Adrenal Insufficiency Due to X-Linked Adrenoleukodystrophy

George Kanakis, MD, PhD
Endocrinologist & Clinical Andrologist (EAA Cert.),
Athens Naval & VA Hospital, Athens 11527
geokan@endo.gr
Corresponding author.

Gregory Kaltsas, Md, PhD
Professor of Endocrinology, Dept of Pathophysiology, Laikon University Hospital, Athens 11527, Greece
gkaltsas@endo.gr
Corresponding author.

ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is an inherited neurodegenerative disorder, involving mainly the white matter and axons of the central nervous system, the adrenal cortex, and the testis and a frequent but under-recognized cause of primary adrenocortical insufficiency. X-ALD is caused by a defect in the gene ABCD1 that maps to Xq 28 locus. The primary biochemical disorder is the accumulation of saturated very long chain fatty acids (VLCFA) secondary to peroxisomal dysfunction. The incidence in males is estimated to be 1:21,000 and in females 1:14,000, without any difference in the prevalence among different ethnicities. At least six distinct phenotypes have been described that differ in the age and severity of clinical presentation; however, there is no correlation between X-ALD phenotype and mutations in the ABCD1 gene. When suspected, the diagnosis is established biochemically and prenatal testing is possible in affected families. Currently, there is no satisfying treatment to prevent the onset or modify the progression of the chronic myelopathy of X-ALD. The administration of a mixture of glyceryl-trioleate and glyceryl- trierucate, also referred as Lorenzo's Oil, has been shown to prevent disease progression in asymptomatic patients with cerebral involvement of X-ALD. Allogeneic hematopoietic stem cell (HSC) transplantation is the treatment of choice for individuals with early stages of the cerebral form of the disease. An alternative option for patients without HLA-matched donors is autologous HSC-gene therapy with lentivirally corrected cells. Once adrenal insufficiency is present, hormonal replacement therapy is identical to that of autoimmune Addison’s disease. For complete coverage of this area and all of Endocrinology, visit www.endotext.org.

INTRODUCTION

Leukodystrophies are inherited neurodegenerative disorders, primary affecting the brain myelin. X-linked adrenoleukodystrophy (X-ALD; OMIM:300100)) is the most common leukodystrophy usually presenting as chronic myelopathy and peripheral neuropathy, a clinical entity called adrenomyeloneuropathy (AMN), frequently accompanied by adrenocortical insufficiency (1). The pattern of inheritance is X-linked and the disease is clinically evident in almost all male patients and in more than 80% of female carriers older than 60 years, though with milder manifestations. Occasionally, male patients and very rarely female carriers may develop a rapidly progressive, devastating cerebral form of the disease known as Cerebral Adrenoleukodystrophy (CALD). The pathophysiological basis of the disease is peroxisome dysfunction and accumulation of very long chain fatty acids (VLCFA, >C22:0) due to impaired VLCFA degradation (2).

In the early 20th century, patients with signs and symptoms belonging in the Leukodystrophies spectrum were grouped under the name “Addison–Schilder disease”. It was not until the 1960s that Blaw introduced the term “adrenoleukodystrophy” as a distinct disease entity with X-linked inheritance (3). In 1976 it was shown that the principal biochemical disorder in X-ALD was the accumulation of VLCFA (4). In 1993, the gene responsible for the disease was identified at Xq28 locus and it was subsequently shown to be the ABCD1 gene, which encodes the Adrenoleukodystrophy Protein (ALDP) (5).

This chapter summarizes the latest data in the literature regarding the progress made in elucidating the pathogenesis of the disease, the strategies for early diagnosis, and the results of established as well as of newer experimental therapies.

GENETICS & PATHOPHYSIOLOGY

X-ALD is associated with the accumulation of saturated VLCFA, particularly hexacosanoic (C26:0) and lignoceric (C24:0) acids, due to impaired degradation by the peroxisomes (6,7).

The gene that is defective is referred to as ABCD1 (GenBank accession number: NM_000033). It is located on Xq28, covers 19.9 kb and contains 10 exons (5). It encodes a peroxisomal trans-membrane protein of 745 amino acids, ALDP, a member of the ATP binding cassette (ABC) transport protein family, which helps to form the channel through which VLCFAs move into the peroxisome as VLCFA-
The mode of inheritance of X-ALD is X-linked recessive, thus the possibility of a son of a female carrier developing X-ALD is 50%, whilst 50% of female off-springs will also be heterozygous carriers. All female off-springs of an affected male will be carriers but none of his male off-springs will be affected. Significant intra-familiar phenotype variability has been observed as different clinical phenotypes can occur even among monozygotic twins (9). Fifty percent of ABCD1 mutations lead to a truncated ALDP, whereas many missense mutations result in the formation of an unstable protein (10). The complete absence of a functional ALDP does not necessarily lead to the severe form of X-ALD, implicating the existence of additional factors that could modify disease’s clinical expression.

Environmental factors, such as moderate head trauma, have been shown to trigger the progression of the disease to the severe central nervous system (CNS) form (11). In contrast, mutations with residual transporter activity or over-expression of ALDP-related protein (ALDRP, ABCD2), the closest homolog of ALDP, might prevent this progression (12). Variations in methionine metabolism have also been associated with the wide phenotypic spectrum of X-ALD (13).

