Biochimie 98 (2014) 135–142

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Mini-review

Pathophysiology of X-linked adrenoleukodystrophy☆

J. Berger[a,*,1], S. Forss-Petter[a], F.S. Eichler[b,1]

[a] Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, A-1090 Vienna, Austria
[b] Department for Neurology, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street ACC 708, Boston, MA 02114, USA

ARTICLE INFO

Article history:
Received 25 September 2013
Accepted 22 November 2013
Available online 4 December 2013

Keywords:
ABC transporter
Axonopathy
Demyelination
Inflammation
Leukodystrophy
Peroxisome

ABSTRACT

Currently the molecular basis for the clinical heterogeneity of X-linked adrenoleukodystrophy (X-ALD) is poorly understood. The genetic bases for all different phenotypic variants of X-ALD are mutations in the gene encoding the peroxisomal ATP-binding cassette (ABC) transporter, ABCD1 (formerly adrenoleukodystrophy protein, ALDP). ABCD1 transports CoA-activated very long-chain fatty acids from the cytosol into the peroxisome for degradation. The phenotypic variability is remarkable ranging from cerebral inflammatory demyelination of childhood onset, leading to death within a few years, to adults remaining pre-symptomatic through more than five decades. There is no general genotype–phenotype correlation in X-ALD. The default manifestation of mutations in *ABCD1* is adrenomyeloneuropathy, a slowly progressive dying-back axonopathy affecting both ascending and descending spinal cord tracts as well as in some cases, a peripheral neuropathy. In about 60% of male X-ALD patients, either in childhood (35–40%) or in adulthood (20%), an initial, clinically silent, myelin destabilization results in conversion to a devastating, rapidly progressive form of cerebral inflammatory demyelination. Here, *ABCD1* remains a susceptibility gene, necessary but not sufficient for inflammatory demyelination to occur. Although the accumulation of very long-chain fatty acids appears to be essential for the pathomechanism of all phenotypes, the molecular mechanisms underlying these phenotypes are fundamentally different. Cell autonomous processes such as oxidative stress and energy shortage in axons as well as non-cell autonomous processes involving axon–glial interactions seem pertinent to the dying-back axonopathy. Various dynamic mechanisms may underlie the initiation of inflammation, the altered immune reactivity, the propagation of inflammation, as well as the mechanisms leading to the arrest of inflammation after hematopoietic stem cell transplantation. An improved understanding of the molecular mechanisms involved in these events is required for the development of urgently needed therapeutics.

1. Introduction and clinical aspects of X-ALD pathophysiology

X-linked adrenoleukodystrophy (X-ALD; OMIM, phenotype MIM number #300100) is the most common inherited peroxisomal disorder. The combined incidence of hemizygotes (all phenotypes) plus heterozygous female carriers is 1:16,800 newborns [1]. One of the key clinical symptoms during aging of X-ALD patients is a slowly progressive axonopathy affecting sensory ascending and motor descending spinal cord tracts with 100% penetrance in men and 65% in heterozygous women by the age of 60 years [2]. Thus, X-ALD represents one of the most common monogenetically inherited neurodegenerative diseases.

The progressive dying-back axonopathy represents the core clinical feature of adrenomyeloneuropathy (AMN) in male patients, with onset usually between 20 and 30 years and in heterozygous females with onset between 40 and 50 years. The initial symptoms include progressive stiffness and weakness of the legs, impaired vibration sense in the lower limbs, sphincter disturbances and impotence, as well as scarce scalp hair (alopecia). About 66% of male AMN patients, but less than 5% of female patients, have adrenocortical insufficiency (Addison disease). Abnormal MRI signals of white matter in the centrum ovale, pyramidal tracts in the brainstem and internal capsules have frequently been observed in AMN, but no gadolinium enhancement is present indicating an intact blood–brain barrier and the absence of an acute inflammatory process [3].

