REVIEW

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CHLORIDE CHANNELS IN AMYOTROPHIC LATERAL SCLEROSIS

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Abstract. *In amyotrophic lateral sclerosis (ALS), human postmortem transcriptomics reveals a consistent shift, with downregulation of neuronal genes and upregulation of glial and inflammatory programs. This establishes a framework where inhibitory synapses and ion transport, particularly chloride channels and transporters that set the neuronal chloride gradient, are mechanistically relevant to motor system vulnerability. This review synthesizes genetic and functional evidence (2010–2025) for the involvement of chloride channels and related transporters in ALS. The evidence converges on several key mechanisms. In SOD1-ALS mouse models, there is an early, cell-type-specific deficit in glycinergic inhibition linked to reduced GLRA1 expression and synaptic loss. In skeletal muscle, ClC-1 (CLCN1) expression and chloride conductance are reduced, a deficit rescued ex vivo by PKC inhibition. In immune cells, CLIC1 is identified as a potential biomarker in peripheral blood mononuclear cells and as an inducible effector of neuroinflammation in microglia. At the synapse, TMEM16F modulates α-motoneuron excitability at C-boutons, and its loss has sex-specific protective effects in SOD1-ALS mice. The ER-resident anion channel CLCC1 is now implicated through both rare genetic variants that impair its function and motor neuron-specific knockout models that trigger ER stress and neurodegeneration. Finally, downregulation of the KCC2 co-transporter in vulnerable motoneurons provides a mechanism for a depolarizing shift in the chloride reversal potential, impairing fast synaptic inhibition. Although coding mutations in chloride channel genes are rare, dysregulation of their expression and function converges on core ALS pathomechanisms, including excitotoxicity, neuroinflammation, and ER stress. Future research requires targeted human studies that couple genetic findings with functional readouts to clarify their therapeutic potential.*

Key words: *amyotrophic lateral sclerosis, chloride channels, glycine receptors, GLRA1, CLCN1, CLIC1, TMEM16F, CLCC1, KCC2, neuroinflammation*

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disorder in which genetic risk, RNA/protein homeostatic failure, glutamate-mediated excitotoxicity, mitochondrial and axonal transport stress, and neuroinflammation converge to produce progressive upper- and lower-motor-neuron degeneration [1]. Because the polarity and strength of fast synaptic inhibition depend on the neuronal chloride gradient set by *KCC2* and *NKCC1*, disturbances of chloride channels and transporters can convert normally hyperpolarizing GABA/glycine currents towards less effective or even depolarizing actions, thereby leading to amplified network hyperexcitability and rendering motoneurons more susceptible to glutamatergic injury [2, 3]. Human postmortem transcriptomics from sporadic ALS (sALS) shows a consistent polarity: neuronal genes are preferentially downregulated and glial and inflammatory programs upregulated in ventral horns, while microarray profiling in the same disease context reports hundreds to thousands of differentially expressed genes with region-specific patterns. This establishes a disease backdrop in which inhibitory synapses and ion transport – including chloride channels and neuronal chloride co-transporters that set E_GABA/E_Gly – are mechanistically relevant to motor-system vulnerability [4, 5]. Against this multifactorial backdrop, human postmortem transcriptomics consistently show downregulation of neuronal programs with reciprocal upregulation of glial/inflammatory pathways in ALS spinal cord, providing the disease context in which inhibitory synapses and chloride handling merit focused scrutiny [4]. For clarity on cohorts, the ventral-horn RNA-seq study by D'Erchia et al. and the anterior-horn/frontal-cortex microarray by Andrés-Benito et al. analyzed independent donor sets; "paired regions" in the latter refer to the same donors within that microarray study, not to the D'Erchia cohort [4, 5]. Because the strength and polarity of fast inhibitory transmission in spinal motor circuits depend on postsynaptic GlyR/GABA_A receptors and presynaptic inputs but are ultimately constrained by intracellular chloride set by *KCC2*/*NKCC1*, we consider both channel and transporter mechanisms alongside ALS-specific evidence [6]. In brief, human spinal cord RNA-seq shows reproducible neuron-down/glia-up polarity, while most chloride channel and co-transporter claims still rest on *SOD1* mice or *in vitro* systems, as discussed below [6-8].

