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Rutherford, H.A. and Hamilton, N. orcid.org/0000-0002-3299-9133 (2019) Animal models of leukodystrophy : a new perspective for the development of therapies. The FEBS Journal, 286 (21). pp. 4176-4191. ISSN 1742-464X

https://doi.org/10.1111/febs.15060

Animal Models of Leukodystrophy: A new perspective for the development of therapies

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Running Title: New and old models of leukodystrophy: the rise of zebrafish

Keywords: Leukodystrophy, neuroinflammation, animal models, myelin, X-linked adrenoleukodystrophy, metachromatic leukodystrophy, Krabbe's disease, Aicardi-Goutières syndrome, Alexander disease, zebrafish

Abbreviations

AAV, adeno-associated viruses; AD, Alexander Disease; AGS, Aicardi-Goutières Syndrome; ASA, arylsulfatase A; BBB, blood brain barrier; CNS, central nervous system; CRISPR, clustered regularly interspaced short palindromic repeats; ERT, enzyme replacement therapy; GALC, galactoceramidase; GFAP, glial fibrillary acidic protein; HSCT, haematopoietic stem cell transplantation; KD, Krabbe's disease; MLD, metachromactic leukodystrophy; MRI, magnetic resonance imaging; TALENs, transcription activator-like effector nucleases; TREX1, three prime repair exonuclease 1; VLCFA, very long chain fatty acids; X-ALD, X-linked adrenoleukodystrophy.

Abstract

The leukodystrophies are a family of heritable disorders characterised by white matter degeneration, accompanied by variable clinical symptoms including loss of motor function and cognitive decline. Now thought to include over fifty distinct disorders, there are a vast array of mechanisms underlying the pathology of these monogenic conditions and, accordingly, a range of animal models relating to each disorder. While both murine and zebrafish models continue to aid in the development of potential therapies, many of these models fail to truly recapitulate the human condition—thus leaving substantial weaknesses in our understanding of leukodystrophy pathogenesis. Additionally, the heterogeneity in leukodystrophy presentation—both in patients and *in vivo* models—often results in a narrow focus on single disorders in isolation across much of the literature. Thus, this review aims to synthesise prominent research regarding the most common leukodystrophies in order to provide an overview of key animal models and their utility in developing novel treatments. We begin by discussing the ongoing revolution across the leukodystrophy field following the rise of next generation sequencing, before focusing more extensively on existing animal models from the mouse and zebrafish fields. Finally, we explore how these pre-clinical models have shaped the development of therapeutic strategies currently in development. We propose future directions for the field and suggest a more critical view of the dogma which has underpinned leukodystrophy research for decades.

Introduction

Derived from the Greek *leuko* (meaning white) and *dystrophy* (meaning wasting), leukodystrophies are family of heritable disorders characterised by white matter degeneration—resulting in variable clinical symptoms including loss of motor function and cognitive decline [1]. There are now thought to be over fifty leukodystrophies, affecting an estimated 31 to 130 cases per million live births [2–4].

The term “leukodystrophy”—first coined in 1928—has evolved with our growing understanding of these disorders [1]. The first formalised definitions—which considered leukodystrophies primarily as myelin disorders—set the tone for decades of research: with a narrow focus on pathogenic mechanisms relating to the myelin sheath and the oligodendrocytes responsible for its formation [5].

However, the rise of genetic sequencing—and with it, the ability to molecularly classify individual patients—has triggered a much-needed shift in the leukodystrophy field: leading to a wider consideration of the white matter components involved in pathogenesis [6]. Yet, with greater knowledge comes greater complexity—the number of distinct, diagnosable leukodystrophies has increased exponentially in recent years, alongside growing heterogeneity in proposed disease mechanisms. As a result, many reviews focus on one disorder or therapy at a time—leading to a fragmented understanding of pathogenesis across this family of disorders [7–9]. However, there is much to be gained from considering the commonalities across the wider leukodystrophy field: particularly in exploring the critical role of animal models in developing novel therapies. While each animal model is specific to a single disease, common limitations may restrict the translational impact of preclinical studies if not

properly addressed. Nonetheless, a broad discussion of the advantages and caveats of existing animal models is lacking from the field.

Hence, this review aims to synthesise prominent literature regarding animal models of the most common leukodystrophies in order to identify future directions in the development of therapies. In order to provide a cohesive overview of current animal models and their utility in developing new treatments, we focus on the five most common leukodystrophies: X-linked adrenoleukodystrophy (X-ALD), metachromatic leukodystrophy (MLD), Krabbe's disease (KD), Alexander disease (AD) and Aicardi-Goutières syndrome (AGS) **(Figure 1)** [2]. We present recent advances in discovering new cellular drivers of these disorders and discuss how these findings, in combination with the development of animal models, have shaped the development of therapies.

The next generation of leukodystrophy research: from oligodendrocytes to astrocytes and neuroinflammation.

Given the role of oligodendrocytes in the formation of the myelin sheath, it is perhaps unsurprising that early leukodystrophy research focussed almost exclusively on this cell type. Indeed, leukodystrophies—by their very nature—are associated with failure of normal myelin formation (hypomyelinating diseases) or progressive loss of myelin (demyelinating disorders), which can be attributed to oligodendrocyte death. For example, metachromatic leukodystrophy (MLD) is a common leukodystrophy associated with mutations in arylsulfatase A (ASA)—a lysosomal enzyme responsible for the breakdown of a key component of the myelin sheath—which are thought to result in substrate accumulation and, thus, oligodendrocyte dysfunction [10]. For many decades, such oligodendrocyte- and myelin-centric mechanisms were thought to be the primary—and perhaps even the sole—drivers of leukodystrophy pathology.

However, as whole exome sequencing has become more widely available, the involvement of other white matter components in pathogenesis has become increasingly apparent—with leukodystrophies associated with mutations in genes extending far beyond those predominantly expressed in oligodendrocytes [6]. Accordingly, proposed classification systems now consider the contribution of other glia cells to white matter pathology **(Figure 1)** [11].