Abbreviations: ABCD, ATP-binding cassette transporter subfamily D; AMN, adrenomyeloneuropathy; CALD, cerebral adrenoleukodystrophy; HSCT, hematopoietic stem cell transplantation; VLCFA, very long-chain fatty acids; X-ALD, X-linked adrenoleukodystrophy.

* Corresponding author. Tel.: +43 1 40160 34300.
E-mail address: johannes.berger@meduniwien.ac.at (J. Berger).

[1] ALD Connect, a consortium comprising scientific investigators, clinicians and patient advocates dedicated to the eradication of adrenoleukodystrophy.

0300-9084/$ – see front matter © 2013 The Authors. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2013.11.023

On the other hand, in a total of about 60% of male X-ALD patients, rapidly progressive, inflammatory cerebral demyelination (cerebral X-ALD, CALD) occurs independent of AMN. The onset of inflammation is most common in children (35–40%), before onset of AMN, and less frequent (20%) in adolescents or adults. The inflammatory demyelination starts most often in the midline of the corpus callosum and progresses relentlessly outward as symmetric, confluent lesion in both hemispheres. Clinically, this coincides with a progressive neurologic decline, leading to a vegetative state or death within 3–5 years. Occasionally, a spontaneous arrest of cerebral disease has been observed. Hematopoietic stem cell transplantation or ex vivo gene correction of autologous hematopoietic stem cells can arrest the inflammation in early stages and, thus, provide an efficient treatment for the inflammatory form of X-ALD [4–6]. For pathophysiological considerations, it is important to note that acute inflammation is only observed in the central nervous system (CNS) and not in other tissues of X-ALD patients [3].

In females, cerebral disease is exceedingly rare and has only been verified in a case where both X chromosomes were affected. In an 8-year-old girl with severe CALD, genetic analysis revealed a *de novo* Xq27-ter deletion on the paternal X-chromosome in addition to the maternally inherited *ABCD1* mutation [7]. Approximately 65% of heterozygous females develop an AMN-like syndrome with an average onset later than in the male patients [8]. In addition, female heterozygotes report prominent diffuse pain and are often misdiagnosed with fibromyalgia. Investigations in blood leukocytes reported a "skewed X-inactivation" in 68% of 22 X-ALD carriers with a significant correlation between the extent of the skewing and the severity of neurologic abnormalities [9]. We hypothesize that the ameliorated symptoms in the majority of heterozygous female carriers compared to male patients are due to the X-inactivation patterns in oligodendrocytes and microglia/macrophages. Here the population of cells bearing the normal allele may provide enough functional ABCD1 activity to protect from CALD. When in female heterozygotes symptoms exceed those of AMN, other explanations should be sought [7,10]. The importance of the proportion of cells carrying a healthy allele is emphasized by the observation that in two CALD patients the arrest of inflammation by autologous hematopoietic stem cell gene therapy could be achieved by long-term correction of only about 16% of $CD34^{+}$-derived cells, as determined by ABCD1-positivity of peripheral granulocytes, monocytes, T and B cells [5,6].

2. Biochemical and genetic aspects of X-ALD pathophysiology

Saturated, unbranched very long-chain fatty acids (VLCFA; fatty acyl-chain length of $\geq$22 carbons) are degraded in the peroxisomal matrix by the sequential reactions of the enzymes (acyl-CoA oxidase 1, D-bifunctional protein and either peroxisomal β-ketothiolase 1 or sterol carrier protein x) of the β-oxidation pathway. In X-ALD patients, saturated VLCFA, in particular C26:0, accumulate in tissues and body fluids serving as a diagnostic marker for X-ALD [3].

