

# ADRENAL INSUFFICIENCY DUE TO X-LINKED ADRENOLEUKODYSTROPHY

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#### ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is a rare inherited neurodegenerative disorder, involving mainly the white matter and axons of the central nervous system and the adrenal cortex and is 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 1:14,700 live births, without any difference among different ethnicities. X-ALD presents with a variable clinical spectrum that includes primary adrenal insufficiency, myelopathy, and cerebral ALD; however, there is no correlation between X-ALD phenotype and specific mutations in the ABCD1 gene. When suspected, the diagnosis is established biochemically with the gold standard for diagnosis being genetic testing (ABCD1 analysis). Currently, there is no satisfying treatment to prevent the onset or modify the progression of the neurologic or endocrine components of the disease. 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.

#### INTRODUCTION

Leukodystrophies are inherited neurodegenerative disorders, primarily 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 clinical presentation. Occasionally, male patients and very rarely female carriers may develop a rapidly progressive, devastating cerebral form of the disease known as Adrenoleukodystrophy (CALD). Cerebral The pathophysiological basis of the disease is peroxisome dysfunction and accumulation of very long chain fatty acids (VLCFA) due to impaired VLCFA degradation (2).

In the early 20th century, patients with signs and symptoms belonging to 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 the 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 newer experimental therapies.

# **GENETICS & PATHOPHYSIOLOGY**

ALD is a rare progressive neurodegenerative disorder with an annual incidence of 1:14,700 live births (considering both hemizygous males and heterozygous females), and no marked difference between males and females (6).

X-ALD is caused by mutations in the ABCD1 gene located on the X chromosome (Xq28), which covers

19.9 kb and contains 10 exons (7) with approximately 900 different mutations reported (8). Mutations in the ABCD1 gene include missense, nonsense, frameshift, and splice-site variants (9). However, identical variants can result in highly diverse clinical phenotypes, suggesting the presence of unknown additional factors that have an impact on the expression of the disease (2). Thus, there is a lack of a genotype-phenotype correlation in ALD (10, 11).

The ABCD1 gene encodes a peroxisomal transmembrane 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-CoA (12). ALDP deficiency leads to an impaired peroxisomal β--oxidation of saturated straight-chain very long-chain fatty acids (VLCFA) (13) resulting in the accumulation of VLCFAs in plasma and tissues, including the white matter of the brain, spinal cord, and adrenal cortex (14). Chronic accumulation of cholesterol with saturated VLCFA in the zona fasciculata and reticularis of the adrenal cortex is believed to result in cytotoxic effects, apoptosis and ultimately atrophy of the adrenal cortex and with loss of cortisol production (15, 16). The pathogenesis of X-ALD is summarized in Figure 1.



Figure 1. The pathogenesis of ALD. Adapted with permission from www.adrenoleukodystrophy.info.

The mode of inheritance of X-ALD is X-linked recessive (figure 2), thus the possibility of a son of a female carrier developing X-ALD is 50%, whilst 50% of female offsprings will also be heterozygous carriers. All female offsprings of an affected male will be carriers but none of his male offsprings will be affected. Since X-ALD is an X-linked inherited disorder, males are more severely affected than females. Some heterozygous X-ALD females can exhibit symptoms due to skewed X-chromosome inactivation or other genetic factors. Females who

carry the defective gene used to be referred to as "carriers" because it was thought that only a small percentage of them will develop clinical symptoms. However, it has been recently shown that 80% of female patients will eventually develop symptoms although milder in severity than males. The most likely explanation for this clinical manifestation is the presence of a normal copy of the *ABCD1* gene on their other X-chromosome that protects women with ALD from developing the brain variant (cerebral ALD) or other still unexplored genetic factors.



#### Figure 2. Adapted with permission from www.adrenoleukodystrophy.info.

Significant intra-familiar phenotype variability has been observed as different clinical phenotypes can occur even among monozygotic twins (17). Fifty percent of ABCD1 mutations lead to a truncated ALDP, whereas many missense mutations result in the formation of an unstable protein (18). 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 the disease's clinical expression. Factors, such as moderate head trauma, have been shown to trigger the progression of the disease to the severe central nervous system (CNS) form (19), but other unknown genetic and environmental factors are likely required for the development of CALD. 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 (20). Variations in methionine metabolism have also been associated with the wide phenotypic spectrum of X-ALD (21).

#### CLINICAL MANIFESTATIONS OF X-ALD

The range of clinical expression of X-ALD varies

widely. The main phenotypes of X-ALD are primary adrenal insufficiency (Addison's disease), myelopathy, and cerebral ALD (CALD), either alone or in any combination.

The most devastating form of ALD is CALD which presents early in life between 4-12 years of age, affecting 1/3 of boys with X-ALD and is rare after 15 years of age (22). It is characterized by inflammatory demyelination mainly of the supratentorial and infratentorial white matter and brain magnetic resonance imaging (MRI) findings usually precede clinical symptomatology (23). The onset of CALD is insidious, with symptoms at school age such as learning, behavioral, and cognitive disabilities often being attributed to Attention Deficit/Hyperactivity Disorder that delay the diagnosis. As the disease overt neurologic deficits become progresses. apparent, including cortical blindness. central deafness, hemiplegia, and guadriparesis. Progression of the disease is often rapid, leading to death within 5 - 10 years following diagnosis (24). Most men who do not develop CALD during childhood develop myelopathy in adult life.

Myelopathy manifests later in life, typically presents in adult males between 20 and 40 years of age, with a median age at onset of 28 years (25). The primary clinical presentation is spinal cord and peripheral nerve dysfunction, leading to progressive spastic paraparesis, abnormal sphincter control, sensory ataxia, and sexual dysfunction. Symptoms are progressive over years or decades, with most patients losing unassisted functionality by the  $5^{th} - 6^{th}$  decade of life. Brain MRI is usually normal but spinal cord atrophy can be detected by conventional T2-weighted MRI sequences. Although myelopathy is a milder form of ALD, cerebral involvement can occur in 27% to 63% of patients (26). Cerebral involvement leads to rapid neurologic deterioration with disabilities and early death in 10% to 20% of adult males (26). Adrenal insufficiency is often present at the time of myelopathy diagnosis, а clinical entity called adrenomyeloneuropathy (AMN).

