Genetics and Molecular Biology, 23, 2, 261-264 (2000)

X-Linked adrenoleukodystrophy: clinical and laboratory findings in 15 Brazilian patients

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Abstract

Adrenoleukodystrophy (X-ALD) is an X-linked recessively inherited peroxisomal disorder, phenotypically heterogeneous, characterized by progressive white-matter demyelination of the central nervous system and adrenocortical insufficiency. We investigated 15 male X-ALD patients varying in age from 7 to 39, diagnosed among 108 suspected patients referred for investigation. Plasma levels of very long chain fatty acids (VLCFA) were measured at our laboratory using gas chromatography (GC). Eleven cases of childhood X-ALD and four cases of adrenomyeloneuropathy (AMN) were diagnosed. Adrenal leukodystrophy insufficiency and limb weakness were the most frequent symptoms, appearing in 12, 8 and 6 of the patients, respectively. Physician awareness of X-ALD seems inadequate to judge by age at diagnosis and lengthy interval between the start of symptoms and diagnosis. This is the first published series of Brazilian patients with X-ALD. We determined signs and symptoms relevant for diagnosis, as early identification seems important for treatment outcome. In addition, diagnosis identifies carriers, who could benefit from genetic counselling and prenatal diagnosis.

INTRODUCTION

X-Linked adrenoleukodystrophy (X-ALD) is the most frequent peroxisomal disorder, with an estimated frequency of 1:20,000 in males (Moser, 1993; Korenke *et al.*, 1995). It should be differentiated clinically and biochemically from neonatal ALD, an autosomal recessive disorder of peroxisome biogenesis in which the function of at least five peroxisomal enzymes is impaired. In X-ALD, a defect in lignoceroyl-CoA ligase causes pathognomonic tissue accumulation of very long chain fatty acids (VLCFA), mainly hexacosanoic ($C_{26:0}$) and tetracosanoic acids ($C_{24:0}$) (Wanders *et al.*, 1988, 1992; Moser, 1993). Fatty acid accumulation seems responsible for adrenal cortex malfunction and nervous system demyelination (Moser *et al.*, 1991; Wanders *et al.*, 1996). Known clinical forms are: classical (or childhood) X-ALD (ALD), adrenomyeloneuropathy (AMN) and isolated Addison's disease (AD). In some cases patients are completely asymptomatic, and these several phenotypes may occur in the same family (Koike *et al.*, 1991; Moser *et al.*, 1991, 1992; Korenke *et al.*, 1995; Wanders *et al.*, 1994, 1995, 1996). ALD and AMN are the two most frequent clinical forms of X-ALD (Moser *et al.*, 1992; Korenke *et al.*, 1995; Wanders *et al.*, 1996). Patients with ALD usually develop the symptoms from age four to eight, including visual and auditory disturbances, decreased school performance, adrenal insufficiency, walking difficulties, demyelinization and leukodystrophy. The disease progresses rapidly and patients usually die approximately two to five years after symptom onset (Moser *et al.*, 1992). In contrast, AMN appears around age 20-30 (Koike *et al.*, 1991) and is characterized by progressive paraparesis and sphincter disturbances, since the spinal cord is affected. Two-thirds of the patients have adrenal insufficiency. Treatment consists of a diet restricted to saturated fatty acids, combined with the use of glyceroltrioleate/glyceroltrierucate (GTO/GTE), known as Lorenzo's oil (Koike *et al.*, 1991; Moser *et al.*, 1994; Korenke *et al.*, 1995; Ruiz *et al.*, 1996). The objective is to normalize VLCFA concentrations, but favorable responses apparently occur only in patients beginning treatment before the appearance of neurological symptoms (Koike *et al.*, 1991; Moser, 1993; Moser *et al.*, 1994; Korenke *et al.*, 1995). Bone marrow transplantation and immunosuppression are also considered therapeutic alternatives (Moser *et al.*, 1992). Lovastatin and sodium phenylacetate are being tested as therapeutic drugs for normalizing VLCFA levels in plasma and skin fibroblasts of X-ALD patients (Singh *et al.*, 1998a,b). We describe 15 male patients with X-ALD, stressing main signs and symptoms and phenotypic differences between ALD and AMN.

