X-LINKED ADRENOLEUKODYSTROPHY: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

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Abstract

Adrenoleukodystrophy (X-ALD) is a rare X-linked peroxisomal disease, which usually presents in affected males with one of three phenotypes: adult-onset adrenomyeloneuropathy (AMN, 45%), childhood-onset cerebral demyelination (CALD, 35%), or primary adrenal insufficiency (PAI, 10%). Occasionally, X-ALD patients may suffer from less specific symptoms resembling those of other neurological conditions. Variability in presentation and age of onset can make the diagnosis challenging. AMN should be considered in patients presenting with spastic paraparesis. Female carriers manifest a late-onset mild form of myelopathy and/or neuropathy. Inclusion of testing for X-ALD in newborn screening programs is expected to expand our understanding of the disorder's natural history. Newborn screening should enable early detection of affected individuals and allow timely therapeutic interventions. Such interventions include corticosteroid replacement therapy for PAI, allogeneic hematopoietic stem cell transplantation for CALD, and approved gene therapy (elivaldogene autotemcel) for suitable candidates with childhood CALD. Currently, no effective treatment exists for the neurological manifestations of AMN, the commonest presentation of X-ALD. Hopefully, in the near future, novel gene therapy approaches, similar to those recently approved for other rare neurogenetic diseases may also be developed for X-ALD.

Key words: X-linked adrenoleukodystrophy; adrenomyeloneuropathy; primary adrenal insufficiency; HSCT; elivaldogene autotemcel

Introduction

X-linked adrenoleukodystrophy (X-ALD, OMIM #300100) is a lipid-storage disease, which primarily affects the nervous system, adrenal cortex, as well as testicular function [1-4]. X-ALD represents the most common peroxisomal disorder, with a recently estimated birth incidence of approximately 1:18,000 [3]. As shown in Box1, clinical manifestations of the disorder vary, with patients presenting with either of the following three basic clinical phenotypes; cerebral X-ALD (CALD), adrenomyeloneuropathy (AMN), and primary adrenal insufficiency (PAI) [4].

X-ALD is due to mutations in the *ABCD1* gene, located on chromosome Xq28, coding for ALDP, a

Box 1. X-ALD Clinical Phenotypes

Males
• Cerebral form (CALD) ○ Childhood ○ Adolescent ○ Adult • Adrenomyeloneuropathy (AMN) o With cerebral involvement o Without cerebral involvement • Primary adrenal insufficiency (PAI), "Addison's only" phenotype • Asymptomatic/Presymptomatic
Females
• AMN-like phenotype • Asymptomatic/Presymptomatic

peroxisomal ATP-binding cassette protein [5-7]. As of June 11, 2021, 3, 247 variants of *ABCD1* have been reported, of which 913 are non-recurrent and 247 are variants of unknown significance (https://adrenoleukodystrophy.info/mutations-biochemistry/mutation-statistics). No correlation between genotype and disease phenotype has been found, even within the same family [8].

ALDP serves as a transmembrane channel which enables the transportation of very long-chain fatty acids ≥ C22:0 (VLCFA) into the peroxisome, for β-oxidation [9]. The defective function of ALDP prevents degradation of VLCFA, resulting in its accumulation in tissues and plasma, which is regarded as X-ALD's biochemical hallmark [10]. VLCFA levels are increased in the plasma of all male carriers and in approximately 80-85% of female carriers [11, 12].

In 2006, Hubbard et al. found raised levels of C26:0 lysophosphatidyl choline, measured via LC-MS/MS assay, in postnatal venous dried blood spots from X-ALD males [13]. This finding has allowed the emergence of a newborn screening diagnostic test for X-ALD with several countries incorporating it in their newborn screening programs [14]. Therapeutic management has also evolved, with corticosteroid replacement therapy and hematopoietic stem cell transplant (HSCT) remaining the mainstay of treatment, but with new therapeutic options, such as gene therapy for eligible candidates suffering from CALD, also emerging [15, 16]. As X-ALD patients have no neurological deficits at birth, early diagnosis through newborn screening has opened a "window of opportunity" for the use of these therapies.

In this review, we intend to enlighten the reader about the disorder's basic pathophysiology and clinical characteristics, as well as provide an update on diagnosis and management of X-ALD.

2. X-ALD Basic Pathophysiology

VLCFA mainly accumulate in the nervous system, adrenal cortex, and testicular Leydig cells, a process already taking place in utero [17-20]. Unidentified molecular events prompt the transition from the metabolic phase, i.e. VLCFA accumulation, to neuroinflammation and demyelination in the brain in CALD, or to axonal degeneration in the spinal cord in AMN [21].

In 2010, Singh and Pujol, in an attempt to describe the mechanisms underlying CALD, proposed the "three-hit hypothesis"; excess of VLCFA and lower plasmalogen levels (antioxidant phospholipids) result in oxidative stress (first hit) that successively, with the contribution of environmental, genetic or epigenetic factors, triggers a neuroinflammatory response (second hit), which further disrupts the peroxisomal function (third hit), leading to a progressive inflammatory demyelinating disease [22-24]. Similarly, regarding the myeloneuropathy's pathophysiology, oxidative stress along with defective mitochondrial function result in the disruption of ATP-dependent axonal transport, inducing a distal non-inflammatory dying-back axonopathy [25].

