X-Linked Adrenoleukodystrophy: Focus on Lipid and Steroid Hormone Metabolism

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ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is an inherited demyelinating disorder characterized by the accumulation of saturated very long chain fatty acids (VLCFA) in plasma and tissues. Adrenocortical insufficiency and symptoms of alopecia and hypogonadism indicate a role of steroid hormones, particularly androgens in this disease. Fibroblasts from X-ALD patients exhibit abnormalities in the function and mRNA abundance of 5alpha-reductase, a key enzyme in testosterone metabolism. Lamellar inclusions rich in VLCFA esterified with cholesterol in the adrenals and testes contribute to the malfunctioning of these tissues. The homeostasis of cholesterol, the precursor of steroid hormones, and some related metabolic pathways are also perturbed in X-ALD.

INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD, OMIM #300100) is an inherited neurological disorder resulting from the accumulation of saturated very long chain fatty acids (VLCFA). Although these fatty acids accumulate in plasma and in all tissues, the main functional abnormalities in X-ALD concern the nervous system with demyelination, adrenocortical insufficiency, and hypogonadism [1,2].

VLCFA metabolism in X-ALD

The accumulation of VLCFA in plasma and fibroblasts is the biochemical marker of X-ALD. In particular, elevated plasma levels of hexacosanoic acid (26:0) and tetracosanoic acid (or lignoceric acid, 24:0) confirm the diagnosis of X-ALD.

VLCFA metabolism is modified in both the catabolic and biosynthetic pathways. Under physiological conditions, VLCFA are first shortened in the peroxisomes by beta-oxidation processes and then further catabolized in the mitochondria. The beta-oxidation enzymes in peroxisomes appear to be normal in X-ALD. Thus, the alteration in VLCFA catabolism is likely further upstream, for example, in the import of VLCFA, which requires the transport protein ALDP encoded by the gene mutated in X-ALD [3].

VLCFA accumulation in X-ALD is also due to alteration of the VLCFA biosynthetic pathway. Tsuj et al. demonstrated that the VLCFA elongation process [4,5], which takes place in the microsomes, is augmented in X-ALD fibroblasts. The metabolism of other fatty acids can

likewise be affected in the disease [6].Other processes such as oxidative stress have a role in the disease [7].

VLCFA, as well as other fatty acids, can be further esterified with cholesterol. As discussed later, the esterification of VLCFA with cholesterol can also present some abnormalities in X-ALD.

The X-ALD gene

X-ALD results from mutations in the ALD (*ABCD1*) gene (MIM 300371), which encodes a peroxisomal protein (ALDP) that exhibits significant homology to subfamily D of the ATP-binding cassette (ABC) family of transporters. The *ABCD1* gene is located on the long arm of the X chromosome (Xq28) [3,8] spans 21 kb and contains 10 exons. Its encoded protein, ALDP, has significant homology to the peroxisomal membrane protein PMP-70. The function of ALDP is thought to be related to VLCFA catabolism but the precise role of this protein is unclear [8].

ALDP is a half-size protein, after integration into the membrane, ALDP must dimerize to form a full functional transporter. Whether ALDP forms a homodimer or a heterodimer has not yet been determined, although indirect evidence favors homodimerization. The ABCD subfamily is composed of four peroxisomal proteins encoded by the *ABCD1–ABCD4* genes. Heterodimerization of peroxisomal ABC proteins appears to occur, at least for the adrenoleukodystrophy-related protein ALDRP (encoded by *ABCD2*), the 70-kDa peroxisomal membrane protein PMP70 (encoded by *ABCD3*), and the 69-kDa peroxisomal membrane protein PMP69 (encoded by *ABCD4*) [9,10].

Mutations

The known mutations in the *ABCD1* gene are listed at http://www.x-ald.nl. A variety of missense mutations, nonsense mutations, single amino acid deletions, frameshifts, and splice acceptor-site defects have been identified, although there is a clustering of mutations in the amino-terminal half of ALDP including TMD1–6, loop1–5, and the NBD. Sixty percent of all mutations are of the missense type [10,11].

Epidemiological studies have indicated that there is no apparent predilection for any particular ethnic group in the manifestation of the various forms of X-ALD, [12] and its estimated incidence is 1:17,000 in all groups [9,13].