Astrocytes

Astrocytes play an important role in maintenance of white matter through supporting oligodendrocyte progenitor cell differentiation and releasing factors which influence myelination [12,13]. Indeed, mutations in astrocyte-specific glial fibrillary protein (GFAP) are thought to cause Alexander disease (AD)—with therapies targeting this protein showing some promise in patients and animal models, as will later be discussed [14,15]. Astrocytic dysfunction is not just limited to AD: mutations in other astrocytic gene products have been linked with Megalencephalic leukoencephalopathy with subcortical cysts, while severe astrocytic pathology has been observed in mouse models and post-mortem tissue from patients with vanishing white matter disorder [16,17].

Microglia and neuroinflammation

Microglia too are thought to play a role in leukodystrophy pathology: perhaps unsurprising, given their roles in supporting myelination, oligodendrocyte progenitor cell survival and differentiation and response to white matter damage [18,19]. Crucially, as the major innate immune cells within the central nervous system (CNS), microglia can also induce oligodendrocyte apoptosis in response to injury [20].

While microglial reactivity has long since been considered a consequential component of leukodystrophy pathology, recent research has suggested that microglial abnormalities may precede myelin loss in several common leukodystrophies. A recent post-mortem study of X-linked adrenoleukodystrophy (X-ALD) and metachromatic leukodystrophy (MLD) reported reductions in overall microglial number alongside increases in activated microglia in areas presenting markers of early white matter lesions: suggesting that such changes precede reductions in oligodendrocyte and myelin viability [21]. While post-mortem studies are somewhat limited in providing a true time-course of pathology progression, it has also been reported that microglial activation arises before astrocyte reactivity and myelin loss in mouse models of Krabbe's disease (KD). Strikingly, markers of the innate immune response were elevated even before microglial activation [22,23]. Together, these data suggest that immune response may be a driver, rather than a consequence, of pathology in some leukodystrophies.

Indeed, several leukodystrophies present with phenotypes that mimic congenital cytomegalovirus infection, and thus have long-since been considered autoimmune in nature: such as Aicardi-Goutières syndrome (AGS) and a rare but similar disorder: RNASET2-deficient cystic leukoencephalopathy [24,25]. In these disorders, the innate immune response is thought to be triggered by the recognition of self-derived nucleic acids as non-self. The above literature suggests that attack of self-derived components may underlie pathology beyond disorders traditionally considered to be autoimmune.

Hence, there are a growing number of proposed mechanisms underpinning leukodystrophy pathology—many of which may be shared across multiple disorders. However, in order to truly understand leukodystrophy pathogenesis and develop effective therapeutic interventions, animal models which recapitulate key aspects of human disease are essential.