In the adrenal glands, VLCFA are preferentially found in the zona fasciculata and zona reticularis, thus glucocorticoid and androgen deficiencies are more common in X-ALD [19, 26, 27]. The suggested mechanisms that elicit the aforementioned deficiencies are summarized as follows: a) VLCFA accumulation exhibiting a direct cytotoxic effect to cells, followed by apoptosis due to oxidative stress [23], b) inadequate free cholesterol availability for steroid hormones' formation owing to the accumulation of cholesterol esters with VLCFA [28], c) incorporation of VLCFA into cell membranes, interfering with adrenocorticotropic hormone's (ACTH) ability to attach to its receptor [29]. A similar pathology is thought to lead to abnormal hormonogenesis in testes' Leydig cells, with low testosterone levels leading to testicular dysfunction [30].

3. X-ALD Clinical Characteristics

Various different X-ALD clinical phenotypes have been described, each of them characterized by specific clinical manifestations (see Table 1). However, considering the disorder's progressive nature and the fact that some phenotypes evolve into others, one could speak of a clinical spectrum of disease (see figure 1). Interestingly, the X-ALD phenotype shows no correlation, either to VLCFA plasma levels or to the *ABCD1* pathogenic variant involved, even in the same family [31-33]. Moreover, in contrast to previous understanding, it is now widely accepted that X-ALD not only affects males, but, female heterozygotes as well, who eventually develop primarily AMN symptoms [34].

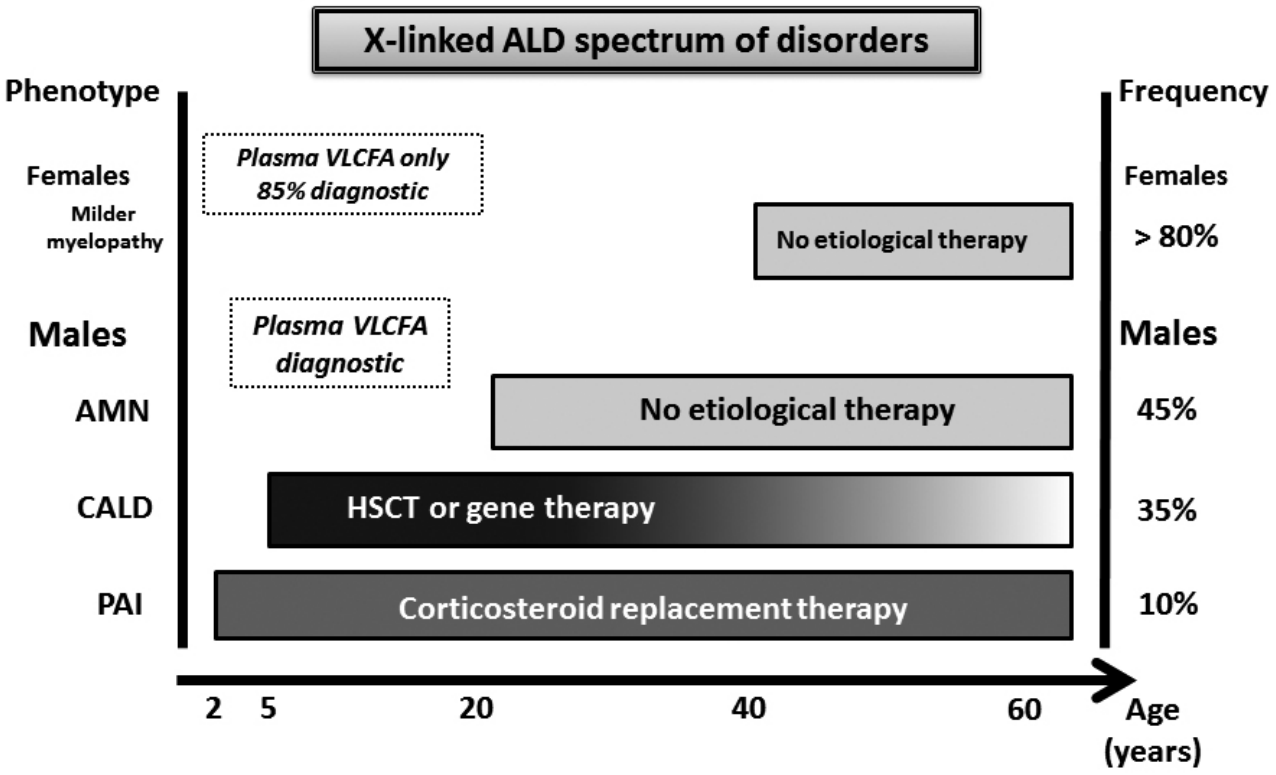
3a. Presentations Most Commonly Seen in Affected Males

The cerebral form of X-ALD can occur at any age (childhood, adolescence, early adulthood), but mostly between four and eight years of age, with the childhood cerebral form occurring in ~35% of affected individuals [35, 36]. Affected schoolboys exhibit cognitive deficits (behavioral and/or learning), which may resemble those of attention deficit hyperactivity disorder. As the demyelinating process progresses, more serious symptoms arise, such as hemiparesis or spastic tetraparesis, apraxia, astereognosia, difficulty in understanding speech despite intact hearing, lack of spatial orientation, visual disturbances, cerebellar ataxia, and seizures [36]. By the time the neurologic disturbances appear, most patients have

