Adrenoleukodystrophy – neuroendocrine pathogenesis and redefinition of natural history

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Abstract | X-Linked adrenoleukodystrophy (ALD) is a peroxisomal metabolic disorder with a highly complex clinical presentation. ALD is caused by mutations in the *ABCD1* gene, which leads to the accumulation of very long-chain fatty acids in plasma and tissues. Virtually all men with ALD develop adrenal insufficiency and myelopathy. Approximately 60% of men develop progressive cerebral white matter lesions (known as cerebral ALD). However, one cannot identify these individuals until the early changes are seen using brain imaging. Women with ALD also develop myelopathy, but generally at a later age than men and adrenal insufficiency or cerebral ALD are very rare. Owing to the multisystem symptomatology of the disease, patients can be assessed by the paediatrician, general practitioner, endocrinologist or a neurologist. This Review describes current knowledge on the clinical presentation, diagnosis and treatment of ALD, and highlights gaps in our knowledge of the natural history of the disease owing to an absence of large-scale prospective cohort studies. Such studies are necessary for the identification of new prognostic biomarkers to improve care for patients with ALD, which is particularly relevant now that newborn screening for ALD is being introduced.

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Identical gene mutations should lead to specific phenotypes; however, this simplistic view does not adequately address the widely varying phenotypes that are commonly observed in many inborn errors of metabolism[1,2]. Indeed, even identical mutations can lead to widely variable clinical phenotypes, which suggests that additional factors modify disease manifestations.

This situation is clearly exemplified by X-linked adrenoleukodystrophy (ALD; OMIM:300100), which is the most common leukodystrophy, but also one of the most puzzling inborn errors of metabolism of the CNS. ALD is a progressive neurodegenerative disease that results from a deficiency of the ATP-binding cassette sub-family D member 1 (also known as ALDP) encoded by the *ABCD1* gene[3]. ALDP deficiency results in impaired peroxisomal β-oxidation of saturated straight-chain very long-chain fatty acids (VLCFA; ≥C22:0)[4–6]. Consequently VLCFAs accumulate in plasma[7] and tissues[8], including the white matter of the brain[9], spinal cord and adrenal cortex[9]. The disease has an estimated incidence of 1 in 17,000 (REF. 10), and has been diagnosed in all geographic regions and ethnic groups[11–14], without any evidence that prevalence varies with ethnicity[15]. ALD is characterized by a striking and unpredictable variation in clinical outcomes among men, ranging from adrenal insufficiency to rapidly progressive and fatal cerebral demyelination (cerebral ALD)[8,16]. In adulthood, virtually all men[16] and 80% of women[17] with ALD develop progressive spinal cord disease (that is, adrenomyeloneuropathy (AMN)). In the absence of a genotype–phenotype correlation, predicting the disease course is impossible, even within individual families[18]. In this Review we discuss the current state of knowledge on the clinical presentation, diagnosis and treatment of ALD, and highlight gaps in our knowledge of the natural history.

History

ALD was originally described as a progressive white matter disorder of the brain, which was associated with adrenal failure in young boys (≤10 years of age)[19], and became known as Schilder's disease in the 1950s[20]. Later that decade, reports of a hereditary spastic paraplegia associated with adrenal failure in adults began to appear[21,22]. After the description in the 1970s of 'intracytoplasmic lamellae and lamellar-lipid inclusions' in electron microscopy studies of the adrenal glands from boys who had died of 'Schilder's disease' (REFS 23,24), closely followed by the discovery that these lipid inclusions consisted of

Key points

- Adrenoleukodystrophy (ALD) is a peroxisomal metabolic disorder owing to mutations in *ABCD1* with a highly complex clinical presentation
- Presenting complaints are usually adrenal insufficiency (80% in childhood) or myelopathy (in adulthood)
- Cerebral ALD can occur at any age and is most likely defined by the interplay between the primary *ABCD1* mutation, a multitude of genetic variants and environmental factors
- Haematopoietic stem cell transplantation (HSCT) remains the only therapeutic intervention for cerebral ALD, but the outcome of the procedure is poor unless performed at an early stage of cerebral disease
- No treatment is currently available for the progressive myelopathy associated with ALD
- Early diagnosis of boys with ALD by screening at birth allows the early detection of adrenal insufficiency to initiate timely adrenal steroid replacement therapy and the early detection of cerebral ALD to offer allogeneic HSCT

