



Pediatric Alexander Disease: Clinical Spectrum, GFAP Genotype–Phenotype Relationships, Molecular Pathogenesis, and Emerging Gene-Targeted Therapies

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Abstract

Alexander disease (AxD) is a rare, dominantly inherited astrocytopathy caused principally by heterozygous pathogenic variants in GFAP, the gene encoding glial fibrillary acidic protein. In children, the disorder spans a continuum from neonatal or early-infantile encephalopathy with severe cerebral white-matter disease, seizures, hydrocephalus and rapid developmental compromise to later childhood phenotypes with mixed cerebral, bulbar, pyramidal and autonomic involvement. The historical age-based categories of infantile, juvenile and adult disease are therefore increasingly complemented by clinically and radiologically grounded cerebral, intermediate and bulbospinal phenotypes. This critical narrative review integrates clinical natural-history data, magnetic resonance imaging patterns, GFAP genotype–phenotype evidence, mechanistic studies in human tissue, induced pluripotent stem cell models and animal models, and the translational development of GFAP-lowering therapies. Evidence published from January 2001 to 8

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August 2026 was prioritised, with older foundational observations considered when necessary. The strongest mechanistic evidence supports a toxic gain-of-function model in which mutant GFAP misassembly and pathological accumulation amplify GFAP transcription, disturb proteostasis, activate stress and inflammatory pathways, alter astrocytic morphology and homeostatic functions, and secondarily impair neurons, oligodendrocytes and myelination. Genotype is informative but not deterministic: recurrent variants at residues such as Arg79 and Arg239 show meaningful differences in age at onset and survival, yet substantial within-variant variability limits individual prognostication. Fluid GFAP is a biologically direct target-engagement biomarker, but its value as an independent clinical outcome biomarker is constrained when GFAP itself is therapeutically suppressed. Antisense oligonucleotide-mediated GFAP reduction has reversed major pathological features in preclinical models and has progressed to a late-stage intrathecal clinical programme. The field is consequently shifting from diagnosis and supportive care towards mechanism-based intervention, but paediatric trial design still requires age-sensitive functional outcomes, validated surrogate markers, genotype-aware stratification and longer-term assessment of developmental trajectories and treatment safety.

Keywords: *Alexander disease; astrocyte; glial fibrillary acidic protein; GFAP; leukodystrophy; paediatric neurology; genotype–phenotype; antisense oligonucleotide.*

1. Introduction

Alexander disease (AxD) occupies an unusual position among childhood white-matter disorders because the primary molecular lesion is in an astrocytic intermediate filament rather than in a myelin protein or a canonical oligodendrocyte pathway. The identification of dominant GFAP variants as the principal cause of AxD transformed the disorder from a neuropathological diagnosis defined by Rosenthal fibres into a genetically diagnosable astrocytopathy with broad age-related and anatomical heterogeneity (Brenner *et al.*, 2001). That conceptual shift is clinically important in paediatrics. A child may present with macrocephaly, developmental delay, seizures and frontal-predominant leukoencephalopathy, but other children show hydrocephalus, episodic deterioration, feeding and bulbar problems, spasticity, ataxia or a mixed phenotype that does not fit the classical infantile–juvenile dichotomy (Prust *et al.*, 2011; Yoshida, 2020; Zhang *et al.*, 2026).

A systematic review and meta-analysis of GFAP variants identified variation in reported age at onset and suggested that specific variant characteristics may contribute to phenotypic differences; however, the aggregated evidence does not establish an individual prognosis (Grossi *et al.*, 2024).

The historical description of AxD as a leukodystrophy remains useful at the bedside because conspicuous white-matter abnormalities are common in early-onset disease. Mechanistically, however, the term can obscure the fact that the initiating lesion resides in astrocytes. GFAP is a type III intermediate filament protein that contributes to the cytoskeletal architecture and stress responsiveness of astrocytes. Disease-associated GFAP variants produce a dominant toxic gain of function rather than a simple deficiency state; GFAP accumulation, filament disorganisation and formation of Rosenthal fibres occur alongside a broad reactive astrocyte programme (Quinlan *et al.*, 2007; Hagemann, 2022). The consequences are non-cell-autonomous. Altered glutamate handling, inflammatory signalling, impaired support of oligodendrocyte progenitors, disturbed extracellular matrix regulation and changes in metabolic homeostasis provide plausible routes by which a primary astrocytic defect becomes a diffuse encephalopathy (Tian *et al.*, 2010; Olabarria *et al.*, 2015; Yi *et al.*, 2025).

Experimental findings from a GFAP-mutant rat model further link astrocyte dysfunction to impaired hippocampal synaptic plasticity and cognitive performance, although these animal observations cannot be directly extrapolated to children with AxD (Berman *et al.*, 2025).

The paediatric literature has expanded substantially in the past decade, yet several interpretive problems remain. First, many genotype–phenotype claims were historically based on isolated cases or small series; only recently have larger variant-specific analyses and paediatric cohorts allowed more defensible comparisons (Messing *et al.*, 2025; Zhang *et al.*, 2026). Second, biomarker development is complicated by the fact that GFAP is both a disease-related analyte and the molecular target of the leading therapy. Third, the emergence of GFAP-lowering antisense oligonucleotides changes the clinical question from whether a pathogenic variant explains a child’s phenotype to when treatment should begin, what outcomes can sensitively detect stabilisation, and whether molecular target engagement predicts meaningful developmental benefit. These issues require integration across clinical neurology, neuroimaging, molecular genetics, cell biology and therapeutic science rather than separate descriptive summaries.

Observations of familial subclinical disease associated with self-silencing GFAP missense alleles also suggest that variant-specific expression may modify clinical manifestations, without establishing a generalizable mechanism for the broader paediatric population (Bachetti *et al.*, 2025).

Taken together, these findings leave a specific gap in the evidence: the extent to which variant-level molecular effects, experimental measures of neural dysfunction and longitudinal paediatric outcomes can be interpreted within a common clinical framework remains insufficiently established. This gap is particularly relevant when assessing whether GFAP reduction produces benefits beyond molecular target engagement.

1.1 Scope, Research Gap and Objectives

This review focuses on genetically confirmed Alexander disease with particular emphasis on childhood-onset disease from the neonatal period through adolescence. It critically examines the clinical and neuroradiological spectrum, the strength and limits of GFAP genotype–phenotype relationships, the molecular sequence linking mutant GFAP to astrocyte dysfunction and downstream

white-matter and neuronal injury, and the evidence supporting emerging gene-targeted treatment. Adult-onset disease is considered where it clarifies biological continuity or variant effects, but it is not the primary focus. The review also evaluates biomarkers and trial-design challenges that are especially relevant to paediatric therapeutic development. The central objective is to distinguish findings supported by replicated human or convergent model evidence from associations that remain provisional, and to identify the translational questions that must be resolved before molecular correction can be equated with durable clinical benefit.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative review design was selected because the questions span heterogeneous evidence types: genotype–phenotype cohorts, radiological series, natural-history studies, biomarker investigations, neuropathology, mechanistic cell and animal experiments, and therapeutic development. A narrowly systematic design would be poorly suited to integrating causal models and translational inferences across these domains, whereas a transparent narrative approach permits explicit appraisal of evidence quality and mechanistic coherence. The conduct and reporting were informed by the principles underlying the Scale for the Assessment of Narrative Review Articles, particularly transparent search description, appropriate referencing, scientific reasoning and presentation of relevant endpoint data (Baethge *et al.*, 2019).

2.2 Information Sources

Literature was identified through searches of PubMed, PubMed Central, Europe PMC, OpenAlex, OpenAIRE, DOAJ, Semantic Scholar and Crossref. Searches were supplemented by backward and forward citation tracing from influential clinical and mechanistic papers, related-article searches, and verification against authoritative registry information for the ongoing gene-targeted clinical programme. Bibliographic metadata and digital object identifiers were cross-checked against PubMed, journal records or DOI-resolving sources before inclusion.

