Four cases of late onset metachromatic leucodystrophy in a family: clinical, biochemical and neuropathological studies

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SUMMARY Four cases of familial metachromatic leucodystrophy are described: the age of onset ranged from 15 to 21 years. Mental deterioration was the earliest clinical sign to be noted and all progressed to severe dementia. The arylsulphatase activity in peripheral leucocytes of the patients was very low, 5 to 15 nmol/h/mg protein, moderately reduced in the heterozygote, 40 nmol/h/mg protein, compared with control values of 60-160 nmol/h/mg protein. Sural nerve biopsies in two cases showed perivascular macrophages filled with metachromatic material and electron microscopy showed typical inclusions in Schwann cell cytoplasm. Necropsy in one of the cases revealed severe demyelination mainly in the cerebral hemispheres with metachromatic material in macrophages and neurons.

Metachromatic leucodystrophy is a familial neurological disorder, characterised by demyelination and accumulation of sulphatide in the central and peripheral nervous system and other tissues especially in the kidney.¹ The storage is due to deficient activity of the lysosomal enzyme cerebroside sulphatase,² which can be measured as arylsulphatase A (ASA) in leucocytes, urine and fibroblasts and also in cultured amniotic fluid cells, thus making prenatal diagnosis possible.^{3,4} The genetic transmission is autosomal recessive.⁵ The cases are usually divided into congenital, late infantile, early juvenile, juvenile and late onset forms.^{6,7} The late onset form is rare, the initial clinical picture is one of psychiatric disorder⁸ and its clinical course is more prolonged than in the other forms. In nerve biopsies segmental demyelination is characteristic of all forms of the disease. There are metachromatic inclusions in the cytoplasm of Schwann cells and in perivascular macrophages. Ultrastructure of the inclusions is variable and not related to the age of onset.⁹ In the central nervous system the striking feature is demyelination with neuronal and axonal loss mainly in the cerebral and cerebellar hemispheres,

basal ganglia, brainstem and spinal cord.

We have recently seen a family with four affected members, all of them with late onset form, and the purpose of this paper is to describe their clinical, biochemical and neuropathological features.

Case reports

Case 1 (II-4) (fig 1)

The patient was born of a normal pregnancy and developed normally in her early years. At the age of 18 years, her parents began to note signs of mental deterioration, and restlessness. At 19, she remained at home, unable to care for herself without help; ataxia was first noticed. At 27, she was admitted for the first time in our hospital. On examination, the patient was alert, and tended to laugh and repeat her name when addressed. No other replies were obtained to questions. The ocular fundi were normal. She followed visual stimuli, and external ocular movements were full without nystagmus. The pupils reacted to light and accommodation. The remaining cranial nerves were also normal. The patient could not stand or walk and was lying in bed with arms adducted and flexed at the elbows. The legs were adducted, extended at the knees. There was a tetraparesis and generalised muscle atrophy. All tendon reflexes were brisk and both plantar responses were extensor. Routine laboratory investigations were normal and a serological test for syphilis was negative. A lumbar puncture yielded clear, colourless, acellular cerebrospinal fluid under a normal pressure; the glucose was 20 mg per 100 ml and the protein 0.5 g/l. Motor nerve conduction velocity was 17 m/s in the left peroneal

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Fig 1 Family pedigree.

nerve and 35 m/s in the left median nerve. The CT scan of the brain showed generalised atrophy with low density in the white matter of both cerebral hemispheres. A diagnosis of metachromatic leucodystrophy was made on sural nerve biopsy and confirmed later by determination of ASA activity in the leucocytes. The patient died at the age of 30 years and necropsy was performed.

Case 2 (II-5)

The first abnormality of this woman was mental deterioration at the age of 21 years, without epilepsy or difficulty in walking. At the age of 26 years, in spite of an obvious mental deterioration, she had no focal signs on neurological examination. The EEG showed generalised theta waves predominantly in right occipital region. CT scan showed generalised cerebral atrophy and zones of low density in the white matter of both hemispheres. A sural nerve biopsy was performed.

Case 3 (II-6)

At the age of 17 years, this patient presented with serious behavioural disturbance and was admitted to a psychiatric hospital with the diagnosis of schizophrenia. His condition deteriorated and at 24 he had the first epileptic seizure. A right hemiparesis and ataxia was noticed. By the age of 26, his speech was limited to monosyllabic replies to questions. He could still walk with help. The fundi were normal. He was able to follow visual stimuli and external ocular movements were full without nystagmus. Pupils reacted to light. The remaining cranial nerves were normal. There were bilateral pyramidal signs and slight distal atrophy of the muscles in the legs.