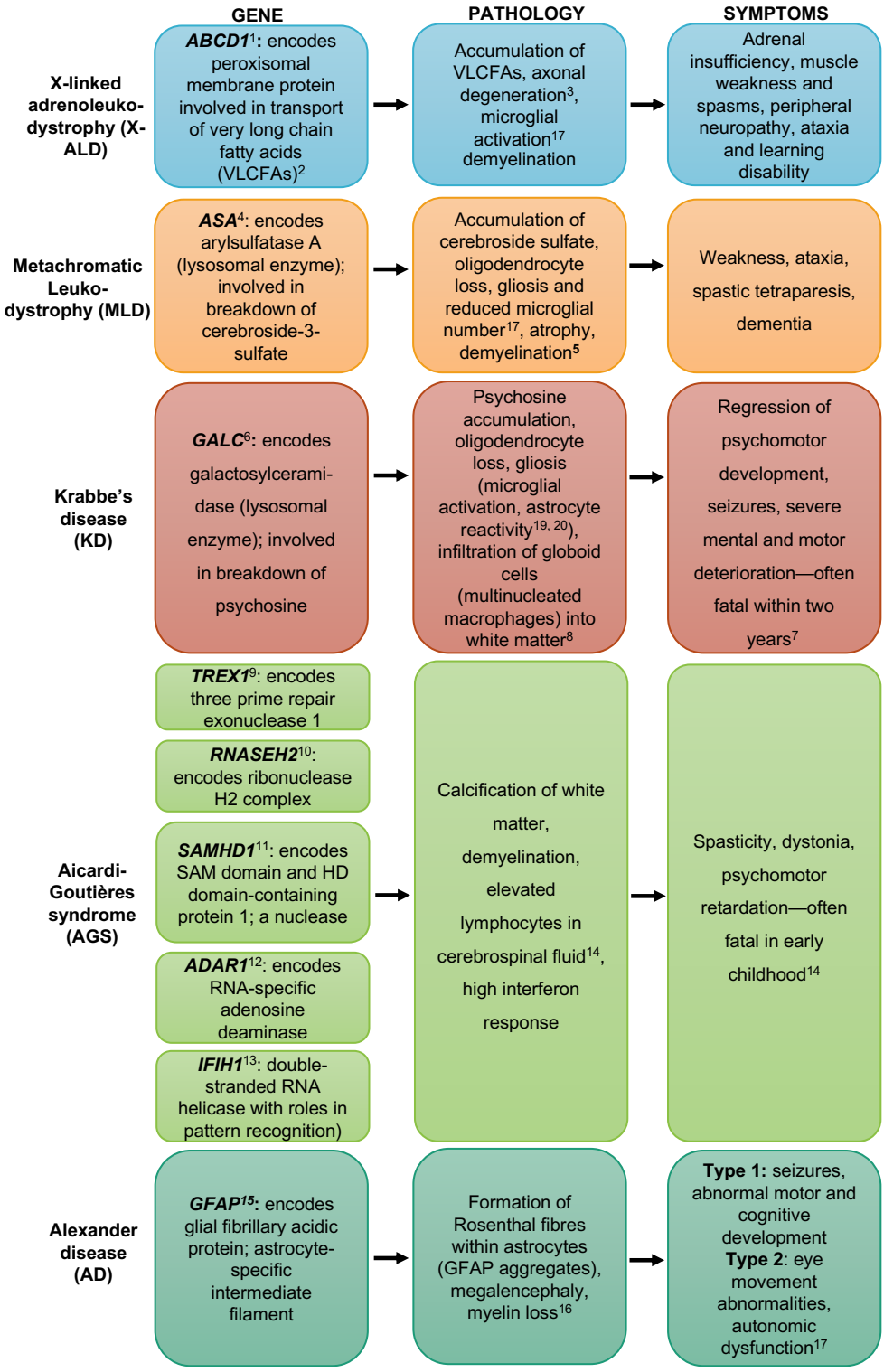

Figure 1: Pathology and clinical manifestations of five common leukodystrophies. AD, Alexander disease; AGS, Aicardi-Goutières syndrome; KD, Krabbe's disease; MLD, metachromatic leukodystrophy; VLCFA, very long chain fatty acids; X-ALD, X-linked adrenoleukodystrophy [10-29].

Animal models of leukodystrophies

The heritable nature of leukodystrophies has led to the development of a range of monogenic animal models: each specific to a single disorder and, often, failing to encapsulate the entirety of the human pathology. While a variety of species have been explored as models of these rare diseases, much of the literature focuses on zebrafish and, in particular, murine models.

Mouse models

The mouse model that has shown perhaps the greatest utility to the field is the twitcher mouse: a naturally occurring model of Krabbe's disease (KD) with a mutation in *Galc* (the gene for galactoceramidase, critical for the breakdown of the lipid psychosine). While this specific mutation is reported in only a subset of KD patients, these mice develop pathology with remarkable similarity to that seen in the human condition: with robust central demyelination and gliosis, alongside clinical symptoms typical of KD patients and significant reductions in survival [22,26,27]. With such strong face and construct validity, the twitcher mouse has allowed us to further our understanding of KD pathology—with recent research in this model suggesting that myelin abnormalities may begin much earlier in development than previously thought [28]. Reduced myelin staining was observed in infant mice long before the surge in oligodendrocyte apoptosis characteristic of the twitcher model, alongside impairments in oligodendrocyte progenitor cell differentiation in twitcher-derived cells—suggesting developmental defects in myelin formation. These findings are the first to suggest that perhaps both hypomyelinating and demyelinating mechanisms underpin KD, which has long-since been considered demyelinating disease—challenging our understanding of pathogenesis.

However, naturally occurring models of disease are rare and, as such, the vast majority of leukodystrophy models are transgenic, knock-in or knockout animals. Many leukodystrophies are associated with loss-of-function mutations: leading to reduced levels of functioning or malfunctioning protein. Hence, the most common mouse models of these disorders are knockouts of disease-associated genes—such as the widely used ASA-deficient model of metachromatic leukodystrophy (MLD), three prime repair exonuclease 1 (TREX1)-deficient model of AGS and ABCD1-deficient model of X-ALD [29–33] (**Table 1, Figure 1**). Yet, these models fail to recapitulate human disease: lacking key aspects of leukodystrophy neuropathology. *Trex1*$^{-/-}$ mice do not develop neuroinflammation, while *ASA*$^{-/-}$ mice present no evidence of demyelination until two years-of-age: a significantly slower disease-course relative to the human condition [29,30,34]. Similarly, ABCD1-deficient mice also fail to present with myelin abnormalities within the brain—despite accumulation of very long chain fatty acids (VLCFA) characteristic of the disorder [31–33,35] (**Table 1**).

There are also notable limitations to using knockouts as models of leukodystrophy. Firstly, complete knockouts have little relevance to the human phenotype—with most patients expressing a low level of functioning or malfunctioning protein. Additionally, such extreme genotypes present a challenge in investigating therapies that aim to reintroduce target proteins, such as gene- or enzyme-replacement therapy, as the novel protein can initiate immune response after being recognised as foreign [36]. This makes it difficult to predict the efficacy of such treatments in patients, who—having some endogenous expression of these genes—will not surmount an immune response to such proteins [10].

Not all leukodystrophies are associated with loss-of-function mutations: several are associated with toxic gain-of-function mutations which can be equally devastating. AD is one such disorder: caused by gain-of-function mutations in glial fibrillary acidic protein (GFAP)[17]. These mutations are thought to lead to GFAP aggregation—forming structures known as Rosenthal fibres—and thus astrocytic cell death and downstream white matter pathology [37]. To mimic the effects of such gain-of-function mutations, knock-in mutations in *GFAP* have been created in several mouse lines [38,39]. However, while these animals develop Rosenthal fibres and astrocyte reactivity in a distribution similar to that seen in human patients, this is not accompanied by white matter abnormalities or reductions in survival—once again failing to truly encapsulate key elements of human pathology.

Table 1: Phenotypes of common mouse models of leukodystrophy

Model	Relevant disease	Phenotype	Ref.
twitcher mouse	Krabbe's disease	Central demyelination, gliosis, infiltration of globoid cells (multinucleated macrophages), progressive hindlimb weakness, reduced survival	[26,27]
***ASA* knockout**	Metachromatic leukodystrophy	Accumulation of cerebroside-3-sulfate, gliosis, neuromotor impairments	[29]
***TREX1* knockout**	Aicardi-Goutières Syndrome	Multi-organ inflammation (including inflammatory myocarditis), reduced survival	[30,34]
***ABCD1* knockout**	X-linked adreno-leukodystrophy	Reduced VLCFA beta-oxidation, elevated levels of saturated VLCFA, myelin abnormalities at 15 months	[31–33,35]
***GFAP* knock-in mice**	Alexander disease	Formation of Rosenthal fibres, increased GFAP expression, astrocyte reactivity	[39]

The rise of zebrafish in leukodystrophy research

Innate immunity studies have shown that there is a significant degree of evolutionary conservation between zebrafish and humans [40–43]. The genetic tractability of the zebrafish has allowed the generation of multiple fluorescent transgenic lines faithfully labelling innate immune cells such as neutrophils and macrophages [44,45]. As such, zebrafish surmount a remarkably similar immune response to several pathogens compared to humans, such as viruses, mycobacterium and staphylococcus to name a few—suggesting the zebrafish by no means invalid as a model of human disease (**Figure 2**) [46–48].