2.3 Search Strategy and Search Period

The principal search period was January 2001 to 8 August 2026, beginning with the molecular identification of GFAP variants as the cause of AxD. Representative combinations included “Alexander disease” AND (GFAP OR glial fibrillary acidic protein) AND (child* OR pediatric OR paediatric OR infant* OR adolescent*), combined with terms for phenotype, MRI, genotype, variant, natural history, Rosenthal fibre, astrocyte, proteostasis, autophagy, inflammation, oligodendrocyte, biomarker, antisense oligonucleotide, gene silencing and therapy. Searches for genotype–phenotype evidence additionally used recurrent residues and variants including Arg79, Arg88, Arg239 and Arg416. Foundational mechanistic work closely linked to the post-2001 molecular model was retained where necessary to explain current concepts.

2.4 Eligibility Criteria

Priority was given to peer-reviewed original research, high-quality reviews, genetically confirmed human cohorts, natural-history studies, and mechanistic experiments directly testing GFAP-dependent pathology. Case reports were used only when they clarified rare presentations or variant effects not represented in larger studies. Studies were excluded when bibliographic identity could not be verified, when the article did not materially address AxD, or when mechanistic claims were based only on indirect analogy. Conference-only efficacy claims were not treated as established evidence. The trial registry was used to describe the design and developmental status of the investigational antisense programme rather than to substitute for peer-reviewed efficacy evidence.

2.5 Screening, Duplicate Handling and Study Selection

Titles and abstracts were screened for direct relevance to paediatric phenotype, GFAP genetics, molecular pathogenesis, biomarkers or gene-targeted treatment. Full text or detailed indexed records were reviewed when necessary to confirm study design, population and claim–source alignment. Duplicate records across scholarly indexes were reconciled by DOI, PMID and title. English-language sources predominated because they were the accessible evidence base used for detailed appraisal. No PRISMA-style record counts are presented because this was not a systematic review with prospectively documented screening totals.

2.6 Critical Appraisal and Evidence Synthesis

Human cohort evidence was appraised for genetic confirmation, sample size, phenotype definition, ascertainment, follow-up and statistical handling of variant groups. Mechanistic studies were judged by model relevance, replication across experimental systems, use of human tissue or patient-derived cells, and the degree to which proposed pathways were shown to influence GFAP accumulation or downstream pathology. Greater confidence was placed in convergent mechanisms reproduced across human samples and more than one model than in single-pathway observations. Therapeutic claims were graded according to developmental stage, target engagement, reversibility of established pathology, and availability of clinically meaningful outcomes. The synthesis was organised around clinical spectrum, genotype–phenotype relationships, mechanistic modules and translational readiness rather than as a study-by-study catalogue.

3. Clinical Spectrum of Pediatric Alexander Disease

3.1 From Age-Based Labels to Phenotype-Based Classification

Classical teaching divided AxD into infantile, juvenile and adult forms according to age at onset. This schema captured an obvious age gradient but implied sharper boundaries than the biology supports. The larger multicentre analysis by Prust *et al.* (2011), which combined 30 new cases with previously reported patients, identified two latent clinical classes. Type I was associated with earlier onset, seizures, macrocephaly, motor delay, encephalopathy, failure to thrive, paroxysmal deterioration and typical cerebral MRI abnormalities, whereas Type II was associated with later onset, autonomic and ocular-motor abnormalities, bulbar dysfunction and more caudal neuraxis involvement. This formulation established that symptom pattern and anatomical distribution may be more informative than age alone.

For paediatric practice, the most useful interpretation is a continuum. At one end are neonates and young infants with diffuse cerebral dysfunction, rapidly progressive developmental compromise and marked white-matter abnormalities. At the other are older children and adolescents whose phenotype may be dominated by bulbar dysfunction, pyramidal signs, ataxia, autonomic disturbance or medullary and upper spinal cord disease. Between these extremes is an intermediate group with substantial overlap. Phenotype-based classification therefore improves description but does not eliminate ambiguity; children may evolve from predominantly cerebral to mixed phenotypes, and a single GFAP variant can produce different ages at onset and rates of progression (Messing *et al.*, 2025; Yoshida, 2020).

The recent paediatric cohort of 81 genetically confirmed patients provides a stronger natural-history anchor than earlier case aggregates because it analysed acquisition and loss of developmental functions longitudinally (Zhang *et al.*, 2026). The cohort confirmed that disease trajectories are heterogeneous and that loss of major motor and communication skills can occur across childhood and adolescence. Its single-centre design and referral characteristics limit generalisability, but the study demonstrates why static labels such as “infantile” are insufficient for therapeutic trials: the clinically relevant outcome is not simply age at onset but the slope and sequence of functional loss.

3.2 Early Cerebral Phenotype: Development, Seizures and Macrocephaly

The early cerebral phenotype remains the most recognisable paediatric presentation. Developmental delay or regression, seizures, pyramidal signs and macrocephaly often coexist with feeding difficulty and impaired growth. Macrocephaly is neither universal nor required for diagnosis; its historical prominence partly reflects ascertainment of severe infantile cases before molecular testing broadened the spectrum. Seizures are clinically important because they may be frequent, difficult to control and associated with acute deterioration, but they should be understood as one component of a diffuse astrocytic encephalopathy rather than a defining feature in isolation (Prust *et al.*, 2011; Zhang *et al.*, 2026).

A particularly severe neonatal or early-infantile presentation may include rapidly enlarging head circumference, marked irritability, bulbar dysfunction, refractory seizures and hydrocephalus. Hydrocephalus has mechanistic plausibility in AxD because Rosenthal fibre-rich astrocytic pathology can involve periventricular and aqueductal regions, but the frequency and determinants of clinically significant cerebrospinal fluid obstruction remain uncertain. Clinical severity can be amplified by intercurrent fever or systemic illness, and episodic worsening has repeatedly been reported in early-onset disease. These observations support careful anticipatory counselling without assuming a uniformly catastrophic course for every child with early onset.

Developmental assessment requires more than a single global milestone score. Gross motor function, fine motor ability, communication, cognition, swallowing and adaptive skills may diverge. In therapeutic trials, the heterogeneity of baseline function creates a major measurement problem: children who never acquire independent ambulation cannot be evaluated with gait-based endpoints, while children with preserved walking may show clinically meaningful stabilisation that is not captured by conventional developmental scales over a short interval. Natural-history work therefore needs age-calibrated domains and individualised functional outcomes in addition to global severity measures.

3.3 Bulbar, Pyramidal, Cerebellar and Autonomic Features in Older Children

Older children and adolescents may present with dysarthria, dysphagia, palatal or pharyngeal dysfunction, spasticity, gait impairment, ataxia and abnormal ocular movements. The clinical pattern reflects the predilection of later-onset disease for the medulla, upper cervical cord and corticospinal pathways. Autonomic symptoms, including bladder dysfunction and orthostatic or other dysautonomic features, are better recognised in Type II disease but can overlap with paediatric intermediate phenotypes (Prust *et al.*, 2011; Yoshida, 2020).

This caudal phenotype is diagnostically vulnerable because white-matter abnormalities may be modest compared with the dramatic cerebral changes seen in infants. An older child with progressive bulbar dysfunction and medullary atrophy may therefore be evaluated for mitochondrial, motor neuron, hereditary spastic paraplegia, spinocerebellar or structural disorders before GFAP testing is considered. The broader lesson is that AxD should not be excluded by the absence of marked supratentorial leukodystrophy. Conversely, GFAP variants of uncertain significance should not be accepted as causal simply because the clinical picture includes spasticity or bulbar signs; radiological concordance, segregation, de novo status, population frequency and, when available, functional evidence remain important.

3.4 Magnetic Resonance Imaging as a Phenotypic Map

MRI remains central because the anatomical distribution of AxD is unusually informative. The classic MRI framework described extensive white-matter signal abnormality with frontal predominance, a characteristic periventricular rim, basal ganglia or thalamic abnormalities, brainstem involvement and contrast enhancement of selected structures (van der Knaap *et al.*, 2001). In the molecular era, these criteria are no longer used as a substitute for genetic confirmation, but they remain valuable for recognising the disease and interpreting variant pathogenicity.

Paediatric cerebral disease often shows frontal-predominant T2 hyperintensity extending into deep and periventricular white matter, with relative posterior sparing early in the course. The periventricular rim and involvement of basal ganglia or thalami may strengthen diagnostic suspicion, while contrast enhancement can reflect active astrocytic pathology and blood–brain barrier disturbance. Over time, signal abnormalities may evolve toward tissue loss and ventricular enlargement. In intermediate phenotypes, supratentorial disease coexists with medullary, upper cervical and cerebellar pathway involvement. The imaging phenotype is therefore dynamic rather than a fixed signature.