# Incidence Of Primary Adrenal Deficiency In X-ALD

The natural history of adrenal insufficiency in ALD is largely unknown because large prospective natural history studies are lacking. However, the loss of adrenal function evolves gradually and initially starts with elevated plasma corticotropin hormone (ACTH) levels before overt adrenal insufficiency with an abnormal cortisol response after cosyntropin stimulation and endocrine symptoms become apparent (10, 27). The average time to adrenal insufficiency or time from initial plasma ACTH elevation to the onset of endocrine symptoms is unknown (27, 28).

The estimated lifetime prevalence of adrenal insufficiency in ALD is considered to be approximately 80% (27, 29, 30). Addison's disease is reported to be

the initial clinical manifestation of ALD in 38% of cases, representing the most common presenting symptom of ALD in childhood (10, 29). ALD has been reported to account for 4% to 35% of cases of idiopathic primary adrenal insufficiency with no detectable steroid-21-hydroxylase antibodies or other obvious cause (31, 32, 33).

Therefore, all boys must be tested for ALD upon diagnosis of adrenal insufficiency if the cause is otherwise not clear. The risk for adrenal insufficiency varies throughout the lifetime and peaks during the first decade of life between 3 and 10 years of age (27, 29). The youngest patients suffering from adrenal insufficiency and ALD have been reported to be as young as 3, 5 and 7 months of age (27, 29, 34). it has therefore been recommended to start adrenal testing in the first six months of life (29).

In a large natural history study of adrenal insufficiency in ALD (29) the cumulative probability of adrenal insufficiency was highest until the age of 10 years, remained prominent until 40 years of age, and decreased substantially thereafter. A timeframe for adrenal testing has been suggested as follows: Besides on-demand testing if endocrine symptoms are present, screening for adrenal insufficiency should be initiated in the first 6 months of life, then routine adrenal testing should be performed every 3 to 6 months until 10 years of age, annual testing thereafter until 40 years of age, and solely on-demand testing in case of endocrine symptoms from age 41 years onward (29).

In this context, International Recommendations for the Diagnosis and Management of Patients with Adrenoleukodystrophy have been recently issued emphasizing the need for early and regular adrenal testing (35, figure 3).

	Male	Screening or diagnostic tool	Screening protocol	Treatment
	Cerebral ALD	MRI	2 years old, 2–12 years old, >12 years old, baseline scan every 6 months yearly	HSCT or gene therapy
	Myeloneuropathy	History and neurologic examination	>18 years old, only in parallel with any other testing	Supportive
	Adrenal insufficiency	Morning fasted cortisol, ACTH, renin, electrolytes	0–6 months, 6 months–10 years old, >10 years old, start screening every 3–6 months yearly	Hormone replacement therapy
	Gonadal insufficiency	Symptoms biomarkers: Testosterone, LH, FSH	No screening test if symptoms	Hormone replacement therapy
	Female	Screening or diagnostic tool	Screening protocol	Treatment
	Myeloneuropathy	History and neurologic examination	>18 years old	Supportive

# Figure 3. Screening and management overview in ALD. Adapted with permission from: International Recommendations for the Diagnosis and Management of Patients with Adrenoleukodystrophy. Neurology 2022.

The most recent Endocrine Society and Pediatric Endocrine Society clinical practice recommendations for the evaluation of adrenal insufficiency are used as guides for establishing cutoff values for ACTH and cortisol (36).

An ACTH value of > 100 pg/mL and a cortisol value of < 10 mcg/dL is suggestive of adrenal insufficiency. Children with normal ACTH and cortisol levels (<100 pg/mL and  $\geq$ 5 mcg/dL respectively) do not require immediate treatment and should be retested in 3 to 4 months. Children with clearly abnormal ACTH (> 300 pg/mL) and inappropriately low cortisol levels should begin daily and stress-dose glucocorticoid replacement. ACTH and cortisol values of 100 - 299 pg/mL and < 10 mcg/dL respectively should prompt high-dose ACTH (cosyntropin) stimulation testing (34). The median time to transition from stress to maintenance dose has been reported as short as 1.46 years (30). The recommended hormonal workup is depicted in figure 4



Figure 4. Suggested hormonal work up for glucocorticoid deficiency in ALD.

Of note, the mineralocorticoid function often remains intact, reflecting the relative sparing of the zona glomerulosa in the adrenal cortex (10). Mineralocorticoid deficiency, leading to salt wasting, is not typically described in patients with ALD, consistent with the preservation of aldosterone production and the lack of VLCFA accumulation (37, 38). As VLCFAs mainly accumulate in the zona fasciculata and reticularis, the relative preservation of the zona aligns with glomerulosa the observation that mineralocorticoid function remains functional in approximately 50% of the patients (29). Therefore, mineralocorticoid replacement therapy should not be initiated unless abnormal signs/plasma renin activity and electrolyte levels become evident.

Once the diagnosis of glucocorticoid deficiency has been made, further evaluation of aldosterone production should be considered in case of symptoms, such as salt craving and hypotension. Because symptoms are difficult to assess in infancy, it is recommended that serum plasma renin activity and electrolytes be tested every 6 months (34). Fludrocortisone should be started when there is evidence of mineralocorticoid deficiency. Infants would also require additional salt supplementation.

Mineralocorticoid deficiency is reported to be present in 40% of patients with ALD with the vast majority presenting in adulthood (30). Given that mineralocorticoid deficiency is less common and generally follows glucocorticoid deficiency, evaluation with plasma renin activity and electrolytes is recommended every 6 months starting after diagnosis of glucocorticoid deficiency (34). The median time until mineralocorticoid replacement therapy has been reported to be 56 years of age in contrast to a much shorter time for glucocorticoid replacement therapy which was 16 years of age (29).

#### **Female Patients**

As ALD is an X-linked disease, women were previously considered to be asymptomatic carriers. It is now known that even though adrenal insufficiency and cerebral disease are rare in women, more than 80% eventually develop progressive spinal cord disease (39, 40); however, the progression rate of myeloneuropathy remains slow (29). Female patients with ALD typically remain asymptomatic in childhood and adolescence, while, myeloneuropathy symptoms usually arise in adulthood.

Fewer than 1% of female patients are reported to develop adrenal insufficiency (30, 35, 39, 41). Therefore, routine monitoring for adrenal insufficiency and MRI of the brain in women are not recommended (34). 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 (42).

with ALD (35, 43, 44). Levels of testosterone in men with ALD are usually in the low-normal range with elevation of luteinizing hormone in some patients (45). These findings indicate primary hypogonadism, possibly due to the toxicity of VLCFA in Leydig cells, but tissue androgen receptor resistance has also been suggested as an alternative hypothesis to explain this finding (46).

