

LESSON OF THE MONTH

X-linked adrenoleukodystrophy with non-diagnostic plasma very long chain fatty acids

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Abstract

Measurement of plasma very long chain fatty acids is widely recognised as a sensitive screening test for X-linked adrenoleukodystrophy (X-ALD). This test has particular importance because of the highly variable clinical expression of X-ALD. In this affected family the progressive childhood form of X-ALD was accompanied by "non-diagnostic" concentrations of plasma very long chain fatty acids. The implications for diagnosis of X-ALD are discussed.

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The phenotype of the progressive childhood form of X-linked adrenoleukodystrophy (X-ALD) was described first by Siemerling and Creutzfeldt¹ and later by Schaumburg *et al.*² This enabled the characteristic pathology³ and associated biochemical abnormality in the metabolism of very long chain fatty acids⁴ to be identified. Since the description by Moser *et al.*⁵ of an increased content of very long chain fatty acids (VLCFAs) in plasma, this assay has been widely used as a screening test for X-ALD and has aided in the identification of presymptomatic and asymptomatic cases as well as several different phenotypes of X-ALD⁶ (see table 1). It is also recognised that women heterozygous for X-ALD may develop a spastic paraparesis.⁷

This is a report of one family with progressive childhood X-ALD in which the characteristic abnormalities of plasma VLCFAs were non-diagnostic and were regarded as normal. The implications for diagnostic evaluation of X-ALD families are discussed.

Case report**HISTORY AND EXAMINATION**

The proband was referred at the age of eight years with a history of longstanding learning difficulties with recent deterioration. There were no neonatal problems but cognitive development was slow from the beginning. He was walking without assistance by the age of one year but was slow to develop imaginative play, dressing and feeding skills, and speech. He attended a school for children with severe learning difficulties. For one year before referral he had seemed to have severe but variable visual impairment. He was

becoming increasingly short tempered and withdrawn. He became unable to complete jigsaw puzzles and his handwriting deteriorated. He continued to enjoy a completely normal diet. In the family history, the patient had a maternal uncle who had died at the age of seven, one year after the onset of an unexplained neurological illness. His maternal grandmother had three siblings, all boys, of whom two had died in childhood.

On examination, the patient was quiet and withdrawn and had difficulty in understanding simple instructions. There was no abnormal skin pigmentation. He could visually fix on a face but was unable to point to a hand held in front of his eyes. Pupillary reactions to light were sluggish and the optic discs were pale. Tone and power in the limbs was normal. Deep tendon reflexes were brisk with spread of the biceps jerk to the fingers. Plantar response testing led to brisk withdrawal. There was no ataxia of the trunk or extremities.

INVESTIGATIONS

CT showed non-enhancing low attenuation in the white matter adjacent to the posterior horns of the lateral ventricles. A working clinical diagnosis of X-ALD was made because of the personal and family history, and findings on neurological examination and CT. Results of measurement of plasma VLCFA concentrations are reported later. Serum cortisol was 187 nmol/l before and 384 nmol/l 30 minutes after synacthen injection, which was interpreted as a normal response. White cell enzyme concentrations in blood and oligosaccharide concentrations in urine were normal. An electroretinogram was of normal amplitude and latency. Visually evoked potentials were delayed with latencies of 133 ms from each eye.

Table 1 Phenotype of X-linked adrenoleukodystrophy (X-ALD): male patients in 617 kindreds

Phenotype	%
Childhood cerebral	48
Adolescent cerebral	5
Adrenomyeloneuropathy	25
Adult cerebral	3
Addisonian only	10
Asymptomatic and presymptomatic	8

Data from Moser *et al.*⁶

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VLCFA CONCENTRATIONS

Plasma VLCFA concentrations were reported as normal by laboratory A. Because of strong clinical suspicion of X-ALD, VLCFA concentrations were measured on a total of five occasions at three laboratories in two countries representing the best standardised assays of plasma VLCFAs available in Europe at that time (table 2). $C_{26:0}$ concentrations and the $C_{26/22}$ ratio were, on one or more measurements, in the upper part of the reference range for unaffected subjects in laboratories A and C and intermediate between normal and X-ALD ranges in laboratory B. The $C_{24/22}$ ratio was at the upper end of the range for unaffected subjects in all three laboratories. The $C_{24/22}$ and $C_{26/22}$ ratios were well below the reference ranges quoted for X-ALD in all three laboratories. Laboratories A and C commented, despite full knowledge of the clinical and family history, that X-ALD had been excluded.

Concentrations of VLCFAs in fibroblasts cultured from skin biopsy were above the normal range and within the range seen in X-ALD patients in three laboratories. The mother's fibroblasts had concentrations intermediate between normal and X-ALD concentrations, confirming carrier status. Laboratory C commented that the plasma and fibroblast samples must have come from different patients unless the patient was on a special diet. The ratio of $C_{24:0}$ (peroxisomal) oxidation to $C_{16:0}$ (mitochondrial) oxidation (again measured in fibroblasts) was one third the ratio seen in unaffected controls, a result typical of males with the X-ALD gene. This confirmed the diagnosis of X-ALD beyond doubt.

CLINICAL COURSE

During six months after diagnosis, the patient deteriorated rapidly to a point where he retained little visual responsiveness. He was able to understand speech but remained mute and in a state of decerebrate rigidity.

