BMC Genomics

RESEARCH Open Access

Circular RNA expression in ALS is progressively deregulated and tissue-dependent

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Abstract

Background There is increasing evidence on the role of circular RNAs (circRNAs) in neuronal and muscular processes. Accordingly, their dysregulation is associated with neurodegenerative diseases and myopathies. We investigated circRNA expression in the central nervous system (CNS) and skeletal muscle, the two main tissues affected in amyotrophic lateral sclerosis (ALS).

Results Based on circRNA sequencing analysis in spinal cord from ALS mice (SOD1G93A) followed by a literature search, 30 circRNAs potentially involved in ALS were tested. All selected circRNAs were downregulated in the SOD1G93A spinal cord, whereas only half of these were quantifiable and were generally upregulated in quadriceps muscle of SOD1G93A mice. Such tissue-dependent expression pattern was observed in both sexes and circRNA abundance in the spinal cord was higher than in the muscle, both in wild type and in SOD1G93A mice. Finally, we assessed the 18 circRNAs with the largest expression differences and the highest degree of interspecies conservation in brain samples from sporadic ALS (sALS) patients and healthy controls. Similar to the mouse model, circRNA levels tended to decrease in the CNS of sALS patients.

Conclusions Expression of circRNAs may be systematically altered in the two tissues most affected by ALS in a progressive and opposed manner. Although more detailed studies are warranted, circRNAs are potentially related to ALS etiopathogenesis and could possibly serve as future biomarkers, therapeutic targets, or customized therapeutic tools to modulate the pathology.

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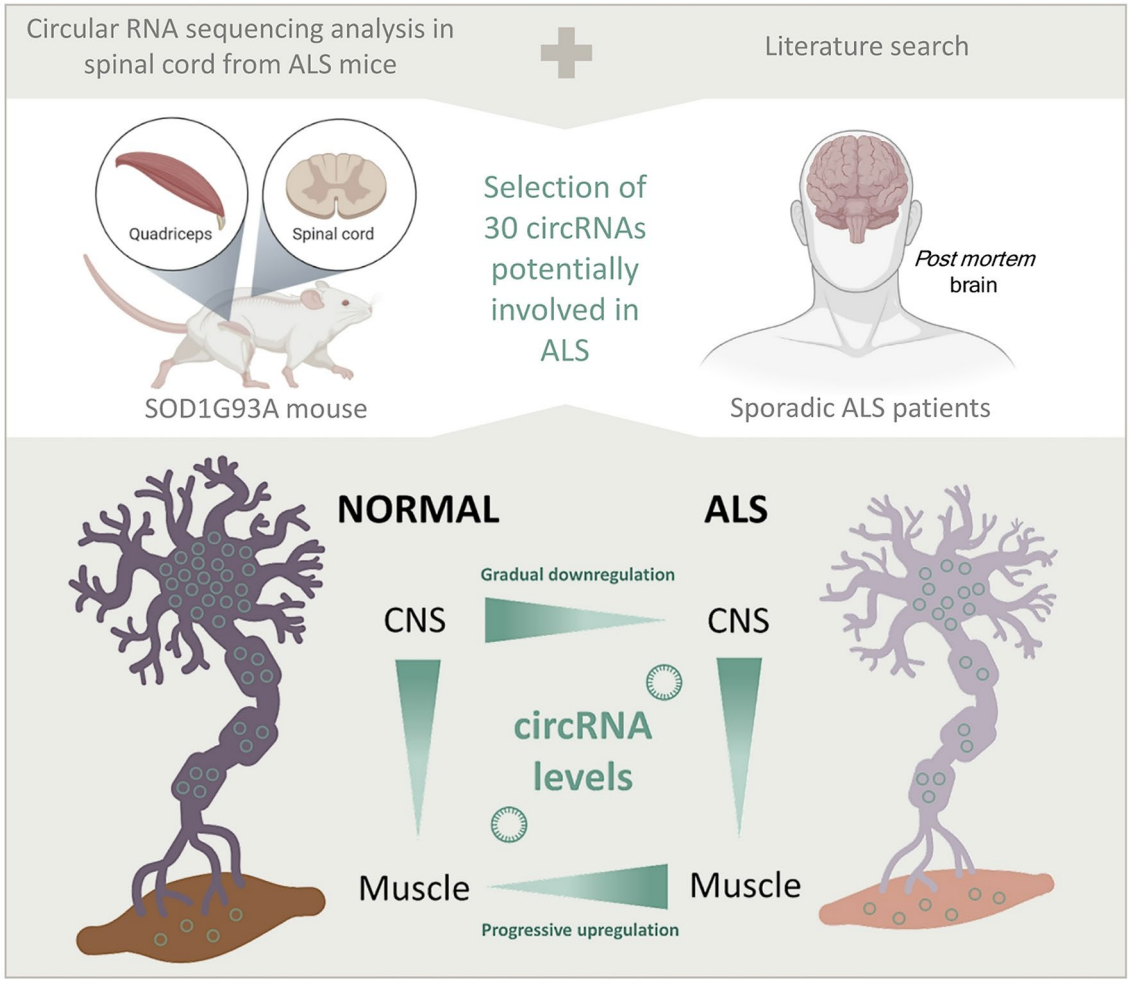
Graphical Abstract



Keywords Non-coding RNA, Circular RNA, Amyotrophic lateral sclerosis, SOD1G93A mice, Neurodegeneration, Central nervous system, Skeletal muscle

Introduction

Splicing of a pre-mRNA transcript can produce linear, protein coding messengers as well as covalently closed single-stranded RNA molecules called circular RNAs (circRNA) that belong to the category of non-coding RNAs (ncRNAs) [1]. The biogenesis of circRNAs normally involves head-to-tail backsplicing, an alternative mRNA splicing process that joins downstream 5' splice donor site of an exon with the 3' upstream splice acceptor site [2, 3]. Such direct backsplicing is mediated by the binding of inverted repeat elements (Alu sequences) or RNA-binding proteins (RBPs) in the flanking regions of circRNAs [2, 3]. These flanking sequences may be modified by adenosine deaminases acting on RNA (ADAR) enzymes, which can alter the stability of RNA secondary structures and, thus, circRNA expression [4]. circRNAs may also be formed by lariat intermediates formed from the exons and/or introns removed during pre- mRNA processing (indirect backsplicing) [2]. Normally such intronic sequences are degraded by RNA lariat debranching enzyme (DBR1), but occasionally they manage to escape degradation giving rise to circRNAs composed uniquely of introns (ciRNAs) [3, 5]. circRNAs may regulate their parental gene expression directly through their interaction with RNA polymerase II or through competition with full-size mRNA production. Additionally, they may cause more inconspicuous pleiotropic effects by sponging microRNAs (miRNA) or RBPs, by regulating splicing and by promoting epigenetic alterations [6]. Despite originally being classified as non-coding RNAs, some circRNAs, such as circZNF609 and circFBXW7, have been shown to be translated into proteins [6,

7]. Although specific functions of some circRNAs have been characterized, a large majority are still poorly understood [8].

circRNAs are unusually stable due to the lack of 5' and 3' ends, often expressed in a tissue- or developmental stage-specific manner, many are evolutionarily conserved, and accumulate in relatively high levels in neuronal and muscle tissues [8, 9]. Indeed, circRNAs have been proposed to play important roles in the development and function of neurons and synapses [10], as well as in skeletal muscle myogenesis [8]. In view of this, it is perhaps not surprising that circRNAs have been found to be dysregulated in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [11], and in myopathies like Duchenne muscular dystrophy (DMD) [12] and myotonic dystrophy type 1 (DM1) [13, 14].