We focus on chloride channels and co-transporters (Figure 1) because convergent human postmortem transcriptomics demonstrate a neuron-down/glia-up polarity with suppression of inhibitory programs, and complementary work shows early degeneration of inhibitory interneurons and pervasive cortical hyperexcitability that together implicate impaired chloride-dependent inhibition in ALS pathophysiology [9, 10]. In line with current understanding, we frame chloride/ polarity imbalance as a disease-amplifying mechanism rather than a universal first cause, while highlighting where human-anchored data are strongest and where evidence remains preclinical.

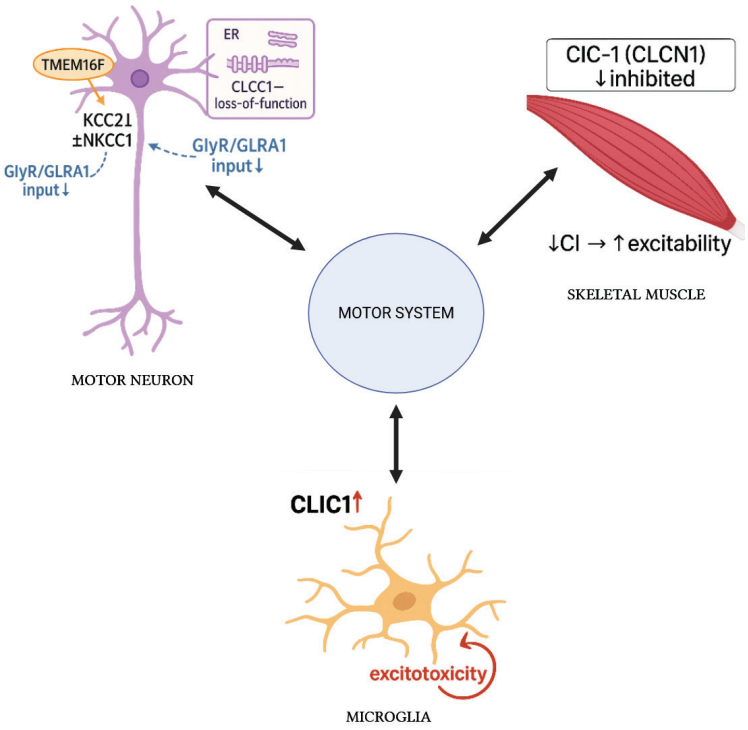

Fig. 1. Schematic overview of chloride-pathway disturbances across motoneurons, skeletal muscle and glial compartments in ALS

NEURONAL COMPARTMENT: CHLORIDE CHANNELS, CO-TRANSPORTERS AND INHIBITORY SYNAPSES

GLRA1 (Glycine Receptor Alpha-1) and Glycinergic Inhibition

Electrophysiology in embryonic spinal motoneurons from *SOD1* G93A mice showed smaller glycine-evoked current densities and reduced glycinergic miniature IPSC amplitudes versus nontransgenic controls, with GABAergic currents unchanged under the same conditions. Single-cell RT-PCR detected reduced GlyRα1 (GLRA1) mRNA and immunocytochemistry showed decreased surface postsynaptic GlyR. Independent anatomy in the same model revealed presymptomatic loss of glycinergic boutons onto motoneurons, with relative

sparing of GABAergic innervation; subtype analyses indicated early effects on large α-motoneurons [11]. These findings document an early, cell-type-biased deficit in glycinergic inhibition at the level of receptor abundance and synaptic input in *SOD1* ALS mice; direct human motoneuron data for GLRA1 remain limited to this date. Consistent with network-level remodelling beyond receptor abundance, in vivo circuit mapping in *SOD1* mice shows early loss of V1 inhibitory interneuron connectivity specifically onto fast α-motoneurons, dovetailing with the GLRA1-centered selective vulnerability described here [12].

Independent anatomical quantification in the same ALS model showed deficient glycinergic innervation of spinal motoneurons at presymptomatic stages, a structural synaptic change preceding motoneuron degeneration. This presymptomatic loss was specific to glycinergic inputs, with GABAergic innervation comparatively spared in that dataset [13]. Subtype analyses further indicated that the glycinergic deficit affects large α-motoneurons early in disease, whereas medium-sized motoneurons did not show altered glycinergic currents in the same embryonic G93A-*SOD1* model, implying a cell-type specificity within the motoneuron pool [14].