Table 1. Clinical manifestations of X-ALD's most common phenotypes and their lifetime prevalence

Phenotypes	Clinical manifestations	Lifetime prevalence
Males		
Cerebral form	cognitive deficits resembling attention-deficit hyperactivity disorder, signs of dementia, visual disturbances, aphasia, apraxia, astereognosia, auditory agnosia, lack of spatial orientation, hemiparesis or spastic tetraparesis, cerebellar ataxia, seizures	~60%
AMN	spastic paraparesis, sensory ataxia, sphincter disturbances, sexual dysfunction	~100%
Adrenocortical insufficiency	increased skin pigmentation, orthostatic hypotension, fatigue, anorexia, weight loss or poor growth, gastrointestinal symptoms	~80%
Females		
AMN-like phenotype	sensory ataxia, sphincter disturbances, sensory symptoms, gait spasticity, neuropathic pain	>80%

Figure 1 legend. Diagram summarizing main diagnostic, phenotypic and therapeutic issues in X-linked adrenoleukodystrophy. Percentages, presented separately for males and females, represent phenotypes at presentation; ALD: adrenoleukodystrophy; AMN: adrenomyeloneuropathy; CALD: cerebral ALD; PAI: primary adrenal insufficiency; HSCT: Hematopoietic stem cell transplantation; gene therapy refers to elivaldogene autotemcel



also developed adrenocortical insufficiency. Eventually, progressive functional decline leads to total disability followed by death, two to four years after symptom onset.

A proportion of affected males suffering from CALD may develop the so-called "chronic or arrested cerebral X-ALD". In such cases, a spontaneous arrest of the demyelinating process is observed, followed by several years of disease stability. Nevertheless, a small number of those patients may, at some point, shift to the progressive form of CALD, reflecting the recurrence of the cerebral demyelinating process [37, 38].

The proportion of affected individuals presenting with symptoms suggestive of adrenomyeloneuropathy is estimated as high as ~40-45% [39]. AMN typically affects men between their second and fourth

decade of life, who develop progressive symptoms such as spastic paraparesis, sensory ataxia, sphincter disturbances, and sexual dysfunction [37]. As made apparent by its clinical manifestations, AMN should be considered in patients with spastic paraparesis of undetermined cause [40, 41]. Adrenal insufficiency is usually already evident as of AMN diagnosis' establishment. Despite initial absence, about 40%-45% of patients suffering from AMN eventually develop some degree of brain involvement, which in some cases becomes severely progressive and may even lead to death [42].

Approximately 10% of male carriers exhibit, at presentation, signs of primary adrenal insufficiency, i.e. increased skin pigmentation, orthostatic hypotension, fatigue, anorexia, weight loss or poor growth, and gastrointestinal symptoms, resulting in a diagnosis of Addison's disease [37]. PAI, if left untreated, may lead to adrenal crisis, a potentially life-threatening situation. Therefore, testing for X-ALD seems reasonable in all male probands showing signs of Addison's disease [43]. Although most affected male individuals will exhibit signs of adrenal dysfunction at some phase of the disease, adrenal function is usually normal in female carriers [44].

Another 5%-10% of affected males present with one the following constellation of symptoms: a) signs of localized brain disease including hemiparesis, aphasia, and visual field defects, and symptoms due to elevated intracranial pressure, such as headache, b) progressive behavioral disturbance, dementia and paresis in an adult, c) ataxia in a child or adult, d) only evidence of sphincter and sexual dysfunction in male carriers [45]. Lastly, a small proportion of male carriers may remain asymptomatic/ presymptomatic. This marked phenotypic heterogeneity has been attributed to the existence of possible disease modifiers, such as environmental triggers but also genetic factors [46]. Numerous reports from studies that aim at identifying modifier genes in X-ALD have been published, an extensive discussion of which is beyond this review's intentions [47].

3b. Presentations Most Commonly Seen in Female Carriers

For a long time, X-ALD was regarded as a disorder that only affects males. However, nowadays, studies have shown that despite adrenocortical insufficiency and CALD affecting less than 1% of female carriers, more than 80% show signs of neurological dysfunction by the age of 60 years [48]. As expected, the disease is less severe and progresses at a slower rate in female carriers. Symptom onset predominantly occurs between the 4th and 5th decade of life, resembling an AMN-like phenotype. Affected women typically present with symptoms suggestive of myelopathy and/or peripheral neuropathy such as sensory ataxia, fecal incontinence, bladder dysfunction, sensory symptoms and gait spasticity [49]. They often suffer from neuropathic pain, a symptom that is not generally present in males with AMN [50]. Women very rarely develop the cerebral form of the disease, this occurring only in the context of two possibilities a) woman carrier of two mutated *ABCD1* alleles, or b) complete inactivation of the normal X-chromosome in all tissue cells [51].