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neuromuscular disorder that has no cure or effective treatment. The primary consequences of ALS are detachment of motoneurons from skeletal muscle which results in muscle atrophy and motor neuron death. Most cases of ALS are sporadic (sALS) and 10% have a family history (fALS) [15]. Around 11% of sALS cases and two-thirds of fALS cases have a genetic etiology [16]. The four most well-established causative genes are *SOD1* (Cu-Zn superoxide dismutase 1), *TARDBP* (transactive response DNA binding protein 43 kDa, TDP-43), *FUS* (fused in sarcoma) and hexanucleotide expansion repeat in *C9ORF72* (Chromosome 9 Open Reading Frame 72) [15, 16]. The exact causes of ALS are largely unknown, but many of the major ALS genes play a critical role in RNA processing, with dysregulated RNA metabolism being a key factor in the pathogenesis [15]. Although very little is known on circRNAs in ALS, RBPs TDP-43 and FUS have been shown, among their other functions, to be involved in the biogenesis of circRNAs [17–19]. To date, the potential of circRNAs as ALS biomarkers has been studied in leukocytes [20] where microarray-based approach combined with quantitative PCR (RT-qPCR) verification identified three circRNAs that could potentially serve as ALS biomarkers. Additionally, recent study based on RNA sequencing of muscle biopsies [21] indicated altered abundance of several circRNAs in ALS patients, some of which were confirmed by RT-qPCR and found to be also altered in motor neurons derived from human induced pluripotent stem cells (iPSCs) harboring ALS mutations and in skeletal muscle ALS mice expressing mutated human SOD1. Interestingly, ADAR2 levels are decreased in ALS and it has been shown that a circRNA lacking typical ADAR2-dependent editing may potentially be detected as ALS-dependent signal in body fluids [22]. It is also possible that some circRNAs could be directly involved in the pathology of ALS, as the case of circ-Hdgfrp3, which is sequestered within FUS-aggregates [23], and a ciRNA derived from *C9ORF72* that serves as the translation template of dipeptide repeat proteins (DPR) [24]. In addition, a possible treatment for ALS has been proposed based on inhibition of RNA lariat debranching enzyme DBR1, which results in an increase of ciRNA levels in motor neurons and decreases TDP-43 toxicity by titration [25].

Here, we investigated the expression levels of selected circRNAs in the most affected tissues in ALS, central nervous system and skeletal muscle, in ALS mouse model expressing mutated human SOD1 (SOD1G93A) and in sporadic ALS patients. Our findings show that levels of circRNAs in ALS are altered in a progressive, tissue-dependent and sex-independent manner.

Results

Selection of circRNAs potentially involved in ALS

To identify circRNAs that may be implicated in ALS pathogenesis, two independent studies were performed: (A) circRNA sequencing (circRNA-seq) analysis in the spinal cord of 50-day-old SOD1G93A and wild type (WT) mice, and (B) an extensive literature search.

Based on the results of the circRNA-seq analysis, 12 circRNAs were selected considering: (1) the largest differences between WT and SOD1G93A mice, (2) function of the host gene, (3) potential involvement in ALS and (4) homologous circRNA present in human. From the literature search, 18 circRNAs were selected. The selection criteria were: (1) circRNAs involved in pathogenic mechanisms altered in ALS (e.g., neuroinflammation, myogenesis, oxidative stress), (2) circRNAs whose host gene may be implicated in ALS, (3) circRNAs highly conserved between species and (4) detection frequency in body fluids.

A summary of the 30 circRNAs selected (alias, circBase identifier, host gene and reason for study) is shown in Table 1.

circRNAs are downregulated in spinal cord from SOD1G93A mice

In SOD1G93A mice, males show accelerated progression of the disease symptoms compared with females. First, the selected circRNAs were analyzed in the spinal cord of WT and SOD1G93A males throughout the course of disease. Specifically, four stages were studied: early presymptomatic (P50), late presymptomatic (P75), symptomatic (P105), and terminal stage (P120).

The levels of all circRNAs studied were significantly altered in one or more stages compared with control mice (Fig. 1). Interestingly, an obvious trend was observed toward a gradual decrease of circRNA levels in spinal cord of SOD1G93A mice, which generally reached the largest fold change at the symptomatic and terminal

Table 1 Characteristics of the 30 circRNAs selected for study in the present work

	Alias	circBase ID	Host gene	Reason for study
Circ-RNA se-quenc-ing analysis	circSOX5	mmu_circ_0013172 hsa_circ_0098181	*SOX5 - SRY-Box Transcription Factor 5*	- Host gene important for neuronal function, with variants observed in AD and ALS patients [26]
	circTTBK2	mmu_circ_0001063 hsa_circ_0034880	*TTBK2 - Tau Tubulin Kinase 2*	- circRNA involved in brain development [27] - TTBK2 proposed as a potential ALS therapeutic target [28]
	circMGA	mmu_circ_0009233 hsa_circ_0000591	*MGA - MAX Dimerization Protein MGA*	- circRNA involved in brain development [27]
	circGRIP1	mmu_circ_0000216 hsa_circ_0027450	*GRIP1 - Glutamate receptor interacting protein 1*	- Host gene involved in glutamate uptake, which is altered in ALS [29] - GRIP1 dysregulation in ALS2 deficient spinal motor neurons leading to neuronal degeneration [30]
	circGRID1	mmu_circ_0000523 hsa_circ_0094310	*GRID1 - Glutamate Ionotropic Receptor Delta Type Subunit 1*	- Host gene involved in glutamate uptake, which is deregulated in ALS [29]
	circEgfem1 circEGFEM1P	mmu_circ_0001116 hsa_circ_0122641	*Egfem1/EGFEM1P - EGF Like and EMI Domain Containing 1, Pseudogene*	- Host gene predicted to enable calcium ion binding activity, with Ca^{2+} being deregulated in ALS [31]
	circVPS13D	mmu_circ_0011390 hsa_circ_0110653	*VPS13D - Vacuolar Protein Sorting 13 Homolog D*	- Host gene involved in regulation of peroxisomal biogenesis; this organelle being impairment in ALS [32]
	circUST	mmu_circ_0002470 hsa_circ_0130993	*UST - Uronyl 2-Sulfotransferase*	- Host gene involved in regulation of axonogenesis and sulfotransferase activity, with sulphates being implicated in ALS [33–35]
	circSpg11	mmu_circ_0009284	*SPG11 - Vesicle Trafficking Associated, Spatacsin*	- Host gene involved in nervous system development - Host gene variants identified in juvenile ALS and sporadic ALS [36]
	circAnks1b	mmu_circ_0000200	*Anks1b - Ankyrin Repeat And Sterile Alpha Motif Domain Containing 1B*	- Host gene has been associated with the age of onset of ALS and encodes a protein involved in neuronal biology and activity, specifically glutamatergic neurotransmission [37]
	circCog7	mmu_circ_0013834	*Cog7- Component of oligomeric Golgi complex 7*	- Host gene required for normal Golgi function; this organelle being fragmented in ALS [38]
	circPcdh15	mmu_circ_0002398	*Pcdh15 - Protocadherin Related 15*	- Host gene involved in calcium-dependent cell-adhesion, with Ca^{2+} being deregulated in ALS [31]