Case 4 (II-11)

This girl presented with behavioural disturbances and progressive intellectual deterioration at the age of 15 years. One year later, although still ambulatory, she was unable to take care of herself. At 17 years neuropsychologic examination showed a frontal syndrome, without any other disturbances. She was alert and cooperative but easily distracted by external stimuli. The ocular fundi and cranial nerves were normal, all tendon reflexes were normal and plantar responses were extensor. No muscular wasting was found and sensation appeared intact. There was a slight tremor and cogwheel rigidity of her right arm. The EEG

Table Arylsulphatases in human leucocytes

Subject	Arylsulphatase A activity nmol/h/mg protein	Arylsulphatase B activity nmol/h/mg protein
CONTROLS RANGE		
n = 20	60-160	150-430
Case 1	14	300
Case 2	15	400
Case 3	5	200
Case 4	13	250
OBLIGATE HETEROZYGOTE	40	350

revealed generalised bilateral theta and delta waves. CT scan of the brain showed atrophy and large white matter hypodensities in both hemispheres.

Other members of the family

Three sisters and a brother (II-7, 8, 9, 13) were also observed; they had normal physical examination and EEG. It was not possible to examine the other two brothers still alive (II-1, 3), but we know that they have normal personal and professional lives at 33 and 28 years of age, respectively.

Four siblings (II-10, 12, 14, 15) died in the first year of life.

BIOCHEMICAL STUDY

Methods Protein was determined using the Lowry's method¹⁰ and the activity of the enzymes was determined according to Roy.¹¹ Electrophoresis⁴ was performed on sheets of cellulose acetate gel at room temperature in an electrophoresis tank with a 0.036 mol/l sodium barbital-0.036 mol/l sodium acetate-acetic acid buffer, pH 7.3, containing 1 mmol/l-mercaptoethanol. Samples of cell homogenates (5-10 µl) were applied to the gel and run for 90 minutes at 180V-5mA. The sheets were then incubated at

Fig 2 Electrophoresis of arylsulphatase A, B and C in case 1 and 2. Normal control values are shown as C.

Fig 3 Histogram showing decreased myelinated fibre diameters. Control values not shaded.

37 °C for 90 minutes in a moist chamber with 15 mmol/l 4-methylumbelliferyl sulfate in 0.5 mol/l sodium acetate-acetic acid, pH 5.4. The reaction was stopped with glycine buffer, pH 10.0 and the electrophoretic pattern was visualised at 363 nm.

Results The table shows the extremely low values of arylsulfatase A in the four patients and an intermediate value in the father (obligate heterozygote). The values for arylsulfatase B are in the normal range. Figure 2 shows the electrophoresis in cellulose acetate gel of arylsulfatases A, B and C in the cases and in a normal control. The band corresponding to arylsulfatase A is lacking in the two patients.

PATHOLOGY

Methods Left sural nerve biopsies of cases 1 and 2 were fixed in glutaraldehyde, postfixed in osmium tetroxide and processed for electron microscopy as usual. The blocks were then embedded in Epon 812. Semi-thin sections (1 µm) were

Fig 4 Myelin sheaths abnormally thin in relation to axon's diameter. Sural nerve biopsy. (Toluidine blue $\times 140$).

Fig 5 Tuffstone inclusion in Schwann cell cytoplasm (EM $\times 40,000$).

cut in a LKB III ultramicrotome and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate. Quantification of myelinated fibres/mm² and fibre diameter histograms were performed in a M.O.P. (Kontrom) associated to a 380 R.M.L. computer. Post mortem material from case 1 was fixed in 10% formal saline and processed routinely into paraffin wax. Sections of cerebral and cerebellar hemispheres were cut and sections from brainstem and spinal cord were also prepared and all were stained with haematoxylin-eosin (H & E), periodic acid Schiff (PAS), Loyez, Luxol fast blue-cresyl violet, Holzer and Glee and Marsland. Sections of the liver, kidney and gall bladder were stained with H & E and PAS. In order to demonstrate metachromasia, frozen sections (5 µ) from sural nerve, frontal white matter and kidney were cut in cryostat, fixed in formal-calcium and stained overnight in 0.1% toluidine blue in McIlvaine buffer (pH 5.0).¹²

Biopsies Left sural nerve of case 1 showed moderate loss of myelinated fibres (3.289/mm², control-9.680/mm²), mainly those of larger diameter (fig 3). The myelin sheaths were abnormally thin in relation to the axon's diameters (fig 4). Some onion bulbs were present. Degenerated fibres were rare. In the cytoplasm of Schwann cells and some perivascular macrophages, there were granules that stained purple-brown with toluidine blue. Electron microscopy showed abnormal separation of the inner lamellae of myelin. In the Schwann cell cytoplasm there were tuffstone inclu-

Fig 6 Section of the left hemisphere showing widespread demyelination in the frontal and temporal white matter (Loyez $\times 1.1$).

sions (fig 5) with either parallel or concentric lamellae. Other inclusions were amorphous and others were osmiophilic bodies. The nerve biopsy of case 2 was similar.