4. X-ALD: Establishing the Diagnosis

The implementation of X-ALD testing in newborn screening programs has undoubtedly led to a new era regarding X-ALD diagnosis in several countries [52]. In such countries, as expected, the burden has now been placed on monitoring of diagnosed infants and extensive discussions are underway, regarding monitoring protocols. Families that give birth to infants with a positive newborn screen are referred to specialized centers for confirmatory testing and genetic advice. The infant is respectively closely followed by pediatric neurologists and endocrinologists [14].

In countries where newborn screening is not available, diagnosis remains a matter of clinical suspicion. When X-ALD is suspected in a male, measurement of VCLFA in blood is diagnostic, with high specificity and sensitivity as all males with X-ALD have elevated VLCFA levels. However, about 15% of female carriers might have normal plasma VLCFA levels, rendering mutation analysis as the diagnostic test of choice in females [35, 53]. In males suffering from primary adrenal insufficiency, the clinician should consider the possibility of X-ALD and test for plasma VLCFA levels [54]. Similarly, boys and young adults with suggestive neurological symptoms, with or without typical lesions on brain MRI, should be considered for X-ALD. Furthermore, both male and female patients with symptoms consistent with chronic myelopathy should be tested for X-ALD, after ruling out more common causes, such as multiple sclerosis, vitamin deficiencies, compressive lesions, radiation, infections, or primary lateral sclerosis, and before or at the same time as embarking on genetic testing for hereditary spastic paraparesis [55]. In males, the coexistence of progressive myelopathy and early baldness may facilitate establishing the diagnosis, as most affected males will already have some signs of adrenal insufficiency by the time they develop myelopathy.

5. Monitoring of X-ALD patients

Once the diagnosis is established in a patient, genetic testing seems reasonable in the entire family. If the affected individual is a male infant/child, the American Pediatric Endocrine Society suggests laboratory screening of cortisol and ACTH levels (every

Box 2. X-ALD diagnosis

Suspect X-ALD
• In males with symptoms and signs compatible with **adrenal insufficiency**. • In males with neurological symptoms and signs compatible with **cerebral** X-ALD. • In patients with **progressive myelopathy** after exclusion of most frequent causes.
If X-ALD is suspected
• Obtain plasma **VLCFA levels**. • Proceed to ***ABCD1* gene mutation analysis**.
Ancillary paraclinical evaluation
• Brain and/or spinal cord MRI. • Laboratory testing for ACTH, cortisol, electrolytes (potassium, sodium), glucose.

Box 3. Sample collection for plasma VLCFA levels determination

According to the Greek Institute of Child Health, sample collection should be carried out as follows:
Time of blood collection: In the morning, fasting blood sample.
Type of sample: Take 5 cc of blood in a heparinized syringe (Heparin sodium) and transfuse in an empty tube.
Transport conditions for samples: Preferably immediate after blood sample collection. If not possible, place samples in cold storage at +2°C to +8°C until shipment via cooled transport (4 °C).
Special cautions: Inform the laboratory in case of prior blood transfusion. In case of hemolysis, obtain a new sample as hemolysis might increase VLCFA levels. Other factors affecting VLCFA levels include: ketogenic diet, liver disease, and dyslipidemia.

3-4 months in the first 2 years and every 4-6 months after 2 years of age) [56]. Regarding MRI surveillance, the following protocol has been recently proposed: a) Obtain an MRI between 12 and 18 months old; b) Obtain a second MRI 1 year after baseline; c) Between 3 and 12 years old, obtain a contrast-enhanced MRI every 6 months; d) After 12 years, obtain an annual MRI. For the time being, no guidelines exist for neuroimaging in adults [57]. T2 hyperintensities can be observed in the involved areas, i.e. corpus callosum, visual pathway, supratentorial white matter and major projection fibers. The presence of gadolinium enhancing lesions reflects the disruption of the blood brain barrier and marks the transition to the demyelinating stage of the disease [38].

The Loes MRI Severity Score is a grading system used to assess the severity of MRI lesions and may range from 0, meaning no disease activity, to 34, which is indicative of the most severe disease. This score is of importance as therapeutic interventions are indicated for subjects with a score between 0.5 and 9 [58]. If the affected individual is female, routine monitoring is not recommended, neither for adrenal insufficiency nor for cerebral ALD. Spinal cord atrophy may be observed amongst individuals suffering from AMN [37]. A recent study showed that patients' spinal cord cross-sectional area correlates with the severity of myelopathy, suggesting that it may serve as a monitoring tool for AMN patients [59].

6. Therapeutic management of X-ALD patients

As expected, a multidisciplinary approach is recommended for the therapeutic management of X-ALD patients. For patients suffering from adrenomyeloneuropathy, no established etiological therapeutic options exist so far. Physical therapy along with treatment of urologic complications and counseling might be of value [60, 61]. If adrenal insufficiency is diagnosed in an affected male, corticosteroid replacement therapy is vital [56, 62]. X-ALD newborn screening in some countries, along with the improvement of imaging modalities, have allowed for more timely intervention, as hematopoietic stem cell transplantation (HSCT) is only indicated for early cerebral disease states. Substantial research is currently underway, using various approaches, in order to identify new effective therapeutic options, offering promising prospects for X-ALD patients [63].