Given that many leukodystrophy patients present with infantile or juvenile onset, zebrafish provide a unique model system in which to observe the developmental component of disease due to their transparency during embryogenesis and early life. Additionally, recent developments have facilitated longitudinal observation of pathology into adulthood—when the fish become opaque—with magnetic resonance imaging (MRI) techniques [49,50]. Indeed, MRI has long since been used to assess white matter pathology with high sensitivity in murine models of leukodystrophy—allowing direct corroboration of findings across all three species of interest (**Figure 2**) [51].

Due to their small size and *ex utero* development, zebrafish are also an ideal model for high-throughput screening—allowing observation of more complex phenotypes than could be seen in cell culture (**Figure 2**) [52]. Zebrafish embryos are particularly useful in the development of drugs for neurological disorders, as the blood brain barrier (BBB) does not mature until three to ten days post fertilisation—allowing identification of candidate drugs which could potentially be optimised for CNS delivery in humans

[53]. Accordingly, the zebrafish is becoming increasingly utilised in neurological research to assess the efficacy and toxicity of small molecules in models of epilepsy and amyotrophic lateral sclerosis [54,55]. These strategies could easily be applied to zebrafish models of leukodystrophy to facilitate relatively simple, low-cost drug screens—critical given the rarity of these disorders.

Furthermore, the genetic strategies available to generate zebrafish mutants result in genotypes that are largely more relevant to the human conditions. Rather than constitutive knockout, anti-sense morpholino approaches are frequently used to transiently knockdown expression, while genome editing strategies (such as transcription activator-like effector nucleases [TALENs] and clustered regularly interrupted short palindromic repeats [CRISPR]/Cas9) have been utilised to generate truncated or malfunctioning protein—such that most models are not entirely deficient in disease-associated protein. Thus, common zebrafish models not only present with a genotype more similar to that of human patients, but also are less likely to surmount an aberrant immune response to proteins of interest if therapeutically reintroduced.

Although fewer in number, zebrafish models reliably present with neurological phenotypes (**Table 2**) therefore increasing their relevance as models of leukodystrophy relative to murine models. For example, *rnaset2* mutants develop white matter lesions in adulthood, and this is accompanied by locomotor and behaviour defects in larval and adult stages therefore placing this animal model at the forefront of pre-clinical models for leukodystrophy [50,56].

Table 2: Phenotypes of zebrafish models of leukodystrophy

Model	Relevant disease	Phenotype	Ref.
***galca/galcb* knockdown**	Krabbe's disease	Disorganised expression of neural markers	[57]
***abcd1* mutant**	X-linked adrenoleukodystrophy	Hypomyelination of spinal cord Reduced oligodendrocytes Impaired motor function Reduced survival	[59]
***samhd1* knockdown**	Aicardi-Goutières Syndrome	Cerebral haemorrhage Upregulation of innate-immune response	[60]
***degs1* knockdown**	Hypomyelinating leukodystrophy (rare)	Reduced myelination Impaired motor function	[58]
***rnaset2* mutant/ knockdown**	RNASET2-deficient cystic leukoencephalopathy (rare and similar to AGS pathology)	White matter lesions associated with axonal damage and astrogliosis Behaviour and locomotion defect in larvae and adults.	[50,56]

However, it is possible such phenotypes may arise due to off-target effects—to which antisense-morpholinos are prone. For example, morpholino-knockdown of zebrafish orthologues of *GALC* led to alterations in neural development without the psychosine build-up which is characteristic of KD [57]. While the authors argue that this suggests a developmental role for *galc* which is independent of psychosine-accumulation, it is entirely possible the observed phenotype arose due to off-target knockdown effects: complicating our understanding of pathogenesis in these models.

Furthermore, not all zebrafish models show white matter-related pathology. While some models show reductions in myelin-related proteins and oligodendrocyte number (such as *degs1* and *abcd1* mutants), others display less relevant phenotypes [58,59].

For example, *samhd1* knockdown only partially recapitulates AGS phenotype: causing brain haemorrhage but not white matter abnormalities [60]. (**Table 2**).

Thus, there remain few models of leukodystrophy that truly recapitulate human pathology. An awareness of the limitations of each model is essential in analysing preclinical literature and assessing the effects of potential treatments.