Table 1 (continued)

	Alias	circBase ID	Host gene	Reason for study
Literature search	circXPO1	mmu_circ_0000246 hsa_circ_0001017	*XPO1– Exportin 1*	- circRNA increased in DM1 [13] and regulates the expression of *RHOB*, which is altered in ALS [39–41] - Host gene identified as modifier of ALS-causal genes [42] and encodes a protein described as a therapeutic target in ALS [43]
	circMORC3	mmu_circ_0006475 hsa_circ_0001189	*MORC3– MORC Family CW-type Zinc Finger 3*	- circRNA detected in body fluids [44] - Host gene codes for an ATPase associated with inflammatory myopathies [45]
	circSPECC1	mmu_circ_0003196 hsa_circ_0000745	*SPECC1 - Sperm Antigen With Calponin Homology And Coiled-Coil*	- circRNA regulates the PI3K-AKT pathway [46,47], which has been identified in several studies as a critical mechanism in the pathogenesis of ALS [48]
	circASH1L	mmu_circ_0010877 hsa_circ_0000137	*ASH1L - ASH1 like Histone Lysine Methyltransferase*	- circRNA involved in brain development [27] - Host gene involved in myoblast fusion and neuronal morphogenesis [49,50]
	circZfp609 circZNF609	mmu_circ_0001797 hsa_circ_0000615	*Zfp609/ZNF609 - Zinc Finger Protein 609*	- circRNA involved in muscle function and deregulated in DMD [12] and DM1 [13,14]
	circANKRD12	mmu_circ_0000807 hsa_circ_0000826	*ANKRD12 - Ankyrin Repeat Domain 12*	- circRNA involved in inflammation [51] and dysregulated in PD [52] and DM1 [13] - Host gene expression and alternative splicing altered in ALS [53]
	circUBXN7	mmu_circ_0006195 hsa_circ_0001380	*UBXN7– UBX Domain Protein 7*	- circRNA involved in inflammation [54] and increased in DM1 [13] - Host gene codes for a ubiquitin protein that bounds to VCP (gene linked to ALS [55])
	circMAPK1	mmu_circ_0006095 hsa_circ_0008870	*MAPK1 - Mitogen-Activated Protein Kinase 1*	- circRNA regulates host gene expression [56] - Host gene involved in neurological processing during brain injuries and proposed as ALS treatment [57]
	circSCLT1	mmu_circ_0010671 hsa_circ_0001439	*SCLT1 - Sodium Channel and Clathrin Linker 1*	- circRNA dysregulated in blood of PD patients [52] - Host gene codes for a linker protein between clathrin and a sodium channel (both involved in ALS [58,59])
	circFBXW7	mmu_circ_0010819 hsa_circ_0001451	*FBXW7 - F-Box and WD repeat domain containing 7*	- circRNA encodes a novel protein that suppresses glioma cell proliferation and the cell cycle [60] - Host gene suppress FUS toxicity in ALS [42]
	circSamd4 circSAMD4A	mmu_circ_0000529 hsa_circ_0004846	*Samd4/SAMD4A - Sterile Alpha Motif Domain Containing 4 A*	- circRNA involved in myogenesis [8] - Host gene identified as modifier of ALS-causal genes [42]
	circSLC8A1	mmu_circ_0000823 hsa_circ_0000994	*SLC8A1 - Solute Carrier Family 8 Member A1*	- circRNA involved in PD through oxidative stress [61] - Host gene implicated in regulation of sodium ion transport, with Na^+ being deregulated in ALS [59]
	circCWC27	mmu_circ_0004423 hsa_circ_0072654	*CWC27 - Spliceosome Associated Cyclophilin*	- circRNA involved in neuroinflammation and altered in AD [62]
	circMAN1A2	mmu_circ_001029 hsa_circ_0000118	*MAN1A2 - Mannosidase Alpha Class 1 A Member 2*	- circRNA upregulated in DM1 muscle [13] - Host gene involved in N-glycan maturation (IgG N-glycan galactosylation proposed as ALS biomarker [63])
	circHIPK2	mmu_circ_0001468 hsa_circ_0001756	*HIPK2 - Homeodomain Interacting Protein Kinase 2*	- circRNA regulates astrocyte activation and myogenesis [64,65]. - HIPK2 promotes endoplasmic reticulum stress in ALS and could be a therapeutic target [57]
	circHIPK3	mmu_circ_0001052 hsa_circ_0000284	*HIPK3 - Homeodomain Interacting Protein Kinase 3*	- circRNA enhances neuroinflammation in PD [66], regulates myoblast differentiation [] and is altered in DM1 muscle [13,]
	ciRS7/ circCDR1as	mmu_circ_0001878 hsa_circ_0001946	*CDR1-AS - Cerebellar Degeneration Related 1 Antisense RNA*	- circRNA involved in brain development [27], neuroinflammation and myogenesis [8, 68] - Host gene presents copy number variations in ALS [69]
	circARF3	mmu_circ_0000650 hsa_circ_0003770	*ARF3 - ADP Ribosylation Factor 3*	- circRNA regulates inflammation through mitophagy [70], which is altered in ALS [71]