Consistent with broader inhibitory-circuit alterations in ALS, independent work reports locomotor deficits in *SOD1* G93A mice paralleled by preferential glycinergic–rather than GABAergic–synaptic loss, and human postmortem spinal-cord studies identify inhibitory-pathway transcript perturbations within the broader neuron-down/glia-up polarity, with subunit-level directionality varying across cohorts [4, 5, 15].

Neuronal Chloride Cotransporters (KCC2/NKCC1) and Inhibitory Reversal Potential

Because inhibitory strength hinges on intracellular (Cl –), neuronal chloride co-transporters are upstream determinants of GlyR/GABA_A receptor efficacy. In ALS-vulnerable lumbar and hypoglossal motoneurons of *SOD1* G93A mice, *KCC2* mRNA and membrane-bound protein decline from late presymptomatic stages onward, while *NKCC1* mRNA remains unchanged; electrophysiology in *SOD1* motoneurons shows depolarized E_GABA/Gly accompanied by slower inhibitory current decay consistent with compensation under reduced *KCC2* function [6]. Emerging, but still limited, translational work suggests *KCC2* loss may feature in human ALS tissues, yet peer-reviewed confirmation remains sparse and variable across regions and cohorts [10]. Human confirmation remains limited: a 2023 preprint reports reduced *KCC2* membrane levels in postmortem motor cortex in ALS, whereas definitive peer-reviewed confirmation in human spinal ventral horn is still lacking; we therefore label cortical *KCC2* data as preprint-level and keep spinal conclusions restricted to animal work [16].

TMEM16F (ANO6) at Motoneuron C-Boutons and ALS

TMEM16F was localized to postsynaptic clusters facing presynaptic cholinergic C-boutons on α-motoneurons in the mouse spinal cord, and recordings showed that TMEM16F underlies a Ca^{2+}-activated chloride conductance in these neurons [7]. Genetic disruption of Tmem16f increased the recruitment threshold of fast α-motoneurons and decreased performance specifically during highly demanding motor tasks, demonstrating a role for this channel in modulating motoneuron excitability during behavior.

In an ALS context, loss of TMEM16F function in *SOD1* G93A mice significantly reduced expression of an activity-dependent early stress marker, reduced muscle denervation, delayed disease onset, and preserved muscular strength – but only in male ALS mice – indicating sex-specific effects of TMEM16F modulation on ALS progression metrics in that model [7]. Human postmortem evidence on C-bouton number or morphology is limited: a small late-1990s series reported fewer cholinergic synapses on ALS motoneurons, while rodent studies variably report enlargement, stability, or loss depending on stage and sex, consistent with both compensatory and pathogenic interpretations [17, 18]. Accordingly, we interpret C-bouton changes as potentially stage-, sex-, and model-dependent rather than uniformly pathogenic in human ALS, with definitive human postmortem quantitation still limited to small cohorts such as the ones mentioned above.

ANO1 (TMEM16A) Signalling in ALS Motor Neurons

In Wang et al., the ANO1–EGFR–CaMKII axis was demonstrated in vitro in motor-neuron models rather than in vivo ALS, and the experiments do not establish adult human spinal motor-neuron expression of ANO1. In an adult single-nucleus atlas with spatial validation, TMEM16A/ANO1 is not highlighted in cholinergic motor-neuron clusters, and a developing-human single-cell atlas characterizes embryonic/fetal – rather than adult – spinal expression dynamics [8,19]. Accordingly, the ANO1–EGFR connection should be framed as biological plausibility rather than a transferable mechanism for human ALS until animal or human validation emerges, despite established EGFR–ANO1 complexing in other mammalian cell systems [20] .

Outside ALS, but relevant to the mechanism, ANO1 has been shown to form functional complexes with EGFR and to regulate EGFR stability and signalling in human squamous cancer cells, lending independent evidence that ANO1 can interface with EGFR signalling modules in mammalian cells. This supports, without broadening, the plausibility of the EGFR–ANO1 axis reported in ALS cellular models [21].

For ANO1, ALS motor neuron models show increased survival and reduced apoptosis via EGFR and CaMKII activation upon ANO1 action, while non-ALS cell systems independently support a physical and functional interaction between ANO1 and EGFR, corroborating the mechanistic axis described in ALS cellular data [8]. Accordingly, in Table 1, we label ANO1 as "Cellular models only" to avoid over-signalling clinical translatability until adult human or in-vivo ALS confirmation emerges.