6a. Allogeneic Hematopoietic stem cell transplantation

Allogeneic Hematopoietic stem cell transplantation (HSCT) is a treatment, suitable for boys and adolescents in early stages of CALD. HSCT is thought to halt neurologic progression when performed in these stages, though the underlying mechanism remains unclear [64, 65]. In contrast to neurologic progression, HSCT has no impact on the progression of ad-

Box 4. Monitoring of X-ALD patients

Upon establishment of X-ALD diagnosis, the following management protocol is proposed:
- **Adrenal insufficiency** surveillance
  - Every 3-4 months, if age ≤ 2 years.
  - Every 4-6 months if age > 2 years.

In case of **abnormal** findings, refer to an **endocrinologis**t for **corticosteroid replacement** therapy.

- **MRI** surveillance
  - Obtain an MRI between 12 and 18 months old.
  - Obtain a second MRI 1 year after baseline.
  - Between 3 and 12 years old, obtain a contrast-enhanced MRI every 6 months.
  - After 12 years, obtain an annual MRI.

In case of **abnormal** findings, refer to an **HSCT/gene therapy** specialized center.

- **Genetic testing** and **counselling** seem reasonable in possibly affected family members.

renal insufficiency. Transplantation, when performed in appropriate candidates, not only offers a survival advantage, but also prevents the development of major functional disability. In order to be considered for HSCT, patients must have few lesions on brain MRI and remain in a good clinical condition, as determined by the ALD-specific Neurologic Function Scale (NFS) and the Loes MRI severity score [58]. HSCT is ineffective in patients with advanced disease and cannot reverse neurologic impairment already present at the time of the procedure. In addition, disease stabilization occurs 3-24 months after HSCT, leading to a possible accumulation of disability in the meantime [15, 66]. As expected, allogeneic HSCT may be associated with acute mortality and late complications, such as failure of donor cell engraftment and graft-versus-host disease [15]. Finally, recent reports have drawn attention to a potential beneficial effect of HSCT even in adult patients with CALD [67, 68].

6b. Gene therapy

For the purpose of overcoming allogeneic HSCT's limitations, i.e. finding of a suitable donor and possibility of developing graft versus host disease, transplantation of autologous, genetically-modified hematopoietic stem/progenitor cells (HSPCs) was proposed [69]. A normal copy of the responsible gene is delivered via gene transfer to the HSPCs, which are then infused into the patient. Before the infusion, busulfan, a myeloablative agent with the ability to facilitate the engraftment of the transplanted HSPCs in the hematopoietic and the central nervous system, is administered to the patient [70].

In July 2021, elivaldogene autotemcel (SKYSONA™, eli-cel; Lenti-D™ gene therapy) received approval for the treatment of early cerebral X-ALD in patients below the age of 18, for whom an HLA-matched sibling-hematopoietic stem cell (HSC) donor is not available. The approval study protocol involved 30 boys aged 4 to 14 years with early CALD. According to the study's results, after two years, 90% of the treated boys showed no signs of major functional disability and approximately 96% of the boys experienced a stable Gross Neurological Function Measure score (a score measuring the developing child's ability to achieve expected motor milestones) after two years. Furthermore, there was evidence of continuing benefit for up to 8 years [71]. Developing gene therapy techniques, such as antisense oligonucleotides and small interfering RNAs, may also have a place in the future treatment of X-ALD [72].

6c. Lipid modulation

Lorenzo's oil is a mixture comprising a 4:1 mix of oleic and erucic acids,that, in conjunction with a low-fat diet, normalizes plasma VLCFA levels. Despite several studies arguing against its efficacy in preventing CALD progression once it already exists, Moser et al., reported that it could prevent the development of CALD in presymptomatic subjects [73]. There have also been some reports of benefit of Lorenzo's oil in males suffering from AMN, but came from studies with questionable methodology [74].

In a recently published paper, Moser et al. reported the VLFCA-lowering effect of the antihypertensive irbesartan in cultured skin fibroblasts from an X-ALD patient, implying a potential beneficial effect in X-ALD that should be further validated [75].

Lastly, it has been recently shown *in vivo*, that metabolic rerouting of saturated to monounsaturated VLCFAs by upregulating the enzyme Stearoyl-CoA Desaturase-1 (SCD1 induction) may decrease lipid toxicity, a strategy that may prove of benefit in X-ALD [69].

6d. Antioxidant therapy

In a small open-label trial, 13 patients suffering from AMN were administered a high dose of α-tocopherol, N-acetylcysteine, and α-lipoic acid in combination. Normalization of biomarkers suggestive of oxidative stress and inflammation was observed, as well as a beneficial effect on the 6-min walk test, justifying larger future placebo-controlled trials [76].

Leriglitazone is a newly developed full PPARγ agonist which can cross the blood brain barrier. It has been proved to decrease oxidative stress, increase adenosine 5'-triphosphate concentrations, as well as exert a neuroprotective effect in animal models of AMN. The study's findings also suggest a potential beneficial role for cerebral X-ALD, as it was shown to prevent the progression to disrupted blood-brain barrier [77].