In all X-ALD patients, mutations affecting the *ATP-binding cassette (ABC) transporter subfamily D member 1* (*ABCD1*) gene, located at chromosome Xq28, have been identified [11]. A summary of more than 643 different *ABCD1* mutations can be found at the web page http://www.x-ald.nl [12]. The *ABCD1* gene encodes the peroxisomal ABC half-transporter ABCD1 (formerly adrenoleukodystrophy protein, ALDP). The ATP-binding domain of ABCD1 is facing the cytosol and substrates are transported from the cytosol into the peroxisome under ATP consumption. It is not clear what determines the substrate specificity of the peroxisomal ABC transporters. For the human ABCD1 protein, CoA-activated VLCFA, such as C26:0-CoA or C24:0-CoA but also C22:0-CoA, are valid substrates; and the degradation of these fatty acids by peroxisomal β-oxidation is strongly reduced in cultured X-ALD fibroblasts [13–15]. In fibroblasts, the peroxisomal import and degradation of C26:0-CoA could be blocked using anti-ABCD1 antibodies [15].

In addition to ABCD1, two other ABC transporters, ABCD2 and ABCD3, are localized in the peroxisomal membrane [16–18]. The functional unit of the ABCD1 transporter is a dimer; *in vivo*, in the peroxisomal membrane, apparently predominantly homodimers are formed, although also heterodimers with ABCD2 or ABCD3, the other two peroxisomal members of the ABCD family are possible [19]. In all tissues investigated so far, the β-oxidation of VLCFA was never completely abolished by ABCD1 mutations, not even by those causing a complete loss of function. We could recently show that in primary fibroblasts of X-ALD patients, the residual activity depends largely on the homologous peroxisomal ABC transporter ABCD3. However, ABCD3 was estimated to be about 45 times less efficient than ABCD1 at mediating a direct or indirect transport of C26:0-CoA, across the peroxisomal membrane [15], which provides an explanation why endogenous ABCD3 is unable to rescue the metabolic defect in X-ALD patients. ABCD2, the closest homolog of ABCD1, would be expected to compensate more efficiently than ABCD3 but is not expressed at relevant amounts in fibroblasts and, thus, under normal conditions does not contribute substantially to the residual β-oxidation activity in this cell type. However, because ABCD1, ABCD2 and ABCD3 could all contribute, directly or possibly indirectly (*via* ω-oxidation), to the transport and, therefore, to the degradation of VLCFA, the extent of the metabolic deficiency in X-ALD probably differs according to the cell type-specific expression profiles of the three peroxisomal ABC transporters (see Chapter 4 of this review).

The lack of a generalized genotype–phenotype correlation in X-ALD becomes clear from three major findings: i) the same *ABCD1* mutation can lead to all possible clinical phenotypes within a single kindred [20]; ii) a complete loss of ABCD1 protein (e.g. early frameshift, nonsense mutations or large deletions); as well as iii) the most common mutation (a two-base pair deletion at c.1415_16delAG in exon 5), has been found in patients with the entire clinical spectrum of X-ALD [21]. However, this does not exclude that individual mutations, which lead to a stable and correctly localized protein that can form dimers and mediate residual transporter activity, can be exclusively associated with the AMN phenotype [22,23].

Interestingly, conventional dietary restriction of VLCFA in X-ALD patients did not lower plasma C26:0 levels [3]. Subsequent studies have demonstrated that the accumulated VLCFA in X-ALD are partially absorbed from the diet but predominantly result from endogenous synthesis through elongation of long- and very long-chain fatty acids [24]. Moreover, it was demonstrated that this elongation system is induced, at least in fibroblasts, of X-ALD patients [24]. This would explain why C22:0 does not accumulate, and often is even slightly reduced, in tissues and cells of X-ALD patients, in spite of the fact that C22:0-CoA is an excellent substrate for ABCD1 [15].

3. Lipidomic aspects of the pathophysiology of X-ALD

In X-ALD, the elongated and insufficiently degraded fatty acids lead to abnormally high levels of VLCFA in various tissues and body fluids. The increased intracellular concentration of VLCFA-CoA esters promotes the incorporation of VLCFA into different complex lipids, where they are normally not enriched. The substrate specificity of different lipid metabolizing enzymes determines the amount of incorporated VLCFA. This varies among different cell types and, importantly, between gray and white matter, thereby effecting crucial regional variation in VLCFA