To date, no trials have been performed to test the outcome of testosterone supplementation in men with ALD. In most men with ALD, fertility seems to be normal (29, 47). No data exists on fertility in women with ALD.

#### Primary Hypogonadism

Gonadal function can also be affected in ALD. Abnormal hormone levels indicating gonadal insufficiency have been described in boys and men Tables 1 and 2 summarize the clinical phenotypes in male and female patients.

Table 1. X-ALD Phenotypes in Males				
Phenotype	Description	Estimated	Adrenocortical	
		Relative Frequency	Insufficiency	
Childhood cerebral	Onset 3-10 years.	31-35%	79%	
	Progressive behavioral,			
	deficits.			
	Total disability often within 3 years.			
Adolescent cerebral	Like childhood cerebral; somewhat slower progression	4-7%	62%	
Adult cerebral	Dementia, behavioral disturbances, focal neurologic deficits without preceding	2-3%	>50%	
	adrenomyeloneuropathy			

Adrenomyeloneuropathy	Onset 28 ± 9 years.	40-46%	50-70%
	Slowly progressive paraparesis, sphincter disturbances		
Addison only	Primary adrenal insufficiency without neurologic involvement.	Varies with age. Up to 50% in childhood	100%
	Most common onset 5-7 years. Most eventually develop AMN or cerebral forms		
Asymptomatic	No demonstrable neurologic or adrenal involvement	Common before 4 years. Diminishes with age.	50% plus with testing

Table 2. Phenotypes In Female X-ALD Carriers				
Phenotype	Description	Estimated relative frequency		
Asymptomatic	No neurologic or adrenal involvement	Diminishes with age		
Mild myeloneuropathy	Increased deep tendon reflexes and sensory changes in lower extremities	Increases with age. ~ 50% at age >40 years.		
Moderate to severe myeloneuropathy	Resembles AMN, but milder and later onset	Increases with age >15% at age >40.		
Clinically evident Addison's disease	Rare at any age	<1%		

# **DIAGNOSIS OF X-ALD**

In patients highly suspected of having ALD, measurement of very long chain fatty acids (VCLFA) in the blood is diagnostic, with high specificity and sensitivity (48). VLCFA levels are already increased on the day of birth and in untreated patients remain

stable 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. Of note, VLCFA levels are also elevated in other peroxisomal disorders whereas they can be falsely elevated in patients with liver insufficiency or on ketogenic diets (49). False negative results may occur in approximately 20% of female patients, thus, any woman with symptoms of myelopathy with or without a family history of ALD should undergo further genetic testing (48). Plasma C26:0/C22:0 and C24:0/C22:0 ratios, although diagnostic for ALD, are not associated with the (agedependent) risk of developing adrenal insufficiency, spinal cord disease, or cerebral disease (50, 51).

However, genetic testing (ABCD1 analysis) is the gold standard for diagnosis.

The diagnosis of X-ALD should be sought (35):

- In boys and men with confluent white matter abnormalities on brain MRI in a pattern suggestive of ALD with or without cognitive and neurologic symptoms
- In adult men and women with symptoms and signs of chronic myelopathy with a normal MRI;
- In boys and men with primary adrenal insufficiency with no detectable steroid-21hydroxylase antibodies or other organ-specific antibodies;
- 4. In all at-risk patients with a relative diagnosed with ALD.

# **Genetic Testing**

To date, more than 800 ABCD1 mutations have been described in the X-ALD database (52). Mutations include missense mutations (49%), large deletions (3%), frameshifts (24%), amino acid insertions/ deletions (6%), and nonsense mutations (12%), leading to decreased or absent ABCD1 protein expression. *De novo* mutation rate is reported to range from 5% to 19% (53). Importantly all clinical phenotypes of X-ALD can occur within the same nuclear family and there is no correlation between

ABCD1 mutation and clinical phenotype except for rare cases such as all reported cases of translation initiation mutations in ABCD1 have presented with an AMN-only phenotype (54, 55).

## **Newborn Screening**

Newborn screening (NBS) is justified for a disorder, provided that therapy is available, and that early diagnosis allows timely implementation. This is particularly relevant for X-ALD as early diagnosis at birth would allow for the early detection of adrenal insufficiency for timely initiation of adrenal steroid replacement therapy and early detection of cerebral ALD would permit hematopoietic stem cell transplantation (HSCT) before severe neurologic impairment is established. Important improvements towards this target were 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 (56).

New York State (NYS) in 2013 was the first authority to include screening for X-ALD in the NBS program and since February 2016, X-ALD has been added to the United States Recommended Uniform Screening Panel (RUSP) (57).

NYS NBS for X-ALD is used by most states in the United States (US) and is based on a 3-tier algorithm: the first tier is tandem mass spectrometry (MS/MS) of C26:0-lysophosphatidylcholine (LPS); the second tier is a confirmatory HPLC-MS/MS; and the third tier is Sanger DNA sequencing of the ABCD1 gene (58). If ABCD1 mutation analysis is negative, then other peroxisomal disorders which are also C26:0-HLPC positive should be sought, such as Zellweger Spectrum Disorders, ACOX1, HSD1B4, ACBD5

deficiency, and CADDS (Contiguous ABCD1 DXS1357/BCAP31 Deletion Syndrome) (57).

As of January 2023, thirty-five US states have successfully added ALD to the conditions screened via NBS with plans to expand to all states (59, 60, 61, 62, 63, 64, 65, 66). Globally, the Netherlands is the only other country that is actively screening for ALD through the Screening for ALD in the Netherlands (SCAN) pilot study, a sex-specific newborn screen for boys (67). Since the implementation of Newborn screening for ALD, data show a rise in the diagnosis of ALD up to ~1 in 10,500 births as well as an earlier diagnosis of adrenal insufficiency (30).

## **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 on whether it should be performed on a routine base. (57).

# Imaging

All individuals with confirmed ALD/AMN complex should undergo neuroimaging to determine if cerebral involvement is present. Brain MRI abnormalities

precede symptoms in patients with the cerebral forms of X-ALD (23). Findings are always abnormal in symptomatic patients, demonstrating cerebral white matter demyelination (Figure 5). 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 previous head trauma has triggered CALD (19). 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 (68). A grading system to assess the degree of MRI abnormalities in X-ALD has been proposed by Loes et al. (69). 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 allogenic hematopoietic stem cell transplantation (70).