7. Conclusion

Increasing understanding of X-ALD pathophysiology has resulted in the emergence of new promising treatment options. Newborn screening for X-ALD enables the identification of patients at high risk for life-threatening adrenal insufficiency and cerebral ALD early in the disease course, allowing early corticosteroid replacement therapy and timely HSCT or gene therapy for suitable candidates. Hopefully, gene therapy approaches similar to those recently approved for other neurologic monogenic diseases, such as spinal muscular atrophy and Duchenne muscular dystrophy, may be developed for X-ALD in the near future.

References

[1] Heubner O. Über diffuse Hirnsclerose. *Charité Ann.* 1897;22:298-310.
[2] Haberfeld W, Spieler F. Zur diffusen Hirn-Rückenmarksklerose im Kindesalter. *Deutsche Zeitschrift Für Nervenheilkunde.* 1910;40:436-463.
[3] Matteson J, Sciortino S, Feuchtbaum L, et al. Adrenoleukodystrophy Newborn Screening in California Since 2016: Programmatic Outcomes and Follow-Up. *Int J Neonatal Screen.* 2021;7.
[4] Moser HW, Moser AB, Naidu S, et al. Clinical aspects of adrenoleukodystrophy and adrenomyeloneuropathy. *Dev Neurosci.* 1991;13:254-261.
[5] Berger J, Molzer B, Fae I, et al. X-linked adrenoleukodystrophy (ALD): a novel mutation of the ALD gene in 6 members of a family presenting with 5 different phenotypes. *Biochem Biophys Res Commun.* 1994;205:1638-1643.
[6] Di Rocco M, Doria-Lamba L, Caruso U. Monozygotic twins with X-linked adrenoleukodystrophy and different phenotypes. *Ann Neurol.* 2001;50:424.
[7] Moser HW. Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. *Brain.* 1997;120 (Pt 8):1485-1508.
[8] Kemp S, Berger J, Aubourg P. X-linked adrenoleukodystrophy: clinical, metabolic, genetic and pathophysiological aspects. *Biochim Biophys Acta.* 2012;1822:1465-1474.
[9] Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res.* 2001;11:1156-1166.
[10] Kemp S, Pujol A, Waterham HR, et al. ABCD1 mutations and the X-linked adrenoleukodystrophy mutation database: role in diagnosis and clinical correlations. *Hum Mutat.* 2001;18:499-515.
[11] Moser HW, Moser AB, Kawamura N, et al. Adrenoleukodystrophy: elevated C26 fatty acid in cultured skin fibroblasts. *Ann Neurol.* 1980;7:542-549.
[12] Moser HW, Moser AB, Frayer KK, et al. Adrenoleukodystrophy: increased plasma content of saturated very long chain fatty acids. *Neurology.* 1981;31:1241-1249.
[13] Hubbard WC, Moser AB, Liu AC, et al. Newborn screening for X-linked adrenoleukodystrophy (X-ALD): validation of a combined liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. *Mol Genet Metab.* 2009;97:212-220.
[14] Eng L, Regelmann MO. Adrenoleukodystrophy in the era of newborn screening. *Curr Opin Endocrinol Diabetes Obes.* 2020;27:47-55.
[15] Raymond GV, 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;25:538-548.
[16] Kanakis G, Kaltsas G. Adrenal Insufficiency Due to X-Linked Adrenoleukodystrophy. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Endotext.* South Dartmouth (MA), 2000.
[17] Johnson AB, Schaumburg HH, Powers JM. Histochemical characteristics of the striated inclusions of adrenoleukodystrophy. *J Histochem Cytochem.* 1976;24:725-730.
[18] Garashi M, Belchis D, Suzuki K. Brain gangliosides in adrenoleukodystrophy. *J Neurochem.* 1976;27:327-328.
[19] Igarashi M, Schaumburg H, Powers J, et al. Fatty acid abnormality in adrenoleukodystrophy. *Journal of neurochemistry.* 1976;26:851-860.
[20] Moser HW, Moser AB, Powers JM, et al. The prenatal diagnosis of adrenoleukodystrophy. Demonstration of increased hexacosanoic acid levels in cultured amniocytes and fetal adrenal gland. *Pediatr Res.* 1982;16:172-175.
[21] Engelen M, Kemp S, Poll-The BT. X-linked adre-