The incidence of the disease is estimated to be 1:17,000 (1:21,000 in males and 1:14,000 in females), a disproportion that may reflect the morbidity related to adrenal insufficiency in males preceding the diagnosis of X-ALD. No difference has been observed in the prevalence among different ethnicities (2,14). More than 1000 different mutations have been identified in X-ALD patients and are updated in the website http://www.x-ald.nl. Of these mutations, 51% are missense mutations, 28% frame-shift mutations, 12% nonsense mutations, 6% point mutations and 3% larger deletions of one or more exons (10). Nine hotspot mutations have been identified, which together account for 20% of all cases; the most common among them being a micro-deletion in exon 5 (p.Gln472Argfs*83) (15). Near 4% of patients are affected by a de novo mutation; however in a recent study from Norway, this figure is reported to be as high as 19% (16).

Accumulation of abnormal VLCFA in affected organs is regarded to represent the underlying pathologic process in X-ALD, leading to cell death due to a combination of disruption of cell membranes as well as an induction of oxidative stress and apoptosis (17). Singh and co-workers have demonstrated that the β-oxidation of C24:0 and C26:0 is reduced in fibroblasts from X-ALD patients to approximately 25% of control levels (6), leading to the accumulation of VLCFA-CoA esters in the cytosol. These esters are prone to further elongation carried out by an elongase specific for VLCFA (ELOVL1), further increasing the intracellular levels of VLCFA (18). Transfection of X-ALD cell lines with normal ABCD1 gene restores their capacity to degrade VLCFA (19). Interestingly, injection of C24:0 complexed to phospholipids (C24:0–lysophosphatidylcholine) into the cortex of wild type mice caused widespread microglial activation and apoptosis (20). Such effects were not produced by injections of long-chain lipids (C16:0–lysophosphatidylcholine), implying a fatty-acyl chain length dependent cytotoxicity. In fact, VLCFA are extremely hydrophobic compounds and experimental data suggest that inclusion of C26:0 in a model membrane can disrupt its structure (21). This effect has been shown to be toxic mainly to the myelin-producing oligodendrocytes and Schwann cells, causing the breakdown or loss of the myelin sheath surrounding the nerve cells in the brain and the peripheral nerves respectively. The pathogenesis of X-ALD is summarized in Figure 1.
The pathogenesis of X-ALD: The mutated ABCD1 gene encodes a defective Adrenoleukodystrophy Protein (ALDP) that impedes Very Long Chain Fatty Acids (VLCFAs) from entering the peroxisome to undergo degradation. This leads to accumulation of VLCFAs in the cytosol, which is further aggravated by the elongation of LCFAs carried out by a specific VLCFA elongase (ELOVL1). VLCFA accumulation of has been shown to be toxic by causing the breakdown of cell membranes and by evoking mitochondrial dysfunction as a result of oxidative stress.

Moreover, it is suggested that VLCFA can induce cell death by disturbing calcium homeostasis and/or evoking oxidative stress-related mitochondrial dysfunction (22,23). The theory of elevated oxidative stress has been supported by experiments showing that lymphocytes of X-ALD patients contain low amounts of total and reduced glutathione, whereas the proportion of oxidized glutathione forms is elevated (24). Oxidative stress may impair mitochondrial function by inducing the formation of the Mitochondrial Permeability Transition Pore (MPTP), which represents an increased permeability of the mitochondrial membranes to molecules of less than 1500 Daltons. Induction of the MPTP is associated with mitochondrial swelling and cell death. Cyclophilin D, the most studied component of MPTP has been found particularly expressed in the affected zones of the brain in patients with X-ALD and in the spinal cord of a mouse model of X-ALD. Notably, these changes can be experimentally reversed by treatment with anti-oxidants (25). In addition, the oxidation of cholesterol and linoleic acid, leads to the formation of cholesterol oxide derivatives oxidized at C7 (7-ketocholesterol (7KC), 7β-hydroxycholesterol (7β-OHC), which further aggravate peroxisomal dysfunction (26).

The pathogenic mechanism that triggers the progression to CALD has not been elucidated as yet. Inflammatory demyelination seems to play a key role and plasmatic VLCFA concentration has been positively correlated to the levels of pro-inflammatory cytokines (27). Moreover, a higher VLCFA content has been reported in the non-affected white matter of patients with CALD compared to patients with non-cerebral ALD, possibly representing a precursor lesion (28). Breakdown of the blood-brain-barrier is also implicated by recent studies that have demonstrated an elevation of the matrix metalloproteinases in the cerebrospinal fluid of CALD compared to AMN patients (29). Progression of the cerebral lesions has also been associated with elevated oxidative stress and impaired plasma antioxidant capacity as expressed by superoxide dismutase (SOD) levels. Plasma SOD levels from patients with CALD demonstrated an inverse correlation to brain magnetic resonance imaging (MRI) severity score, while longitudinal samples from the same patients showed a decrease in plasma SOD activity prior to and at the time of diagnosis (30).

Regarding the adrenal gland, abnormal VLCFA accumulation is believed to cause apoptosis and ultimately atrophy of the adrenal cortex. Increased esterification of cholesterol with VLCFAs may further impair cortisol secretion due to a relative shortage of substrate for steroidogenesis (31). Impaired cortisol response to ACTH stimulation usually precedes frank hypocortisolism indicating that loss of adrenal function is a gradual, progressive phenomenon (Figure 2). A possible explanation for this early adrenal dysfunction may be the incorporation of VLCFAs into the adrenocortical cell membrane, which may impair the stimulatory effects of adrenocorticotropic hormone (ACTH) on the adrenocortical cells (32).