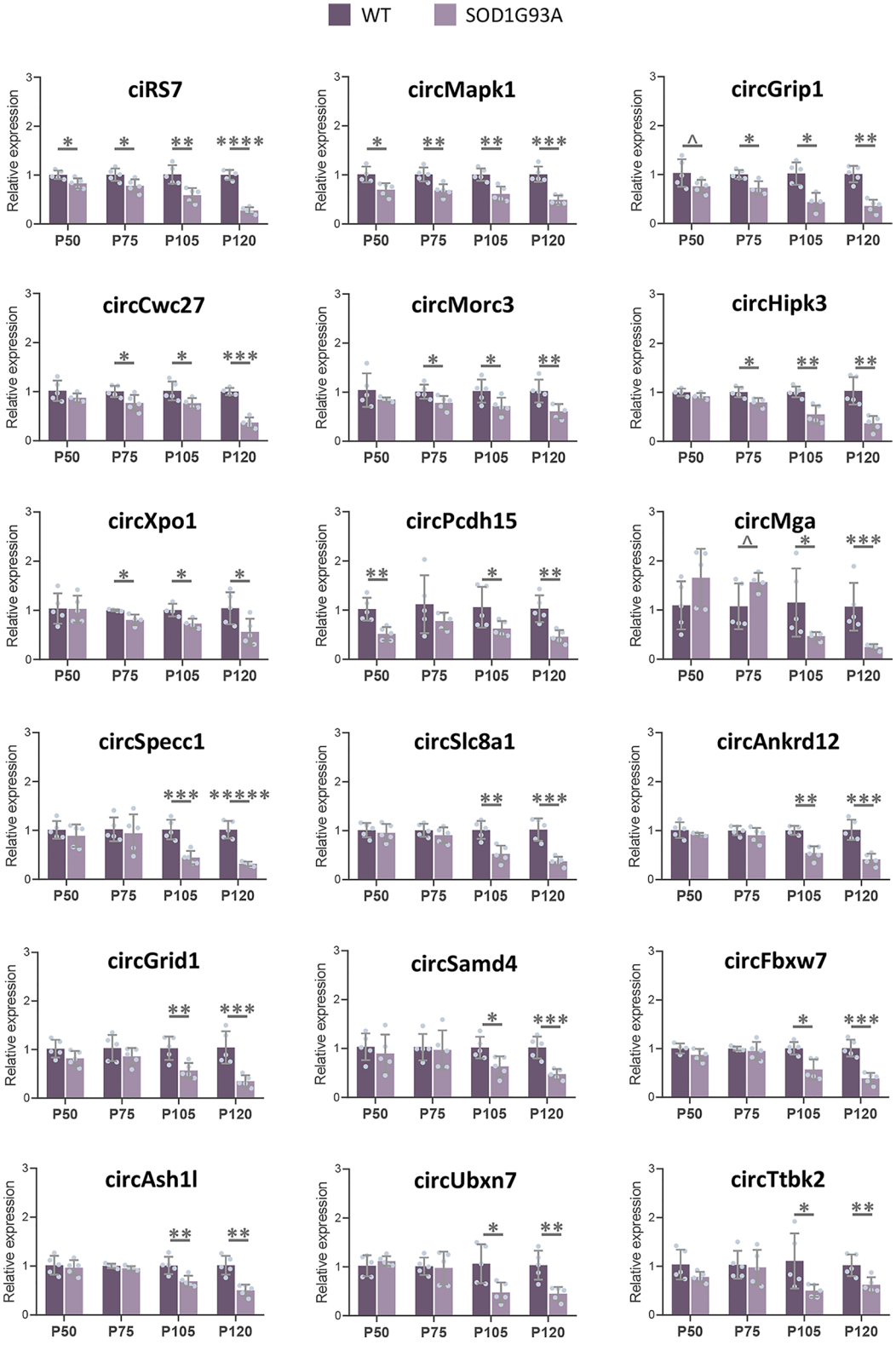


Fig. 1 Relative expression of the 30 circRNAs selected in the spinal cord of SOD1G93A mice. A total of 40 animals were used (10 male mice for each stage: WT $n=5$, SOD1G93A $n=5$). CircRNA levels were normalized using Actb and relative expression is shown in each stage with control mice set to 1. Light blue dots indicate 2−ddCt value obtained for each individual male mouse. Bars represent mean ± standard deviation. Statistical significance was assessed by student's t-test (unpaired two-tailed). ^$p<0.1$ (close to significance), *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, *****$p<0.00001$

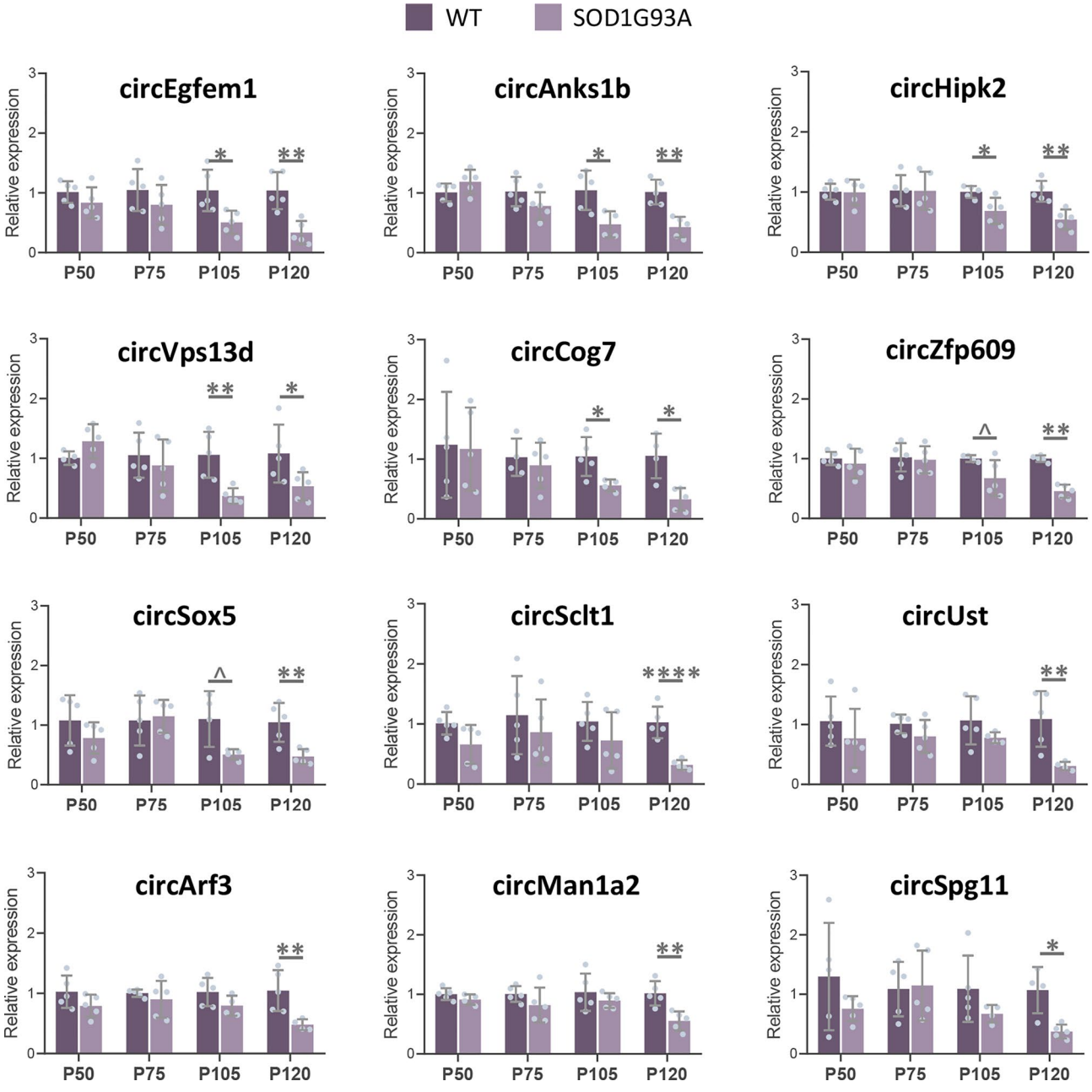


Fig. 1 (continued)

stages of the disease. The relative expression of three (P50), seven (P75), 23 (P105) and all 30 circRNAs (P120) was decreased in the different disease stages.