CLCC1 as an ER Anion Channel Implicated in ALS

CLCC1 is now established as a pore-forming ER anion channel: purified CLCC1 reconstituted into lipid bilayers forms an anion conductance; ALS-linked variants reduce channel currents; and motor-neuron–specific CLCC1 deletion in mice induces ER stress and progressive neurodegeneration, establishing that normal CLCC1 function is required for motor neuron survival. In a Central South China cohort, 4 heterozygous missense variants among 1,005 ALS patients [0.40%] were identified (Q92P, R380K, T415M, R443Q), each predicted to be damaging and novel in ALS at the time of report [22].

Additional work referencing the motor neuron conditional knockout corroborated that loss of CLCC1 in motor neurons triggers ER stress and neurodegeneration, reinforcing the ER stress linkage to CLCC1 insufficiency in neuronal tissue, though the cited context was outside ALS models per se [23]. For CLCC1, ER channel function is established with ALS-linked variants impairing conductance, and motor neuron–selective CLCC1 loss produces ER stress and neurodegeneration; an independent patient cohort reports CLCC1 variants that reduce channel currents, together providing genetic and functional evidence for CLCC1 involvement in ALS. Independent loss-of-function studies show that CLCC1 deficiency triggers ER-stress-linked neurodegeneration in mice [24], and recent work extends CLCC1 biology to ER lipid flux and nuclear pore complex assembly, with ALS-linked variants such as S263R and W267R exhibited ~40–50% lower slope conductance than human WT CLCC1 under matched conditions, with unchanged open probability, directly indicating channel hypofunction [25].

GABAergic Transcripts [GAD2, GABRE] in Human ALS Spinal Tissue

Bioinformatic analysis comparing gene expression in spinal versus oculomotor tissues from control individuals and sALS patients found that GAD2 and GABRE were downregulated in spinal tissues of ALS patients, whereas their endogenous levels were higher in oculomotor tissues relative to spinal tissues. The authors interpreted this pattern as aligning with the vulnerability of spinal motor systems and the relative resilience of oculomotor neurons, within the limits of their dataset [15]. The PubMed summary for the same study explicitly states the downregulation of GAD2 and GABRE in ALS spinal tissue and their relatively higher endogenous levels in oculomotor tissues, restating the core observation without adding claims beyond the original analysis. For GABA-pathway transcripts in human ALS, spinal tissue analyses report downregulation of GAD2 and GABRE in sALS, contrasting with higher expression in oculomotor tissue, aligning with differential vulnerability profiles documented across human motor systems. Across larger human ventral-horn RNA-seq datasets the inhibitory-pathway changes sit within the overarching neuron-down/glia-up polarity, and current cohort-level resources have not linked these inhibitory transcript shifts to clinical phenotypes such as spasticity or site of onset [26].

SKELETAL MUSCLE COMPARTMENT: CIC-1/CLCN1 AND ALTERED G_CL

CLCN1 (ClC-1) and Skeletal Muscle Chloride Conductance

The central role of ClC-1 in setting human skeletal-muscle g_Cl is well established, and emerging clinical pharmacology that targets ClC-1 for neuromuscular transmission diseases underscores the translational interest in modulating this conductance [27]. In skeletal muscle-restricted *SOD1* models, ClC-1 protein expression and resting chloride conductance (g_Cl) are significantly reduced; the reduced g_Cl is restored ex vivo by the PKC inhibitor chelerythrine and augmented by acetazolamide. These data implicate PKC-dependent suppression of ClC-1 activity in the ALS muscle phenotype. Mechanistic biophysics outside ALS shows acetazolamide directly shifts ClC-1 gating via intracellular acidification, aligning with the ex vivo conductance increases, though clinical ALS data are lacking and all drug effects examined are ex vivo [28]. To date, neither acetazolamide nor PKC inhibitors such as chelerythrine have been evaluated in randomized or observational ALS patient studies,

and any future trial would require routine acid–base, electrolyte, renal, and ventilatory monitoring, as carbonic anhydrase inhibitors are associated with dose-dependent risks of paresthesias, dysgeusia, polyuria, and fatigue in randomized trials [29]. Reviews of ALS clinical trials likewise do not list ClC-1 modulators among tested agents, underscoring the preclinical status of this approach [30]. As of August 10, 2025, we did not identify any registered ALS interventional trials testing acetazolamide or chelerythrine on ClinicalTrials.gov or the EU CTR, consistent with their current preclinical status in ALS.