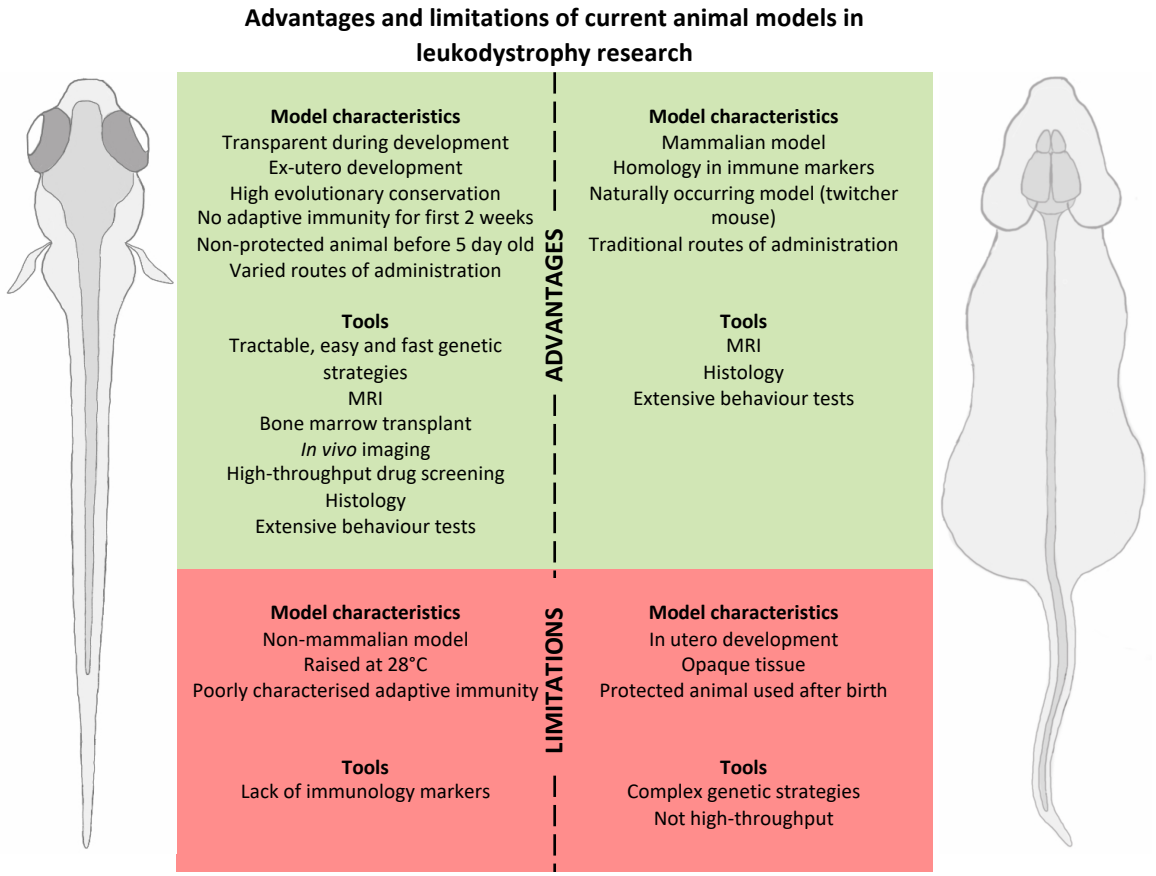

Figure 2: A summary of the general advantages and disadvantages of current animal models in leukodystrophy research.

Therapies emerging from preclinical models

Conventional approaches in the treatment of leukodystrophies aim to halt disease progression by regaining homeostasis in the CNS—restoring the activity of the deficient or malfunctioning protein and thus minimising cell death. Nonetheless, such treatments are lacking: failing to reliably alter disease-course and improve survival across animal models and patients. Emerging treatments have begun to target previously overlooked pathogenic mechanisms—yet, their wider therapeutic benefit remains unclear due to limited preclinical testing. In this section, we discuss one such treatment—anti-reverse transcriptase therapy—alongside more conventional therapies: enzyme replacement therapy, gene therapy, antisense oligonucleotide therapy and haematopoietic stem cell transplant. We highlight the role of preclinical models in the continuing development of these therapies and suggest future directions which may enhance their translational impact (**Figure 3**).

Enzyme replacement therapy

First used to treat Gaucher's disease, enzyme replacement therapy (ERT) has been successful in the treatment of several disorders caused by enzymatic dysfunction [61]. Enzyme administration can restore enzymatic function within cells, as endocytosis mediated by the mannose-6-phosphate receptor allows extracellular enzyme to replace its endogenous counterpart [62].

ERT has been particularly explored in lysosomal storage leukodystrophies – such as MLD and KD. Yet, despite strong theoretical rationale, the success of ERT has been limited by failure to increase enzyme activity in the CNS: with the blood brain barrier (BBB) presenting a significant obstacle for intravenous application.

In recent years, however, insightful enzyme delivery strategies have been explored to combat this challenge: such as the use of nanoparticles. Due their small size and the increasing availability of modifications to optimise drug delivery, nanoparticles are able to cross the BBB—presenting a truly appealing drug-delivery strategy. Yet, results have been disappointing thus far, with nanoparticle-delivery of arylsulfatase (ASA) failing to increase cerebral enzymatic activity to a greater level than free-ASA application in a mouse model of MLD [63]. It has been hypothesised that the fusion of ASA to the particle-surface may have altered the trafficking of the particle into the CNS, and thus encapsulation of the enzyme within the nanoparticle may be a more desirable strategy.

Indeed, the administration of nanoparticle-encapsulated palmitoyl-protein thioesterase 1 (deficient in a rare lysosomal storage disorder) elevated enzymatic activity in a fibroblast model, with preliminary results suggesting that similar results may be seen for nanoparticle-formulations of GALC [64]. Yet, this study fails to truly demonstrate an advantage over free-enzyme administration: most notably, the use of an *in vitro* model is minimally informative about the ability of these particles to traverse the BBB.

While *in vivo* models remain the best system in which to assess the efficacy of enzyme replacement, there are potential limitations to the use of some animal models in this context: in particular, knockout animals. As previously discussed, reintroducing functioning proteins into animals entirely deficient in the protein of interest—such as the ASA-deficient model of MLD—can trigger an immune response, as the novel construct is recognised as foreign [36]. Thus, future work may continue to explore

enzyme replacement in models with greater relevance to the human genotype: such as in the existing zebrafish mutants discussed above. Alternatively, genome editing strategies—such as those utilised to generate the GFAP knock-in model of AD—could also be utilised in the mouse to generate models which are not entirely deficient in the protein of interest: perhaps expressing a truncated or malfunctioning protein (**Figure 3**).