Regarding AMN, MRI of the spinal cord is unremarkable on standard sequences, it can however show atrophy in advanced cases (71). Contrast enhancement is not observed in AMN, since inflammation is not a feature of extra-cerebral lesions.

Brain F18 fludeoxy-glucose positron emission tomography (PET) may reveal hypometabolic regions particularly in the cerebellum and temporal lobe areas, before lesions emerge in MRI (72). In contrast, hypermetabolism may be evident in the frontal lobes, related to the clinical severity of the disease (73).



Figure 5. MRI of a patient with cerebral ALD, showing reduced volume and increased signal intensity of the white matter localized mainly at the parieto-occipital regions. The anterior white matter is spared. (<u>http://en.wikipedia.org/w/index.php?title=Adrenoleukodystrophy&oldid=506277486</u>).

# THERAPY

#### **Dietary Treatment**

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 is allowed on a daily basis (Table 3).

Table 3. Dietary Restrictions In X-ALD. Adopted Form Ref. 2				
Foods rich in VLCFAs	Foods rich in saturated fat			
Vegetable oils	Vegetable oils			
Fatty fish and meat	Fatty fish and meat			
Plant cover and cuticle	Milk and milk products			
Fruit peel and seeds	Egg yolk			
Grains and nuts	Industrial pastry			

However, since the majority of VLCFA are of endogenous origin (74), this approach is not sufficient. A mixture of oleic acid [C18:1] and erucic acid [C22:1], also referred to as Lorenzo's Oil (LO), has also been applied (75). LO has been shown to halt the elongation of VLCFA by inhibiting ELOVL1, the primary enzyme responsible for VLCFA synthesis.

LO in combination with a low-fat diet nearly normalizes plasma VLCFA levels within four weeks and in a study involving 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 (76). However, in patients who are already symptomatic, controlled clinical trials failed to show improved neurological or endocrine function, nor did it arrest the progression of the disease (35, 77, 78). 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 docosahexaenoic acid, which is essential for neurocognitive development.

## Allogenic Hematopoietic Stem Cell Transplantation (HSCT)

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 improvement of neurological status (77, 79, 80). Stem cells can be harvested from peripheral blood, bone marrow, and umbilical cord blood of immunecompatible 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 (81). 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 (82).

Allogeneic HSCT has been shown to increase 5-year survival compared to no transplant (95% versus 54%) and arrest the progression of the neurologic disease when undertaken early in the course of cerebral disease (44). In contrast, hematopoietic stem cell transplantation is not effective in patients with advanced cerebral ALD, therefore the general criteria for eligibility are a genetically and/or clinically confirmed diagnosis of ALD and the presence of cerebral disease that is not advanced, based on neurological symptoms and brain MRI findings (83). Eligibility of a patient for transplantation can be assessed using the ALD-specific Neurologic Function Scale (NFS) and the Loes MRI severity score (54). The NFS scale is a 25-point, ALD-specific tool that assesses the severity of neurological disability according to the severity of symptoms, but no score absolutely determines the decision for HSCT. HSCT affects not only survival, but also the long-term functional status of patients. Studies have shown that post-transplant survival and major functional disability (MFD)-free survival are superior in patients with lower NFS and Loes score (84, 85). A recent multi-center analysis showed that in early-stage transplanted patients the overall survival at 5 years from CALD diagnosis was 94% and the MFD -free survival was 91%, whereas in patients with advanced disease the overall survival and the MFD-free survival were 90% and 10% respectively (83).

Allogeneic HSCT has its limitations. Transplantation is not effective in patients with advanced disease. Neurologic findings present at the time of HSCT do not reverse and symptoms can progress after HSCT as cerebral disease stabilization is not achieved before 3 to 24 months after stem cell infusion (54). Furthermore, the identification of an acceptable donor for HSCT could be very challenging. Significant risks associated with HSCT include acute mortality (10% at day 100 from transplant), failure of donor cell engraftment (5% risk), and graft-versus-host disease (GVHD) (10-40% risk of acute GVHD and 20% risk of chronic GVHD) (85).

HSCT has not been tested systematically in AMN because of concerns that the risk-benefit ratio may not be favorable: up to 50% of AMN patients will never develop cerebral involvement, whereas it is highly unlikely that HSCT will affect the non-inflammatory distal axonopathy which is the main pathological feature in AMN (86). 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 (87).

Although data are limited, HSCT is unlikely to affect adrenal insufficiency (35). The proposed underlying

mechanism is that VLCFAs accumulation in the adrenal cortex has already reached a critical point that is irreversible by the time of transplant, whereas cerebral ALD has a considerable progressive inflammatory component that is stabilized by the transplant (88).

#### **Gene Therapy**

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 (89). 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 (90). The procedure called Lenti-D gene therapy resulted in clinical disease and imaging stabilization according to neurological symptoms and brain MRI findings in the vast majority of enrolled patients. An ongoing study recruiting for a phase III trial that has been recently opened across the US and Europe (NCT03852498) will further evaluate the efficacy and safety of Lenti-D gene therapy in participants with CALD. 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 (91, 92). 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 (93).

Regarding adrenal function, similarly to HSCT, there is no evidence for the reversal of adrenal failure after autologous HSC gene therapy (88). Advances in gene therapy could offer new treatment options for ALD. Potential therapies include a) antisense oligonucleotides which target specific mutations to exclude pathogenic variants or to establish a normal reading frame shift, b) gene editing through the use of endonucleases that allows permanent modifications to specific DNA segments and c) targeted viral vector therapy that could deliver a normal copy of the ABCD1 gene to steroidogenic and to microglial cells to prevent disease adrenal and neurological dysfunction respectively (54).

# 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.

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.

#### **Experimental Therapies**

Experimental treatment options include 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

peroxisomes. ALDP must dimerize with one of these half-transporters to form a functional full transporter (94). Over-expression of ABCD2, the gene producing ALDRP has been shown to compensate for ABCD1 deficiency and ameliorate VLCFA production from X-ALD cell series (95). 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 (96). 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, which are being tested in vitro and in vivo for the treatment of X-ALD (97, 98, 99). Lately, it has been shown that AMPactivated protein kinase (AMPKa1) is reduced in X-ALD, raising the question if metformin, a well-known AMPKa1inducer, may have a therapeutic role for X-ALD (100).