MATERIAL AND METHODS

We studied 15 male patients (from 12 families) aged between 7 and 39 with confirmed diagnosis of X-ALD, selected from 108 patients referred for investigation due to suggestive symptoms from pediatric, endocrinology and neurology services from various Brazilian cities. A refer-

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ence group of 30 healthy individuals was also studied to establish normal values of VLCFA plasma concentrations.

VLCFA were analyzed at our laboratory following the Moser technique (Moser and Moser, 1991). Internal standard ($C_{27:0}$ - heptacosanoic acid) was added to 100 µl of plasma samples. Chloroform-methanol (1:1) was added for plasma extractions. After centrifugation, the supernatant was mixed with distilled water and chloroform for removal of precipitated proteins. After further centrifugation the lower phase was dried under N_2 and chloroform-methanol (2:1) was used to rinse down dried lipids. Methanolic HCl (1 N) was added to each dry total lipid extract and the tubes kept at 75ºC for 16 h to perform acid methanolysis. The fatty acid methyl esters were then purified by thin-layer chromatography. The plates (0.25 µm silica gel G 20 x 20) were pre-washed in chloroform: methanol:acetic acid:water (52:20:7:3) and hexane: ether:acetic acid (90:10:1). The plate was developed in toluene:ether (97:3). After air drying during 16 h, the samples were exposed to iodine vapors. Extraction of VLCFA methyl esters from silica was performed using hexane (3 times). After drying with N_2, the samples were redissolved with 50 µl hexane for analysis by gas chromatography. We used Varian GC equipment with an HP-5 column (cross-linked 5% phenyl methyl silicone, 0.33 µm-thick film, 0.2 mm in internal diameter and 25 m long), flame ionization detector, split/splitless injector, and helium as carrier gas. Concentrations of $C_{22:0}$ (docosanoic acid), $C_{24:0}$ (tetracosanoic acid) and $C_{26:0}$ (hexacosanoic acid) were measured, and $C_{26:0}/C_{22:0}$ and $C_{24:0}/C_{22:0}$ ratios were also calculated.

Statistical analysis

Fishers's exact test was employed to compare the signs and symptoms found in the patients with the two different clinical forms of X-ALD. The criterion for significance was $P < 0.05$.

RESULTS AND DISCUSSION

The 15 patient samples included 11 cases of ALD and 4 of AMN. Symptom frequency of these two clinical forms of X-ALD was determined for these cases (Table I).

The $C_{26:0}$ concentration, $C_{26:0}/C_{22:0}$ ratio and $C_{24:0}/C_{22:0}$ ratio were determined (Figure 1). These were from the first plasma sample collected for diagnosis from each patient. The $C_{26:0}$ plasma concentration (Figure 1A) was above normal in all patients. In addition, the $C_{26:0}/C_{22:0}$ ratio was high in all patients (Figure 1B). Due to the increased concentration of $C_{24:0}$, the $C_{24:0}/C_{22:0}$ ratio was high in all patients (Figure 1C), except one. In fact, the elevations in $C_{26:0}$ plasma concentration and $C_{26:0}/C_{22:0}$ plasma ratio are X-ALD pathognomonic.

ALD patients were diagnosed on average at age 10 although first symptoms were observed at age eight, thus the average interval between suspicion and diagnosis was two years. For AMN patients average age at diagnosis was 27, while symptom onset began at 20, resulting in an average seven-year delay. Patients were diagnosed only when neurologic symptoms appeared. Both age at diagnosis and lapse between first symptoms and diagnosis aggravated prognosis since response to treatment is known to depend on precocious diagnosis and absence of neurological symptoms. Diagnosis delay in our cases could be attributed mainly to physicians unawareness of X-ALD, since adrenal insufficiencies are frequently simply diagnosed as Addison's disease, and patients are not investigated for VLCFA accumulation.

Leukodystrophy, adrenal insufficiency, limb weakness, impaired vision and learning difficulties were the main symptoms presented by the X-ALD patients as a whole. Leukodystrophy, impaired vision, school difficulty and poor handwriting were more frequently found in ALD patients. Although not significant ($P = 0.09231$), a tendency to impaired vision was observed in ALD patients. Adrenal insufficiency occurred with equal frequency in both clinical forms. Limb weakness associated with paraparesis was much more frequent in AMN patients ($P = 0.02564$). Adrenal insufficiency, hyperpigmentation, seizures, tremor, sphincter and gait disturbances and speech difficulties appeared in both clinical forms with almost equal intensity.