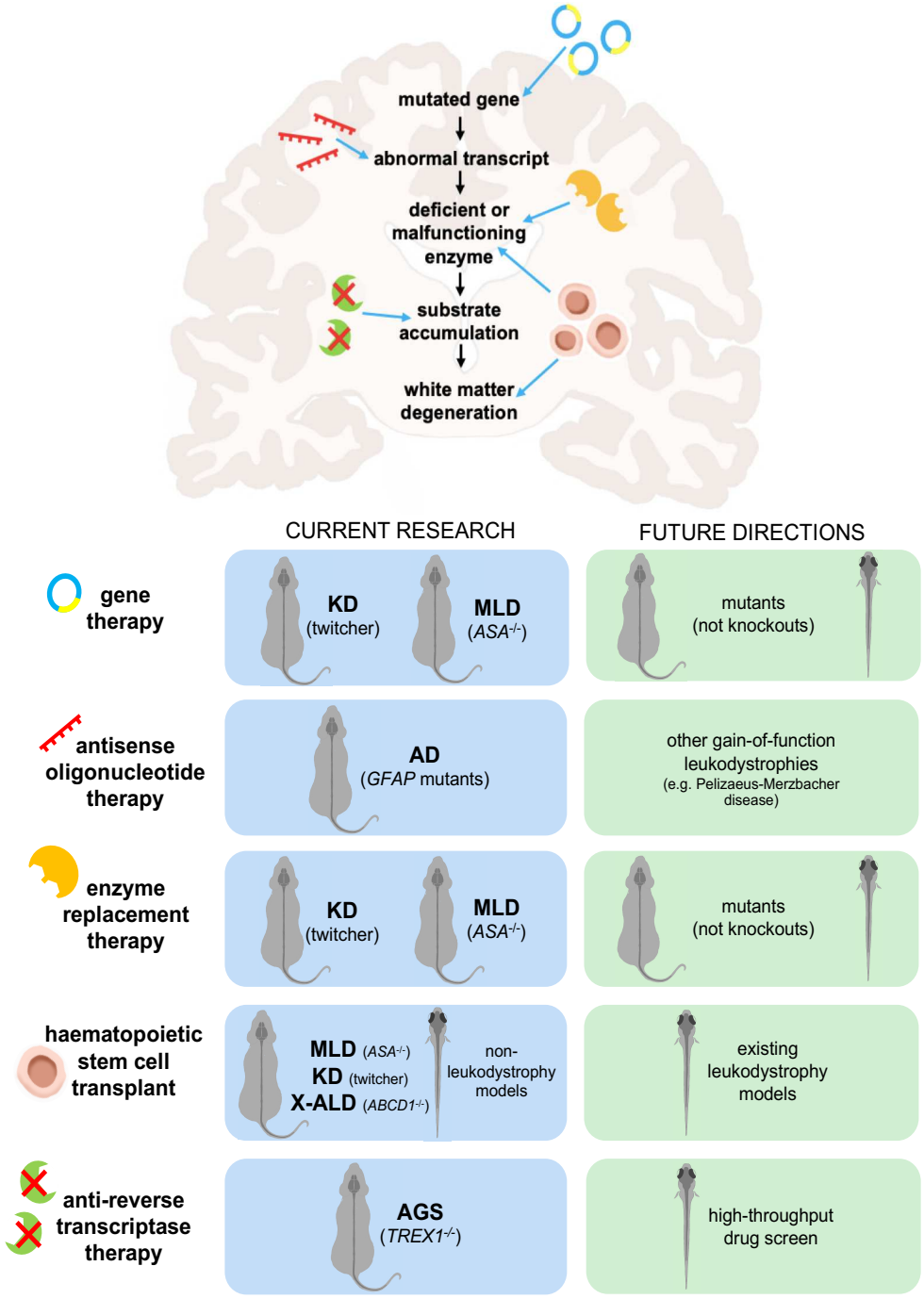

Figure 3: Therapeutic strategies explored in animal models – current work and future directions. Schematic summarising the interactions of treatment strategies with leukodystrophy pathology, alongside the animal models in which they have been trialled. Gene therapy seeks to increase expression of functioning gene, while enzyme replacement therapy aims to elevate enzymatic activity. Haematopoietic stem cell transplant may ameliorate pathology by elevating levels of functioning enzyme, supporting host cells or replacing white matter components. In contrast, anti-reverse

transcriptase therapy may reduce substrate (nucleotide) accumulation by preventing reverse transcription. Future directions are suggested for each therapy.

Gene therapy

While the challenges of ERT have limited its utility in the clinic, the theoretical rationale still stands—elevating levels of functioning protein has the potential to improve pathology in leukodystrophies. Thus, gene therapy has been explored as a strategy to elevate protein expression within the CNS: with adeno-associated viruses (AAVs) largely dominating this field [65].

Preclinical studies have suggested promising results: with a recent study reporting that administration of a *GALC*-encoding AAV-vector elevated enzymatic activity to levels exceeding wild type in twitcher mice, accompanied by improved survival, motor function and even reduced CNS demyelination-rate [66].

Yet, the success of AAV-mediated gene delivery has not been ubiquitous. A similar study in *ASA*$^{-/-}$ mice found that delivery of AAV-vectors elicited a minimal increase in enzyme concentration in the cerebrospinal fluid, which decayed over the weeks following administration—paralleled by increasing levels of anti-human-ASA antibodies within the plasma [36]. Although these findings are merely correlative, this suggests the failure to elevate ASA was not due to a lack of genomic integration, but rather immune response to the novel enzyme; reflecting a key limitation of the model, as previously discussed.

However, the variable success of these studies could also be attributed to differences in methodology: most notably, the time of vector application. While *ASA*$^{-/-}$ mice were

treated after the emergence of histological symptoms, twitcher mice were injected pre-symptomatically with greater success: a common theme across the literature. Yet, this is not always feasible in patients. Due to the recessive nature of inheritance and often rapid progression, diagnosis may not occur until symptom presentation: limiting the wider utility of such interventions. Indeed, while gene therapy has led to some—albeit varied—positive outcomes across several leukodystrophies in clinical trials, complete reversal of neurological phenotypes is yet to be achieved (reviewed elsewhere [8,9]).