Similarly, male patients may present with testicular insufficiency due to the toxicity of VLCFAs on Sertoli and Leydig cells. Testosterone levels are usually in the lower–normal range with elevated luteinizing hormone, while the response to human chorionic gonadotropin is blunted, indicating primary hypogonadism (33). However, in a recent case report, hypogonadism was attributed to tissue specific androgen resistance rather than to primary testicular failure, probably mediated through VLCFA accumulation at the androgen receptor and/or post-receptor levels (34).
PATHOLOGY

In the CNS, ALD is mostly expressed in oligodendrocytes, microglia, astrocytes and endothelial cells, but not in most neurons (35). Lipid inclusions containing cholesterol, phospholipids and gangliosides esterified with saturated VLCFA have been found in all affected tissues, even in morphologically normal regions, indicating that the biochemical abnormality precedes histopathological changes (17). Lesions of the spinal cord and peripheral neural system observed in AMN have been traditionally characterized as a non-inflammatory distal axonopathy with minimal myelin changes (36). Nevertheless, recent studies have shown that affected spinal cord microglia is also vulnerable to phagocytosis, allowing the injury of neurons that reside within an altered metabolic milieu (37). The regions that are mostly affected in AMN are the dorsal columns in the cervical cord segments and the cortico-spinal tracts in the lower thoracic and lumbar segments of the spinal cord (38). AMN can also insult peripheral nerves and this is evident in the epidermis where low nerve fiber densities can be found, indicating a loss of the thin unmyelinated nerve fibers (39). Such alterations may also appear in the optic nerve and can be detected by optical coherence tomography as thinning of the retinal nerve fiber layer (stratum opticum) and of the macula (40).

On the other hand, brain lesions in CALD are evident as large areas of demyelination of the white matter, while the cortex is typically spared. The parieto-occipital regions are affected in 85% of cases, with asymmetric progression of the lesions towards the frontal or temporal lobes, whereas the frontal lobes are involved in only 5% of cases (41). In general, arcuate fibers are spared, except in chronic cases, where axonal loss may be considerable, but myelin loss is usually greater. Lesions may sometimes involve the brainstem, especially the pons, whereas the spinal cord is usually spared, except in cases of bilateral cortico-spinal tract degeneration (42). Occasionally, demyelinated areas may be seen in the cerebral white matter of asymptomatic patients, however, these are scattered in a patchy manner and without signs of inflammation.

The severe and rapidly progressive cerebral form of X-ALD is associated with the evolution of an inflammatory process besides demyelination. Upon microscopic examination, these inflammatory lesions of CALD consist of three distinct concentric zones. The most outward zone contains many lipid-laden macrophages and destruction of myelin albeit with axonal sparing. The second zone also contains many macrophages and a mixture of myelinated and demyelinated axons. A hallmark finding in this zone is perivascular infiltration with lymphocytes (43,44). The pathological process involving this zone is responsible for the gadolinium enhancement observed on MRI scans. The third zone is the innermost and largest one, consisting of a dense grid of glial fibrils and scattered astrocytes. This distinct zonal pattern of CALD lesions can be also detected on MRI scans (45).

VLCFA accumulation is also evident in the adrenal cortex of patients with X-ALD, particularly in the zona reticularis and the zona fasciculate, with a relative sparing of the zona glomerulosa. Microscopically adrenocortical cells become balloononed and striated due to the accumulation of lamellae and lamellar-lipid profiles, which consist of cholesterol molecules esterified with saturated VLCFA (31). These distinct pathological features can also be demonstrable in the fetal adrenal gland, indicating that accumulation of VLCFA is present already in utero. Similar lesions can be found in the testes of X-Ald men, primary affecting Leydig cells that are responsible for steroidogenesis (46).

CLINICAL MANIFESTATIONS OF X-ALD

The range of clinical expression of X-ALD varies widely. Tables 1a and 1b summarize the principal clinical phenotypes. A hallmark that distinguishes X-ALD from other inherited neurodegenerative diseases is that patients are asymptomatic at birth (1); however, in male patients adrenocortical insufficiency may develop even during the first year of life (47). In contrast, AMN the most frequent form of the disease is rarely present before adulthood and reaches 100% penetration over the age of 55 years. The presence of asymptomatic males beyond this age is exceptional. In fact, ALD is more accurately considered as a progressive disorder and this phenotypic classification is due to systematic reasons: approximately 60% of male patients, including 20% of those initially diagnosed as AMN, will eventually present CALD during their lifespan with the childhood form being the most severe (48). The clinical course of the disease and particularly the presence of CALD is thought to be the result of an interplay between genetic and environmental factors (Figure 3).
Adrenal Insufficiency Due to X-Linked Adrenoleukodystrophy

Adrenomyeloneuropathy

AMN is a disorder that affects mainly the long tracts of the spinal cord characterized by an absent or mild inflammatory response (36,38) and such patients may survive to the eighth decade of life. The disease onset is usually between the third and fourth decade and in two-thirds of patients, the neurologic disability progresses slowly over a span of 10-15 years. In the remaining, a more rapid progression is observed within 3–5 years. The primary manifestation is gait disorder due to spastic paraparesis and sensory ataxia with impaired vibration sense, which mainly affects the lower limbs; loss of dexterity or strength in the arms is exceptional (49). Sphincter dysfunction initially presenting as urge complaints, progressing to full incontinence as well as impotence are accompanying features; whereas in some cases a characteristic diffuse hair loss is observed (50). Signs of peripheral neuropathy may also be present, however they are usually masked by the most prominent clinical features of myelopathy (51). The course of AMN is gradually progressive, with most patients losing ambulation by the 6th decade of life (52).