Restriction of dietary VLCFA and Lorenzo's oil is recommended to decrease fatty acid accumulation (Moser *et al.*, 1994; Korenke *et al.*, 1995; Ruiz *et al.*, 1996). In addition, treatment requires supplements with the essential linoleic and linolenic acids. Six of the 15 patients followed this recommendation (one died after five years of treatment). However, as previously mentioned, more favorable prognosis depends on treatment beginning before neurological symptoms appear (Moser *et al.*, 1994; Moser, 1995; Korenke *et al.*, 1995). In 15 Italian ALD boys and 20 American ALD boys presenting neurological symptoms, Lorenzo's oil therapy failed to change the clinical course of the disease (Wanders *et al.*, 1992; Moser *et al.*, 1992). Korenke *et al*. (1995) studied 16 patients treated with Lorenzo's oil during 19 months. Six of nine neurologically symptomatic patients deteriorated during therapy, and MRI alterations worsened in all patients with clinical deterioration. None of the seven neurologically asymptomatic patients developed neurological symptoms. In none of the 6 patients with normal cranial MRI at start of therapy did MRI deterioration occur during therapy. Follow-up of the neurologically asymptomatic children supports the hypothesis that Lorenzo's oil therapy can prevent development of neurological symptoms. Our own experience with a small number of ALD symptomatic patients, however, agrees with the hypothesis that Lorenzo's oil lacks efficacy in these late diagnosed patients.

Adrenal leukodystrophy insufficiency and limb weakness appeared in 12, 8 and 6 of our 15 patients, respectively. These signs are important in investigating X-ALD

Table I - Frequency of signs and symptoms in the 15 X-linked adrenoleukodystrophy Brazilian patients.

Signs and symptoms	Adrenoleukodystrophy patients (N = 11)	Adrenomyeloneuropathy patients (N = 4)
Leukodystrophy	10	2
Adrenal insufficiency	6	2
Limb weakness*	2	4
Impaired vision	6	-
Learning difficulty	5	1
Speech difficulty	4	1
Hyperpigmentation	4	1
Seizures	4	1
Paraparesis*	1	3
Poor handwriting	4	-
Tremor	3	1
Behavioral disturbances	2	2
Gait disturbances	2	1
Sphincter disturbances	2	1
Abdominal pain	2	-
Neuronal deterioration	2	-
Impaired hearing	1	1
Hyperactivity	1	1
Vomiting and prostration	2	-
Atypic facies	1	-
Progressive ataxia	1	-
Cyanosis	1	-
Attention deficit	1	-
Headache	1	-
Microcephaly	1	-
Hypotrophy	-	1
Aphasia	1	-
Cerebral edema	1	-
Enuresis	1	-
Hyponatremy	1	-
Hemiparesy	1	-
Pneumonia	1	-
Gastroesophagic bleeding	1	-
Mild dementia	1	-
Average age for presenting signs (years)	8	20
Average age at diagnosis (years)	10	27

*($P < 0.05$, Fisher's exact test).