Notably, two circRNAs (ciRS7 and circMapk1) were significantly decreased from early presymtomatic to terminal stage, and five circRNAs (circGrip1, circCwc27, circMorc3, circHipk3 and circXpo1) from late presymtomatic to terminal stage. The most significant downregulation was seen for circSpecc1 ($p = 0.000004$), circSclt1 ($p = 0.00007$) and ciRS7 ($p = 0.00007$) at terminal stage.

Upon normalization to P50 (Fig. S1a), our analysis revealed that most circRNAs predominantly increased with age in WT animals, while they showed a decreasing trend in SOD1G93A mice.

circRNA levels tend to increase in skeletal muscle of SOD1G93A mice

Next, the expression of the selected circRNAs was studied in quadriceps of SOD1G93A and WT mice throughout the disease course using muscle tissue derived from the same male mice as in the spinal cord.

Fourteen out of the 30 circRNAs could not be quantified in mouse quadriceps. These circRNAs that are presumably CNS-specific or below the limit of detection in

skeletal muscle included all those selected from circRNA-seq analysis in spinal cord of 50-day-old mice and two additional circRNAs (circSpecc1 and circSclt1). All 16 circRNAs detectable in quadriceps showed significant differences at some stage of the disease (Fig. 2). In contrast to what we observed in spinal cord (Fig. 1), circRNA levels tended to increase gradually towards the terminal stage in SOD1G93A mice. The relative expression of two

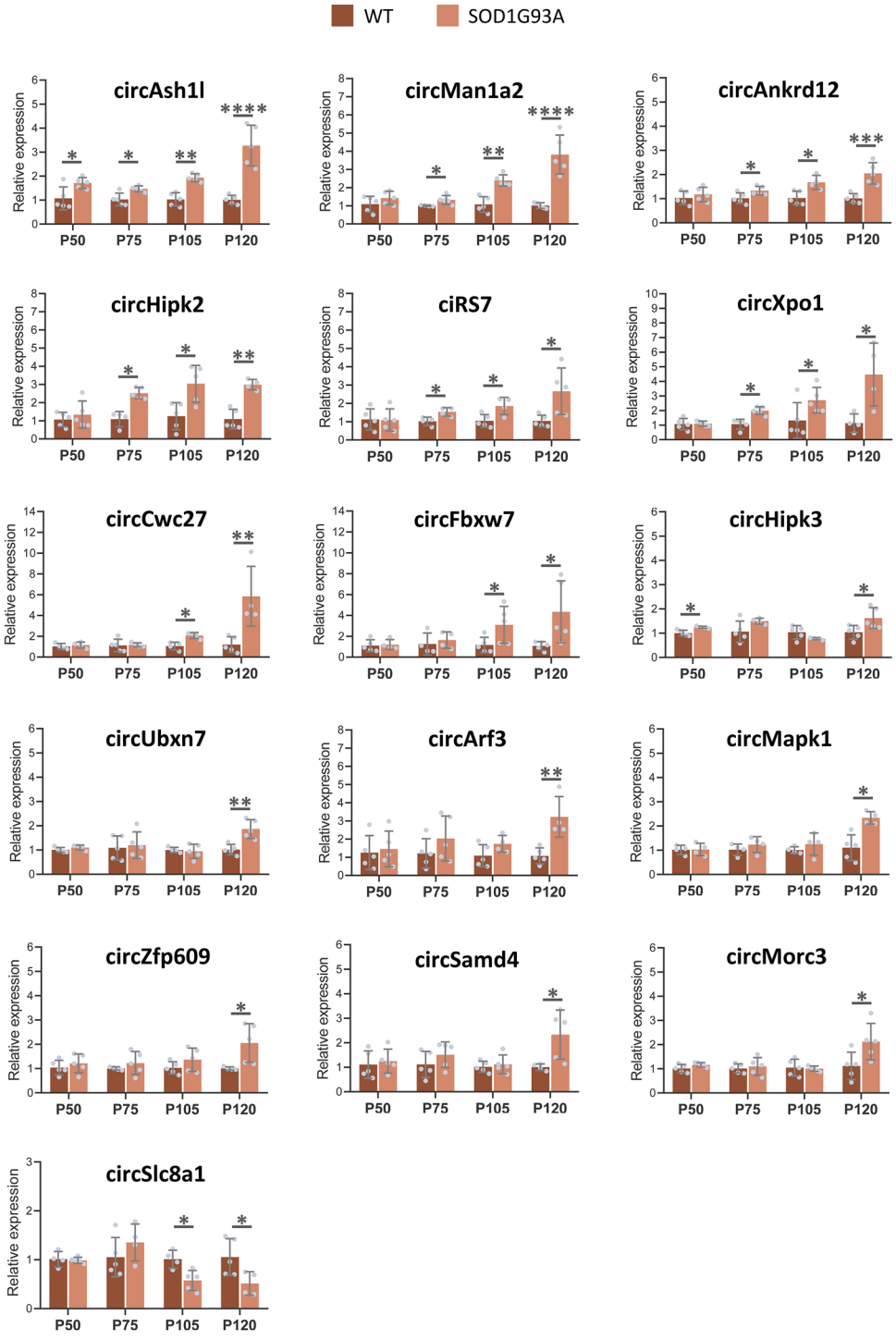


Fig. 2 Relative expression profiles of the detectable circRNAs in quadriceps from SOD1G93A mice. A total of 40 animals were used (10 male mice for each stage: WT $n=5$, SOD1G93A $n=5$). Relative circRNA levels were normalized using *Gapdh* and relative expression is shown in each stage with control mice set to 1. Light blue dots indicate 2^{-ddCt} value obtained for each male mouse. Bars represent mean ± standard deviation. Statistical significance was assessed by Student's t-test (unpaired two-tailed). ^$p < 0.1$ (close to significance), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

(P50), six (P75), nine (P105) and all 16 circRNAs (P120) was significantly altered in the different disease stages and were generally increased. Only one circRNA (circSlc8a1) presented the opposite pattern, decreasing significantly in P105 and P120, as in the spinal cord (Figs. 1 and 2).