Up to 63% of AMN patients are reported to have additional cerebral demyelination (53) and subtle cerebral manifestations are often present. The rate of depressive illness appears to be elevated at least two-fold (54) and mood change fluctuations according to the hormonal replacement of adrenal insufficiency frequently occur. Approximately 20-30% of the AMN patients may develop at a later stage progressive cerebral involvement in which the inflammatory response is present (55). In such cases, the survival is reported to be as poor as in childhood cerebral adrenoleukodystrophy. However, this risk decreases markedly after the age of 45 years.

Cerebral ALD

The risk of a newborn male carrier of the ABCD1 mutation developing CALD is 35 – 40% between the ages of 5 and 12 years. Disease onset prior to 3 years of age is rare and this risk is substantially lower among boys whose brain MRI remains normal until 7 years of age (56). The earlier the onset of disease, the more rapid the progression is, whereas patients may remain asymptomatic as long as demyelinating lesions are not visible on brain MRI. Generally, the onset of CALD is insidious, and can be confused with the Attention Deficit Hyperactivity Disorder which is characterized by hyperactivity, impulsiveness and an abrupt decline in school performance. Cognitive deficits can be accompanied by neurologic deficits such as hemiplegia or quadripareisis, cerebellar ataxia, impaired central auditory discrimination, visual field defects, cortical blindness, and often seizures (1).

The presentation in adults is similar and initially may appear as a psychiatric disturbance resembling the manifestations of obsessive-compulsive personality disorder (57). These psychiatric symptoms may precede frank motor or cognitive changes by some years. Infections or head trauma may trigger the onset of CALD, but usually no extrinsic factor is identified (58). Nevertheless, once the disease becomes inflammatory, as evidenced by the post-contrast enhancement of the borders of the brain lesions as shown in MRI, it usually progresses rapidly to a devastating form, leading to a vegetative state within two to five years (59). Interestingly, 10% of males with imaging evidence of CALD may never enter into the active inflammatory stage, a phenotype referred to as “chronic or arrested cerebral X-ALD” (60); however it is possible that these lesions may be reactivated many years later.
Female Heterozygotes

Contrary to previous beliefs, that considered female heterozygotes as being asymptomatic, it is now accepted that approximately 65% of such individuals will develop an AMN-like syndrome by the age of 60. In general, the onset of neurologic symptoms occurs at a later age than in males, and there is a strong association between the onset of symptoms and age. Typically symptoms appear in the fourth to fifth decade of life and disease manifestations are less severe with a notable occurrence of early fecal incontinence (61). Scanty scalp hair can also be found in females (62). Only a few females have been reported to develop CALD and this has been attributed to skewed inactivation of the X-chromosome carrying the mutated ABCD1 gene (63).

Incidence of Primary Adrenal Deficiency in X-ALD

The incidence of primary adrenal insufficiency (PAI) in males with X-ALD has been reported to be 50-86% and the corresponding figures in the various phenotypes are shown in Tables 1 and 2. The incidence of PAI in the patients with the childhood cerebral forms of ALD appears to be higher than in the AMN patients. While many patients have both neurologic involvement and adrenal insufficiency, a considerable number has only one or the other. The patients with the "Addison only" phenotype by definition are free of demonstrable neurologic involvement; however, due to the progressive nature of the disease, many individuals in this category will later develop neurologic involvement. ALD is the cause for up to 20 percent of male cases of idiopathic Addison’s disease. Biochemical evidence of adrenal insufficiency can be present for up to two years before the development of relevant clinical signs and the youngest boy detected with subclinical PAI was 5 months of age (47). Elevated ACTH levels and impaired cortisol response to ACTH administration are the most frequent findings. Frank hypoaldosteronism with salt wasting is not frequent, but impaired aldosterone response to ACTH may be observed in approximately one third of men with X-ALD (64).

Addison’s disease is rare in women heterozygous for X-ALD (1% or less), and considerably less frequent than the AMN-like syndrome, which develops in approximately 50% of women in middle age or later. Even though it is rare for heterozygous women to show clinically evident adrenal insufficiency, post-mortem studies have revealed adrenal abnormalities resembling those in affected males (65). When more subtle tests of adrenal function, such as the response to ovine corticotropin-releasing-hormone, were performed, subnormal responses were demonstrated in five of eight women with previously normal ACTH stimulation tests (66).