MRI is also likely to become a treatment-response tool, but its optimal role is unsettled. Conventional lesion burden may change slowly and may not map linearly onto function. Quantitative volumetry, diffusion metrics and brainstem morphometry could provide more sensitive measures, yet they require age-specific reference data and harmonisation across scanners. A paediatric biomarker programme should distinguish structural damage that is unlikely to reverse from imaging signals that might reflect active, treatment-responsive astrocyte dysfunction.

The diagnostic value of MRI is strongest when pattern recognition is integrated with age and clinical trajectory. In an infant with a rapidly progressive encephalopathy, frontal predominance and a periventricular rim can make AxD a high-priority molecular diagnosis, whereas a similar degree of T2 signal abnormality without the characteristic distribution has a much broader differential. In older children, medullary and upper cervical atrophy can be more discriminating than total supratentorial lesion burden. These age-dependent shifts explain why rigid application of a single radiological checklist can reduce sensitivity at the edges of the spectrum (van der Knaap *et al.*, 2001; Prust *et al.*, 2011).

Serial imaging also raises a distinction between disease activity and accumulated damage. Enhancement, evolving signal abnormalities and diffusion changes may represent active tissue stress, while established atrophy more plausibly reflects irreversible structural loss. This distinction matters for trials because a molecular therapy could suppress the initiating astrocytic stress without rapidly reversing existing volume loss. Longitudinal MRI protocols should therefore separate measures expected to respond over months from structural outcomes that may require years to demonstrate stabilisation. The available human literature has not yet validated a single imaging surrogate that reliably predicts later functional benefit, so quantitative MRI should remain complementary to clinical outcomes rather than replacing them (Waldman *et al.*, 2026).

Table 1. Clinically useful phenotype framework for pediatric Alexander disease

Phenotype	Typical paediatric context	Dominant manifestations	Characteristic imaging emphasis	Key limitations	Supporting references
Early cerebral / Type I	Neonatal period to early childhood	Developmental delay or regression, seizures, macrocephaly, spasticity, feeding difficulty, episodic deterioration	Frontal-predominant cerebral white matter, periventricular rim, deep grey involvement, enhancement	Macrocephaly and seizures are not universal; severity varies within the same variant	Prust <i>et al.</i> , 2011; van der Knaap <i>et al.</i> , 2001; Zhang <i>et al.</i> , 2026
Intermediate / mixed	Childhood	Combined cerebral and bulbospinal features; variable motor and communication loss	Supratentorial abnormalities plus medullary/brainstem or upper cervical involvement	Boundaries are descriptive and may shift with disease evolution	Yoshida, 2020; Waldman <i>et al.</i> , 2026
Bulbospinal / Type II-like	Later childhood or adolescence, but possible earlier	Bulbar dysfunction, spasticity, ataxia, ocular-motor and autonomic symptoms	Medulla and upper cervical cord atrophy/signal change; less dramatic cerebral leukodystrophy	May mimic other neurodegenerative, mitochondrial or motor-system disorders	Prust <i>et al.</i> , 2011; Yoshida, 2020
Rapid early-onset severe course	Neonatal or early infancy	Severe encephalopathy, seizures, bulbar compromise, hydrocephalus, early functional loss	Diffuse cerebral disease with periventricular and brainstem involvement	Evidence is enriched for referral and severe cases	Zhang <i>et al.</i> , 2026

Note. Phenotype terms are descriptive rather than mutually exclusive biological subtypes. MRI = magnetic resonance imaging

Imaging-genotype relationships are similarly probabilistic. Severe early variants are enriched among children with extensive cerebral disease, but the same recurrent residue can be associated with different substitution-specific phenotypes, and imaging severity can evolve with age. MRI can strengthen interpretation of a likely pathogenic GFAP variant, particularly when the pattern is characteristic, but it should not be used to force an atypical child into a genotype-derived phenotype. The most informative

approach is longitudinal: molecular diagnosis establishes cause, while serial MRI provides an anatomical record of how that cause is expressed across development.

The evidence summarised in Table 1 shows that paediatric AxD is best understood as a spectrum defined jointly by age, dominant anatomical compartment, functional trajectory and MRI pattern. No single classification captures all clinically relevant variation, so phenotype labels should support rather than replace longitudinal description.

4. GFAP Genetics and Genotype–Phenotype Relationships

4.1 Molecular Genetics and Inheritance

GFAP is the canonical disease gene for AxD. The landmark demonstration of heterozygous coding variants established a dominant genetic mechanism and showed that many paediatric cases arise *de novo* (Brenner *et al.*, 2001). Most established pathogenic changes are missense variants that alter conserved residues within the GFAP protein, although the mutational spectrum includes changes that influence splicing and isoform expression. The disease mechanism is not explained by simple haploinsufficiency: knockout models do not reproduce the full AxD phenotype, whereas overexpression and mutant protein accumulation recapitulate key pathological features. The genetic lesion therefore acts predominantly through toxic gain of function and dosage-sensitive accumulation (Quinlan *et al.*, 2007; Hagemann, 2022).

The practical consequence for paediatric counselling is that parental testing is important even when family history is negative. A *de novo* variant substantially lowers, but does not eliminate, recurrence risk because of possible parental germline mosaicism. In inherited families, penetrance and expressivity can vary, and an apparently mildly affected parent does not guarantee a mild course in a child. Genetic reports should therefore avoid deterministic language based only on inheritance pattern. Variant interpretation is strongest when the molecular change has been observed repeatedly in AxD, affects a functionally constrained residue, has appropriate rarity, and is concordant with clinical and MRI features.

Alternative splicing adds further complexity. Helman *et al.* (2020) demonstrated that exonic changes can alter GFAP pre-mRNA processing and increase expression of a GFAP lambda isoform in Type II disease. This finding is conceptually important for children with atypical variants because the pathogenic effect may not be predictable from amino-acid substitution alone. It also cautions against therapy designs that assume all disease biology is carried exclusively by the canonical GFAP-alpha transcript.

4.2 Recurrent Residues and Variant-Specific Effects

Early genotype–phenotype analyses recognised recurrent mutations at residues including Arg79, Arg88, Arg239 and Arg416, but small numbers and pooling of different substitutions limited inference. Prust *et al.* (2011) found associations between specific recurrent variants and age at onset, yet the data still contained substantial overlap. More recent analysis by Messing *et al.* (2025) improved statistical resolution by compiling variants represented in at least seven unrelated cases and separating substitutions at the same residue. Their results support three broad patterns: variants with consistently earlier onset, variants with later onset, and variants with wider variability. Importantly, p.Arg239His was associated with earlier onset than p.Arg239Cys, illustrating that residue-level grouping can conceal clinically meaningful substitution-specific effects.

The 2025 analysis also found differences in survival across variants, but it should not be converted into individual prediction tables without caution. Cases were assembled from the literature and natural-history sources across eras with different diagnostic practices and follow-up. Age at recognition is vulnerable to retrospective interpretation, and treatment remains largely supportive, so survival estimates may be influenced by respiratory, nutritional and seizure management. Moreover, only a minority of the many reported GFAP variants have enough unrelated cases for robust modelling. The strongest conclusion is therefore qualitative: genotype contributes materially to phenotype, but the relationship is probabilistic rather than deterministic.

The paediatric cohort reported by Zhang *et al.* (2026) adds prospective clinical relevance by linking recurrent Arg239 variation with poorer outcome in a multivariable analysis. The association is biologically plausible and consistent with prior recognition of severe early phenotypes involving this region, but the confidence interval was wide and the study was from a single centre. Replication across geographically diverse cohorts is needed before Arg239 status is used as a formal stratification factor for treatment allocation or family-level prognostication. Genotype may be most useful when integrated with age at onset, baseline developmental attainment, MRI phenotype and biomarker profile.

4.3 Why Genotype Does Not Fully Determine the Paediatric Phenotype

Several sources of variability can weaken genotype–phenotype concordance. First, GFAP toxicity is dosage sensitive, and the level of mutant and total GFAP can be influenced by transcriptional feedback and cellular stress responses. Second, post-translational modifications alter solubility and aggregation; phosphorylation, oxidation, crosslinking and ubiquitination can differ between cellular contexts (Battaglia *et al.*, 2019; Lin *et al.*, 2024). Third, astrocyte maturation and regional identity change across development, creating age-dependent vulnerability. Finally, modifier genes, epigenetic regulation, inflammatory state and environmental stressors may influence the threshold at which astrocyte dysfunction becomes clinically apparent.