Separate biophysical work in human skeletal muscle demonstrated that acetazolamide can directly influence the voltage-dependent gating of ClC-1 through intracellular acidification linked to carbonic anhydrase inhibition, mechanistically supporting the observed increase in chloride conductance upon acetazolamide exposure, although that particular study did not address ALS tissue [31]. Clinicians should also note that carbonic anhydrase inhibitors can precipitate metabolic acidosis and exacerbate CO_2 retention in hypercapnic respiratory failure, and they carry dose-dependent risks such as electrolyte disturbances and nephrolithiasis, caveats that are particularly relevant in ALS with ventilatory compromise [29]. A focused review of therapeutic targets in ALS that included experimental data from the same muscle-restricted *SOD1* models summarized the reduction of ClC-1 expression and the restoration of reduced g_Cl by chelerythrine in MLC/*SOD1* G93A muscles, reinforcing the PKC–ClC-1 inhibitory axis as a modifiable contributor to muscle hyperexcitability in ALS contexts [30].

GLIAL AND IMMUNE COMPARTMENT

CLIC1 (Chloride Intracellular Channel-1) in Immune Cells and Neuroinflammation Contexts

Proteomic discovery and validation in peripheral blood mononuclear cells (PBMCs) identified *CLIC1* among a small panel of proteins (including CALR, IRAK4, GSTO1, and CypA) that were significantly associated with ALS in univariate logistic analysis; the study verified specificity against non-ALS neurological controls and reported *CLIC1* as part of an ALS-associated multiprotein biomarker signature [32]. However, sample sizes were modest and multi-site, and prospective replication has not been shown. Outside ALS, inflammatory stimuli such as Aβ recruit *CLIC1* to the microglial membrane and unmask a *CLIC1*-mediated chloride conductance coupled to reactive species and cytokine production, directly documenting inducible *CLIC1* biology in neuroinflammation [32]. No independent ALS cohort has yet prospectively reproduced the PBMC *CLIC1* signal reported by Nardo et al., and contemporary blood-biomarker reviews for ALS continue to prioritize neurofilament assays over *CLIC1* [33].

In microglia, Aβ exposure upregulated *CLIC1* protein and unmasked a *CLIC1*-mediated chloride conductance at the plasma membrane, responses that were barely detectable in quiescent cells before stimulation; induction of *CLIC1* coincided with increased production of reactive nitrogen intermediates and TNF-α, demonstrating stimulus-dependent *CLIC1* expression and function in innate immune cells. Although this work was done in Aβ-stimulated microglia rather than ALS tissue, it directly documents *CLIC1* membrane recruitment and conductance under inflammatory stressors relevant to neurodegeneration [34].

Complementing these findings, a separate study showed acute translocation of *CLIC1* from cytosol to the microglial plasma membrane in response to Aβ in vitro and in vivo, and linked *CLIC1* function to microglia-mediated neurotoxicity in Alzheimer-related paradigms, expanding direct evidence that *CLIC1* is an inducible microglial effector channel under neuroinflammatory challenge [35].

KCNN4 (KCA3.1/IK) AS A CALCIUM-ACTIVATED POTASSIUM CHANNEL IN ALS-RELEVANT CELLS

Microglia isolated from *SOD1* G93A mice exhibited increased KCNN4 expression, reported as a 7.54-fold rise compared with age-matched wild-type, and pharmacological inhibition of KCa3.1 with TRAM-34 reduced microglial activation markers and modulated disease-relevant microglial functions in that ALS model, providing direct evidence that KCNN4 is upregulated in ALS microglia and is pharmacologically targetable in vivo [36].

Hyperexcitability of motoneurons has been independently observed in ALS patients and in iPSC-derived motoneurons from familial ALS genotypes, supporting the relevance of channels that shape afterhyperpolarization and excitability; while these studies did not isolate KCNN4 specifically in motoneurons, they document the excitability phenotype to which calcium-activated K + channels, including KCa3.1, can theoretically contribute. The statements here are limited to what is shown in those studies [37].