Regarding the use of antioxidative treatments, experimental data show that treatment of ABCD1 null mice with a combination of antioxidants containing αtocopherol, N-acetyl-cysteine and α-lipoic acid reversed oxidative damage, axonal degeneration, and locomotor impairment (101). 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 (102). 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 (103).

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

REFERENCES

- 1. Moser HW. Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. Brain. 1997;120 (Pt 8:1485–508.
- Engelen M, Kemp S, Poll-The BT. X-linked adrenoleukodystrophy: pathogenesis and treatment. Curr Neurol Neurosci Rep. 2014;14(10):486.
- Blaw ME, Osterberg K, Kozak P, Nelson E. Sudanophilic Leukodystrophy and Adrenal Cortical Atrophy. Arch Neurol. 1964;11:626–31.
- Igarashi M, Schaumburg HH, Powers J, Kishmoto Y, Kolodny E, Suzuki K. Fatty acid abnormality in adrenoleukodystrophy. J Neurochem. 1976;26(4):851–60.
- Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, et al. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature. 1993;361(6414):726–30.
- Moser A, Jones R, Hubbard W, Tortorelli S, Orsini J, Caggana M, Vogel B, Raymond G. Newborn screening for X-linked adrenoleukodystrophy. Int J Neonatal Screen. 2016;2(4):15.
- Kemp S, Berger J, Aubourg P. X-linked adrenoleukodystrophy: clinical, metabolic, genetic and pathophysiological aspects. Biochim Biophys Acta 2012;1822 (9):1465–74.
- 8. Orphanet. X-linked adrenoleukodystrophy.
- 9. ALD info website. ProMED-mail website https://adrenoleukodystrophy.info/mutations-and-variantsin-abcd1.
- Kemp S, Huffnagel IC, Linthorst GE, Wanders RJ, Engelen M. Adrenoleukodystrophy - neuroendocrine pathogenesis and redefinition of natural history. Nat Rev Endocrinol. 2016;12(10):606-615.
- 11. Di Rocco M, Doria-Lamba L, Caruso U. Monozygotic twins with X-linked adrenoleukodystrophy and different phenotypes. Ann Neurol. 2001;50(3):424.
- 12. Higgins CF. ABC transporters: from microorganisms to man. Annu Rev Cell Biol. 1992;8: 67–113.
- 13. Kemp, S. & Wanders, R. Biochemical aspects of Xlinked adrenoleukodystrophy. Brain Pathol. 2010; 20, 831–837.
- Moser, H. W., Smith, K. D., Watkins, P. A., Powers, J. & Moser, A. B. in The Metabolic and Molecular Bases of Inherited Disease 3257–3301 (McGraw Hill, 2001)

other immunomodulators have been used without success (104, 105). Promising results have been extracted by the use of the antioxidant N-acetyl-Lcysteine as adjunctive therapy to HSCT in patients with advanced CALD (106, 107).

- Powers, J. M., Schaumburg, H. H., Johnson, A. B. & Raine, C. S. A correlative study of the adrenal cortex in adreno-leukodystrophy — evidence for a fatal intoxication with very long chain saturated fatty acids. Invest. Cell Pathol 1980;3: 353–376.
- Johnson, A. B., Schaumburg, H. H. & Powers, J. M. Histochemical characteristics of the striated inclusions of adrenoleukodystrophy. J. Histochem Cytochem 1976;24: 725–730.
- Pereira Fdos S, Matte U, Habekost CT, de Castilhos RM, El Husny AS, Lourenco CM, et al. Mutations, clinical findings and survival estimates in South American patients with X-linked adrenoleukodystrophy. PLoS One. 2012;7(3):e34195.
- Kemp S, Pujol A, Waterham HR, van Geel BM, Boehm CD, Raymond G V, et al. ABCD1 mutations and the X-linked adrenoleukodystrophy mutation database: role in diagnosis and clinical correlations. Hum Mutat. 2001;18(6):499–515.
- Raymond G V, Seidman R, Monteith TS, Kolodny E, Sathe S, Mahmood A, et al. Head trauma can initiate the onset of adreno-leukodystrophy. J Neurol Sci. 2010;290(1–2):70–4.
- Netik A, Forss-Petter S, Holzinger A, Molzer B, Unterrainer G, Berger J. Adrenoleukodystrophy-related protein can compensate functionally for adrenoleukodystrophy protein deficiency (X-ALD): implications for therapy. Hum Mol Genet. 1999;8(5):907–13.
- Hudspeth MP, Raymond G V. Immunopathogenesis of adrenoleukodystrophy: current understanding. J Neuroimmunol. 2007;182(1–2):5–12.
- 22. Liberato AP, Mallack EJ, Aziz-Bose R, et al. X-linked adrenoleukodystrophy. Neurology. 2019;92(15):1698–1708.
- Moser HW, Loes DJ, Melhem ER, Raymond G V, Bezman L, Cox CS, et al. X-Linked adrenoleukodystrophy: overview and prognosis as a function of age and brain magnetic resonance imaging abnormality. A study involving 372 patients. Neuropediatrics 2000;31(5):227–39.
- Zhu J, Eichler F, Biffi A, Christine N, Williams D, Majzoub J. The Changing Face of Adrenoleukodystrophy. Endocrine Reviews, 2020; 41(4):577–593.