Necropsy Case 1 The brain weight was 1005 g and the sulci were moderately widened. The optic nerves were normal. The brain stem and cerebellum looked normal. On coronal slicing, the white matter was rubbery mainly in the centrum ovale. Grey patches were seen in the periventricular white matter, corpus callosum and internal capsules; the occipital white matter was less affected. The U fibres, optic tracts, cerebellar white matter and white matter in the brain stem looked normal. The cerebral cortex, basal ganglia hippocampus, cerebellar cortex and brain stem looked normal. There was mild dilatation of the ventricular system. The cause of death was bronchopneumonia. No other abnormality was seen.

Histology Demyelination (fig 6) was widespread through the white matter of cerebral and cerebellar hemispheres. Macrophages with PAS positive material were quite numerous mainly in subcortical areas. In frozen sections, this material stained red with toluidine blue. PAS positive macrophages were also seen in the cerebellar white matter and pons. Perivascular lymphocytic cuffing was also seen scattered in the white matter. In the cerebral cortex, there was loss of nerve cells and their processes and reactive astrocytes were quite numerous. Some metachromatic material was seen in macrophages here and also in macrophages and

neurons in the globus pallidus and putamen. Electron microscopy showed that the inclusions consisted of collection of lamellae either parallel or concentric (fig 7). PAS positive material was found in peritubular cells in the kidneys. Liver and gall bladder were normal.

Discussion

Metachromatic leucodystrophy is classified according to the age of onset. The late infantile form is the most frequently reported and patients with onset at 1–2 years of age constitute 70% of all cases.¹³ The age of onset of juvenile metachromatic leucodystrophy is between 3 and 15 years. The majority of cases present with a fall-off in school performance but gait disturbances, clumsiness, slurred speech and signs of pyramidal dysfunction rapidly appear and the child is normally bedridden in about a year.¹⁴

Adult metachromatic leucodystrophy is reported in cases as young as 16 years.¹⁷ In contrast to the juvenile form, the clinical picture of adult metachromatic leucodystrophy is mainly characterised by progressive mental deterioration, and other neurological symptoms may appear later in the course of the disease. A 5 to 10 year survival is common and the disease may even progress slowly over various decades. In 1970 Muller¹⁸ described 19 cases of adult onset metachromatic leucodystrophy with age of onset between 19 and 46 years. At the onset psychiatric symptoms alone were present in 14 of the cases while mixed psychiatric and neurological symptoms occurred in one case. In most of the cases a diagnosis was made only post mortem. Similar results are reported in more recent cases.^{14–18}

All our cases were diagnosed during life and one patient died after 12 years of evolution, all the others are still alive. Although in one of the patients (case 4) the age of onset was 15 years, we decided to classify this family in the adult onset group because of the initial symptoms and their clinical evolution.

There is some clinical intrafamilial variation in this family; case 3 with 8 years of evolution was already unable to walk with severe muscle atrophy and a spastic quadriplegia; her sister, case 2, with 5 years of evolution has a severe dementia with a normal neurological examination.

The level of residual ASA activity in leucocytes and cultured fibroblasts does not help to differentiate between the clinical forms but ASA value in urine is significantly higher in patients with adult and juvenile forms than in the infantile form of the disease.^{19–23} Perhaps the difference in residual ASA activity can explain the interfamilial variation.²⁴

In late infantile metachromatic leucodystrophy a neuropathy is first observed preceding central nervous system involvement.^{25,26} In adult onset metachromatic leucodystrophy, however, overt clinical

Fig 7 White matter inclusions consisting of concentric and parallel lamellae in macrophages (EM $\times 200,000$).

signs of neuropathy are not usually described. In contrast with this lack of clinical signs electrophysiology shows evidence of peripheral nerve involvement in nearly all of the adult cases tested.^{13 27} Case 1 has a severe demyelination neuropathy and a severe atrophy at neurological examination; however case 2 in spite of an abnormal biopsy has no clinical signs of neuropathy.

Our observation suggests that metachromatic leucodystrophy should be considered in the differential diagnosis of dementia in young adults and a sural nerve biopsy and ASA activity are important contributions to diagnosis.

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