cholesterol, phospholipids and gangliosides esterified with VLCFA[9], a diagnostic biomarker became available[7]. The biochemical signature of ALD is the abnormal accumulation of hexacosanoic acid (C26:0)[7]. Following these results, Schilder's disease and hereditary spastic paraparesis with adrenal insufficiency were clearly part of one clinical spectrum of the same metabolic disorder characterized by VLCFA accumulation[24,25]. In the late 1970s and 1980s, the clinical classification of ALD was improved. At the Kennedy Krieger Institute in Baltimore, Hugo Moser and his group made great contributions to the clinical characterization of ALD and created awareness of the disease among physicians[8]. ALD was now considered a disease with distinct phenotypes, either manifesting as rapidly progressive cerebral demyelination in childhood (that is, childhood cerebral ALD), or as progressive myelopathy in adulthood (known as adrenomyeloneuropathy (AMN))[25,26]. Other categories of ALD, such as 'Addison only', 'adolescent cerebral' and 'adult cerebral' were described but are considered rare[8]. Although female carriers of ALD were known to also develop signs and symptoms of the disease[27–32], a systematic study on disease manifestations in women was only published in 2014 (REF. 17). The aforementioned terminology of ALD phenotypes is still widely used; however, ALD is more accurately considered as a progressive disorder. This brief history demonstrates how the term 'adrenoleukodystrophy' evolved, but it might be considered a misnomer as not all patients have or will develop cerebral or adrenal involvement (especially women). The term '*ABCD1*-deficiency', therefore, seems to be a more accurate description for ALD; however, the disease terminology might take decades before it is revised.

Clinical features and diagnosis

ALD is a progressive disease. Detailed large-scale prospective natural history studies are currently not available, although some are in progress. Based on published data and expert opinion, one can reasonably assume that all patients with ALD are born presymptomatic (FIG. 1). The youngest symptomatic patient reported was ~3 years old[8]. The first manifestations of the disease in male patients with ALD are usually adrenal insufficiency, typically observed in childhood[33]. In adulthood, signs of myelopathy invariably develop[8,16]. Progressive cerebral demyelination can occur, both in childhood and adulthood, either as the first manifestation of ALD or in addition to adrenal insufficiency or myelopathy[34,35] (FIG. 1). Women with ALD develop signs of myelopathy[17], yet other manifestations (such as adrenal insufficiency or cerebral demyelination) are very unusual (<1%)[31,36]. Women with ALD are also affected and not merely carriers of the *ABCD1*-deficiency, as >80% of these individuals have developed signs and symptoms of myelopathy by the age of 60 years[17]. The risk of experiencing symptoms increases with age (from 18% below 40 years of age to >80% by 60 years of age.)[17]. ALD should, therefore, be considered a progressive metabolic disease.

Adrenal insufficiency in ALD. In a prospective evaluation of adrenal function in a cohort of 49 neurologically presymptomatic boys (age range 5 months to 13 years), 80% already had biochemical evidence of otherwise clinically silent adrenal insufficiency at the time of ALD diagnosis[33]. The youngest boy with clinically silent adrenal insufficiency was 5 months of age[33]. Primary adrenal insufficiency is a prominent feature of ALD and is characterized by high levels of adrenocorticotropic hormone (ACTH) and low levels of cortisol[37]. Current endocrine tests cannot discriminate between adrenal insufficiency owing to ALD and other causes, such as those associated with an autoimmune response, which is the most prevalent, especially in adults[38]. Patients with ALD have hyperpigmentation, as seen in all individuals with primary adrenal insufficiency, owing to high levels of ACTH (FIG. 2). The nature of adrenal gland toxicity and its relationship with increased VLCFA levels is poorly understood. However, accumulation of cholesterol with saturated VLCFA in the zona fasciculata-reticularis has been reported[9,39], and is already present in fetal adrenal glands[40]. This chronic accumulation of cholesterol with saturated VLCFA is believed to result in apoptosis and ultimately atrophy of the adrenal cortex, with loss of cortisol production[41]. Notably, adrenal insufficiency has also been reported in patients with a Zellweger spectrum disorder[42,43], who also accumulate VLCFA in their adrenal glands. This observation, therefore, lends further support to the notion that VLCFA accumulation is important in the pathophysiology of adrenal insufficiency.

Owing to the lack of detailed prospective natural history studies of the disease, the penetrance of adrenal insufficiency in ALD is not known. The investigators of some studies report a penetrance of 50–100%[44], but in our own prospective ALD cohort several men in their sixties maintain normal adrenal function (M. Engelen, unpublished data). In some patients with ALD a slightly abnormal result of the ACTH stimulation test, without any clinical symptoms related to hypocortisolism, seems to support the hypothesis that a loss of adrenal function is a gradual, progressive phenomenon, similar to the myelopathy and peripheral neuropathy (M. Engelen, unpublished data). Patients with ALD, but without clinical