In particular, circAsh1l was significantly upregulated from early presymptomatic to terminal stage, five (circMan1a2, circAnkrd12, circHipk2, ciRS7 and circXpo1) from P75 to P120. The most significantly overexpressed circRNAs in the terminal stage were circMan1a2 ($p = 0.00007$), circAsh1l ($p = 0.00008$), circAnkrd12 ($p = 0.0006$) and circUbxn7 ($p = 0.001$).

When we adjusted our data relative to P50 (Fig. S1b), most circRNAs increased with age in SOD1G93A, while their levels remained more stable in WT animals.

Alterations of circRNAs are generally consistent in male and female SOD1G93A mice

Given the marked and consistent alterations of circRNAs in male SOD1G93A mice, we considered the possibility that these may be influenced by the sex, given that the disease pathology is generally delayed in ALS females compared with males. Therefore, the levels of the 14 most deregulated circRNAs in each tissue of male mice were measured in terminal stage WT and SOD1G93A females.

In spinal cord (Fig. 3a), all circRNAs studied were significantly decreased in SOD1G93A females. Like observed in males at P120 (Fig. 1), circSpecc1 ($p = 0.0003$) and ciRS7 ($p = 0.0005$) were the first and third most significantly downregulated circRNAs. In quadriceps (Fig. 3b), the trend of increased levels of circRNAs was

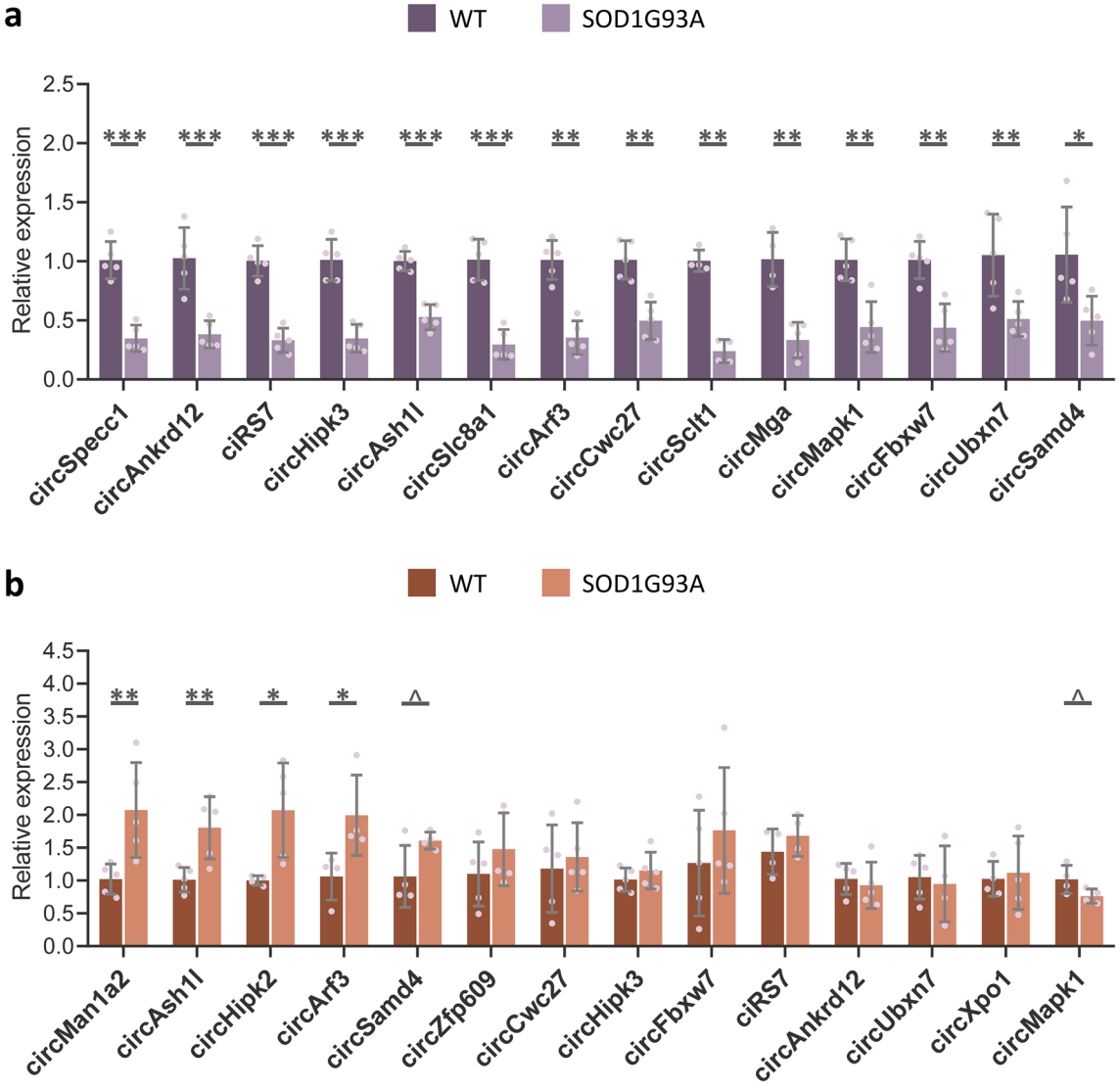


Fig. 3 Relative circRNA expression in (**a**) spinal cord and (**b**) quadriceps of female SOD1G93A mice. A total of 10 animals were used (WT $n = 5$, SOD1G93A $n = 5$) at terminal stage (P120). Relative levels were normalized using Actb and Gapdh as control. Light pink dots indicate 2−ddCt value obtained for each female. Bars represent mean ± standard deviation. Statistical significance was assessed by Student's t-test (unpaired two-tailed). ^$p < 0.1$ (close to significance), $p < 0.05$, **$p < 0.01$, ***$p < 0.001$

unchanged in SOD1G93A females. However, only four circRNAs were significantly upregulated. Similar to the males (Fig. 2), the lowest p values were seen for circMan1a2 ($p = 0.008$) and circAsh1l ($p = 0.008$).

Host genes exhibit concomitant expression trend with their respective circRNAs

circRNAs can regulate transcriptional levels of their parental genes, most of which in this study are involved in neuronal and muscular processes (Table 1). Considering that circRNAs may exert distinct functions in different tissues, we examined the expression of 14 host genes whose corresponding circRNAs were altered in both the spinal cord and quadriceps.

In the spinal cord of terminal-stage mice (Fig. 4a), 10 host genes were significantly downregulated and one (*Samd4*) exhibited a near-significant decrease. In contrast, in the quadriceps (Fig. 4b), 11 host genes were significantly upregulated and one showed a trend toward significance (*Zfp609*).