These mechanisms help explain why the same nominal mutation can occur in children with different ages at onset, MRI burdens and functional trajectories. They also suggest that molecular therapy could reduce the impact of an adverse genotype by lowering the shared downstream driver—excess GFAP—rather than correcting each variant individually. This is a major strategic advantage

of transcript-lowering therapy, but it increases the need to establish whether different variants require different degrees or timing of GFAP suppression.

Table 2 synthesises recurrent genotype–phenotype patterns without implying that any variant alone determines prognosis. The strongest evidence exists for a relatively small set of recurrent variants; rare variants still require case-level interpretation.

Table 2. GFAP genotype–phenotype relationships with relevance to childhood-onset Alexander disease

GFAP feature	Observed phenotype tendency	Strength of evidence	Interpretive caution	Supporting references
p.Arg239 substitutions	Often early onset; p.Arg239His earlier than p.Arg239Cys in aggregated data; Arg239 variation linked to poorer outcome in one paediatric cohort	Moderate to strong for age-at-onset difference; emerging for prognosis	Substitution-specific effects matter; referral and cohort effects remain	Messing <i>et al.</i> , 2025; Zhang <i>et al.</i> , 2026
p.Arg79 substitutions	Several variants associated with early or variable onset depending on amino-acid substitution	Moderate	Do not collapse all changes at the same residue into one category	Messing <i>et al.</i> , 2025
p.Arg416Trp	Broad age-at-onset variability	Moderate	Wide expressivity limits individual prognostication	Messing <i>et al.</i> , 2025
R70W and N386S	Tend toward later onset in compiled variant-specific data	Moderate	Paediatric relevance is mainly in differential diagnosis and counselling	Messing <i>et al.</i> , 2025
Splice-altering exonic variants	Can increase non-canonical GFAP isoform expression and produce Type II phenotypes	Mechanistically strong but based on few families	RNA consequences may be missed by protein-only interpretation	Helman <i>et al.</i> , 2020
Total GFAP expression level	Higher expression and accumulation correlate with greater model severity	Strong preclinical support	Human clinical severity is not reducible to one concentration at one time point	Jany <i>et al.</i> , 2013; Hagemann, 2022

Note. Associations are probabilistic and should be integrated with age, phenotype, MRI findings and longitudinal course. GFAP = glial fibrillary acidic protein

5. Molecular Pathogenesis: From Mutant GFAP to Multicellular CNS Dysfunction

5.1 The Core Toxic-Gain-of-Function Model

The most coherent model of AxD begins with a qualitative and quantitative disturbance of GFAP. Disease-associated mutations can impair normal intermediate-filament assembly, increase the propensity for aberrant filament–filament interactions, and promote accumulation of poorly soluble GFAP. At the same time, reactive transcriptional programmes increase GFAP expression. The result is a positive feedback loop in which an abnormal cytoskeletal protein induces cellular stress that drives further production of the same protein. This distinguishes AxD from a simple structural deficiency and explains why lowering total GFAP, including wild-type transcript, can be therapeutic in model systems (Quinlan *et al.*, 2007; Hagemann *et al.*, 2018).

Rosenthal fibres are the histopathological hallmark of this process but should not be assumed to be the sole toxic species. They contain GFAP, alphaB-crystallin, HSP27, ubiquitin and numerous additional proteins. Proteomic analysis expanded the catalogue of associated proteins and suggested links to stress-granule biology, but did not support a simple model in which disease arises because essential proteins are quantitatively sequestered into aggregates (Heaven *et al.*, 2016). Microscopy studies indicate that small granular GFAP-rich deposits can develop along intermediate filaments and enlarge over time; astrocytes with abundant inclusions can show striking distortion of cellular processes and nuclear morphology (Sosunov *et al.*, 2017). These observations favour a model in which aggregate formation is one visible component of a broader proteostatic and cytoskeletal crisis.

The distinction between aggregate burden and toxicity matters therapeutically. If large Rosenthal fibres were purely toxic, treatment success could be judged by aggregate clearance alone. If, instead, soluble or oligomeric intermediates, altered filament mechanics and chronic reactive signalling are major drivers, then meaningful target engagement requires normalisation of GFAP abundance and astrocyte physiology. The dramatic reversal of Rosenthal fibres after antisense-mediated GFAP suppression in mouse models is encouraging, but the clinically relevant question is whether neuronal, oligodendroglial and developmental consequences can also stabilise or recover once established.

5.2 Proteostasis Failure, Stress Kinases and Autophagy

GFAP accumulation interacts bidirectionally with protein-quality-control systems. Tang *et al.* (2006) demonstrated a reinforcing relationship among GFAP accumulation, stress-activated JNK signalling and proteasomal impairment. In this framework, accumulating GFAP reduces proteasome function, proteasomal stress activates kinase pathways, and those pathways further increase GFAP accumulation. Such feedback offers a plausible explanation for non-linear disease progression: once GFAP burden crosses a threshold, cellular clearance capacity and stress signalling may become mutually destabilising.

Autophagy appears to be partly compensatory. Mutant GFAP accumulation activates p38/MAPK signalling, inhibits mTOR signalling and induces macroautophagy (Tang *et al.*, 2008). This response can help clear aggregated proteins but is unlikely to restore homeostasis when the upstream production of GFAP remains high. The therapeutic implication is that pharmacologically stimulating degradation may have limited durability unless the transcriptional and translational input is also reduced. Earlier experiments with lithium and other proteostasis modifiers provided proof of concept for GFAP reduction but also highlighted tolerability and specificity limitations compared with a direct transcript-targeted approach.

Protein turnover measurements reinforce the idea that accumulation reflects both production and clearance. Altered GFAP turnover kinetics in AxD models show that mutant and wild-type GFAP exist within a disturbed proteostatic environment rather than behaving as independent species (Moody *et al.*, 2017). This may explain dominant effects of mutant protein on a mixed filament network. It also argues against therapeutic strategies that target only a single visible aggregate conformation while leaving the overall GFAP pool and stress-responsive transcription intact.

5.3 Post-Translational Modification, Oxidative Chemistry and Cytoskeletal Instability

Recent studies have added a chemical dimension to the pathogenesis model. In patient tissue and induced pluripotent stem cell-derived astrocytes, site-specific phosphorylation of GFAP at Ser13 and caspase-6-associated cleavage were linked to severe early disease, suggesting that post-translational state may integrate the effects of different primary mutations (Battaglia *et al.*, 2019). These findings are provocative because they offer a route by which genetically diverse variants could converge on a common aggregation-prone species. Nevertheless, the association with severity was derived from limited human tissue and requires validation in longitudinal biomarkers or larger collections.

Oxidation and crosslinking provide another convergence mechanism. Lin *et al.* (2024) identified high-molecular-weight GFAP species in Rosenthal fibres and showed that cysteine-generating mutations can promote cysteine-dependent oxidation, abnormal crosslinking and reduced solubility. Complementary work on the p.Arg239Cys mutant demonstrated increased susceptibility to lipoxidation, altered assembly and mitochondrial dysfunction in cellular systems (Viedma-Poyatos *et al.*, 2022). These data support a feed-forward interaction between mutant protein chemistry and oxidative stress, but they do not establish that antioxidant treatment alone would modify clinical disease. Oxidative stress may be a downstream amplifier rather than the initiating lesion.

The apparent benefit of Nrf2 overexpression in a mouse model is consistent with a role for stress-response pathways but also illustrates mechanistic complexity. Forced astrocytic Nrf2 expression reduced GFAP and Rosenthal fibres and improved body weight, yet the effect was not explained by restoration of glutathione (LaPash Daniels *et al.*, 2012). The result suggests that transcriptional reprogramming can influence GFAP burden through mechanisms beyond canonical antioxidant defence. Such pleiotropy complicates translation of pathway modifiers, because broad stress-response activation could have developmentally dependent effects that are difficult to control in children.