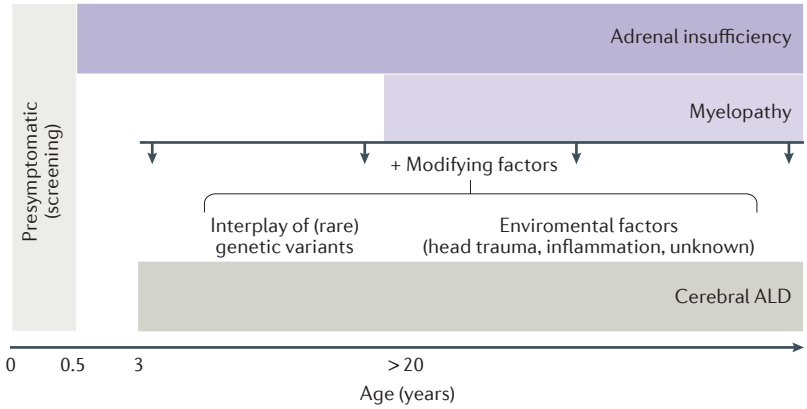

Figure 1 | **The clinical spectrum of ALD in men.** Patients with ALD are presymptomatic at birth. The coloured bars indicate the age-range of onset for adrenal insufficiency (purple bar), myelopathy (mauve bar) and cerebral ALD (grey bar). Onset of adrenal insufficiency can be as early as 5 months of age. In adulthood, men develop a chronic progressive myelopathy. Cerebral ALD can occur at any age, but the youngest reported patient was 3 years of age. Patients with adrenal insufficiency and/or myelopathy are at risk of developing cerebral ALD. Initiation of cerebral ALD is most likely defined by the interplay of the primary *ABCD1* gene defect and a multitude of genetic variants and environmental factors.

signs of adrenal insufficiency, should therefore, have periodic assessments of endogenous cortisol production (preferably after ACTH administration).

Gonadal function can also be affected in ALD. Levels of testosterone in men with ALD are usually in the lower-normal range with elevation of luteinizing hormone in some patients[45,46]. This observation is indicative of primary hypogonadism, possibly due to the toxicity of VLCFA in Leydig and Sertoli cells, but tissue androgen receptor resistance has also been suggested as an alternative hypothesis to explain this finding[45,47]. Importantly, decreased libido and erectile dysfunction might be related to myelopathy and/or the presence of a chronic disease and might not necessarily reflect hypogonadism[45]. To date, no trials to test the outcome of testosterone supplementation in men with ALD have been performed. Historically, some patients have been prescribed dehydroepiandrosterone-sulfate, but this does not seem to have a beneficial effect[48]. In men with ALD, fertility seems to be normal[49]; no data exists on fertility in women with ALD.

In a 2016 multicentre study in children (48 girls and 47 boys) with primary adrenal insufficiency of unknown cause, two of 47 boys had *ABCD1* mutations consistent with ALD, while at the time of study no neurological symptoms were present that indicated underlying ALD[50]. This finding highlights the need to include VLCFA assessment in any child with primary adrenal insufficiency. In adult patients with primary adrenal insufficiency, VLCFA assessment is often recommended[8,16,33], but usually not routinely performed. For example, in a study in 153 men from Norway who had primary adrenal insufficiency, no previously unknown cases of ALD were found[51], an observation supported by our own clinical experience. Screening for ALD, therefore, is important in all boys with primary adrenal insufficiency, whereas in adults with concomitant neurological symptoms, the physician should consider ALD.

The myelopathy of ALD. The signs and symptoms of the chronic progressive myelopathy in ALD are not specific. Patients report a slowly progressive gait disorder due to spastic paraparesis and sensory ataxia[16]. Neurogenic bladder dysfunction with urinary urgency is also present[16]. On neurologic examination spasticity and paresis, brisk reflexes with Babinski sign, and prominent dorsal column dysfunction are seen. Although on examination brisk tendon reflexes in the arms (and sometimes a positive Hoffman-Trömner sign) can also occur, patients do not report loss of dexterity or strength in the arms[16]. Neurologic bladder dysfunction is almost invariably present, initially presenting as urge complaints, progressing to full incontinence[16]. In male patients with ALD, typical age of myelopathy onset is in the 3rd decade of life, but in some cases can be earlier (2nd decade of life)[52], or much later (up to the 5th decade)[52]. Progression of myelopathy occurs over years or decades, with most patients losing unassisted ambulation by the 6th decade[16,34]. This description is based on expert opinion and retrospective data, as large prospective natural history studies with quantitative outcome parameters have not been done[8,16,34,52]. In a retrospective study, the progression of physical disability (which is quantified with the modified Rankin scale) was reported for 60 men with ALD[34]. Approximately 16 years after the first manifestation of myelopathy, patients with AMN require assistance to walk and might even be confined to a wheelchair[34]. Conventional MRI of the spinal cord shows atrophy of the cervical spinal cord in advanced myelopathy. Abnormalities on diffusion tensor imaging or magnetization transfer imaging become apparent much earlier than in MRI[53,54]. In women with ALD the myelopathy occurs at a later age than in men (around the 5th decade) and progression is slower than in men[17]. However, faecal incontinence is also a frequent complaint in women with ALD[17]. The diagnosis of ALD in women with a slowly progressive myelopathy is not always considered, and in our cohort two women were initially misdiagnosed with spondylogenic cervical myelopathy[17].

Peripheral neuropathy in ALD. Nerve conduction studies in patients with ALD often reveal a peripheral neuropathy, usually a sensorimotor axonal neuropathy[17,55]. There are reports of the peripheral neuropathy fulfilling criteria of a demyelinating neuropathy[56,57]. Small nerve fibre neuropathy is probably also common[58]. Clinically, the more disabling and prominent signs of myelopathy usually mask signs of concomitant neuropathy. In addition, some signs are difficult to attribute; for example, loss of vibration sense can indicate dorsal column dysfunction or peripheral neuropathy. In some patients signs of peripheral neuropathy can be prominent[55].