Next, we analyzed the correlation between circRNAs and their corresponding host genes (Fig. 4c, d and Fig. S2), finding that 11 out of 14 circRNAs exhibited a significant positive correlation with their parental genes. Specifically, four circRNAs (circMorc3, circAsh1l, circMapk1 and circMan1a2) showed consistent correlations in both tissues. Three circRNAs (circZfp609, circHipk3 and circArf3) correlated exclusively in the spinal cord, whereas four (circXpo1, circAnkrd12, circUbxn7 and circCwc27) correlated only in the quadriceps. Three circRNAs (circHipk2, circFbxw7, and circSamd4) did not show significant correlations. Nevertheless, circHipk2 and circFbxw7 showed a trend toward correlation in the spinal cord (0.076 and 0.051, respectively), while circSamd4 had a similar trend in muscle tissue (0.068).

circRNAs are more abundant in spinal cord than in skeletal muscle

As the levels of circRNAs were downregulated in spinal cord and generally upregulated in quadriceps in both sexes of SOD1G93A mice, we next determined whether their expression would be generally higher in skeletal muscle than in the CNS. For this purpose, we compared the abundance of circRNAs between spinal cord and quadriceps of mice at the stage that reached the largest fold change (P120) (Fig. 5).

CircRNA levels were substantially higher in spinal cord than in quadriceps from WT mice (Fig. 5a). The same was observed in SOD1G93A mice, even though in ALS circRNA expression tended to increase in muscle and decrease in CNS compared to healthy controls (Fig. 5a). Again, females presented the same profile as males, both WT and SOD1G93A (Fig. 5b).

Several circRNAs are decreased in the brain from sALS patients

Homologous human circRNA was identified for 24 of the 30 mouse circRNAs tested (Table 1). To assess whether the circRNA expression patterns found in the SOD1G93A mice are also observed in ALS patients, our objective was to validate circRNAs in the spinal cord and muscle of ALS patients. However, due to the limitations related to sample availability, we were only able to obtain brain tissue from human subjects. We then analyzed the expression of 18 human circRNAs homologs in samples derived from *post mortem* cortex and brainstem of sporadic ALS patients and healthy controls. The selection was made considering three criteria: (1) the largest significant differences observed in the mouse model, (2) the highest degree of conservation between species, and (3) the potential importance in CNS biology or in ALS (Table 1).

Among the 18 circRNAs assessed (Fig. 6), nine were downregulated in the brain samples from sALS patients compared to healthy controls and two were close to differential expression. None of the circRNAs were upregulated in ALS brains, consistent with the expression pattern observed in the spinal cord of the murine model. The most deregulated circRNAs in ALS brain were circXPO1 ($p = 0.00009$), circSCLT1 ($p = 0.0005$), circMAPK1 ($p = 0.001$), circTTBK2 ($p = 0.002$), and circSAMD4A ($p = 0.003$) (Fig. 6). Figure 7 shows a summary of all the data obtained in the murine model and in sALS patients, reflecting the expression patterns of circRNAs in each tissue and how they are maintained in both species.

Discussion

The work presented here suggests that circRNA expression may be profoundly impaired in CNS and skeletal muscle, the two tissues most affected by ALS. The observed dysregulation of circRNA levels seems to argue for a pattern that is tissue-dependent: whereas in the ALS spinal cord and brain circRNAs are generally downregulated, in the skeletal muscle their abundance tends to increase (Figs. 7 and 8).

The abundance of circRNAs can be altered mainly due to three causes: (1) circRNA biogenesis (regulated by RBPs, Alu elements and ADAR enzymes), (2) circRNA degradation (DBR1, RNase L, m6a modification, among others), and (3) release of circRNAs in extracellular vesicles [3].

CircRNA downregulation in CNS could be due to an altered function of RBPs, a common ALS hallmark [72]. Motor neurons silenced or mutated for FUS exhibit decreased levels of circRNAs [18, 19]. Whereas FUS WT binds to the flanking introns of circRNAs and regulates their expression, it seems that FUSP525L mutant could preferentially bind to introns with inverted Alu repeats