noleukodystrophy: pathogenesis and treatment. *Curr Neurol Neurosci Rep.* 2014;14:486.
[22] Singh I, Pujol A. Pathomechanisms underlying X-adrenoleukodystrophy: a three-hit hypothesis. *Brain Pathol.* 2010;20:838-844.
[23] Fourcade S, Lopez-Erauskin J, Galino J, et al. Early oxidative damage underlying neurodegeneration in X-adrenoleukodystrophy. *Hum Mol Genet.* 2008;17:1762-1773.
[24] Wanders RJ. Metabolic functions of peroxisomes in health and disease. *Biochimie.* 2014;98:36-44.
[25] Powers JM, DeCiero DP, Ito M, et al. Adrenomyeloneuropathy: a neuropathologic review featuring its noninflammatory myelopathy. *J Neuropathol Exp Neurol.* 2000;59:89-102.
[26] 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;76:481-491.
[27] Schaumburg HH, Powers JM, Raine CS, et al. Adrenoleukodystrophy. A clinical and pathological study of 17 cases. *Arch Neurol.* 1975;32:577-591.
[28] Powers JM. Adreno-leukodystrophy (adrenotesticulo-leukomyelo-neuropathic-complex). *Clin Neuropathol.* 1985;4:181-199.
[29] Whitcomb RW, Linehan WM, Knazek RA. Effects of long-chain, saturated fatty acids on membrane microviscosity and adrenocorticotropin responsiveness of human adrenocortical cells in vitro. *J Clin Invest.* 1988;81:185-188.
[30] Powers JM, Schaumburg HH. The testis in adreno-leukodystrophy. *Am J Pathol.* 1981;102:90-98.
[31] 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;45:100-110.
[32] Takano H, Koike R, Onodera O, et al. Mutational analysis and genotype-phenotype correlation of 29 unrelated Japanese patients with X-linked adrenoleukodystrophy. *Arch Neurol.* 1999;56:295-300.
[33] Pan H, Xiong H, Wu Y, et al. ABCD1 gene mutations in Chinese patients with X-linked adrenoleukodystrophy. *Pediatr Neurol.* 2005;33:114-120.
[34] Campopiano R, Femiano C, Chiaravalloti MA, et al. A Large Family with p.Arg554His Mutation in ABCD1: Clinical Features and Genotype/Phenotype Correlation in Female Carriers. *Genes (Basel).* 2021;12.
[35] Moser HW, Mahmood A, Raymond GV. X-linked adrenoleukodystrophy. *Nat Clin Pract Neurol.* 2007;3:140-151.
[36] Liberato AP, Mallack EJ, Aziz-Bose R, et al. MRI brain lesions in asymptomatic boys with X-linked adrenoleukodystrophy. *Neurology.* 2019;92:e1698-e1708.
[37] Engelen M, Kemp S, de Visser M, et al. X-linked adrenoleukodystrophy (X-ALD): clinical presentation and guidelines for diagnosis, follow-up and management. *Orphanet J Rare Dis.* 2012;7:51.
[38] Mallack EJ, van de Stadt S, Caruso PA, et al. Clinical and radiographic course of arrested cerebral adrenoleukodystrophy. *Neurology.* 2020;94:e2499-e2507.
[39] Griffin JW, Goren E, Schaumburg H, et al. Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. I. Clinical and endocrinologic aspects. *Neurology.* 1977;27:1107-1113.
[40] Koutsis G, Lynch DS, Tucci A, et al. A novel ABCD1 mutation detected by next generation sequencing in presumed hereditary spastic paraplegia: A 30-year diagnostic delay caused by misleading biochemical findings. *J Neurol Sci.* 2015;355:199-201.
[41] Lynch DS, Koutsis G, Tucci A, et al. Hereditary spastic paraplegia in Greece: characterisation of a previously unexplored population using next-generation sequencing. *Eur J Hum Genet.* 2016;24:857-863.
[42] Huffnagel IC, van Ballegoij WJC, van Geel BM, et al. Progression of myelopathy in males with adrenoleukodystrophy: towards clinical trial readiness. *Brain.* 2019;142:334-343.
[43] Eng L, Regelmann MO. Early Onset Primary Adrenal Insufficiency in Males with Adrenoleukodystrophy: Case Series and Literature Review. *J Pediatr.* 2019;211:211-214.
[44] Huffnagel IC, Laheji FK, Aziz-Bose R, et al. The natural history of adrenal insufficiency in X-linked adrenoleukodystrophy: an international collaboration. *The Journal of Clinical Endocrinology & Metabolism.* 2019;104:118-126.
[45] Raymond GV, Moser AB, Fatemi A. X-Linked Adrenoleukodystrophy. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R)).* Seattle (WA), 1993.
[46] Palakuzhiyil SV, Christopher R, Chandra SR. Deciphering the modifiers for phenotypic variability of X-linked adrenoleukodystrophy. *World J Biol Chem.* 2020;11:99-111.
[47] Schuldiner M, Zalckvar E, Kim PK, et al. *Peroxisome Biology: Breakthroughs, Challenges and Future Directions.* Lausanne: Frontiers Media SA, 2021.
[48] Habekost CT, Pereira FS, Vargas CR, et al. Progression rate of myelopathy in X-linked adrenoleukodystrophy heterozygotes. *Metab Brain Dis.* 2015;30:1279-1284.