Table 1.
X-ALD Phenotypes in Males

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Description</th>
<th>Estimated Relative Frequency</th>
<th>Adreno-cortical Insufficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood cerebral</td>
<td>Onset 3-10 years. Progressive behavioral, cognitive, neurologic deficits.</td>
<td>31-35%</td>
<td>79%</td>
</tr>
<tr>
<td></td>
<td>Total disability often within 3 years.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent cerebral</td>
<td>Like childhood cerebral; somewhat slower progression</td>
<td>4-7%</td>
<td>62%</td>
</tr>
<tr>
<td>Adult cerebral</td>
<td>Dementia, behavioral disturbances focal neurologic deficits without preceding adrenomyeloneuropathy</td>
<td>2-3%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Adrenomyeloneuropathy</td>
<td>Onset 28 ± 9 years. Slowly progressive paraparesis, sphincter disturbances</td>
<td>40-46%</td>
<td>50-70%</td>
</tr>
<tr>
<td>Addison only</td>
<td>Primary adrenal insufficiency without neurologic involvement.</td>
<td>Varies with age. Up to 50% in childhood</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Most common onset 5-7 years. Most eventually develop AMN or cerebral forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>No demonstrable neurologic or adrenal involvement</td>
<td>Common before 4 years. Diminishes with age.</td>
<td>50% plus with testing</td>
</tr>
</tbody>
</table>

Table 2.
Phenotypes in Female X-ALD Carriers

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Description</th>
<th>Estimated relative</th>
</tr>
</thead>
</table>
X-ALD appears to be a more frequent cause of Addison's disease in males than is generally recognized. Lauretti et al. found that 5 of 14 male patients between 12 and 45 years of age, previously diagnosed as having PAI, had abnormally high plasma VLCFA levels in the setting of X-ALD (67). Jorge et al. diagnosed X-ALD in ten of 37 patients with idiopathic Addison’s Disease (27%), and found that the incidence was 100% in patients in whom adrenal insufficiency became evident before 7.5 years of age (68). These findings are of great clinical importance, as prompt diagnosis of X-ALD has profound implications for prognosis, therapy and genetic counseling. It is therefore important that screening for X-ALD is carried out in all male patients with idiopathic Addison’s Disease. The need to do so is particularly relevant in patients in whom PAI was manifested before 7.5 years of age.

**DIAGNOSIS OF X-ALD**

The plasma assay for VLCFA is the hallmark diagnostic procedure as it is very reliable for the identification of affected males (70). VLCFA levels are already increased on the day of birth and in untreated patients remain approximately the same throughout life. Testing, typically includes three VLCFA parameters: the level of hexacosanoic acid (C26:0) and tetracosanoic acid (C24:0), and the ratio of these two compounds to docosanoic acid (normal values of C24:0/C22:0 ratio <1.0 and C26:0/C22:0 ratio <0.02). Hexacosanoic acid is the one most consistently elevated, and is therefore considered to be diagnostic of the disease. It should be noted though, that VLCFA levels are also elevated in some other peroxisomal disorders, whereas they can be falsely elevated in patients on ketogenic diets (71). On the other hand, grape-seed and mustard-seed oils may cause false negative results. So far, no correlation has been established between the degree of VLCFA elevation and the severity of the disease or the onset of certain manifestations (72). The assay can also be used to identify asymptomatic patients by screening members of the extended family (49). Notably, false negative results may occur in approximately 15 to 20% of obligate female heterozygotes. In such patients, mutation analysis by molecular genetic testing of the ABCD1 gene locus is the most accurate method for a definitive diagnosis (73). Nevertheless, in some cases mutation analysis may reveal a sequence variant of the ABCD1 gene with unknown clinical significance, presenting a diagnostic conundrum for the clinician.

The diagnosis of X-ALD should be sought in:

1. Boys with progressive behavioral, cognitive or neurologic disturbances beginning at 3 years of age or later.
2. Males with Addison's disease in whom the etiology has not been defined (e.g. absent auto-antibodies against adrenal antigens). Since the plasma VLCFA assay is non-invasive, and the practical and genetic implications of the diagnosis of X-ALD are significant, the VLCFA assay could be part of the routine initial evaluation of male patients with Addison’s disease.
3. Men and women with progressive myelopathy. AMN is often misdiagnosed as multiple sclerosis. Nevertheless, a relapsing and remitting evolution is never seen in AMN. The diagnosis of X-ALD should be considered even when there is no clinical or biochemical evidence of PAI. In a large series from Germany adrenal function was normal in 20 of 41 men with AMN, and PAI occurred in less than one percent of women with and AMN-like syndrome (74).
4. Patients in whom PAI occurs in combination with neurologic disability (Table 3).
5. Patients who are at genetic risk of having X-ALD on the basis of pedigree. Because X-ALD is X-linked recessive, a large number of relatives in the nuclear and extended family are at genetic risk. Detection of asymptomatic patients is particularly important, since
therapeutic interventions have the greatest chance of success when clinical manifestations are still mild.

**Table 3.**

Conditions in Which Adrenocortical Insufficiency is Associated with Neurologic Dysfunction.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Nature of Neurologic Disturbance</th>
</tr>
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<tbody>
<tr>
<td>X-linked adrenoleukodystrophy</td>
<td>See text</td>
</tr>
<tr>
<td>Neonatal adrenoleukodystrophy</td>
<td>Autosomal recessive; early onset; dysmorphic features, multiple organ involvement</td>
</tr>
<tr>
<td>Triple A syndrome (OMIM 231550)</td>
<td>Achalasia, alacrima, adrenal insufficiency</td>
</tr>
<tr>
<td></td>
<td>Peripheral neuropathy, cerebellar ataxia</td>
</tr>
<tr>
<td>Glycerol kinase deficiency</td>
<td>Mild dementia, autosomal recessive, gene defined</td>
</tr>
<tr>
<td></td>
<td>Autosomal recessive. Psychomotor retardation</td>
</tr>
</tbody>
</table>

