 4A 17% SDS-PAGE of brain storage material isolated from patients with adult NCL .

The isolated storage material (10 [left] and 20 µg [right] of protein per lane) showed a major protein band of 12-14 kDa in molecular weight in silver stain. Molecular weight markers are shown on the left.

Western blot using α -SAP D antiserum

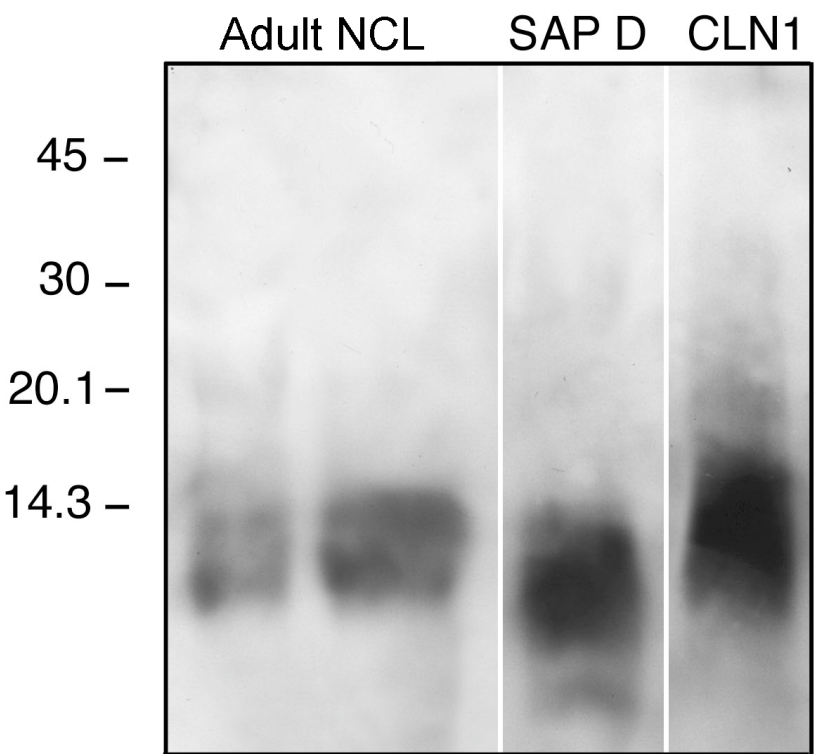


Fig 4B Western blotting of isolated brain storage material using anti-SAP D antiserum.

The major 12-14 kDa protein band of isolated storage material reacts strongly with SAP D antiserum (lanes 'adult NCL', 10 [left] and 20 μ g [right]). Purified SAP D (lane SAP D) and CLN1 storage material (lane CLN1) are shown as controls.

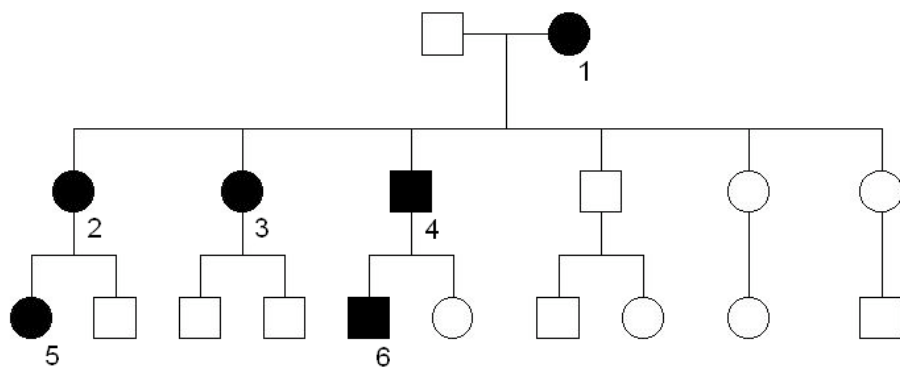


Fig 5 Family pedigree, indicating autosomal dominant inheritance. (Black symbols = affected).

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