[49] Engelen M, Barbier M, Dijkstra IM, et al. X-linked adrenoleukodystrophy in women: a cross-sectional cohort study. *Brain.* 2014;137:693-706.
[50] Huffnagel IC, Dijkgraaf MGW, Janssens GE, et al. Disease progression in women with X-linked adrenoleukodystrophy is slow. *Orphanet J Rare Dis.* 2019;14:30.
[51] Finsterer J, Lasser S, Stophasius E. Dementia from the ABCD1 mutation c.1415-1416delAG in a female carrier. *Gene.* 2013;530:155-157.
[52] Lee S, Clinard K, Young SP, et al. Evaluation of X-Linked Adrenoleukodystrophy Newborn Screening in North Carolina. *JAMA Netw Open.* 2020;3:e1920356.
[53] Boehm CD, Cutting GR, Lachtermacher MB, et al. Accurate DNA-based diagnostic and carrier testing for X-linked adrenoleukodystrophy. *Mol Genet Metab.* 1999;66:128-136.
[54] 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;83:3163-3168.
[55] Wong SH, Boggild M, Enevoldson TP, et al. Myelopathy but normal MRI: where next? *Pract Neurol.* 2008;8:90-102.
[56] Regelmann MO, Kamboj MK, Miller BS, et al. Adrenoleukodystrophy: Guidance for Adrenal Surveillance in Males Identified by Newborn Screen. *J Clin Endocrinol Metab.* 2018;103:4324-4331.
[57] Mallack EJ, Turk BR, Yan H, et al. MRI surveillance of boys with X-linked adrenoleukodystrophy identified by newborn screening: Meta-analysis and consensus guidelines. *J Inherit Metab Dis.* 2021;44:728-739.
[58] Loes DJ, Fatemi A, Melhem ER, et al. Analysis of MRI patterns aids prediction of progression in X-linked adrenoleukodystrophy. *Neurology.* 2003;61:369-374.
[59] van de Stadt SIW, van Ballegoij WJC, Labounek R, et al. Spinal cord atrophy as a measure of severity of myelopathy in adrenoleukodystrophy. *J Inherit Metab Dis.* 2020;43:852-860.
[60] Casasnovas C, Ruiz M, Schluter A, et al. Biomarker Identification, Safety, and Efficacy of High-Dose Antioxidants for Adrenomyeloneuropathy: a Phase II Pilot Study. *Neurotherapeutics.* 2019;16:1167-1182.
[61] Marchetti DP, Steffens L, Jacques CE, et al. Oxidative Imbalance, Nitrative Stress, and Inflammation in C6 Glial Cells Exposed to Hexacosanoic Acid: Protective Effect of N-acetyl-L-cysteine, Trolox, and Rosuvastatin. *Cell Mol Neurobiol.* 2018;38:1505-1516.
[62] Miller BS, Spencer SP, Geffner ME, et al. Emergency management of adrenal insufficiency in children: advocating for treatment options in outpatient and field settings. *J Investig Med.* 2020;68:16-25.
[63] Turk BR, Moser AB, Fatemi A. Therapeutic strategies in adrenoleukodystrophy. *Wien Med Wochenschr.* 2017;167:219-226.
[64] Baumann M, Korenke CG, Weddige-Diedrichs A, et al. Haematopoietic stem cell transplantation in 12 patients with cerebral X-linked adrenoleukodystrophy. *European journal of pediatrics.* 2003;162:6-14.
[65] Mahmood A, Raymond GV, Dubey P, et al. Survival analysis of haematopoietic cell transplantation for childhood cerebral X-linked adrenoleukodystrophy: a comparison study. *Lancet Neurol.* 2007;6:687-692.
[66] Yalcin K, Çelen SS, Daloglu H, et al. Allogeneic hematopoietic stem cell transplantation in patients with childhood cerebral adrenoleukodystrophy: A single-center experience "Better prognosis in earlier stage". *Pediatric Transplantation.* 2021:e14015.
[67] 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;140:953-966.
[68] Waldhuter N, Kohler W, Hemmati PG, et al. Allogeneic hematopoietic stem cell transplantation with myeloablative conditioning for adult cerebral X-linked adrenoleukodystrophy. *J Inherit Metab Dis.* 2019;42:313-324.
[69] Shahryari A, Saghaeian Jazi M, Mohammadi S, et al. Development and Clinical Translation of Approved Gene Therapy Products for Genetic Disorders. *Front Genet.* 2019;10:868.
[70] Capotondo A, Milazzo R, Politi LS, et al. Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation. *Proc Natl Acad Sci U S A.* 2012;109:15018-15023.
[71] Keam SJ. Elivaldogene Autotemcel: First Approval. *Molecular Diagnosis & Therapy.* 2021:1-7.
[72] Bradbury AM, Ream MA. Recent Advancements in the Diagnosis and Treatment of Leukodystrophies. *Semin Pediatr Neurol.* 2021;37:100876.
[73] Moser HW, Raymond GV, Lu SE, et al. Follow-up of 89 asymptomatic patients with adrenoleukodystrophy treated with Lorenzo's oil. *Arch Neurol.* 2005;62:1073-1080.
[74] Aubourg P, Adamsbaum C, Lavallard-Rousseau M-C, et al. A two-year trial of oleic and erucic acids ("Lorenzo's oil") as treatment for adrenomyeloneuropathy. *New England Journal of Medicine.* 1993;329:745-752.
[75] Moser AB, Liu Y, Shi X, et al. Drug discov-

ery for X-linked adrenoleukodystrophy: An unbiased screen for compounds that lower very long-chain fatty acids. *J Cell Biochem.* 2021;122:1337-1349.

[76] Raas Q, van de Beek MC, Forss-Petter S, et al. Metabolic rerouting via SCD1 induction impacts X-linked adrenoleukodystrophy. *J Clin Invest.* 2021;131.

[77] Rodríguez-Pascau L, Vilalta A, Cerrada M, et al. The brain penetrant PPARγ agonist leriglitazone restores multiple altered pathways in models of X-linked adrenoleukodystrophy. *Science Translational Medicine.* 2021;13.