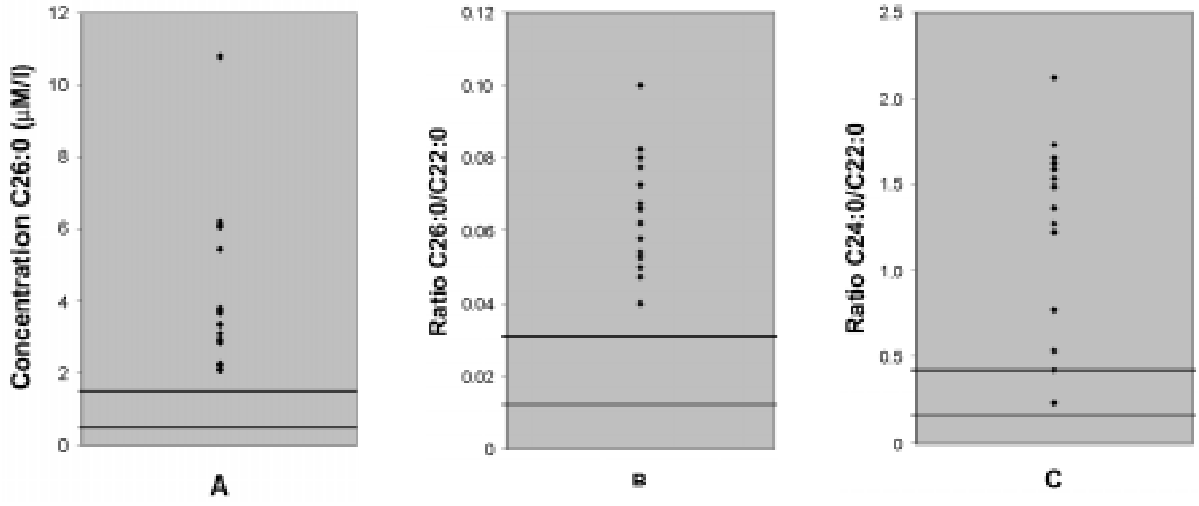

Figure 1 - Very long chain fatty acid levels in 15 Brazilian patients with X-linked adrenoleukodystrophy: **A**, C26:0 plasma concentration; **B**, C26:0/C22:0 ratio; **C**, C24:0/C22:0 ratio. The horizontal solid lines indicate upper (X + 2 SD) and lower (X - 2 SD) reference values, calculated with samples from 30 healthy subjects. SD = Standard deviation.

and when they occur in males should call for VLCFA level determination. Patients with adrenal insufficiency but no neurological symptoms always require VLCFA plasmatic analysis, since Lorenzo's oil could prevent or delay clinical manifestation of X-ALD.

The VLCFA plasma level determination in at-risk families should be done independently of patient age or symptomatology presence. Detection of index cases in families is important for: a) detection of further X-ALD cases; b) treatment of asymptomatic or barely symptomatic cases to avoid or delay symptom appearance, c) detection of heterozygotes; d) providing genetic counselling and prenatal diagnosis for at-risk subjects. Although VLCFA analysis is still an important tool for diagnosis of patients and for the treatment follow-up, it is being progressively replaced, for carrier detection and prenatal diagnosis, by the more specific molecular analysis of the X-ALD gene.

ACKNOWLEDGMENTS

We are indebted to Dr. R.J. Wanders and to Dr. P. Vreken, from University Hospital Amsterdam, who kindly provided necessary training for establishing our VLCFA assay methods and for laboratory assistance in investigating some difficult cases. We are also grateful to Dr. Moacir Wajner for valuable comments and suggestions, and to the physicians who referred patients for evaluation. This work was partially supported by CNPq, CAPES, FIPE/HCPA, PROPESP UFRGS, FAPERGS and PRONEX (grant 76.97.1025.00).

RESUMO

Adrenoleucodistrofia (X-ALD) é uma desordem peroxissomal com padrão de herança ligada ao X, fenotipicamente heterogênea, caracterizada por uma progressiva desmielinização da substância branca do sistema nervoso central e por insuficiência adrenal. Foram investigados por nós 15 pacientes do sexo masculino com sinais clínicos sugestivos de X-ALD, com idade entre 7 e 39 anos, diagnosticados entre 108 pacientes encaminhados para investigação por suspeita clínica. Os níveis plasmáticos dos ácidos graxos de cadeia muito longa (VLCFA) foram dosados em nosso laboratório através de cromatografia gasosa (GC). Onze (73%) casos da forma infantil de X-ALD (ALD) e 4 (27%) casos de adrenomieloneuropatia (AMN) foram diagnosticados. Insuficiência leucodistrofia adrenal e fraqueza muscular foram os sinais mais freqüentes, aparecendo em 80, 53 e 40% dos casos, respectivamente. O conhecimento dos médicos sobre a possibilidade da X-ALD parece ser pequeno, o que pode ser concluído a partir da elevada idade no diagnóstico e do grande intervalo entre o início dos sintomas e o diagnóstico. Neste trabalho, que relata a primeira série brasileira de pacientes com X-ALD, procuramos enfatizar os sinais e sintomas que são relevantes para a suspeita diagnóstica, uma vez que a identificação precoce dos casos parece ser importante para o sucesso do tratamento. Além disso, o diagnóstico permite a identificação de portadores, os quais podem se beneficiar do aconselhamento genético e do diagnóstico pré-natal.

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(Received February 21, 2000)