Antisense oligonucleotide therapy

While the therapies described above aim to elevate levels of a deficient or malfunctioning protein, a different strategy is required to tackle gain-of-function mutations – such as those seen in Alexander disease. A recent preclinical study has explored the use of antisense oligonucleotides—single-stranded RNA which silence gene expression by binding to transcripts and preventing their translation—to knockdown GFAP expression [15]. Strikingly, intracerebral injection of antisense oligonucleotides reduced levels of GFAP transcripts and protein, alongside markers of gliosis, across several GFAP-mutant Alexander disease mouse models. While this is the first study to explore the potential of such a therapy in leukodystrophy models, similar promise has been shown in mouse models of Huntington's disease and antisense oligonucleotides have been FDA-approved for the treatment of spinal muscular atrophy 1 [67–69].

The main disadvantage of this approach, however, is that antisense oligonucleotides remain unable to cross the BBB: hence requiring invasive intracerebral or intrathecal injection [70]. However, injection of these constructs in early development—before the

formation of the BBB barrier—could overcome this limitation. While both challenging and dependent on prior diagnosis, *in utero* treatments could facilitate effective pre-symptomatic interventions for leukodystrophies with the earliest onsets. Indeed, antisense oligonucleotides have been shown to effectively downregulate gene expression when injected into the amniotic cavity of mouse embryos *in utero*, or as routinely used into the yolk of single-cell zebrafish embryos [71,72]. Hence, antisense oligonucleotides may present a compelling avenue for *in utero* interventions.

Haematopoietic stem cell transplant

A further strategy for the treatment of leukodystrophies is haematopoietic stem cell transplant (HSCT). Transplantation of cells derived from the bone marrow or umbilical cord of healthy individuals (allogenic transplantation) is a theoretically appealing therapy: transplanted cells could provide source of functioning enzyme, support degenerating cells or even replace them functionally (**Figure 3**). Indeed, preclinical studies have suggested that transplanted cells can migrate into the CNS and differentiate following bone marrow transplant—both in healthy animals and mouse models of MLD [73–75].

However, clinical studies investigating allogenic HSCT have shown highly variable results. While there is some evidence that HSCT may provide a viable treatment option for pre-symptomatic individuals with MLD, outcomes for those treated after symptom presentation are much poorer [76,77]. Curiously—despite successfully rescuing ASA activity—HSCT fails to reliably improve pathology or symptoms: with many patients showing continued deterioration, albeit at perhaps a slower rate than untreated individuals [78–80].

The potential of autologous HSCT - the transplantation of genetically-corrected cells derived from the patient themselves - was first highlighted in mouse models. Following host irradiation, transplantation at 6-weeks old of genetically-corrected haematopoietic stem cells derived from MLD mice led to normalisation of enzyme activity and, strikingly, prevention of motor and cognitive deficits, and myelin abnormalities [73].

Following this preclinical success, a recent clinical trial reported that infusion of lentivirus-transfected haematopoietic stem cells halted disease progression in a modest sample of MLD patients—crucially, with no serious adverse effects [81]. However, given that all successfully treated patients were either pre-symptomatic or in the earliest stages of disease, it remains unclear whether autologous HSCT provides greater therapeutic efficacy than its allogenic counterpart. Nonetheless, such studies demonstrate the critical role of animal models in informing clinical trials.

It must be noted, however, that HSCT in mice is a time-consuming and severe procedure—requiring irradiation or immunosuppression to minimise the probability of transplant rejection [82]. However, the need for these procedures is abolished if HSCT is performed on embryos before maturation of the adaptive immune system. While such interventions could be performed on mice, the zebrafish presents an ideal opportunity to explore HSCT in early development. Zebrafish develop transparent embryos *ex utero* with no adaptive immunity until 2 weeks of age, allowing transplant of foreign cells without the need of harmful irradiation procedures. Recent research has demonstrated that zebrafish and human primary stem cells are able to engraft within the haematopoietic niche of transplanted zebrafish embryos [83–86]. Hence,

zebrafish models may provide a simpler alternative to their murine counterparts in exploring the potential efficacy of HSCT in embryos (**Figure 3**).

Anti-reverse transcriptase therapy

While the above therapies are somewhat conventional in their approach, recent clinical strategies have approached the treatment of leukodystrophies from an arguably more obscure angle. As mentioned above, the innate immune response which underlies the pathology of autoimmune leukodystrophies is thought to be stimulated by endogenous nucleic acids incorrectly identified as non-self [24,25]. A growing body of literature has suggested that these immunostimulatory nucleic acids may arise from the transcription of endogenous retrotransposons: genetic elements which, after transcription, undergo reverse transcription from RNA to DNA [87].

This phenomenon has been noted to be of particular relevance to AGS as several of the genes underpinning the disorder have been implicated in restricting reverse transcription (**Figure 4**): leading to the hypothesis that it may be possible to treat AGS with reverse-transcriptase inhibitors (RTIs). Indeed, the application of RTIs to human stem cells with TREX1 mutations was found to both decrease the level of single stranded RNA and reduce apoptosis [88]. However, preclinical studies have shown limited success. Initial findings revealed that the use of a single RTI failed to extend the survival of *Trex1*$^{-/-}$ mice [89], with subsequent research utilising a combination of RTIs also proving disappointing. While one group report that a cocktail of RTIs reduced mortality and cardiac inflammation in *Trex1*$^{-/-}$ mice, further research has suggested that RTI treatment had no such effect [90,91]. The reasons for this discrepancy are unclear, though it has been suggested that housing effects may be particularly

prominent in such studies, as the microbiome has been reported to influence activity of endogenous retroelements: resulting in heterogeneity in the mean survival of *Trex1*$^{-/-}$ mice [92].