Cerebral disease in ALD. Men with ALD are at risk of developing demyelinating lesions in the cerebral white matter[8,16,35]. Onset of these lesions has never been reported before the age of 3 years[8]. Cerebral ALD was considered to be rare in adolescence (4–7%) and adulthood (2–5%)[8], but this finding might be the result of diagnostic bias. In our current knowledge, we cannot

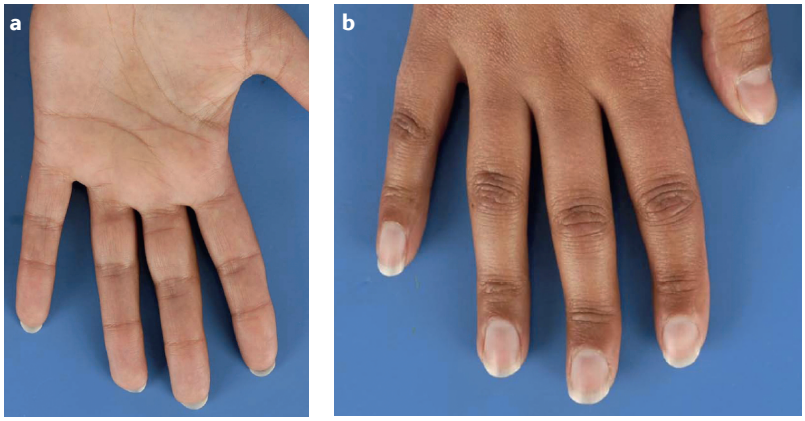

Figure 2 | **Hyperpigmentation of the hand of a white patient with primary adrenal insufficiency.**

predict if and when a patient will develop cerebral ALD. A possible environmental trigger is head trauma[59–61], but other modifiers (both genetic and environmental) have not yet been identified (discussed later in the text). White matter lesions on MRI can precede symptoms, and typically begin in the splenium of the corpus callosum before gradually expanding into the periventricular and occipital white matter[62]. In ~15% of cerebral ALD cases the lesion starts from the genu of the corpus callosum and spreads into the frontal white matter[63,64]. In 1–2% of cases, lesions can begin in the cerebellar white matter or even the brainstem[8,62,64]. Lesions show gadolinium enhancement just behind the leading edge of the lesion[65] (FIG. 3). Cerebral white matter lesions in ALD can be scored with the classification system that scores the severity of white matter lesions on a scale of 0 (normal on MRI) to 34 (severely abnormal)[66].

The symptoms associated with cerebral ALD depend on the site of the initial lesions. In elementary school aged boys, the first symptoms are usually cognitive deficits and behavioural problems manifesting as a decline in school performance[8]. These early clinical symptoms are often initially attributed to other disorders such as attention deficit hyperactivity disorder, which can delay the diagnosis of ALD[8]. In adults, initial symptoms are often psychiatric as well, especially if the lesions are located in the frontal lobes, and can resemble a depression or psychosis[67,68]. In these patients, the diagnosis of ALD is often delayed; especially when no family history of ALD is present and when clinical symptoms of adrenal insufficiency are absent. When lesions progress, pyramidal tract signs, central visual impairment and sometimes seizures occur[8]. Cerebral ALD is considered relentlessly progressive and when untreated leads to severe disability and death on average 2 years after the onset of symptoms[8]. Spontaneous stabilization of cerebral ALD lesions with disappearance of gadolinium enhancement (known as arrested cerebral ALD) has been reported[69], but is considered rare[8,62]. In an ongoing prospective natural history study including 46 boys and men with ALD at the Academic Medical Center in Amsterdam, 31% of male patients had typical white matter involvement without gadolinium enhancement, suggesting that arrested cerebral ALD lesions occur rather frequently. Moreover, these preliminary data suggest that the natural history of cerebral ALD might not always be as rapidly progressive as initially claimed (M. Engelen, unpublished data). The occurrence of symptoms of cerebral ALD in women is exceedingly rare. Some isolated cases have been reported[70–72], and are possibly associated with unfavourable non-random X-inactivation[18,70]. MRI abnormalities suggestive of cerebral demyelination are also rare in women with ALD[73].

Other features. Patients with ALD often have thin and scant scalp hair and develop male type balding early in life, usually already in their twenties[74]. *ABCD1* is highly expressed in hair follicles[75], but the association between alopecia of the scalp and ALDP deficiency has not been investigated. Patients with ALD who do not have cerebral demyelination have been reported to often exhibit neuropsychiatric symptoms and can have neuropsychological deficits[76,77].