Phenotypes

Clinical manifestations of X-ALD can be divided into two main subgroups: the rapidly progressive cerebral form (CCALD), which shows confluent cerebral demyelination and, usually, childhood onset and the slowly progressive adrenomyeloneuropathy (AMN) phenotype, characterized by axonopathy of the pyramidal and somatosensory tracts and peripheral neuropathy later in life. There is no general correlation between the type of *ABCD1* gene mutation and the clinical phenotype, although polymorphisms related to methionine metabolism can be correlated with demyelination [14].

The diversity of clinical presentations of X-ALD makes an accurate laboratory diagnosis indispensable. As in many genetic diseases, it is important to reliably identify not only affected patients but also heterozygous carriers. When X-ALD is suspected, the first diagnostic step consists of analysis of VLCFA in plasma. The diagnosis of ALD and AMN is established by measurement of the absolute levels of C26:0 as well as by calculation of the C24:0/C22:0 and C26:0/C22:0 ratios. C22:0 is used as a standard because its metabolism, and consequently its plasma and tissue levels are normal in X-ALD [15].

Therapy

Lorenzo's oil represents the most important therapeutic approach available for X-ALD. This therapy was developed by the extraordinary efforts of Augusto and Michaela Odone, parents of the affected boy Lorenzo. The contribution of the late Hugo Moser strongly stimulated research into Lorenzo's oil.

At the beginning of their studies, Rizzo et al. [16] supplemented skin-fibroblasts with oleic acid. Subsequently, a mixture of two monounsaturated fatty acids, oleic acid and erucic acid (cis-13-docosenoic acid, 22:1) was studied. Lorenzo's oil is composed of a mixture of these two fatty acids, administered to patients as glyceryl trioleate and glyceryl trierucate (4:1) [17]. These monounsaturated fatty acids compete with saturated fatty acids for the same microsomal elongating enzymes, thus decreasing the synthesis of VLCFA [18].

The combination of dietary restriction and supplementation with Lorenzo's oil produces significant reductions in plasma VLCFA levels and appears to have a preventive effect in asymptomatic boys whose brain MRI is normal [12,19]. Due to the lack of a control group in the clinical studies performed thus far, it is difficult to evaluate the efficacy of the therapy in the progression of the disease. Current therapies for X-ALD include hormonal replacement therapy for

adrenal insufficiency or hypogonadism, which is needed for all patients with impaired adrenal function, but does not significantly arrest neurological progression [2].

Another therapeutic option is bone marrow transplantation, which has shown to be of long-term benefit in the inflammatory forms of X-ALD [20]. Other therapeutic approaches are under investigation. These include phenylbutyrate, which showed promising results in a mouse model of X-ALD, arginine butyrate and lovastatin. Because overexpression of *ABCD1*'s closest homologue, *ABCD2*, has been shown to partially compensate for ALDP deficiency, there is particular interest in developing approaches that increase the expression of *ABCD2*, and in long-term gene replacement therapy [9, 21, 22, 23, 24].

Androgen And Lipid Metabolism In X-ALD

Although VLCFA accumulate ubiquitously in plasma and tissues of X-ALD patients, specific organs such as the nervous system, the adrenal cortex, and the testes, are particularly affected. Lamellar inclusions, rich in VLCFA esterified with cholesterol, have been found in the adrenals and testes of X-ALD patients [25,26]. The specific involvement of these organs indicates a role of steroidogenesis in X-ALD.

Cholesterol esters are key metabolites of lipid and steroid hormone metabolic pathways

The adrenals are very rich in the esterified form of cholesterol. These esters can be considered crucial metabolites in X-ALD metabolic pathways. After hydrolysis, cholesterol and VLCFA follow distinct pathways. Due to entrapment of cholesterol in VLCFA esters, cholesterol homeostasis is perturbed in X-ALD. This observation is particularly relevant considering that cholesterol is the precursor of all steroid hormones. As discussed above, the metabolism of VLCFA after its hydrolysis, is impaired in X-ALD.

In steroidogenic tissues, free cholesterol can be obtained in three ways: after cholesterol ester hydrolysis, de novo synthesis from acetate, or mainly imported from lipoproteins by specific receptor-mediated pathways [27]. In the adrenals, this mechanism is mediated by adrenocorticotropic hormone (ACTH).