**Newborn Screening**

Newborn screening (NBS) is justified for a disorder, provided that a therapy is available and that early diagnosis allows timely implementation. This is particularly relevant for X-ALD after the promising results of hematopoietic stem cell transplantation (HSCT): early diagnosis at birth would allow the early detection of PAI in order to initiate timely adrenal steroid replacement therapy, whereas early detection of CALD would permit HSCT before severe neurologic impairment is established. Important improvements towards this target was the development of mass spectrometry methods to assess the presence of VLCFA in dried-blood spots as well as a combined liquid chromatography/tandem mass spectrometry (LC-MS/MS) high-throughput assay that could measure VLCFA enriched lysophosphatidylcholine (lysoPC), thus providing the technical background for NBS (75). Eventually, New York State (NYS) in 2013 was the first authority to include screening for X-ALD in the NBS program, while more states are expected to add screening for X-ALD to their own NBS program since it has been added to the Recommended Universal Screening Panel (RUSP) (76).

NYS NBS for X-ALD is based on a 3-tier algorithm. The first tier, refers to all newborns and includes C26:0 VLCFA assessment in dried blood spots. In case of a pathological result, the second more specific tier, measuring C26:0-lyso-PC is employed. If the C26:0 lyso-PC is also elevated, then sequencing of the 10 exons of the ABCD1 gene is performed as part of the third tier of screening. If sequencing reveals a relevant mutation, a confirmatory VLCFA analysis should be ordered in an independent laboratory. If ABCD1 mutation analysis is negative, then a rare peroxisomal disorder should be sought (76). The whole procedure is reported to be both highly sensitive and specific and might be used as a template to diagnose X-ALD in symptomatic patients. It is however; still premature to draw conclusions about the health and social impact that NBS has on the diagnosed individuals and their families.

**Genetic Counseling**

As soon as an index case is detected either as a consequence of symptoms or as a result of NBS, genetic counseling should be offered to the family. If the index case is male, testing should be offered to his mother and female offspring. If the mother is confirmed to be a carrier for an ABCD1 mutation, testing should also include all the male siblings of the index case. If the index case is female, initial testing should include both parents. Regarding mutation testing of minor females of an affected family, there is no consensus whether it should be performed on a routine base. (76).

Prenatal diagnosis is an option for women who are heterozygous carriers of the ABCD1 gene (77). Recently, a non-invasive prenatal determination of fetal sex being able to detect Y chromosome sequences in maternal blood by molecular techniques (78). However, since a significant number of heterozygous women will develop AMN in adulthood, prenatal diagnosis may also be considered for a female fetus. Sex determination along with ABCD1 mutational analysis can be performed on a fresh chorionic villus sample (CVS) at 11–13 weeks of pregnancy. Amnioparacentesis can still be performed at 15–18 weeks of gestation; however, this option might delay the decision-making process since amniotic cell culture requires an additional 2–3 weeks. If the ABCD1 gene mutation has not been recognized but the maternal phenotype is highly suspicious, prenatal diagnosis of a male fetus can be done by the measurement of VLCFA levels in cultured CVS cells or amniotic cells (79). Preimplantation genetic diagnosis is an additional option, particularly useful for heterozygous female carriers who have already had pregnancy interruption due to prenatal diagnoses of an X-ALD male fetus.

**Imaging**

All individuals with confirmed ALD/AMN complex should undergo neuroimaging to determine if cerebral involvement is present. Brain MRI is the procedure of choice and should be performed every 6 months in pre-symptomatic male patients between 3 and 12
years of age and yearly after that up to 45 years (80). Brain MRI abnormalities precede symptoms in patients with the cerebral forms of X-ALD (56). Findings are always abnormal in symptomatic patients, demonstrating cerebral white matter demyelination (Figure 4). The lesions typically begin in the splenium of the corpus callosum before gradually expanding to the occipito-parietal region and they are usually bilateral, but occasionally can be limited to only one side, particularly if a previous head trauma has triggered CALD (11). The presence of contrast enhancement just behind the outermost edge of the lesions as seen in T1-weighted images (WI), heralds the progression to inflammatory devastating form of CALD (59). Loes et al. has introduced a grading system to assess the degree of MRI abnormalities in X-ALD (81). This is a 32-point scale score (0: normal, 32: most severe) that assesses the degree and extent of hyperintense lesions on FLAIR or T2W images as well as the degree of regional atrophy, and has proven to have predictive value for the response to HSCT (82). Regarding AMN, MRI of the spinal cord is unremarkable on standard sequences, it can however show atrophy in advanced cases (83). Contrast enhancement is not observed in AMN, since inflammation is not a feature of extra-cerebral lesions.

Functional imaging such as Proton MR Spectroscopy may detect white matter abnormalities that are not apparent on conventional MR imaging and may predict disease progression (84). A decrease of N-acetyl-aspartate (NAA)/creatinine ratio is observed, reflecting axonal loss and an increase of choline / creatinine and myo-inositol/creatine ratios associated lipid turnover changes (85). Brain F18 fludeoxy-glucose positron emission tomography (PET) may reveal hypometabolic regions particularly in cerebellum and temporal lobe areas, before lesions emerge in MRI (86). In contrast, hypermetabolism may be evident in the frontal lobes, related to the clinical severity of the disease (57).

Figure 4.

MRI of a patient with CALD, showing reduced volume and increased signal intensity of the white matter localised mainly at the parieto-occipital regions. The anterior white matter is spared.