5.4 Reactive Astrocyte State, Inflammation and Loss of Homeostatic Function

AxD astrocytes do not merely accumulate protein; they change state. In mouse models, protoplasmic astrocytes lose fine distal processes, become hypertrophic and can acquire abnormal nuclear features (Sosunov *et al.*, 2013). These structural changes are likely to disrupt the highly localised contact that astrocytic processes normally maintain with synapses, vessels and myelin. The phenotype is therefore better described as maladaptive astrogliosis than as passive storage of an abnormal filament.

Inflammatory signalling is a prominent downstream consequence. Olabarria *et al.* (2015) showed marked microglial activation and strong induction of chemokines, including CXCL10 and CCL2, in an AxD mouse model; human tissue also demonstrated microglial activation. This work supports a causal sequence in which primary astrocyte stress can generate a secondary inflammatory environment without a primary immune trigger. Whether inflammation is predominantly injurious, compensatory or stage-dependent remains unresolved. Direct anti-inflammatory therapy could suppress harmful amplification but might not correct the GFAP-driven initiating process.

STAT3 is a particularly important node because it links reactive astrogliosis with GFAP transcription. Conditional genetic reduction of STAT3 signalling in AxD models lowered GFAP accumulation, reactive astrocyte markers and microglial activation even when pathology was already established (Hagemann *et al.*, 2023). These experiments strengthen the concept of a transcriptional feedback loop and identify STAT3 as a plausible pharmacological target. Yet broad STAT3 inhibition is less specific than GFAP transcription lowering and could affect immune and developmental signalling, so the translational risk–benefit balance is uncertain.

5.5 Non-Cell-Autonomous Injury: Glutamate, Oligodendrocytes and Myelin

The defining clinical consequences of AxD—seizures, developmental impairment, spasticity and white-matter disease—cannot be explained by astrocyte morphology alone. A key mechanistic bridge is impaired glutamate transport. Tian *et al.* (2010) found reduced GLT-1/EAAT2 expression in affected human and mouse tissue and demonstrated impaired glutamate uptake in astrocytes expressing mutant GFAP. Neurons co-cultured with mutant astrocytes were more vulnerable to glutamate challenge. This provides a plausible route from astrocyte dysfunction to excitotoxic neuronal stress and seizure susceptibility, although in vivo seizure generation is likely multifactorial.

Oligodendroglial injury offers a second bridge to the leukodystrophy phenotype. Patient-derived induced pluripotent stem cell models have shown that AxD astrocytes can impair oligodendrocyte progenitor proliferation and myelination, and gene correction of the GFAP mutation reverses these effects. Such systems are valuable because conventional mouse models incompletely recapitulate human white-matter disease. More recent paediatric iPSC-derived astrocytes have also shown extracellular matrix abnormalities, including increased chondroitin sulphate proteoglycan-related signatures, suggesting that the extracellular environment created by mutant astrocytes may itself become inhibitory to neural repair (Yi *et al.*, 2025).

These findings support a network model: mutant GFAP produces an abnormal astrocyte state; astrocytes then alter neurotransmitter clearance, inflammatory signalling, extracellular matrix composition and trophic or metabolic support; neurons and oligodendroglial cells become secondarily dysfunctional; and tissue-level white-matter and motor-system abnormalities emerge. The model is mechanistically coherent but still incomplete. The relative contribution of each pathway almost certainly differs by developmental stage and neuroanatomical region, which may help explain why infants predominantly develop cerebral disease while later-onset patients often show bulbospinal involvement.

Table 3 groups mechanistic evidence according to causal proximity. The most secure therapeutic target is the upstream excess of GFAP; downstream pathways are biologically credible but generally supported by more limited or model-dependent evidence.

Table 3. Mechanistic modules in Alexander disease and their translational interpretation

Mechanistic module	Evidence	Likely role	Therapeutic implication	Supporting references
GFAP misassembly and accumulation	Human genetics, tissue, cell and animal models	Primary toxic gain of function and initiating proteostatic stress	Direct GFAP lowering addresses the most upstream shared driver	Brenner <i>et al.</i> , 2001; Hagemann, 2022; Quinlan <i>et al.</i> , 2007
Rosenthal fibre formation	Human pathology and models; proteomic characterisation	Marker and participant in disturbed cytoskeleton/proteostasis; not proven to be sole toxic species	Aggregate clearance is encouraging but should not be the only efficacy criterion	Heaven <i>et al.</i> , 2016; Sosunov <i>et al.</i> , 2017
Proteasome–JNK and autophagy responses	Cell and mouse experiments	Self-amplifying stress with partly compensatory degradation	Downstream modifiers may complement but are less direct than transcript lowering	Tang <i>et al.</i> , 2006; Tang <i>et al.</i> , 2008
Post-translational modification and oxidation	Human tissue, iPSC and cell models	May promote crosslinking, insolubility and severity-modifying chemistry	Potential adjunct targets; causal hierarchy not fully established	Battaglia <i>et al.</i> , 2019; Lin <i>et al.</i> , 2024; Viedma-Poyatos <i>et al.</i> , 2022
Reactive signalling and inflammation	Mouse and human tissue evidence	Astrocyte-to-microglia amplification and increased GFAP transcription	STAT3/inflammatory pathways are plausible adjunct targets with broader safety concerns	Hagemann <i>et al.</i> , 2023; Olabarria <i>et al.</i> , 2015
Loss of astrocyte support to neurons and oligodendroglia	Human tissue, co-culture and iPSC models	Impaired glutamate clearance, myelination support and extracellular environment	Clinical recovery may lag behind molecular correction and may be stage dependent	Tian <i>et al.</i> , 2010; Yi <i>et al.</i> , 2025

Note. iPSC = induced pluripotent stem cell; JNK = c-Jun N-terminal kinase; STAT3 = signal transducer and activator of transcription 3

5.6 Regional Vulnerability, Rosenthal Fibres and Developmental Context

A persistent mechanistic question is why a broadly expressed astrocytic protein produces such striking regional and age-dependent pathology. One possibility is that vulnerability tracks with baseline GFAP abundance and the intensity of local astrocyte stress responses. Another is that regional differences in astrocyte function make particular circuits less tolerant of intermediate-filament disruption. The frontal white matter of young children, for example, is undergoing active maturation and depends on coordinated astrocyte-oligodendrocyte interactions, whereas later bulbospinal disease may expose distinct dependencies of long motor and autonomic pathways. Current evidence supports regional heterogeneity but does not establish a single anatomical determinant, and models that explain all phenotypes by GFAP concentration alone are too simple (Hagemann, 2022; Waldman *et al.*, 2026).

Rosenthal fibres illustrate the difference between a disease marker and a disease mechanism. They are highly characteristic accumulations containing GFAP, small heat-shock proteins and other components, and proteomic analyses confirm that they represent complex stress-associated structures rather than pure GFAP deposits (Heaven *et al.*, 2016; Sosunov *et al.*, 2017). Yet their precise contribution to toxicity remains uncertain. Aggregation could sequester abnormal filament material and initially be partly protective, while extensive inclusions may later distort astrocyte architecture and proteostasis. Therapeutic interpretation should therefore focus on the upstream burden of abnormal GFAP and the accompanying cellular state rather than assuming that visible Rosenthal fibre number is a linear measure of neurotoxicity.

Developmental timing may determine whether the downstream phenotype is primarily loss of function, failed acquisition of function or both. In early cerebral disease, astrocyte dysfunction occurs while white-matter tracts and neural networks are still maturing, so the same molecular insult can interfere with developmental construction as well as damage established tissue. This helps explain why removal of the initiating toxic protein might stabilise pathology without immediately normalising cognition or motor skills. In later-onset disease, where established circuits have developed more normally, slowing secondary degeneration may

yield a different pattern of benefit. These distinctions are central to paediatric endpoint selection and to interpretation of apparent treatment non-response.

The mechanistic literature also cautions against a purely aggregate-centric view. Mutant GFAP can perturb filament organisation, kinase signalling, autophagy, redox state and transcription before large inclusions dominate histology. Reactive astrocytes undergo phenotypic conversion and acquire altered structural and signalling properties, while inflammation and impaired support functions expand injury beyond the initially affected cells (Sosunov *et al.*, 2013; Olabarria *et al.*, 2015). A useful causal hierarchy therefore places abnormal GFAP production and handling upstream, with Rosenthal fibres as one conspicuous manifestation within a broader maladaptive astrocyte programme.