- 25. Van Geel BM, Bezman L, Loes DJ, et al. Evolution of phenotypes in adult male patients with X-linked adrenoleukodystrophy. Ann Neurol. 2001;49(2):186–94.
- 26. De Beer M, Engelen M, van Geel BM. Frequent occurrence of cerebral demyelinaion in adrenomyeloneuropathy. Neurology. 2014;83(24):2227– 2231.
- Dubey P, Raymond GV, Moser AB, Kharkar S, Bezman L, Moser HW. Adrenal insufficiency in asymptomatic adrenoleukodystrophy patients identified by very longchain fatty acid screening. J Pediatr. 2005;146(4):528–532.
- Blevins LS Jr, Shankroff J, Moser HW, Ladenson PW. Elevated plasma adrenocorticotropin concentration as evidence of limited adrenocortical reserve in patients with adrenomyeloneuropathy. J Clin Endocrinol Metab. 1994;78(2):261–265.
- 29. Huffnagel IC, Laheji FK, Aziz-Bose R, et al. The Natural History of Adrenal Insufficiency in X-Linked Adrenoleukodystrophy: An International Collaboration. J Clin Endocrinol Metab. 2019;104(1):118-126.
- Alcantara JR, Grant NR, Sethuram S, et al. Early Detection of Adrenal Insufficiency: The Impact of Newborn Screening for Adrenoleukodystrophy. J Clin Endocrinol Metab. 2023;108(11):1306-1315.
- Laureti S, Casucci G, Santeusanio F, et al. X-linked adrenoleukodystrophy is a frequent cause of idiopathic Addison's disease in young adult male patients. J Clin Endocrinol Metab. 1996;81(2):470–4.
- Laureti S, Aubourg P, Calcinaro F, et al. Etiological diagnosis of primary adrenal insufficiency using an original flowchart of immune and biochemical markers. J Clin Endocrinol Metab. 1998;83(9):3163–3168.
- Guran T, Buonocore F, Saka N, et al.Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort. J Clin Endocrinol Metab. 2016;101(1):284–292.
- Regelmann MO, Kamboj MK, Miller BS, et al. Adrenoleukodystrophy: Guidance for Adrenal Surveillance in Males Identified by Newborn Screen. J Clin Endocrinol Metab. 2018;103(11):4324-4331.
- Engelen M, van Ballegoij WJC, Mallack EJ, et al. International Recommendations for the Diagnosis and Management of Patients with Adrenoleukodystrophy: A Consensus-Based Approach. Neurology. 2022;99(21):940-951.
- Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and treatment of primary adrenal insufficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016;101(2):364–389.

- Powers JM, Schaumburg HH. Adreno-leukodystrophy (sex-linked Schilder's disease). A pathogenetic hypothesis based on ultrastructural lesions in adrenal cortex, peripheral nerve and testis. Am J Pathol 1974;76(3):481– 491.
- Schaumburg HH, Powers JM, Raine CS, Suzuki K, Richardson EP Jr. Adrenoleukodystrophy. A clinical and pathological study of 17 cases. Arch Neurol. 1975;32(9):577–591.
- Engelen M, Barbier M, Dijkstra IM, Schur R, de Bie RM, Verhamme C, Dijkgraaf MG, Aubourg PA, Wanders RJ, van Geel BM, de Visser M, Poll-The BT, Kemp S. X-linked adrenoleukodystrophy in women: a cross-sectional cohort study. Brain. 2014; 137:693–706.
- Habekost CT, Schestatsky P, Torres VF, de Coelho DM, Vargas CR, Torrez V, Oses JP, Portela LV, Pereira Fdos S, Matte U, Jardim LB. Neurological impairment among heterozygote women for X-linked Adrenoleukodystrophy: a case control study on a clinical, neurophysiological and biochemical characteristics. Orphanet J Rare Dis. 2014; 9:6.
- 41. Schmidt S, Traber F, Block W, et al. Phenotype assignment in symptomatic female carriers of X-linked adrenoleukodystrophy. J Neurol. 2001;248(1):36-44
- 42. Maier EM, Kammerer S, Muntau AC, Wichers M, Braun A, Roscher AA. Symptoms in carriers of adrenoleukodystrophy relate to skewed X inactivation. Ann Neurol. 2002;52(5):683–8.
- Kuhl JS, Suarez F, Gillett GT, et al. Long-term outcomes of allogeneic haematopoietic stem cell transplantation for adult cerebral X-linked adrenoleukodystrophy. Brain. 2017;140(4):953-966.
- Mahmood A, Raymond GV, Dubey P, Peters C, Moser HW. Survival analysis of haematopoietic cell transplantation for childhood cerebral X-linked adrenoleukodystrophy: a comparison study. Lancet Neurol. 2007;6(8):687-692.
- Brennemann, W., Kohler, W., Zierz, S. & Klingmuller, D. Testicular dysfunction in adrenomyeloneuropathy. Eur. J. Endocrinol. 1997;137: 34–39.
- Karapanou, O. et al. Xlinked adrenoleukodystrophy: are signs of hypogonadism always due to testicular failure? Hormones (Athens) 13, 146–152 (2014).
- Stradomska, T. J., Kubalska, J., Janas, R. & TylkiSzymanska, A. Reproductive function in men affected by Xlinked adrenoleukodystrophy/ adrenomyeloneuropathy. Eur. J. Endocrinol. 2012;166: 291–294.
- Moser AB, Kreiter N, Bezman L, et al. Plasma very long chain fatty acids in 3,000 peroxisome disease patients and 29,000 controls. Ann Neurol. 1999;45(1):100–110.
- 49. Stradomska TJ, Bachański M, Pawłowska J, et al. The impact of a ketogenic diet and liver dysfunction on serum

very long-chain fatty acids levels. Lipids. 2013;48(4):405–409.

- Korenke GC, Roth C, Krasemann E, H<sup>\*</sup>ufner M, Hunneman DH, Hanefeld F. Variability of endocrinological dysfunction in 55 patients with X-linked adrenoleucodystrophy: clinical, laboratory and genetic findings. Eur J Endocrinol. 1997;137(1):40–47.
- Tran C, Patel J, Stacy H, Mamak EG, Faghfoury H, Raiman J, et al. Long-term outcome of patients with X-linked adrenoleukodystrophy: A retrospective cohort study. Eur J Paediatr Neurol (2017) 21(4):600–9.
- 52. ALD info website. ProMED-mail website. https:// adrenoleukodystrophy.info/.
- Horn MA, Retterstøl L, Abdelnoor M, et al. Adrenoleukodystrophy in Norway: high rate of de novo mutations and age-dependent penetrance. Pediatr Neurol. 2013;48(3):212–219.
- Zhu J, Eichler F, Biffi A, Duncan CN, Williams DA, Majzoub JA. The Changing Face of Adrenoleukodystrophy. Endocr Rev. 2020;41(4):577-593.
- 55. O'Neill GN, Aoki M, Brown RH Jr. ABCD1 translationinitiator mutation demonstrates genotype-phenotype correlation for AMN. Neurology. 2001;57(11):1956–1962.
- Hubbard WC, Moser AB, Tortorelli S, Liu A, Jones D, Moser H. Combined liquid chromatography-tandem mass spectrometry as an analytical method for high throughput screening for X-linked adrenoleukodystrophy and other peroxisomal disorders: preliminary findings. Mol Genet Metab. 2006;89(1–2):185–7.
- Vogel BH, Bradley SE, Adams DJ, Aco KD, Erbe RW, Fong C, et al. Newborn screening for X-linked adrenoleukodystrophy in New York State: Diagnostic protocol, surveillance protocol and treatment guidelines. Mol Genet Metab. 2015;114(4):599–603.
- Kemper AR, Brosco J, Comeau AM, et al. Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation. Genet Med. 2017;19: 121–126.
- Prinzi J, Pasquali M, Hobert JA, et al. Diagnosing X-Linked Adrenoleukodystrophy after Implementation of Newborn Screening: A Reference Laboratory Perspective. Int J Neonatal Screen. 2023;9(4):64.
- Wiens K, Berry SA, Choi H, Gaviglio A, Gupta A, Hietala A, et al. A report on state-wide implementation of newborn screening for Xlinked Adrenoleukodystrophy. Am J Med Genet. 2019; 179:1205–13.
- Tang H, Matteson J, Rinaldo P, Tortorelli S, Currier R SS. The clinical impact of CLIR tools toward rapid resolution of post-newborn screening confirmatory testing for X-linked adrenoleukodystrophy in California. Int J Neonatal Screen. 2020; 6:62.