Diagnosis

ALD has distinct clinical and radiological features that are recognizable by the clinician. The combination of primary adrenal insufficiency and neurological signs is a diagnostic clue that will result in an ALD diagnosis even after a superficial literature search. Patients with ALD are usually diagnosed by a paediatrician, an internist, endocrinologist, or a neurologist. Inclusion of ALD in diagnostic algorithms for chronic myelopathy in adults and adrenal insufficiency in boys and men has definitively increased the likelihood of rapid diagnosis[78]. If ALD is suspected, the diagnosis can be confirmed by biochemical and genetic laboratory tests. In men and boys, unambiguous diagnosis of ALD can be achieved by demonstrating elevated levels of VLCFA in plasma[7,8]. Importantly, 10–15% of women with ALD have normal VLCFA levels in plasma[79,80] and fibroblasts[17]. Consequently, *ABCD1* mutation analysis is recommended in women suspected of ALD[81]. This is easier if the disease-causing *ABCD1* mutation has already been identified in the family or if mutation analysis yields a known pathogenic mutation. However, if mutation analysis identifies a sequence variant with unknown clinical significance, and in combination with a normal VLCFA profile, clinicians can have a diagnostic dilemma. For example, a 2016 report describes two women with a clinical presentation compatible with ALD, an *ABCD1* variation of unknown significance, a normal VLCFA profile and a negative history for ALD[82]. In that publication, a diagnostic test is described to investigate the pathogenicity of *ABCD1* variants of unknown significance[82].

Genetics and biochemistry

All patients with ALD carry a mutation in *ABCD1* (REF. 3). This disorder is inherited in an X-linked manner, and ~4% of patients are affected by a *de novo* mutation, which indicates that the mutation occurred in the germ line[83]. Forty three percent of kindreds with ALD have a unique mutation; 750 non-recurrent mutations have

been catalogued in the ALD database [15]. Nine hotspot mutations have been identified, which together account for 20% of all cases; the most common mutation, a microdeletion in exon 5 (p.Gln472Argfs*83)[84], has been identified in 105 of 1,750 kindreds investigated.

The lack of a simple genotype–phenotype correlation in ALD is clearly exemplified by the fact that patients with the deleterious p.Gln472Argfs*83 mutation can present with all of the different clinical variants of ALD[84,85], and the observation that six brothers with ALD with a p.Pro484Arg mutation had five different ALD phenotypes[86]. Monozygotic twins have been reported in which only one sibling was affected by cerebral ALD[87–89]. These data indicate that the primary defect in *ABCD1,* and the storage of VLCFA in tissues, results in adrenal insufficiency and myelopathy, but that additional environmental triggers and/or genetic factors are required for the initiation of cerebral demyelination (FIG. 1). This hypothesis is further supported by the fact that AMN has a near 100% lifetime penetrance: virtually all men and up to 80% of women with the disease develop myelopathy[17,90,91]. Segregation analysis has suggested the involvement of an autosomal modifier gene that has a major role in determining the clinical manifestation of ALD[92,93]. Ever since the identification of the *ABCD1* gene as the genetic cause of ALD, investigators have been hunting for modifier genes. To date, modifier studies using the candidate gene and genome-wide association approaches have been largely unsuccessful; reviewed elsewhere[94]. In several cases a positive association with clinical outcome was found, such as with genes involved in methionine metabolism[95,96]; however, this association could not be validated in another ALD cohort[97]. An inherent challenge associated with identifying modifying genes in any rare disease is the relatively small number of patients. Many linkage studies might have been performed in underpowered sample collectives, which makes a definitive conclusion difficult. Moreover, as ALD is a progressive disease, when patients are studied to identify certain modifying factors these groups represent a single point in time. For example, whereas cerebral ALD is a clearly distinct phenotype, patients classified as 'myelopathy only' at the time of study might develop cerebral ALD later in life.

Taken together, these currently available data strongly indicate that the existence of a single modifier gene or locus is highly unlikely. Clinical manifestations of ALD will likely be defined by the interplay of a multitude of rare genetic variants and environmental factors (FIG. 1).