Discussion

The diagnosis of X-ALD in this family seems certain yet plasma VLCFA concentrations were atypical with most or all variables in the

reference range of the reporting laboratories for unaffected persons.

Concentrations of VLCFAs rise in plasma and fibroblasts because of the block in the β -oxidation pathway and are therefore an indirect measure of the biochemical defect. Direct measurement of oxidation in fibroblasts of the proband confirmed that the defect in peroxisomal β -oxidation of fatty acids was of the same degree as is usually seen in X-ALD. Because the mother had biochemical evidence of carrier status for X-ALD, it is very likely that the condition also affected the maternal uncle and great uncles of the proband.

The phenotype of childhood X-ALD classically presents with progressive school difficulties and motor problems but the clinical range is wide. Thus in the absence of a family history, clinical diagnosis of the childhood form of X-ALD can sometimes be difficult. Adult phenotypes of X-ALD broaden the range of clinical presentation further⁶ and both extremes of the phenotypic range may be expressed in a single kindred. Delayed cognitive development from infancy, as seen in the proband, has been seen in a few boys with X-ALD (Moser HW, unpublished observations) but it is not clear whether this is part of the expression of that gene.

These clinical variations do not prevent a correct diagnosis being reached, provided that the possibility of X-ALD is considered and a reliable laboratory screening test is undertaken. Measurement of plasma VLCFA has been thought to fulfil this function but the proband reported here suggests that this test, as currently interpreted in Europe, is less than 100% sensitive for X-ALD. Had we been guided by the laboratory's interpretation of the plasma VLCFA result in the proband, the illness of the affected boy might well have been classified as an unknown, and probably autosomal recessive, leukodystrophy and inappropriate genetic counselling would have been given to the family.

There were no peculiarities in the patient's diet to explain the unexpectedly "normal" plasma VLCFA concentrations. The fact that plasma VLCFA concentrations were similar on several occasions in three laboratories makes a technical error unlikely. Plasma VLCFA concentrations are not related to phenotype among patients with X-ALD. Furthermore the defect of β -oxidation in the proband was quantitatively no different to that typically seen in X-ALD. It therefore seems unlikely that he belongs to a clinically or biochemically distinct subgroup of X-ALD. It is nevertheless clear from the laboratories' comments that the possibility of X-ALD occurring with such a pattern of plasma VLCFA was not widely recognised in Europe at the time the patient presented, nor are we aware of any previous published reports of this. Similarly, data collected in North America at the J F Kennedy Institute, Baltimore, on 800 ALD hemizygotes is thought to suggest that plasma VLCFA measurement is 99.9% specific and close to 100%

Table 2 VLCFA concentrations in plasma for proband

	$C_{26:0}$ (μ g/ml)	$C_{24/22}$	$C_{26/22}$	Laboratory comment
Laboratory A: 16 January 1990	0.41 (0.13–0.90)	0.99 (0.32–0.92)	0.023 (0–0.03)	Not consistent with ALD Lower than normally seen in ALD
26 June 1990	0.76 (0.16–0.5)	0.99 (0.68–1.0)	0.043	
Laboratory B: 19 February 1990	0.96 (0.27 (0.09))	0.85 (0.72 (0.09))	0.037 (0.01 (0.003))	Not typical for ALD
2 April 1990	0.71	0.93	0.027	No comment
ALD range for Laboratory B	1.52 (0.32)	1.46 (0.14)	0.06 (0.01)	
Laboratory C: 5 July 1990	0.51 (0.36 (0.19))	0.96 (0.71 (0.15))	0.02 (0.01 (0.01))	ALD excluded
ALD range for Laboratory C	1.52 (0.56)	1.40 (0.15)	0.07 (0.03)	

Figures in parentheses indicate the laboratory's reference range; for laboratory A they are quoted as -2 to $+2$ SDs; for laboratories B and C they are quoted as mean (SD) at the time of testing, for unaffected persons.

sensitive for the detection of ALD, using as a diagnostic criterion values raised by more than two SDs above the control for both $C_{26:0}$ concentration and the $C_{24/22}$ ratio and the $C_{26/22}$ ratio. Many of the 11 000 persons whose plasma VLCFA ratios did not show this degree of abnormality were not followed up so the absence of "false negatives" amongst them remains uncertain (Moser HW *et al*, unpublished observations).

We suggest that the diagnostic failure in this case resulted from insufficient recognition of the degree of overlap that exists between plasma VLCFA concentrations in ALD hemizygotes on the one hand and unaffected patients on the other. Results for plasma VLCFA in X-ALD should be divided into three categories: "typical of ALD", "normal" and "in need of further study". The delineation of this third category has been hindered by the diversity of reference ranges and their method of application by different laboratories.

In patients either with clinical abnormalities suggesting the diagnosis (including adrenomyeloneuropathy or presenile dementia), or at increased risk because of a positive family history, the possibility of X-ALD must be considered when plasma VLCFA concentrations are borderline, suggesting the need for further study. We suggest that such "borderline" results should include values at or

above two SDs above controls in any one of the three values measured—namely, $C_{26:0}$, $C_{24/22}$ or $C_{26/22}$. With such a criterion, all three laboratories would have classified our case as "in need of further study". Advice based on plasma VLCFA concentrations that can be classified as "normal" should be secure but even in this case, consideration should still be given to skin biopsy for VLCFA measurement in cultured fibroblasts if clinical suspicion of X-ALD is strong.

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