6. Diagnosis, Biomarkers and Current Clinical Management

6.1 Diagnostic Integration of Clinical Phenotype, MRI and Molecular Testing

The diagnostic standard in contemporary paediatric practice is molecular confirmation in the context of a compatible phenotype. MRI often raises the suspicion, but genetic testing determines aetiology and avoids brain biopsy in typical cases. The classical MRI criteria remain highly instructive, especially in children with frontal-predominant white-matter disease and characteristic periventricular or brainstem changes (van der Knaap *et al.*, 2001). With broad exome and genome testing, the inverse scenario is increasingly common: a GFAP variant is identified first, and the clinician must decide whether it explains the child's presentation. In that setting, phenotype–imaging concordance is essential to prevent overdiagnosis from a variant of uncertain significance.

Differential diagnosis depends on age and anatomical pattern. Severe infantile cases overlap clinically with other leukodystrophies, metabolic encephalopathies and developmental epileptic disorders. Later childhood bulbospinal disease can resemble hereditary spastic paraplegia, spinocerebellar disorders, mitochondrial disease, motor neuron disease or structural cervicomedullary pathology. The combination of characteristic imaging and a pathogenic GFAP variant is usually decisive, but atypical cases benefit from careful reassessment of the variant and alternative diagnoses rather than reflexive attribution.

6.2 Fluid GFAP and the Biomarker Paradox

GFAP is an intuitively attractive biomarker because it is the disease protein and can be measured in cerebrospinal fluid (CSF) and blood. CSF concentrations are elevated in AxD and correlate broadly with brain GFAP burden in model systems (Jany *et al.*, 2013, 2015). Plasma GFAP is also measurable, and modern assays have improved sensitivity. The 2026 phenotype study found higher CSF and plasma GFAP in AxD than in controls and demonstrated differences across cerebral, intermediate and bulbospinal phenotypes, with distinct longitudinal patterns in younger versus older participants (Waldman *et al.*, 2026). These results support GFAP as a marker of biological disease activity and, importantly, of target engagement when a therapy directly reduces GFAP.

The same property creates a paradox. If an antisense oligonucleotide suppresses GFAP transcription, a fall in CSF or plasma GFAP can occur even if downstream neuronal injury or function does not improve. GFAP therefore cannot serve alone as a surrogate for clinical benefit. Biomarker panels need treatment-independent measures. Plasma neurofilament light, tau and related neurodegeneration markers have been explored, but their specificity and longitudinal behaviour in AxD remain insufficiently established (Ashton *et al.*, 2024). Imaging measures, functional scales and patient-centred outcomes must therefore accompany GFAP concentrations in trials.

Age is likely to be a major biomarker modifier. Young children may have rapidly changing GFAP levels during active cerebral disease, whereas older individuals can show different trajectories as tissue becomes chronically damaged or atrophic. A single threshold cannot therefore be assumed to have the same meaning across the paediatric age range. Longitudinal within-child change may be more informative than comparison with a cross-sectional reference distribution.

6.3 Supportive Care in the Pre-Disease-Modifying Era

Until disease-modifying therapy is established, management remains supportive and multidisciplinary. Seizures are treated according to epilepsy phenotype and tolerability rather than with an AxD-specific antiseizure regimen. Spasticity, dystonia and gait impairment require physiotherapy, orthotic support and pharmacological or procedural management when appropriate. Dysphagia and bulbar dysfunction warrant repeated swallowing assessment, nutritional support and aspiration-risk management. Respiratory evaluation becomes increasingly important in children with advanced bulbar or motor disease.

Hydrocephalus requires standard neurosurgical assessment when progressive ventricular enlargement and intracranial pressure are clinically relevant. Communication support, educational planning, augmentative devices and psychosocial support are essential because cognitive, motor and speech trajectories can diverge. Care plans should be anticipatory but not nihilistic: genotype and early presentation influence risk, yet individual trajectories remain variable. The emergence of targeted treatment also strengthens the case for early referral to centres able to perform natural-history assessment and trial screening.

Table 4 highlights the biomarker hierarchy that is most defensible for paediatric trials. The central principle is separation of molecular target engagement from evidence that neural function or tissue integrity has been preserved.

Table 4. Biomarkers and outcome domains for paediatric Alexander disease trials

Measure	What it captures	Strength	Key limitation	Best use	Supporting references
CSF GFAP	Direct disease-protein concentration	Strong biological specificity	Falls directly with GFAP-targeted therapy and cannot alone prove clinical benefit	Target engagement and pharmacodynamic response	Jany <i>et al.</i> , 2015; Waldman <i>et al.</i> , 2026
Plasma GFAP	Less invasive GFAP measure	Practical for serial sampling	Age and phenotype influence levels; same target-engagement paradox	Frequent pharmacodynamic monitoring	Waldman <i>et al.</i> , 2026
Plasma neurofilament light / tau	Downstream neuronal injury markers	Potentially treatment-independent	Limited AxD-specific validation and uncertain paediatric reference ranges	Exploratory injury biomarkers	Ashton <i>et al.</i> , 2024
MRI lesion burden / volumetry	Anatomical disease distribution and tissue loss	Objective and mechanistically relevant	Slow change; scanner and age effects; irreversible atrophy may not recover	Structural progression and regional response	van der Knaap <i>et al.</i> , 2001
Motor, bulbar and developmental outcomes	Clinical function meaningful to child and family	Direct clinical relevance	Floor/ceiling effects and heterogeneous baseline ability	Primary or key secondary efficacy outcomes	Prust <i>et al.</i> , 2011; Zhang <i>et al.</i> , 2026
Individualised symptom/global impression measures	Patient- or caregiver-prioritised change	Captures heterogeneous disease burden	Susceptible to expectation and reporting effects	Complement to objective functional endpoints	Clinical trial context

Note. CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; MRI = magnetic resonance imaging; AxD = Alexander disease

7. Emerging Gene-Targeted and Molecular Therapies

7.1 Why GFAP Lowering is the Leading Strategy

A disease caused by dominant toxic gain of function is an attractive target for transcript reduction when the affected protein is not absolutely required at full normal abundance. AxD meets these criteria unusually well. Disease severity in models tracks with GFAP accumulation, overexpression can itself produce Rosenthal fibre pathology, and reduction of GFAP should lower both mutant and wild-type protein within the same filament network. This strategy bypasses the need to design a separate agent for each pathogenic variant and is therefore suitable for a genetically heterogeneous rare disease (Jany *et al.*, 2013; Hagemann, 2022).

The key preclinical experiment used antisense oligonucleotides (ASOs) directed against Gfap. In mutant mouse models, a single central nervous system administration produced profound and durable transcript and protein reduction, clearance of Rosenthal fibres, improvement in molecular stress responses and microglial activation, improved body condition and rescue of impaired hippocampal neurogenesis (Hagemann *et al.*, 2018). Particularly important for translation, treatment was effective in adult mice with established pathology rather than only when delivered pre-symptomatically. This supports biological reversibility, although the degree of reversibility in the developing human brain remains unknown.

The preclinical study also showed that CSF GFAP fell after treatment, creating a translational pharmacodynamic biomarker. This is a major advantage in a rare disease where brain tissue cannot be sampled serially. Yet the result also reinforces the biomarker paradox described above: a lower GFAP concentration is expected if the drug engages its target and therefore must be linked to outcomes that reflect neural integrity and function.

7.2 Zilganersen and the Clinical Translation of Antisense Therapy

The clinical translation of GFAP lowering is represented by zilganersen (formerly ION373), an intrathecally administered antisense therapy designed to reduce GFAP RNA. The registered programme includes a phase 1–3, randomised, controlled design followed by open-label treatment and long-term extension (ClinicalTrials.gov, n.d.). The broad age range reflects the biological continuity of AxD but creates substantial endpoint challenges because functional abilities differ markedly between a two-year-old with severe cerebral disease and an ambulant adolescent or adult with bulbospinal disease.

As of the review search end date, the trial programme had advanced to late-stage clinical development, but the most rigorous peer-reviewed evidence available for publication remained far less mature than the preclinical mechanistic evidence. This distinction is crucial. Positive target engagement or public reporting of trial outcomes should not be equated automatically with established long-term paediatric efficacy. Durable benefit must be demonstrated against disease-relevant functional trajectories, with careful assessment of intrathecal procedural burden, laboratory abnormalities, neurological adverse events and developmental safety.