I*n vitro* studies have demonstrated that the membrane microviscosity of cultured human adrenocortical cells exposed to VLCFA is altered, decreasing their ability to respond to ACTH [28]. In X-ALD patients the ACTH-induced response of the adrenals is decreased. Consequently, the use

of cholesterol by lipoproteins is reduced, thus impairing the utilization of cholesterol for steroid hormone synthesis.

The esterification process can be relevant, for X-ALD and other disorders because, steroid hormones can utilize similar enzymatic systems as cholesterol and can be esterified with fatty acids or VLCFA [29].

Involvement of lipoproteins in neurodegeneration

The highest content of cholesterol is found in the nervous system, especially in myelin membranes, where cholesterol together with sphingomyelin and selected glycolipids (cerebrosides and sulphatides) plays a major structural role.

The impairment of mechanisms, involving lipoproteins and related receptor-mediated mechanisms, also plays roles in other neurodegenerative disorders such as Alzheimer's disease. In X-ALD, these deficits may be relevant to the demyelinating processes. Apolipoprotein E (Apo E) is the main apolipoprotein in the nervous system, where it plays a central role in cholesterol utilization.

Apo E is present in steroidogenic tissues such as the adrenals and the testes [30], and its high expression in adrenocortical cells suggests possible roles in modulating the availability of cholesterol for steroidogenesis and in cholesterol ester storage [31]. Androgens can have a positive effect on the nervous system similar to the better known effects of estrogens [32]. The interactions between androgens and Apo E have been considered always more important [33,34].

Role of androgens in X-ALD

The synthesis of steroid hormones from cholesterol is a complex process that leads to the production of molecules with highly diversified biological activity.

Some X-ALD symptoms point to a particular role of androgens in this disease. Alopecia, a symptom related to alterations in the testosterone metabolic pathway, can develop in X-ALD patients [35,36]. Clinical observations have evidenced cases arising even before the onset of puberty. Hypogonadism affects a certain percentage of X-ALD patients and testosterone is used as a therapy but its effects on VLCFA accumulation and clinical symptomatology need to be assessed [37].

A key enzyme in the metabolic route of androgens is 5alpha-reductase (5alpha-R), present in isoforms 1 and 2. Alopecia, affecting a certain percentage of X-ALD patients, is related to

androgen perturbation and to altered activity of 5alpha-R [38]. This enzyme converts testosterone into its main metabolite dihydrotestosterone (DHT), which acts on the same androgen receptors and is 10 times more powerful than testosterone. 5alpha-R also utilizes other steroid hormones as substrates, whose potential roles in X-ALD have never been investigated.

DHT and its metabolite 5alpha-androstan-3alpha,17beta-diol (3alpha-diol), but not testosterone itself, incubated in X-ALD fibroblasts, were able to reduce VLCFA accumulation in a certain percentage of cases in an acute *in vitro* study (6-hour incubation) [39]. The rapid effects of steroid hormones on a variety of biologicl parameters are due to their particular mechanism of action. The same effect of decreased VLCFA accumulation was observed for dehydroepiandrosterone, the main androgen in the adrenals, in a chronic *in vitro* study [40].

When we tested the activity of 5alpha-R by using labeled testosterone, its conversion into DHT was significantly reduced in all X-ALD cases compared with controls. This metabolic conversion is the sum of the activities of both isoforms.

When we evaluated the mRNA abundance of the distinct isoforms, isoform 2 was overexpressed with respect to controls in some X-ALD fibroblasts, whereas isoform 1 was not affected [38]. The expression of the proteins for both isoforms was not published because of the low specificity of the human antibodies tested. In X-ALD fibroblasts, 5alph-R might not be functioning properly and poor DHT production could be the cause of the enzyme's overexpression.

If the activity of 5alpha-R is impaired in X-ALD patients, testosterone given as therapy for hypogonadism could exhibit altered conversion into DHT and its metabolites.

CONCLUSION

X-ALD, which arises from mutations in the gene encoding a peroxisomal protein transporter (ALDP), is a complex disease whose molecular mechanisms and metabolic pathways have not been fully elucidated. Not only is VLCFA metabolism altered but cholesterol homeostasis and lipoprotein-related mechanisms are also perturbed. Lipoprotein metabolic pathways need to be clarified due to their function in transporting both lipids and steroid hormones. The role of androgens requires further research for a better understanding of the pathogenesis of X-ALD.

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