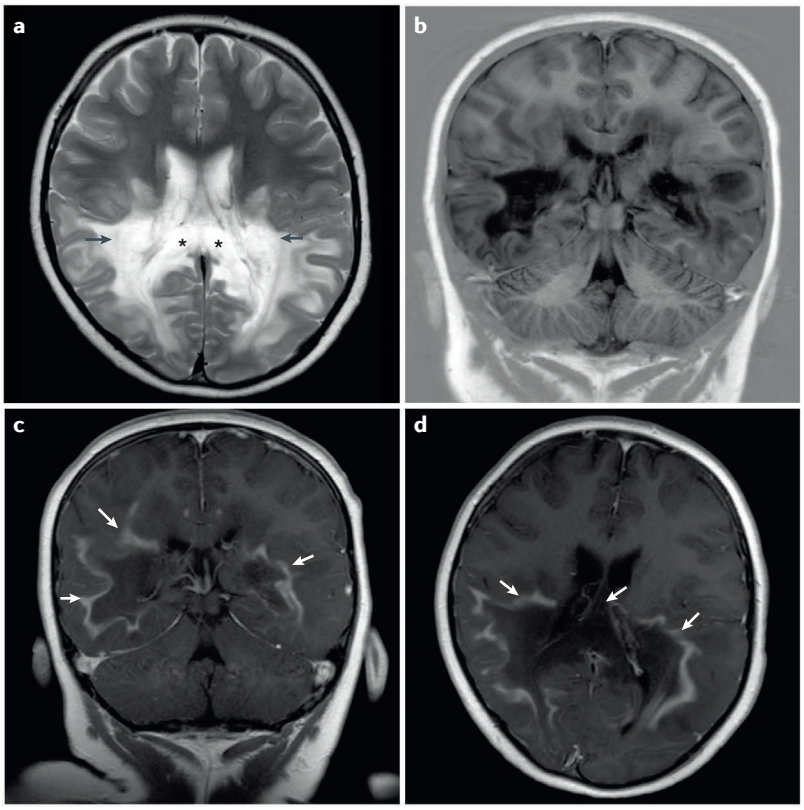

Figure 3 | **MRI of a 6-year-old boy with cerebral ALD.** Note the extensive white matter lesions involving the parieto-occipital white matter (arrows) and splenium of the corpus callosum (asterisks). **a** | The lesion shows increased signal intensity on T2-weighted images. **b** | the lesion has decreased signal on T1-weighted images. **c** | extensive gadolinium enhancement just beyond the leading edges of the lesion on coronal. **d** | axial T1-weighted images after gadolinium administration.

The ALD protein

ABCD1 spans 19.9 kb and 10 exons and encodes a peroxisomal transmembrane protein of 745 amino acids with the general structure of an ATP-binding cassette (ABC) transporter[98]. ALDP is an integral peroxisomal membrane protein with the ATP-binding domain located towards the cytoplasmic surface of the peroxisomal membrane[99]. ALDP transports VLCFA-CoA across the peroxisomal membrane into the peroxisomal matrix[100], which then undergoes degradation by peroxisomal β-oxidation[4,5] (FIG. 4). β-oxidation activity of VLCFA in ALDP-deficient fibroblasts is ~15–25% of the levels seen in normal fibroblasts[101,102], which is explained by the presence of ATP-binding cassette sub-family D member 3 (also known as PMP70), encoded by *ABCD3*, which can also transport VLCFA-CoA into peroxisomes[103]. This finding is consistent with the demonstration that overexpression of either *ABCD2* or *ABCD3* in ALDP-deficient cells rescues peroxisomal VLCFA β-oxidation[104,105]. Although ALDP is an integral peroxisome membrane protein, 65% of missense mutations affect protein folding and stability, which results in a marked reduction of ALDP from the membrane[106,107]. Some of these 'unstable' mutants can be rescued by low temperature culture of fibroblasts, which in some cases normalizes VLCFA levels[108]. Studies aimed at the identification of small-molecules that correct aberrant protein folding might, therefore, have therapeutic relevance for ALD.

A deficiency in ALDP reduces VLCFA-CoA import into peroxisomes[100], and impairs the breakdown of VLCFA. This effect leads to a rise in the levels of cytosolic VLCFA-CoA[109], further chain lengthening by elongation of very long chain fatty acids protein 1 (ELOVL1), the human VLCFA-specific elongase[109], and enhanced incorporation of VLCFA into complex lipids[110] (FIG. 4).

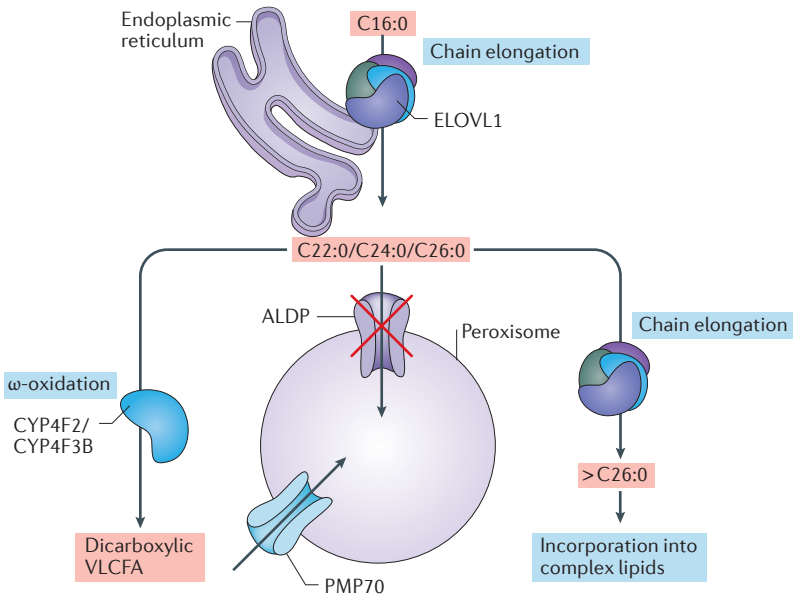

Figure 4 | **Key enzymes in VLCFA homeostasis.** Very long-chain fatty acids (VLCFA) are synthesized via chain-elongation of long-chain fatty acids by ELOVL1, the human VLCFA-specific elongase. A deficiency in ALDP impairs peroxisomal import and β-oxidation of VLCFA. The resulting rise in cytosolic VLCFA levels results in further fatty acid chain-elongation by ELOVL1, and enhanced incorporation of VLCFA into complex lipids. ω-oxidation by CYP4F2/CYP4F3B provides a possible escape route as dicarboxylic-VLCFA are transported into peroxisomes via PMP70.