Paediatric treatment timing may be decisive. If early cerebral disease reflects a rapidly self-amplifying GFAP stress response, intervention before major tissue loss could offer the greatest opportunity to preserve development. Conversely, very early treatment

raises uncertainties about the physiological role of GFAP in the maturing brain and about long-term consequences of sustained reduction. The preclinical observation that extensive suppression is tolerated in mice is reassuring but cannot fully resolve human developmental safety. Dose selection must therefore balance sufficient suppression of toxic accumulation against preservation of astrocyte resilience over years of growth.

ASO pharmacology also shapes the practical treatment model. Intrathecal administration is used because systemically delivered oligonucleotides do not reliably achieve therapeutic concentrations throughout the central nervous system. Repeated dosing permits adjustment over time, an advantage in a growing child whose disease stage and safety profile may evolve. The same feature creates burden: lumbar procedures, anaesthesia or sedation in some young children, travel to specialist centres and repeated monitoring can affect feasibility independently of molecular efficacy. These implementation outcomes should be measured rather than treated as incidental, because a lifelong therapy that is biologically effective but difficult to deliver may produce unequal real-world benefit.

The strongest preclinical argument for GFAP lowering is not simply that it reduces a biomarker. Transcript suppression changes the upstream variable that integrates diverse pathogenic variants, and in mice it reverses multiple downstream readouts, including Rosenthal fibre burden and stress-associated molecular responses (Hagemann *et al.*, 2018). That breadth distinguishes causal target engagement from pathway-specific symptomatic modulation. Nevertheless, species differences in astrocyte complexity, brain size, dosing distribution and developmental timing limit direct extrapolation. The key human experiment is therefore whether a defined degree and duration of GFAP reduction predicts slower functional decline after adjustment for age, baseline severity and genotype.

Paediatric efficacy assessment will also require attention to response lag. A fall in CSF GFAP may occur relatively quickly, whereas changes in swallowing safety, motor development or adaptive function may emerge only after sufficient time has elapsed to diverge from the untreated trajectory. A trial can therefore be biologically positive before it is clinically separable, particularly in slowly progressive or mixed phenotypes. Conversely, transient functional improvement without sustained molecular control would not establish durable disease modification. Integrated longitudinal modelling of pharmacodynamic, imaging and functional trajectories is likely to be more informative than a single end-of-study comparison.

7.3 Alternative Molecular Targets and Combination Strategies

Direct GFAP lowering has the clearest causal rationale, but downstream pathways may eventually become adjunctive targets. STAT3 inhibition could interrupt the reactive transcriptional feedback that sustains GFAP overexpression (Hagemann *et al.*, 2023). Modulation of protein-quality-control pathways could enhance clearance of residual aggregates, and interventions aimed at oxidative crosslinking or pathological post-translational modification might reduce formation of particularly insoluble GFAP species (Battaglia *et al.*, 2019; Lin *et al.*, 2024). Anti-inflammatory strategies could dampen microglial and chemokine amplification, while approaches that restore glutamate transport or oligodendrocyte support could address non-cell-autonomous injury.

The evidence for these strategies is currently less direct than for ASO-mediated GFAP suppression. Each downstream intervention risks treating a consequence rather than the driver, and broad pathway modulation may have off-target effects. Combination therapy may become rational if substantial neurological progression continues despite robust GFAP lowering, but this should be demonstrated empirically rather than assumed. Mechanistic biomarkers could help identify residual disease modules after target suppression—for example, persistent neurofilament elevation despite marked GFAP reduction might indicate ongoing neuronal injury that requires additional intervention.

Gene editing and vector-based gene silencing are conceptually attractive because they could provide durable suppression after a single intervention. However, the dominant-negative versus toxic-gain-of-function architecture, astrocyte targeting requirements, irreversible nature of editing and need to control expression across the central nervous system create substantial safety and delivery barriers. In children, a titratable ASO has an important advantage: exposure can be adjusted or stopped if safety or developmental concerns emerge. Durable one-time strategies will require a substantially higher evidentiary threshold.

7.4 Translational Bottlenecks for Paediatric Efficacy

The central translational problem is outcome sensitivity. A trial that enrolls children across a broad age and severity range cannot rely on one motor metric. Gait speed is irrelevant for non-ambulant children, while developmental scales may have floor effects in severely affected participants and ceiling effects in milder adolescents. Composite or stratified endpoints must preserve clinical meaning without creating statistical fragility in a very small population. Individualised most-bothersome-symptom approaches can capture heterogeneity but require protection against expectation bias.

A second problem is disease-stage irreversibility. Lowering GFAP may quickly normalise a molecular biomarker while myelin loss, axonal injury or severe developmental impairment persists. Early treatment could therefore show larger functional effects than late treatment even with comparable target engagement. This creates a strong rationale for age- and stage-stratified analyses and for long-term extension studies that assess whether stabilisation prevents expected future loss rather than only producing short-term gains.

A third problem is genotype and phenotype imbalance in tiny samples. Recurrent severe variants may cluster in younger participants and confound comparisons if treatment groups are not balanced. Trial interpretation should therefore incorporate pre-specified stratification or modelling by baseline phenotype, age, ambulatory status and recurrent genotype where sample size permits. The goal is not to create genetically deterministic strata but to reduce variance from known prognostic factors.

Table 5. Emerging molecular treatment strategies for Alexander disease

Strategy	Molecular rationale	Developmental status	Potential advantages	Main uncertainties	Supporting references
GFAP-targeting antisense oligonucleotide	Reduce mutant and wild-type GFAP RNA, lowering the shared toxic protein burden	Strong preclinical evidence; late-stage intrathecal clinical programme	Variant-agnostic, titratable, measurable target engagement	Long-term paediatric efficacy, developmental safety, optimal timing and degree of suppression	ClinicalTrials.gov, n.d.; Hagemann <i>et al.</i> , 2018
STAT3 pathway inhibition	Interrupt reactive transcriptional amplification of GFAP	Preclinical genetic proof of concept	Targets feedback mechanism and may reduce inflammatory activation	Pathway is pleiotropic; pharmacological specificity and paediatric safety uncertain	Hagemann <i>et al.</i> , 2023
Proteostasis/autophagy modulation	Improve clearance of accumulated or aggregated GFAP	Mechanistic/preclinical	Could complement partial transcript suppression	May not overcome ongoing GFAP production; systemic effects	Tang <i>et al.</i> , 2006; Tang <i>et al.</i> , 2008
Oxidation/PTM-directed therapy	Reduce pathological crosslinking, phosphorylation or aggregation-prone GFAP states	Early mechanistic stage	May target toxic protein chemistry downstream of diverse variants	Causal importance in vivo and druggability not established	Battaglia <i>et al.</i> , 2019; Lin <i>et al.</i> , 2024; Viedma-Poyatos <i>et al.</i> , 2022
Anti-inflammatory / microglial modulation	Reduce secondary chemokine and microglial amplification	Preclinical rationale	Could address persistent secondary injury	May leave primary GFAP toxicity intact; stage-specific effects possible	Olabarria <i>et al.</i> , 2015
Vector or editing-based silencing/correction	Durable reduction or correction of pathogenic GFAP	Conceptual/early preclinical direction	Potential one-time treatment	Irreversibility, delivery, dosage control, immunogenicity and developmental risk	Mechanistic inference from GFAP gain-of-function evidence

Note. GFAP = glial fibrillary acidic protein; PTM = post-translational modification. Developmental status reflects the evidence base up to 8 August 2026

Table 5 places the major therapeutic strategies on an evidence gradient. The most mature strategy is direct transcript reduction; other molecular approaches remain principally mechanistic or preclinical and should not be presented as equivalent alternatives.

8. Integrated Critical Synthesis

The clinical and molecular literature now supports a coherent disease model, but the strength of evidence differs substantially across its components. The causal link between heterozygous GFAP variants and AxD is secure. The toxic-gain-of-function interpretation is strongly supported by the absence of a comparable phenotype in simple GFAP deficiency, by the effects of GFAP overexpression, by mutant-protein aggregation and by the therapeutic reversal produced by transcript suppression. The conclusion that excessive GFAP is a central driver is therefore more robust than any single downstream pathway.

