X-linked adrenoleukodystrophy in patients with idiopathic Addison disease

Abstract  The two main causes of primary adrenal disease are tuberculosis and auto-immune adrenal destruction. The latter is responsible for about 70% of the cases of primary adrenal insufficiency (Addison disease). Commonly referred to as a rare cause of adrenal failure is X-linked adrenoleukodystrophy (ALD), a demyelinating peroxisomal disorder affecting 1: 20000 Caucasian males. Albeit primary adrenal insufficiency is a rare entity per se, we decided to study patients with idiopathic Addison disease and establish the frequency of ALD as a cause of adrenal insufficiency. The biochemical defect of ALD was found in 5 out of 24 patients. The small number of cases in our series led us to include in our analysis the published results of two other groups of investigators. This analysis indicates that the proportion of cases in which Addison disease is attributable to ALD is age dependent. It is highest when the adrenal insufficiency manifests before 15 years. This study clearly demonstrates that the proportion of ALD in patients presenting primary adrenal insufficiency has been underestimated.

Conclusion  Addison disease manifesting during the first decade of life has a high likelihood of being the first sign of X-linked adrenoleukodystrophy.

Key words  Addison disease  Adrenomyeloneuropathy  Phenotypic variability  Very long chain fatty acids  X-Linked adrenoleukodystrophy

Abbreviations  AD Addison disease  ALD X-linked adrenoleukodystrophy  VLCFA very long chain fatty acids

Introduction

X-linked adrenoleukodystrophy (ALD) is a demyelinating peroxisomal disorder, presenting with marked clinical variability even within the same kindred. It includes childhood ALD, the most frequent phenotype, with serious neurological impairment that can lead to death a few months after appearance of the first symptoms, usually between 4 and 8 years of age, and a milder variant called adrenomyeloneuropathy involving mainly the spinal cord and peripheral nerves, with an average age of onset of 28±7 years and a course that may extend over decades [9, 13]. AlD leads to a pathognomonic accumulation of very long chain fatty acids (VLCFA) in brain white matter, adrenal gland, fibroblasts and plasma, caused by the impaired-oxidation of these fatty acids in peroxisomes [6]. The metabolic defect is expressed most severely in the brain, adrenal cortex and testis. The reason why these tissues are particularly susceptible to the accumulation of VLCFA remains unclear [11].

Primary adrenal insufficiency is characterised by easy fatiguability, weakness, anorexia, nausea and vomiting, abdominal pain, weight loss, drowsiness, and occasionally coma. When chronic hyperkalaemia is present this may occasionally bring on an attack of acute areflexic
paralysis, which may include respiratory, facial, or bulbar weakness [3].

There are two main causes of primary adrenal disease: tuberculosis and auto-immune adrenal destruction. The latter is a component of the polyendocrine deficiency syndrome [8]. As tuberculosis is becoming less frequent in the industrialised nations, the polyendocrine deficiency syndrome is associated with the majority of cases of Addison disease (AD) [18]. ALD, where the primary adrenal insufficiency may precede, co-exist or develop after neurological dysfunction, is frequently referred to as a rare cause of AD. The adrenocortical insufficiency in ALD appears to be directly attributable to the accumulation of VLCFA whereas the pathogenesis of the nervous system lesions appears to be more complex and possibly includes immunological mechanisms [16].

Since ALD occurs more often than was previously realised and because it may be the causal mechanism in a significant proportion of male patients with primary adrenal insufficiency, we screened for ALD in 24 male patients with primary adrenal disease of unknown cause.

Previous results [5, 14] led us to investigate if the age at onset of primary adrenal insufficiency in patients with idiopathic AD is related to the existence or absence of the biochemical defect of ALD.

**Patients and methods**

**Patients**

Biochemical studies were conducted in 24 males of different families with idiopathic AD. All patients were being treated for primary adrenocortical insufficiency. Patients with multiple endocrine gland deficiencies were excluded, since it has been shown previously that such patients have normal levels of VLCFA [15].

**Family members**

Family studies by VLCFA analysis were also performed in obligate heterozygous and other females and males at risk.

**Biochemical analysis**

The biological samples used for this study were blood plasma and cultured skin fibroblasts [4].

Plasma samples were sent to us from the endocrinology departments of different Hospitals in Portugal, and were kept at -20°C before processing. A skin biopsy for fibroblast culture was performed when an abnormal VLCFA profile was found in plasma.

**Procedure**

Total lipids were extracted according to a modification of the method of Folch et al. [2]. Methylation of plasma lipids was carried out in methanol/HCl (3 mol/l) at 75°C for 16h and that of fibroblasts in boron trichloride/methanol 12% (w/v) at 80°C for 1 h. After thin-layer chromatographic separation of the VLCFA, methyl esters were then dissolved in 80 μl of n-hexane and an aliquot (1 μl) was injected into a gas-chromatograph, equipped with a splitless injection system, a flame ionisation detector and a fused Silica Gel capillary column [10].

The C24:0/C22:0 and C26:0/C22:0 ratios were calculated by determining the ratio of the corresponding area of each fatty acid. The absolute amount of a particular VLCFA was determined by comparison of the corresponding peak area, relative to C27:0 fatty acid methyl ester, used as internal standard.

**Results**

The clinical and biochemical data of the 24 male patients with idiopathic AD are summarised in Table 1. All patients had markedly increased plasma ACTH concentrations and low levels of cortisol after ACTH stimulation (data not shown), and are now under replacement therapy. At the time of the study none showed neurological involvement.

Elevated plasma C24:0/C22:0 and C26:0/C22:0 (data not shown) ratios and C26:0 concentrations were found in 5 of the 24 patients confirming the ALD status. Increases in the C26:0 level (μg/mg protein) and C26:0/C22:0 (data not shown) ratio were also present in cultured skin fibroblasts (Table 1).

Patients classified as “Addison only” (Table 1) continue to present normal neurological findings, including MRI. Patient 22 with the juvenile form, showed a rapid deterioration including paraparesis, seizures and vision loss leading to a vegetative state and death, a few months after the diagnosis of ALD.

Family studies by VLCFA analysis revealed 12 heterozygotes and 5 additional hemizygotes some with distinct phenotypes including a patient with the adult form of ALD and pre-symptomatic status, demonstrating the heterogeneity of the disease.

Ages at manifestations of AD in patients with or without ALD are presented in Fig. 1. To test the hypothesis of different manifestation ages, a contingency table was used revealing that the two samples (ALD+ and ALD-) seem to be different (Chi-square