- Lee S, Clinard K, Young SP, Rehder CW, Fan Z, Calikoglu AS, et al. Evaluation of X-linked adrenoleukodystrophy newborn screening in North Carolina. JAMA Netw Open. 2020;3: 1–12.
- Hall PL Li H, Hagar AF, Caleb Jerris S, Wittenauer A, Wilcox W. Newborn screening for X-linked Adrenoleukodystrophy in Georgia: experiences from a pilot study screening of 51,081 newborns. Int J Neonatal Screen. 2020;6: 81.
- 64. Matteson J, Sciortino S, Feuchtbaum L, Bishop T, Olney RS, Tang H. Neonatal screening adrenoleukodystrophy newborn screening in California since 2016: programmatic outcomes and follow-Up. Int J Neonatal Screen. 2021; 7:22.
- Moser AB, Raymond G V, Burton BK, Hickey R, Hitchins L, Shively V, et al. Neonatal screening newborn screening for X-linked adrenoleukodystrophy: the initial Illinois experience. Int J Neonatal Screen. 2022; 8:6.
- Priestley JRC, Adang LA, Drewes Williams S, Lichter-Konecki U, Menello C, Engelhardt NM, et al. Newborn screening for X-linked adrenoleukodystrophy : review of data and outcomes in Pennsylvania. Int J Neonatal Screen. 2022; 8:24.
- Barendsen RW, Dijkstra IME, Visser WF, Alders M, Bliek J, Boelen A, et al. Adrenoleukodystrophy newborn screening in the Netherlands (SCAN Study): the X-factor. Front. Cell Develop. Biol. 2020; 8:499.
- Melhem ER, Loes DJ, Georgiades CS, Raymond G V, Moser HW. X-linked adrenoleukodystrophy: the role of contrast-enhanced MR imaging in predicting disease progression. Am J Neuroradiol. 2000;21(5):839–44.
- Loes DJ, Hite S, Moser H, Stillman AE, Shapiro E, Lockman L, et al. Adrenoleukodystrophy: a scoring method for brain MR observations. Am J Neuroradiol. 1994;15(9):1761–6.
- McKinney AM, Nascene D, Miller WP, Eisengart J, Loes D, Benson M, et al. Childhood cerebral X-linked adrenoleukodystrophy: diffusion tensor imaging measurements for prediction of clinical outcome after hematopoietic stem cell transplantation. Am J Neuroradiol. 2013;34(3):641–9.
- Dubey P, Fatemi A, Huang H, Nagae-Poetscher L, Wakana S, Barker PB, van Zijl P, Moser HW, Mori S, Raymond GV. Diffusion tensor-based imaging reveals occult abnormalities in adrenomyeloneuropathy. Ann Neurol. 2005; 58:758-66.
- Renard D, Castelnovo G, Collombier L, Kotzki PO, Labauge P. Brain fludeoxyglucose F 18 positron emission tomography hypometabolism in magnetic resonance imaging-negative x-linked adrenoleukodystrophy. Arch Neurol. 2011;68(10):1338–9.

- Salsano E, Marotta G, Manfredi V, Giovagnoli AR, Farina L, Savoiardo M, et al. Brain fluorodeoxyglucose PET in adrenoleukodystrophy. Neurology. 2014;83(11):981–9.
- Tsuji S, Sano T, Ariga T, Miyatake T. Increased synthesis of hexacosanoic acid (C23:0) by cultured skin fibroblasts from patients with adrenoleukodystrophy (ALD) and adrenomyeloneuropathy (AMN). J Biochem. 1981;90(4):1233–6.
- Rizzo WB, Leshner RT, Odone A, Dammann AL, Craft DA, Jensen ME, et al. Dietary erucic acid therapy for X-linked adrenoleukodystrophy. Neurology. 1989;39(11):1415–22.
- Moser HW, Raymond G V, Lu SE, Muenz LR, Moser AB, Xu J, et al. Follow-up of 89 asymptomatic patients with adrenoleukodystrophy treated with Lorenzo's oil. Arch Neurol. 2005;62(7):1073–80.
- 77. Aubourg P, Blanche S, Jambaque I, Rocchiccioli F, Kalifa G, Naud-Saudreau C, et al. Reversal of early neurologic and neuroradiologic manifestations of X-linked adrenoleukodystrophy by bone marrow transplantation. N Engl J Med. 1990;322(26):1860–6.
- 78. Van Geel BM, Assies J, Haverkort EB, Koelman JH, Verbeeten B. J, Wanders RJ, et al. Progression of abnormalities in adrenomyeloneuropathy and neurologically asymptomatic X-linked adrenoleukodystrophy despite treatment with "Lorenzo's oil." J Neurol Neurosurg Psychiatry. 1999;67(3):290–9.
- 79. Shapiro E, Krivit W, Lockman L, Jambaque I, Peters C, Cowan M, et al. Long-term effect of bone-marrow transplantation for childhood-onset cerebral X-linked adrenoleukodystrophy. Lancet. 2000; 356:713–8.
- Miller WP, Rothman SM, Nascene D, Kivisto T, DeFor TE, Ziegler RS, et al. Outcomes after allogeneic hematopoietic cell transplantation for childhood cerebral adrenoleukodystrophy: the largest single-institution cohort report. Blood. 2011;118(7):1971–8.
- Stradomska TJ, Drabko K, Moszczynska E, Tylki-Szymanska A. Monitoring of very long-chain fatty acids levels in X-linked adrenoleukodystrophy, treated with haematopoietic stem cell transplantation and Lorenzo's Oil. Folia Neuropathol. 2014; 52:159–63.
- Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science. 1988; 239:290–2.
- Raymond G, Aubourg P, Paker A, et al. Survival and functional outcomes in boys with cerebral adrenoleukodystrophy with and without hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2019;25(3):538–548
- 84. Orchard, P. J. et al. Hematopoietic cell therapy for metabolic disease. J. Pediatr. 151, 340–346 (2007).
- 85. Peters, C. et al. Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation

experience from 1982 to 1999. Blood 104, 881-888 (2004).