Genotype–phenotype relationships are real but incomplete. Earlier scepticism arose partly because rare variants were pooled and because single case reports dominated the literature. Variant-specific analyses now show reproducible differences in age at onset and survival for recurrent substitutions, and paediatric cohort data identify potentially adverse recurrent variants. Yet the effect size of genotype is moderated by developmental stage, total GFAP expression, post-translational state and probably additional modifiers. Precision counselling should therefore use genotype as one layer in a multivariable prognosis rather than as a deterministic forecast.

The most important conceptual shift is from leukodystrophy to astrocytopathy. White-matter injury remains clinically central, especially in children, but multiple lines of evidence indicate that myelin damage is secondary to a dysregulated astrocyte state. This explains why the disease can affect neurons, brainstem motor systems, autonomic pathways and development in ways that exceed a simple demyelinating disorder. It also explains why direct astrocyte-targeted therapy is biologically credible.

The largest remaining uncertainty concerns reversibility. Preclinical ASO treatment reverses molecular and histopathological abnormalities even after pathology is established, but paediatric brains carry accumulated developmental consequences that may not be reversible by removing the initiating stressor. A child who has never acquired a skill because of early network disruption may respond differently from a child who recently lost a previously acquired skill. Clinical success may therefore include stabilisation, slower decline or preservation of future developmental gains rather than dramatic recovery. Trial interpretation must be calibrated to that reality.

Biomarker strategy also requires restraint. GFAP is an excellent measure of target engagement and a potentially useful index of biological activity in untreated disease, but once GFAP is pharmacologically suppressed it cannot independently validate efficacy. Downstream biomarkers such as neurofilament light, quantitative MRI and functional outcomes are needed to triangulate benefit. The ideal surrogate would change early after treatment, predict later functional stabilisation, be minimally affected by age and be independent of direct drug action. No such biomarker has yet been fully validated in paediatric AxD.

Finally, rarity magnifies every methodological weakness. Single-centre cohorts, referral bias, publication of severe or unusual variants, inconsistent historical classification and heterogeneous follow-up can all distort apparent natural history. International harmonisation of phenotype definitions, common outcome measures and longitudinal sampling is therefore not ancillary infrastructure; it is a prerequisite for determining whether molecular therapy truly changes the course of the disease.

9. Future Directions

The immediate research priority is a prospective, internationally harmonised paediatric natural-history framework that is explicitly designed to serve therapeutic trials. It should enrol genetically confirmed children across the full phenotype spectrum and capture motor, communication, swallowing, respiratory, autonomic, cognitive and adaptive domains at intervals appropriate to disease velocity. Analyses should model age and baseline function continuously rather than forcing all children into historical onset categories. Recurrent GFAP variants should be pre-specified as covariates where numbers allow, but rare variants should remain in phenotype-driven analyses to avoid underpowered genetic subgrouping.

A second priority is validation of treatment-independent biomarkers. CSF and plasma GFAP should continue to be measured because they are valuable pharmacodynamic indicators, but they need to be paired with neuroaxonal markers, quantitative MRI and other measures that can predict clinical trajectories. Longitudinal studies should determine whether neurofilament light or other proteins change before irreversible functional decline and whether their trajectories differ between cerebral and bulbospinal phenotypes. Paediatric normative data are essential because biomarker concentrations can change with age and neurodevelopment independently of AxD.

A third priority is to define the therapeutic window. The key question is not only whether GFAP lowering works, but when lowering produces the largest clinically meaningful effect. Comparisons of very young children, older ambulant children and advanced non-ambulant patients can clarify whether molecular target engagement translates into developmental gain, stabilisation or merely slower decline at different stages. Study designs should include sufficiently long observation to distinguish transient practice effects from altered disease slope.

A fourth priority is mechanistic sampling before and after GFAP lowering. Patient-derived iPSC astrocytes and, where feasible, molecular biomarker panels can test whether inflammatory activation, extracellular matrix abnormalities, oxidative modifications and oligodendrocyte-support deficits normalise in parallel with GFAP. Persistence of specific downstream abnormalities despite

strong target suppression would identify rational combination targets. This strategy is preferable to developing adjunct therapies solely from untreated model systems.

A fifth priority is developmental safety. Long-term suppression of GFAP in children should be evaluated for effects on astrocyte resilience during infection, injury and brain maturation. Registries and extension studies need to capture outcomes over years, not only treatment-emergent adverse events over months. Dose optimisation should seek the minimum degree of sustained suppression required to control toxic accumulation rather than assume that near-complete GFAP elimination is always desirable.

A sixth priority is to make outcome measurement developmentally intelligent. Conventional neurological scales were not designed for a disorder in which an infant may be acquiring head control while an adolescent is losing gait endurance or swallowing efficiency. Future studies should establish age-banded core outcome sets that combine directly observed function with caregiver-reported abilities and objective measures such as instrumented gait, swallow studies or respiratory assessments where relevant. Repeated measures should be interpreted against expected developmental acquisition as well as decline: failure to gain an age-appropriate skill can be a clinically meaningful negative trajectory even when an absolute score appears stable. This is especially important for early treatment, where preserved development may be the principal benefit.

A seventh priority is equitable ascertainment and access. The published AxD literature is dominated by specialist centres with molecular diagnostics, advanced MRI and capacity for repeated intrathecal treatment. Children in settings with limited genetic testing may be diagnosed late or not at all, which can distort estimates of phenotype frequency and exclude them from natural-history cohorts. International networks should therefore support confirmatory GFAP testing, harmonised MRI acquisition where feasible, remote collection of selected patient-reported outcomes and referral pathways for trial participation. Broader ascertainment would improve both external validity and fairness without lowering diagnostic standards.

Longer-term research may explore durable gene silencing or editing, but such approaches should follow, rather than precede, clearer definition of the therapeutic window and safe level of GFAP reduction from titratable ASO experience. For an irreversible intervention, the evidentiary standard must include robust astrocyte targeting, control of expression across relevant CNS regions, low off-target editing, and a credible rescue strategy if unanticipated developmental effects occur. The near-term opportunity is therefore to use the current antisense programme as both therapy development and a human experiment that tests the central disease model.

10. Conclusions

Pediatric Alexander disease is a genetically defined astrocytopathy with a continuous clinical spectrum ranging from rapidly progressive cerebral disease in infancy to mixed or bulbospinal phenotypes in later childhood and adolescence. GFAP genotype contributes meaningfully to age at onset and prognosis for several recurrent variants, but it does not determine outcome in isolation. The most defensible mechanistic model places toxic accumulation of mutant and total GFAP upstream of disrupted intermediate-filament organisation, proteostatic stress, maladaptive astrocyte reactivity, inflammatory amplification and failure of astrocytic support for neurons and oligodendrocytes. This hierarchy explains both the white-matter phenotype and the broader neurological manifestations of the disorder. Direct GFAP transcript reduction has the strongest disease-modifying rationale and has progressed from robust preclinical reversal of pathology to late-stage clinical development. The major challenge is now evidentiary rather than conceptual: paediatric trials must show that molecular target engagement translates into preservation of meaningful function across highly heterogeneous developmental stages. Genotype-aware stratification, longitudinal natural history, treatment-independent biomarkers and long-term developmental safety assessment will determine whether GFAP lowering becomes not merely a successful molecular intervention but a durable alteration of childhood disease trajectory.

11. Limitations

This critical narrative review is intrinsically vulnerable to selection and interpretive bias despite the use of a transparent search, source-verification and evidence-appraisal process. The literature on Alexander disease is small and heterogeneous, and many mechanistic conclusions depend on animal models, patient-derived cell systems or limited human tissue rather than direct paediatric *in vivo* experimentation. Human genotype-phenotype studies combine cases accrued across different diagnostic eras and referral settings, creating potential ascertainment and publication bias. Natural-history data remain geographically concentrated and are not yet uniformly replicated across independent international cohorts. Outcome definitions, MRI methods and follow-up intervals vary, limiting direct comparison and precluding quantitative meta-analysis across the full clinical spectrum. Biomarker studies are relatively recent and include modest sample sizes, while the therapeutic field is developing rapidly; consequently, clinical-trial evidence available by the search end date was less mature than the preclinical evidence for GFAP lowering. English-language accessibility also shaped detailed appraisal. These limitations mean that variant-level prognosis, biomarker surrogacy and long-term treatment benefit should be regarded as evolving rather than settled.

Consent

It is not applicable.

Ethical Approval

It is not applicable.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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