Despite limited preclinical data, results from a small clinical trial have suggested that a battery of three RTIs is effective in lowering interferon signalling in AGS patients: indicating reduced immune activation [93]. However, no data demonstrating the effects on neurological symptoms have been released thus far. Likewise, issues with study design limit the conclusions that can be drawn from this trial—with no blinding or controls, a patient group of just eight and substantial heterogeneity in the patient interferon score both at baseline and after treatment—indicating a larger controlled study is necessary.

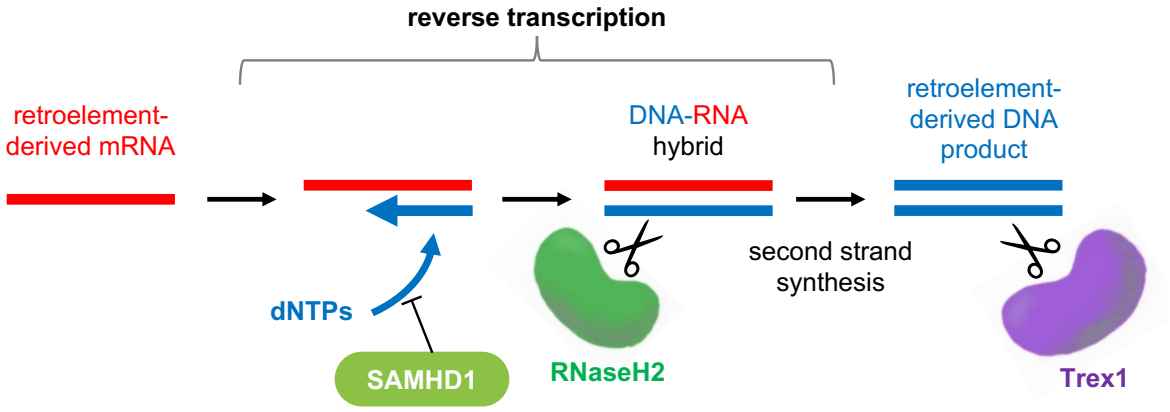

Figure 4: Schematic depicting the role of AGS-associated genes in restricting reverse transcription. AGS-associated enzymes are proposed to limit reverse transcription by acting at multiple stages. SAMHD1 is thought to regulate retrotransposon transcription by degrading the deoxynucleotides needed for complementary DNA-strand formation. The ribonuclease RNaseH2 is proposed to degrade the RNA component of DNA-RNA hybrids formed during the reverse transcriptase process, while the exonuclease Trex1 is hypothesized to metabolize retroelement-derived nucleotides [24,89,110]. dNTP, deoxyribonucleotide triphosphate.

Nonetheless, these results provide an early indication that anti-reverse transcription therapy may present a promising clinical avenue in AGS and possible proof-of-concept for the role of endogenous retroelements in autoimmune conditions. Indeed, RTIs may have potential in treating autoimmune conditions such as systematic lupus erythematosus, which itself has been associated with *TREX1* mutations [94]. Strikingly, recent findings suggest that RTIs may minimise some age-associated inflammatory markers in wild type mice: suggesting this therapy may show utility in conditions like Alzheimer's disease, in which neuroinflammation is thought to have a key role [95]. However, until RTIs are tested in models of such disease, their translational potential remains unclear.

Conclusion

Leukodystrophies are rare but debilitating conditions characterised by white matter degeneration, loss of motor function and cognitive decline. Although recent decades have made substantial progress in understanding their pathogenesis, these disorders remain an unmet clinical need: with few treatments permitted for widespread clinical use across multiple disorders.

This failure in translation is partly due to a lack of appropriate animal models of these disorders. By discussing the most common leukodystrophies, we have highlighted common weaknesses in the animal model literature—namely that the majority of models fail to recapitulate the key component of human disease: white matter pathology.

Likewise, poor study design limits the translation of such research. As discussed throughout, many preclinical studies explore pre-symptomatic intervention—a strategy that is not always possible in patients. Similarly, the doses utilised are often inappropriate for use in humans, who may require long-term treatment in order to halt disease progression. Furthermore—due to the rare nature of these disorders—clinical studies are often underpowered: using small numbers of patients or even reporting findings on a case-by-case basis.

Additionally, it is possible that our understanding of disease mechanisms is flawed. The primary event for many leukodystrophies is considered to be enzymatic dysfunction and, as such, the end-point of many studies and therapies is normalisation of enzyme activity or substrate accumulation. Similarly, the accumulation of the relevant substrate is often deemed sufficient to consider many knockout animals models of leukodystrophy, despite a lack of neurological symptoms. Yet, lowering substrate concentration does not always halt disease progression [65,78,96]. It could be argued that continued degeneration is simply the result of secondary pathological processes—such as neuroinflammation and immune response—progressing in the absence of the primary event. Yet, animal models treated even before the onset of histological symptoms have been reported to show progressive motor impairment despite minimal substrate accumulation [65]. Thus, it may be time for us to reconsider our understanding of leukodystrophy pathogenesis and, with it, the criteria by which we assess our animal models—simply presenting with the relevant biochemical profile is no longer enough.

While several of the conventional therapies discussed in this review have not yet yielded widespread clinical results, innovative approaches to the challenges they pose certainly warrant further development. However therapeutic strategies and research that challenges our understanding of the causes of leukodystrophy are needed going forward. Anti-retroviral therapy is one such intervention that begins to challenge the framework within which we think about leukodystrophies. As such, the greatest therapeutic advancements may arise by asking more unconventional questions about the causes of these rare diseases.

Acknowledgements:
NH is supported by a fellowship from the European Leukodystrophy Association (ELA 2016-012F4). We thank Professor Stephen Renshaw for valuable feedback and constant support, Dr Julie Simpson for critical feedback and Jacob Rudman for helpful discussion on mouse immunology.

Author contributions:
HAR performed the literature search, wrote and revised the manuscript, and designed the graphical artwork.. NH conceived the study, critically revised and finally approved the manuscript.

Conflict of interest: The authors declare no conflict of interest

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