- Powers JM, DeCiero DP, Ito M, Moser AB, Moser HW. Adrenomyeloneuropathy: a neuropathologic review featuring its noninflammatory myelopathy. J Neuropathol Exp Neurol. 2000;59(2):89–102.
- van Geel BM, Poll-The BT, Verrips A, Boelens JJ, Kemp S, Engelen M. Hematopoietic cell transplantation does not prevent myelopathy in X-linked adrenoleukodystrophy: a retrospective study. J Inherit Metab Dis. 2015; 38(2):359-61
- 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 Transplant. 2012;47(10):1377–1378.
- Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Bougneres P, Schmidt M, Kalle C V, et al. Lentiviral hematopoietic cell gene therapy for X-linked adrenoleukodystrophy. Methods Enzym. 2012; 507:187– 98.
- Eichler F, Duncan C, Musolino PL, Orchard PJ, De Oliveira S, Thrasher AJ, et al. Hematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy. N Engl J Med. 2017;377(17):1630–8.
- Rothe M, Modlich U, Schambach A. Biosafety challenges for use of lentiviral vectors in gene therapy. Curr Gene Ther. 2013;13(6):453–68.
- Engelen M. Optimizing Treatment for Cerebral Adrenoleukodystrophy in the Era of Gene Therapy. N Engl J Med. 2017;377, 1682–1684.
- Kahraman, S. et al. Successful haematopoietic stem cell transplantation in 44 children from healthy siblings conceived after preimplantation HLA matching. Reprod. Biomed. Online 29, 340–351 (2014).
- 94. Kemp S, Berger J, Aubourg P. X-linked adrenoleukodystrophy: Clinical, metabolic, genetic and pathophysiological aspects. Biochim Biophys Acta. 2012;1822(9):1465–74.
- Netik A, Forss-Petter S, Holzinger A, Molzer B, Unterrainer G, Berger J. Adrenoleukodystrophy-related protein can compensate functionally for adrenoleukodystrophy protein deficiency (X-ALD): implications for therapy. Hum Mol Genet. 1999;8(5):907–13.
- Fourcade S, Ruiz M, Guilera C, Hahnen E, Brichta L, Naudi A, et al. Valproic acid induces antioxidant effects in X-linked adrenoleukodystrophy. Hum Mol Genet. 2010;19(10):2005–14.
- 97. Jang J, Kim HS, Kang JW, Kang HC. The genetically modified polysialylated form of neural cell adhesion molecule-positive cells for potential treatment of X-linked adrenoleukodystrophy. Yonsei Med J. 2013;54(1):246–52.

- Gondcaille C, Genin EC, Lopez TE, Dias AM, Geillon F, Andreoletti P, et al. LXR antagonists induce ABCD2 expression. Biochim Biophys Acta. 2014;1841(2):259–66.
- 99. Park CY, Kim HS, Jang J, Lee H, Lee JS, Yoo JE, et al. ABCD2 is a direct target of beta-catenin and TCF-4: implications for X-linked adrenoleukodystrophy therapy. PLoS One. 2013;8(2): e 56242.
- 100. Singh J, Olle B, Suhail H, Felicella MM, Giri S. Metformininduced mitochondrial function and ABCD2 up-regulation in X-linked adrenoleukodystrophy involves AMP-activated protein kinase. J Neurochem. 2016;138(1):86–100.
- 101. Galea E, Launay N, Portero-Otin M, Ruiz M, Pamplona R, Aubourg P, et al. Oxidative stress underlying axonal degeneration in adrenoleukodystrophy: A paradigm for multifactorial neurodegenerative diseases? Biochim Biophys Acta. 2012;1822(9):1475–88.
- Morato L, Galino J, Ruiz M, Calingasan NY, Starkov AA, Dumont M, et al. Pioglitazone halts axonal degeneration in a mouse model of X-linked adrenoleukodystrophy. Brain. 2013;136(Pt 8):2432–43.
- 103. Engelen M, Tran L, Ofman R, Brennecke J, Moser AB, Dijkstra IME, et al. Bezafibrate for X-Linked

Adrenoleukodystrophy. Baud O, editor. PLoS One. 2012 Jul 20;7(7): e41013

- Berger J, Pujol A, Aubourg P, Forss-Petter S. Current and future pharmacological treatment strategies in X-linked adrenoleukodystrophy. Brain Pathol. 2010;20(4):845–56.
- 105. Horvath GA, Eichler F, Poskitt K, Stockler-Ipsiroglu S. Failure of repeated cyclophosphamide pulse therapy in childhood cerebral X-linked adrenoleukodystrophy. Neuropediatrics. 2012;43(1):48–52.
- 106. Casasnovas C, Montserrat R, Schlüter A, Naudí A, Fourcade S, Veciana M, Castañer S, Albertí A, Bargalló N, Johnson M, Gerald V, Raymond G, Fatemi A, Moser A, Villarroya F, Portero-Otín M, Artuch R, Pamplona R, Aurora Pujol A. Biomarker Identification, Safety, and Efficacy of High-Dose Antioxidants for Adrenomyeloneuropathy: a Phase II Pilot Study. Neurotherapeutics (2019) 16:1167– 1182.
- 107. Zhou J, Terluk M, Orchard P, Cloyd J, Kartha R. N-Acetylcysteine Reverses the Mitochondrial Dysfunction Induced by Very Long-Chain Fatty Acids in Murine Oligodendrocyte Model of Adrenoleukodystrophy. Biomedicines. 2021 Dec; 9(12): 1826