**Testing of Adrenal Function**

Adrenal function should be evaluated as soon as the diagnosis of X-ALD is set by measurement of basal (8:00 AM) plasma ACTH and cortisol concentrations. A combined ACTH value more than twice the upper limit of normal (>100 pg/mL) with a cortisol value of less than 10 mcg/dL (270 nmol/L) make the diagnosis of PAI high likely and should prompt the initiation of proper cortisol replacement therapy (87). If results are equivocal (e.g. normal cortisol levels but elevated ACTH), a formal stimulation test following ACTH / cosyntropin administration should be offered. A response of cortisol less than 18 mg/dL, 60 minutes after the administration of cosyntropin is also indicative of PAI, which requires replacement therapy at least in situations of physical stress (surgery, acute febrile illness, vomiting etc.). In case the diagnosis of X-ALD is made in infancy, cosyntropin stimulation test is also indicated to diagnose PAI, since ACTH and cortisol production is not predictable until 6 to 12 months of age (88). Regarding asymptomatic patients with X-ALD, according to the NYS NBS guidelines, screening for PAI should be repeated every 6 months (76). Evaluation for mineralcorticoid deficiency is not currently recommended, due to the relative sparing of the zona glomerulosa, however it should be considered in the presence of symptoms, such as salt-craving and polyuria (89).

**THERAPY**

**Allogeneic Hematopoietic Stem Cell Transplantation**

Therapy of the neurologic aspects of X-ALD is a major challenge. Currently, there is no satisfying treatment to prevent the onset or modify the progression of the chronic myelopathy of X-ALD. Allogeneic HSCT is the treatment of choice for individuals with early stages of cerebral involvement of X-ALD, which may increase disease specific survival and can lead to long-term stabilization and occasionally improvement (90–92). Stem cells can be harvested from peripheral blood, bone marrow, and umbilical cord blood of immune-compatible donors. Although the mechanism of this effect is still unclear, bone marrow cells do express the ABCD1 gene and plasma VLCFA levels are reduced after bone marrow transplantation, offering a useful biomarker for the assessment of engraftment, graft failure, or rejection (93). It has been shown that bone marrow-derived cells do enter the brain-blood barrier and that a portion of perivascular microglia is gradually replaced by donor derived cells (94). HSCT may also diminish the brain inflammatory response as well as lipid peroxidation and protein damage. Stabilization of the disease is usually evident about 6 months after the transplantation. The outcomes of allogeneic HSCT for CALD have been mainly studied among adolescents: the 5-year survival among boys of Loes score < 10 is as high as 89%, whereas in those with a score ≥10 is only 40%. On the other hand, the cumulative incidence of transplantation-related mortality is 8% (92). A recent study has also evaluated the potential long-term neurological benefits and the complications of allogeneic HSCT in adult CALD, with less compelling results (95).

Current strategy is to monitor asymptomatic patients by MRI at 6-month to yearly intervals depending on their age and consider HSCT when the MRI abnormality is advancing and clinical disability is still mild (96). Because HSCT carries a substantial mortality risk (5%), it is not recommended for patients who already have advanced cerebral involvement (e.g. IQ<80 and a Loes score ≥10), because there is evidence that such an approach may not reverse severe deficits and in some instances may accelerate disease progression (97). HSCT has not been tested systematically in AMN because of concern that the risk-benefit ratio may not be favorable: up to 50% of AMN patients will never develop cerebral involvement, whereas it is high unlikely that HSCT will affect the non-inflammatory distal axonopathy which is the main pathological feature in AMN (36). Moreover, in retrospective series of patients who successfully underwent HSCT for CALD in childhood, it was shown that it could not prevent the onset of AMN in adulthood (98). It is still a question whether the progression of myelopathy in X-ALD might be slowed down by HSCT.

In case of patients without HLA-matched donors or adult patients with CALD (given the higher mortality risk of allogeneic HSCT compared to children), an alternative option is autologous HSC-gene therapy with lentivirally corrected cells (19). In this procedure, CD34+ cells from X-ALD patients are transfected ex vivo using a lentiviral vector encoding the wild-type ABCD1 cDNA. As a result of this therapy, 7–14% of granulocytes, monocytes, T and B lymphocytes express the lentivirally encoded ALDP. In a recent phase 2-3 study including 17 boys, short-term clinical outcomes were reported to be comparable to that of allogeneic HSCT (19). Nevertheless, concerns regarding long-term efficacy, biosafety of lentiviral vectors, as well as the high cost of this therapy need to be taken into account (100,101). An alternative approach is performing allogeneic HSCT from healthy siblings conceived after preimplantation HLA matching, which offers the possibility of selecting unaffected embryos that are HLA compatible with the sick child (102). Regarding adrenal function, there is no evidence for the reversal of adrenal failure after either HSCT or autologous HSC gene therapy (103).