VLCFA and pathophysiology

The exact role of VLCFA in the pathogenesis of ALD remains largely unresolved. VLCFA are extremely hydrophobic with different physiological properties than long-chain fatty acids. For example, the desorption rate of C26:0 from a phospholipid bilayer model membrane is 10,000 times slower than that of C16:0 and C18:0 fatty acids[111]. Microcalorimetric measurements have shown that inclusion of C26:0-containing lipids in a plasma membrane can disrupt its structure[111]. These measurements agree with the finding that, compared with controls, membrane microviscosity is increased in erythrocytes from patients with ALD[112], and the observation that adding C26:0 to the culture medium of adrenocortical cells leads to increased membrane microviscosity and a decreased response to ACTH stimulation[113]. Some insight into the cytotoxic properties of C26:0-containing lipids comes from the demonstration that exposure of rat oligodendrocytes and astrocytes to C26:0, but not C16:0, induced apoptosis[114]. Furthermore, C26:0 exposure induced high reactive oxygen species generation, depolarization of the mitochondria *in situ,* and deregulation of intracellular calcium homoeostasis[114–116]. In mice, injection of C24:0-lysophosphatidylcholine, but not C16:0-lysophosphatidylcholine, into the brain resulted in widespread microglial activation and apoptosis[117]. In the spinal cord of *Abcd1* knockout mice, oxidative damage to proteins is present long before the first neuropathological lesions or onset of clinical signs manifest[115]. These data indicate that a disturbed fatty acid homeostasis and/or an excess amount of C26:0 in specific lipid classes has a critical role in the pathogenesis of ALD. However, new findings suggest that ALDP-deficiency might affect other cellular functions beyond VLCFA metabolism alone[118]. siRNA-mediated *ABCD1* silencing in cultured human brain microvascular endothelial cells leads to increased expression of adhesion molecules and a decrease in tight junction proteins, which thereby facilitates the transmigration of monocytes across the endothelium[118]. Importantly, these effects were seen before an increase in VLCFA levels was demonstrated in these *ABCD1*-silenced cells.

Current treatment strategies

Treatment of ALD with glucocorticoid supplementation is similar to that of patients with other causes of primary adrenal insufficiency. In our own cohort, supplementation of mineralocorticoids is unnecessary in all patients with ALD, as mineralocorticoid function seems to be preserved in some patients. As adrenal insufficiency can be the presenting symptom of ALD, endocrinologists should, therefore, test for VLCFA accumulation in boys and men when tests for adrenal cortex autoantibodies are negative, or when signs of myelopathy are present[51]. Haematopoietic stem cell transplantation (HSCT) remains the only therapeutic intervention for cerebral ALD, but outcome of the procedure is poor (5-year survival 60% with extensive neurological deficits) unless performed at an early stage of cerebral disease (5-year survival >90%)[119–121], usually defined as few lesions on brain MRI (for example, a score of ≤9 according to the criteria of Loes *et al.*)[64] and good clinical condition (that is, no serious focal deficits and performance IQ of ≥80)[122]. If HSCT is successful, lesions will stabilize 6–12 months after engraftment[119–121], therefore the outcome of HSCT is poor if performed in advanced cases of cerebral ALD (generally defined as a score >9)[120,122]. HSCT-related mortality in specialized centres is 5–10%, and is dependent on factors like availability of a matched related donor[119,120,122]. Virtually all the literature on HSCT for cerebral ALD describes the procedure in children. Now that MRI follow-up is becoming routine for adult patients with ALD[16], cerebral ALD is clearly not as rare as previously assumed. No pathophysiological reason exists to assume that HSCT would not be effective for cerebral ALD in adults. In preliminary data from France and Germany, HSCT seems to be as effective in adults as it is in children, but that transplant-related death is probably higher[123]. Men with advanced myelopathy (with severe motor symptoms and incontinence) are also likely to be at higher risk of complications, possibly related to poor clinical condition before transplant[123]. In the near future, transplantation with genetically corrected autologous haematopoietic stem cells might become an alternative to allogeneic HSCT, once the highly encouraging results reported in the first two treated patients have been extended to a larger number of patients with cerebral ALD[124]. However, importantly, HSCT performed in childhood does not seem to prevent the onset of myelopathy and peripheral neuropathy in adulthood. In a study of five adult men (age range 18 to 25 years) who

underwent HSCT in childhood (age range 4 to 9 years) for the treatment of cerebral disease, three patients developed signs of myelopathy during follow-up[125]. Whether HSCT has any disease-modifying effect on AMN remains to be determined, and longer follow-up of a large group of transplanted and untransplanted patients is needed. Such a study has been initiated on behalf of the Center for International Blood and Marrow Transplant Research. The finding that transplanted boys still can develop myelopathy in adulthood indicates that HSCT only halts the inflammatory component of cerebral ALD without addressing the underlying biochemical defect[125]. Consequently, the chronic myelopathy that affects all men and most women with ALD is related to chronic VLCFA toxicity[8,41,126].