OTHERS

CFTR Splicing as a Prototypical TDP-43–Sensitive Chloride Channel Transcript

TDP-43 was identified as a nuclear factor that binds UG-rich sequences in CFTR intron 8 and promotes

skipping of exon 9, a splicing event that produces a nonfunctional CFTR chloride channel isoform in cystic fibrosis contexts; antisense inhibition or RNAi-mediated depletion of TDP-43 increased exon 9 inclusion in cellular models, demonstrating a direct, bidirectional control of CFTR exon definition by TDP-43. These findings establish CFTR as a chloride channel transcript whose splicing is directly regulated by TDP-43 levels and binding to UG repeats [38, 39]. To date, no studies have reported altered CFTR splicing or expression in ALS tissues.

CONCLUSIONS

For clinical practice today, no chloride-modulating therapy is trial-ready in ALS, and we are not aware of any acetazolamide, chelerythrine, or other ClC-1/*KCC2*-targeting trials imminently enrolling; routine care remains centered on multidisciplinary management, ventilatory support, and biomarker awareness (e.g., neurofilament) rather than chloride-targeted interventions. For practice, the main human-anchored message is the robust neuron-down/glia-up polarity in ALS spinal tissue, whereas channel- or synapse-specific mechanisms (ClC-1 pharmacology, TMEM16F/C-boutons, ANO1–EGFR) remain preclinical or debated and should be framed cautiously until replicated in human material or animals with clear translational endpoints. GLRA1/GlyR-related inhibitory deficits are well supported in embryonic and early presymptomatic *SOD1* mice but are not yet proven in human motoneurons. In skeletal muscle, depressed ClC-1 expression and g_Cl with ex vivo rescue by PKC inhibition and acetazolamide are robust preclinical observations. TMEM16F at C-boutons modulates α-motoneuron excitability and male-specific disease readouts in *SOD1* mice, though the generality of C-bouton remodeling remains debated. ANO1's EGFR–CaMKII axis is currently cell-based; in vivo ALS confirmation is outstanding despite strong mechanistic support for ANO1–EGFR coupling in non-ALS systems. CLCC1 now offers convergent genetics-to-mechanism evidence, from pore-forming ER anion channel biophysics to ALS-linked hypofunction and motor-neuron conditional loss phenotypes. Human transcriptomics consistently indicates neuronal-to-glial polarity with inhibitory-pathway perturbations, and animal studies provide a coherent chloride-homeostasis framework via *KCC2* decline and depolarized E_GABA/Gly. A practical 'watch-list' for the next 2–3 years is therefore limited to human confirmation of *KCC2* alterations in defined regions with cell-composition control, replication of CLCC1 genetics in multi-ancestry cohorts coupled to standardized channel biophysics and carefully monitored first-

Table 1. Summary of current knowledge and evidence levels on the involvement of chloride channels in ALS pathology

Mechanism/Target	Human postmortem	In vivo animal	Cellular	Reference
Human ventral-horn transcriptomic polarity (neuron↓/glia↑)	Yes	No	No	(4)
GLRA1/GlyRα1 & glycinergic inhibition in MNs	No (direct MN data lacking)	Yes	Yes	(11)
Skeletal muscle ClC1 (gCl) reduction; PKC-dependent suppression	No (ALS muscle not established)	Yes	Yes (ex vivo muscle; human biophysics)	(27)
TMEM16F/ANO6 at Cboutons (fast αMN excitability; sex – specific effects)	No (C – bouton human evidence limited)	Yes	Yes	(35)
ANO1/TMEM16A → EGFR/CaMKII survival axis (ALS cellular models)	No	No (ALS)	Yes	(19)
KCC2/NKCC1 and depolarized E_GABA/Gly in vulnerable MNs	Emerging (cortex preprint; spinal not definitive)	Yes	Yes	(6)
CLCC1 (ER anion channel) – ALS – linked variants; MN – conditional loss → ER stress	No (genetic association; not PM)	Yes	Yes	(23)
CLIC1 in PBMCs/microglial inducible conductance	No (human blood biomarker)	No (ALS)	Yes	(31)
GABApathway transcripts (GAD2, GABRE) in human ALS spinal tissue	Yes	No (primary evidence human)	No	(38)
CFTR exon9 splicing as a TDP – 43–sensitive chloridechannel transcript	No (not studied in ALS tissue)	No	Yes	(39)
KCNN4/KCa3.1 upregulation in ALS microglia (targetable with TRAM – 34)	No (ALS PM not primary)	Yes	Yes	(37)

'Yes' under Human postmortem denotes direct analyses of human CNS tissue; genetic association or blood-based biomarker studies are not counted as 'Human postmortem'."

in-human studies only after robust preclinical packages emerge.

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