**Dietary Treatment**

Other therapeutic options include dietary therapies with restriction of fat intake and particularly of VLCFAs and saturated fats to avoid their accumulation. In order to achieve this, total fat intake is restricted to 15% of the total calorie supply and a maximum of 5-10 mg of C26:0 are allowed on a daily basis (Table 4). However, since the majority of VLCFA are of endogenous origin (104), this approach is not sufficient. A mixture of glycerol trioleate and glycerol trierucate, also referred as Lorenzo's Oil (LO), which is shown to halt the elongation of VLCFA by inhibiting ELOVL1, has also been applied (105).
This therapy normalizes plasma VLCFA levels within four weeks and in a recent study involving 89 asymptomatic X-ALD patients with normal brain MRI, dietary treatment with LO resulted in a twofold or greater reduction in the risk of developing the childhood cerebral form of X-ALD (106). However, its therapeutic effects in patients who are already symptomatic has been disappointing. Besides, it is widely admitted that LO therapy does not improve adrenal function (52). The daily dosage of LO is 2-3 mL/kg/day and is usually well tolerated. Its most severe side effects are thrombocytopenia and lymphopenia, which usually revert to normal after treatment discontinuation. Treatment with LO may be continued for an indefinite time until disease progression and/or severe side effects occur. It is not recommended in children under one year of age, as it causes a decrease in the levels of other fatty acids, particularly of docosahexaenoic acid, which is essential for neurocognitive development.

### Table 4.

Dietary restrictions in X-ALD. Adopted form ref. 2.

<table>
<thead>
<tr>
<th>Foods rich in VLCFAs</th>
<th>Foods rich in saturated fat</th>
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</thead>
<tbody>
<tr>
<td>Vegetable oils</td>
<td>Vegetable oils</td>
</tr>
<tr>
<td>Fatty fish and meat</td>
<td>Fatty fish and meat</td>
</tr>
<tr>
<td>Plant cover and cuticle</td>
<td>Milk and milk products</td>
</tr>
<tr>
<td>Fruit peel and seeds</td>
<td>Egg yolk</td>
</tr>
<tr>
<td>Grains and nuts</td>
<td>Industrial pastry</td>
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### Experimental Therapies

Current research on novel treatment options for X-ALD is focused on a) agents that bypass the defective ALDP by inducing alternative pathways for VLCFA degradation, b) combinations of antioxidants that diminish oxidative stress, c) agents that halt VLCFA elongation, and d) the use of neurotrophic factors.

Apart from ALDP, three additional closely related ABC half-transporters exist: ALDRP, PMP70 and PMP69, which are located on the membrane of peroxysomes. ALDP must dimerize with one of these half-transporters to form a functional full-transporter (107). Overexpression of ABCD2, the gene producing ALDRP has been shown to compensate for ABCD1 deficiency and ameliorate VLCFA production from X-ALD cell lines (12). Valproic acid (VPA), a widely used anti-epileptic drug, 4-phenylbutyrate, and other histone deacetylase inhibitors, are known inducers of the expression of ALDRP. In a 6-month pilot trial of VPA in X-ALD patients marked correction of the protein oxidative damage was observed (108). Other agents known to evoke induction of the ABCD2 gene are ligands to several nuclear receptors: fibrates for PPAR alpha, thyroid hormones and thyromimetics, retinoids, and lately LXR antagonists and are being tested in vitro and in vivo for the treatment of X-ALD (109–111). Lately, it has been shown that AMP-activated protein kinase (AMPKα1) is reduced in X-ALD, raising the question if metformin, a well-known AMPKα1 inducer, may have a therapeutic role for X-ALD (112).

Regarding the use of antioxidative treatments, experimental data show that treatment of ABCD1 null mice with a combination of antioxidants containing α-tocopherol, N-acetyl-cysteine and α-lipoic acid reversed oxidative damage, axonal degeneration, and locomotor impairment (22). Similar results have been observed with the oral administration of pioglitazone, an agonist of the PPAR gamma receptor, which restored oxidative damage to mitochondrial proteins and DNA, and reversed bioenergetic failure. Lately, bezafibrate, a PPAR pan agonist has been demonstrated to reduce VLCFA levels in X-ALD fibroblasts (113). The mechanism for this action is by decreasing the synthesis of C26:0 through a direct inhibition of ELOVL-1 and subsequent fatty acid elongation activity. Unfortunately, these actions could not be confirmed in vivo as in a recent clinical trial, bezafibrate was unable to lower VLCFA levels in plasma or lymphocytes of X-ALD patients (114).

The options for treatment of the advanced progressive form of CALD remain limited. Even though the presence of inflammatory lesions is well recognized, trials of immunosuppressive therapies have yielded poor results. Cyclophosphamide, interferon, IVIG, and other immunomodulators have been used without success (80,115). Promising results have been extracted by the use of the antioxidant N-acetyl-L-cysteine as adjunctive therapy to HSCT in patients with advanced CALD (116).

### Treatment of Adrenal Insufficiency and Hypogonadism

For those patients with X-ALD who have impaired adrenal function glucocorticoid replacement therapy is mandatory. Glucocorticoid replacement requirements are generally the same as in other forms of PAI whereas most patients may not require mineralocorticoid replacement. While there is one report of substantial improvement of neurologic function when replacement therapy was administered to a patient with AMN (117), the general impression is that adrenal replacement therapy does not alter neurologic progression.

Male patients who present clinical manifestations of hypogonadism and confirmed low serum testosterone levels, should be treated with...
testosterone. Nevertheless, careful evaluation should be warranted, since impotence, in most instances may imply spinal cord involvement or neuropathy, rather than testosterone deficiency.

REFERENCES

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103. Petryk A, Polgreen LE, Chahla S, Miller W, Orchard PJ. No evidence for the reversal of adrenal failure after hematopoietic cell transplantation in X-linked adrenoleukodystrophy. Bone Marrow Transpl. 2012; [PMC free article: PMC4547590] [PubMed: 22388279]