This finding highlights the need to develop an effective treatment that lowers VLCFA levels in the CNS. Lorenzo's oil is a dietary therapy based on oral administration of oleic acid (C18:1) and erucic acid (C22:1), both in triglyceride form, that normalizes plasma C26:0 levels within 1 month in most patients with ALD[127,128]. Importantly, this treatment does not affect levels of C26:0 in the nervous system[129]. However, in several open-label trials, Lorenzo's oil failed to improve neurological or endocrine function in patients with ALD nor did it arrest the progression of the disease[130–132]. A cohort of 89 boys with ALD, who were presymptomatic and had a normal MRI, was followed for several years (mean 6.9 years)[133]. In this cohort, C26:0 levels in plasma were reduced with Lorenzo's oil and an association was seen between a reduction in plasma C26:0 levels and a reduced risk of developing cerebral ALD during childhood[133]. However, in this cohort 24% of boys still developed MRI abnormalities that were suggestive of cerebral demyelination. An important limitation of the study is that the investigators used historical controls instead of a placebo group. In the historical data, the expected percentage of boys developing cerebral ALD is 37%[8], but this figure might be an overestimation because of selection bias towards severe clinical presentations. Indeed, other investigators reported a lower percentage (31%) of boys developing cerebral ALD before the age of 21 years[52]. This study, therefore, offers no definitive evidence for the efficacy of Lorenzo's oil in the prevention of the onset of cerebral ALD in boys with ALD. The use of a strict low-fat diet and Lorenzo's oil is not without effect on quality of life. Potential adverse effects include mild increases in liver enzyme levels (55%), thrombocytopenia (55%), gastrointestinal complaints (14%) and gingivitis (14%)[132]. The costs and potential benefits of Lorenzo's oil are weighed differently around the world. Lorenzo's oil is now seldomly used in Europe, but many patients in the USA often use Lorenzo's oil during childhood based on the aforementioned study[133].

Lovastatin, a cholesterol-lowering drug, can reduce levels of plasma VLCFA[134]. However, in a placebo-controlled trial, levels of C26:0-containing lipids in blood cells remained unaffected with lovastatin treatment[135]. In plasma, VLCFA are transported as cholesterol esters in lipoprotein particles. The effect of lovastatin on VLCFA was, therefore, considered to be a nonspecific effect owing to the decrease in plasma levels of LDL cholesterol[135].

VLCFA are synthesized from long-chain fatty acids by ELOVL1 (REF. 109) (FIG. 4), and siRNA-mediated ELOVL1 knockdown reduced both C26:0 synthesis and levels in human ALD fibroblasts[109]. Consequently, pharmacological inhibition of ELOVL1 activity might be an attractive therapeutic option[109]. The lipid-lowering drug, bezafibrate can reduce *de novo* C26:0 synthesis in human ALD fibroblasts to normal levels[136], by competitively inhibiting ELOVL1[137]. Unfortunately, bezafibrate did not lower C26:0 levels in leukocytes from patients with ALD, which was most likely attributable to the inability of reaching adequate drug levels *in vivo* to have an effect[138]. Interestingly, VLCFA can also undergo ω-oxidation by phylloquinone ω-hydroxylase CYP4F2 and docosahexaenoic acid ω-hydroxylase CYP4F3 (isoform CYP4F3B), which generates dicarboxylic-VLCFA[139–141] (FIG. 4). The β-oxidation of dicarboxylic-VLCFA is normal in ALD patients[142], because their import into peroxisomes involves PMP70 (REF. 143). The stimulation of this existing metabolic pathway might, therefore, provide an escape route, because it can compensate for the deficient peroxisomal β-oxidation of VLCFA in patients with ALD[144]. Experimental evidence also suggests that the onset of neurological symptoms in *Abcd1*/*Abcd2* double knockout mice can be prevented by treatment with an antioxidant cocktail (*N*-acetyl-cysteine, α-lipoic acid, and α-tocopherol)[145]. A clinical trial with a mixture of *N*-acetyl-cysteine, lipoic acid and vitamin E is currently ongoing in Spain for patients with ALD[146].

Conclusions

In February 2016, ALD was added to the Recommended Uniform Screening Panel in the USA, which is the federal list of all genetic diseases recommended for state newborn screening programs. The state of New York initiated screening for ALD in newborns in 2014 (REF. 147), and in Europe, clinicians in the Netherlands will start ALD newborn screening in the near future. Screening at birth enables prospective monitoring and early intervention to treat ALD[147,148]. Early diagnosis of boys with ALD is important for two reasons: for the early detection of adrenal insufficiency to initiate timely adrenal steroid replacement therapy; and the early detection of cerebral ALD to offer allogeneic HSCT. Screening for ALD at birth enables the creation of prospective cohorts that can be followed throughout life. Such cohorts will be of key importance for the successful identification of predictive biomarkers. Currently, follow-up is identical for all men and boys with ALD, who have frequent follow-up with cerebral MRI[16]. An algorithm for the follow-up of patients with ALD has been published previously[16]. The discovery of predictive biomarkers or risk factors for the development of cerebral ALD is needed to stratify individuals at low or high-risk of developing the disease. These markers will, therefore, enable the development of personalized treatments and possibly even preventive HSCT before the onset of cerebral ALD.

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FURTHER INFORMATION
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