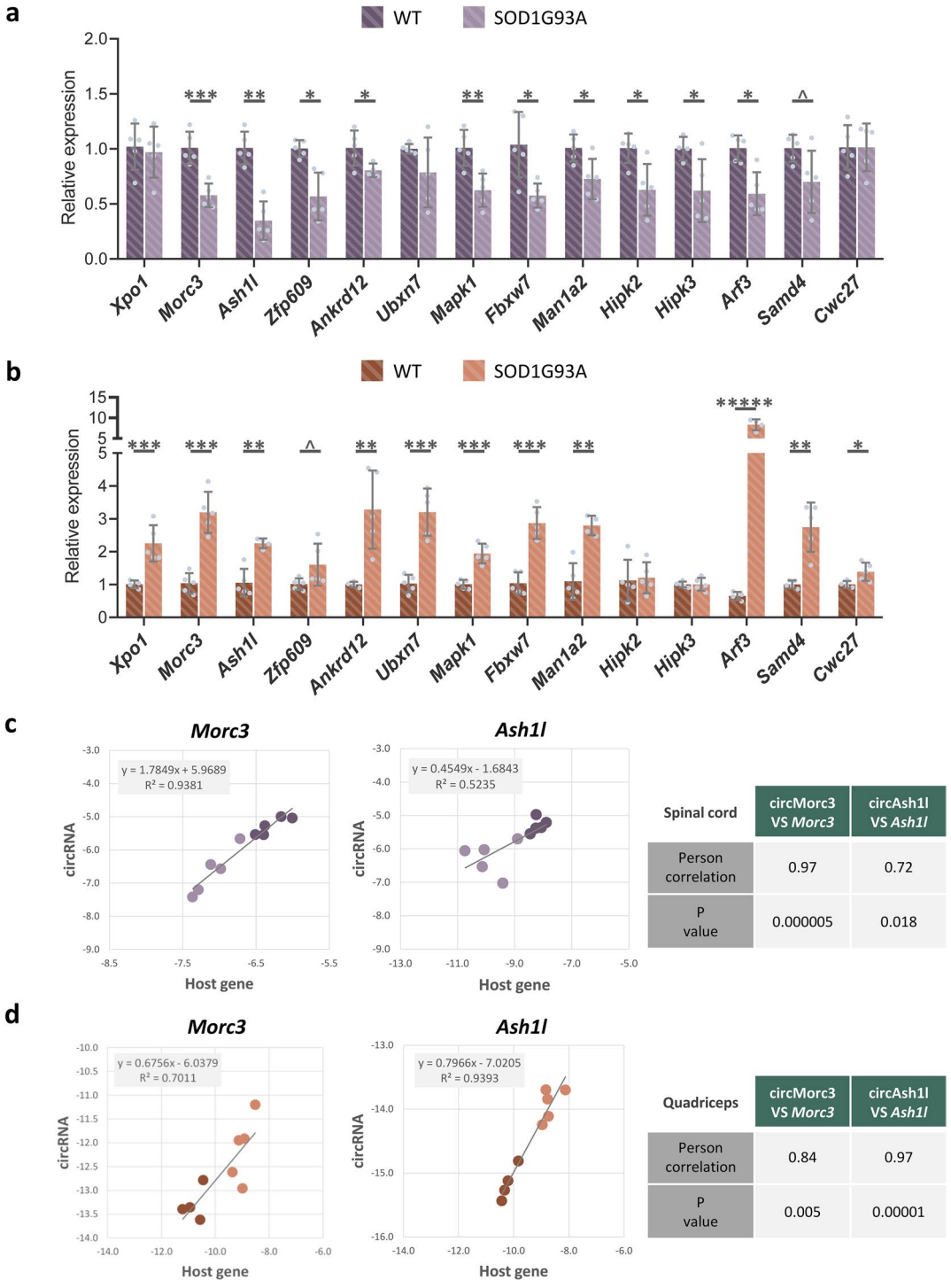


Fig. 4 Expression of host genes and their correlation with circRNA levels. Relative parental gene expression in **a** spinal cord and **b** quadriceps of male mice at the terminal stage (P120). Light blue dots indicate 2 – ddCt value obtained for each mouse. Bars represent mean ± standard deviation. Statistical significance was assessed by Student's t-test (unpaired two-tailed). ^$p < 0.1$ (close to significance), $p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Scatter plots of circMorc3 and circAsh1l levels with mRNA expression of their respective host genes in **c** spinal cord and **d** quadriceps of 120-day-old male mice. The Y-axis represents the levels of a circRNA (-dCt), while the X-axis shows the expression levels of its parental gene (-dCt). The dark purple and dark orange dots represent the spinal cord and quadriceps of WT mice, respectively, while the light purple and light orange dots represent the same tissues from SOD1G93A mice. Relationship between circRNA and host gene expression levels were analyzed using Pearson's correlation coefficient, with the corresponding P-values displayed on the right. The remaining correlations can be found in Figure S2

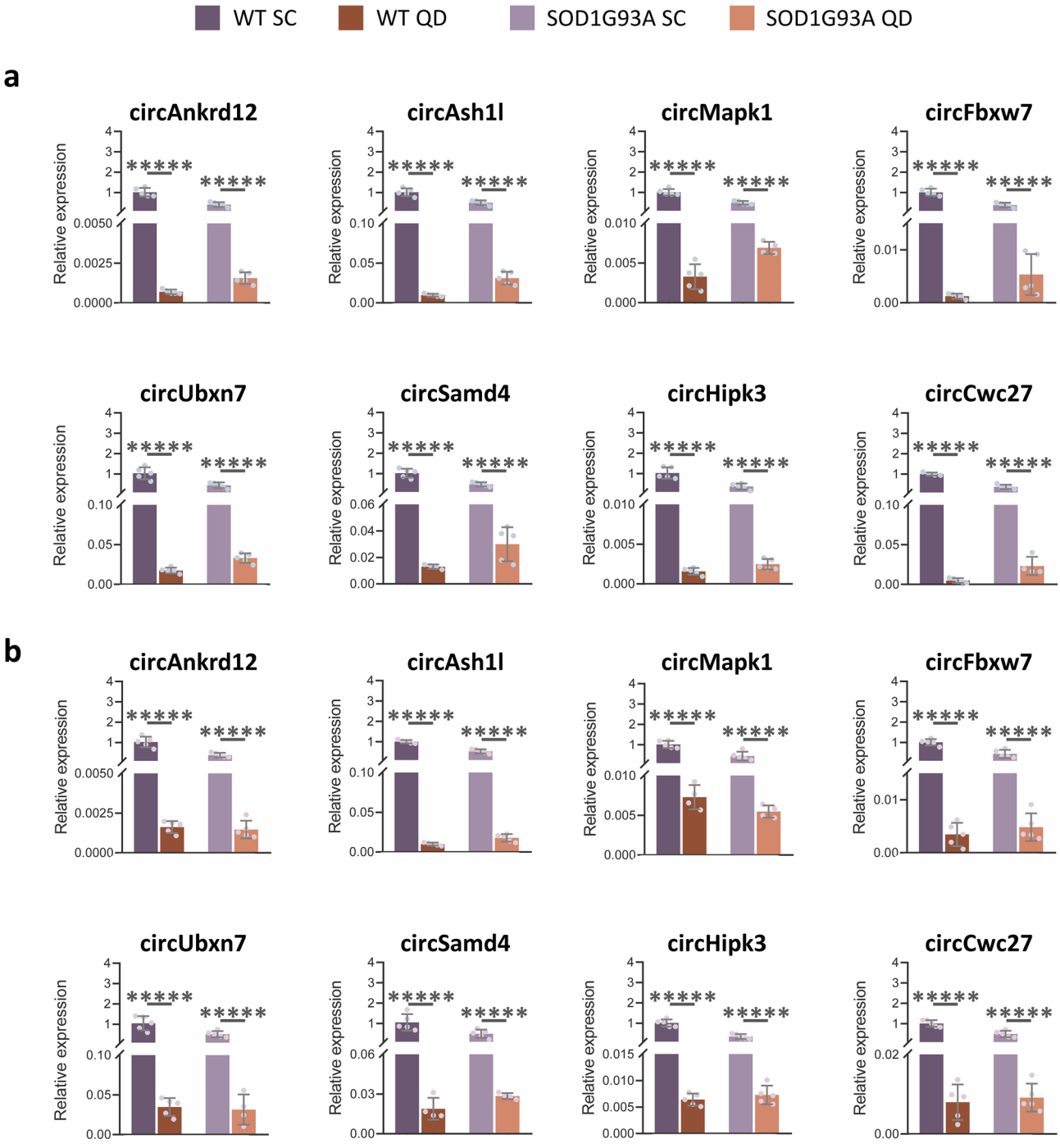


Fig. 5 Comparison of circRNA abundance between spinal cord and quadriceps from WT and SOD1G93A mice. RT-PCR analyses assessed in a male and b female mice at the terminal stage (P120). Relative levels were normalized using Actb and Gapdh as control. Dots indicate 2– ddCt value obtained for each mouse (light blue for males, light pink for females). Bars represent mean ± standard deviation (purple for spinal cord, orange for quadriceps). Statistical significance was assessed by Student's t-test (unpaired two-tailed). *****$p < 0.00001$. Abbreviations: SC (spinal cord), QD (quadriceps), WT (wild type)

flanking circRNAs which would inhibit circRNA biogenesis [19]. Decreased levels of CNS circRNAs both in the SOD1G93A mice and in sporadic ALS patients, as observed here, could be a consequence of a common etiopathogenic mechanism, such as misfolded proteins (FUS, TDP-43, SOD1). On the other hand, opposing alterations in circRNA levels between CNS and muscle could be due to the different response of neuronal and muscle cells to misfolded proteins in ALS [73–77]. It has been shown that muscle cells more efficiently eliminate misfolded proteins than neurons [73–76]. Thus, depending propensity for aggregate formation or management of

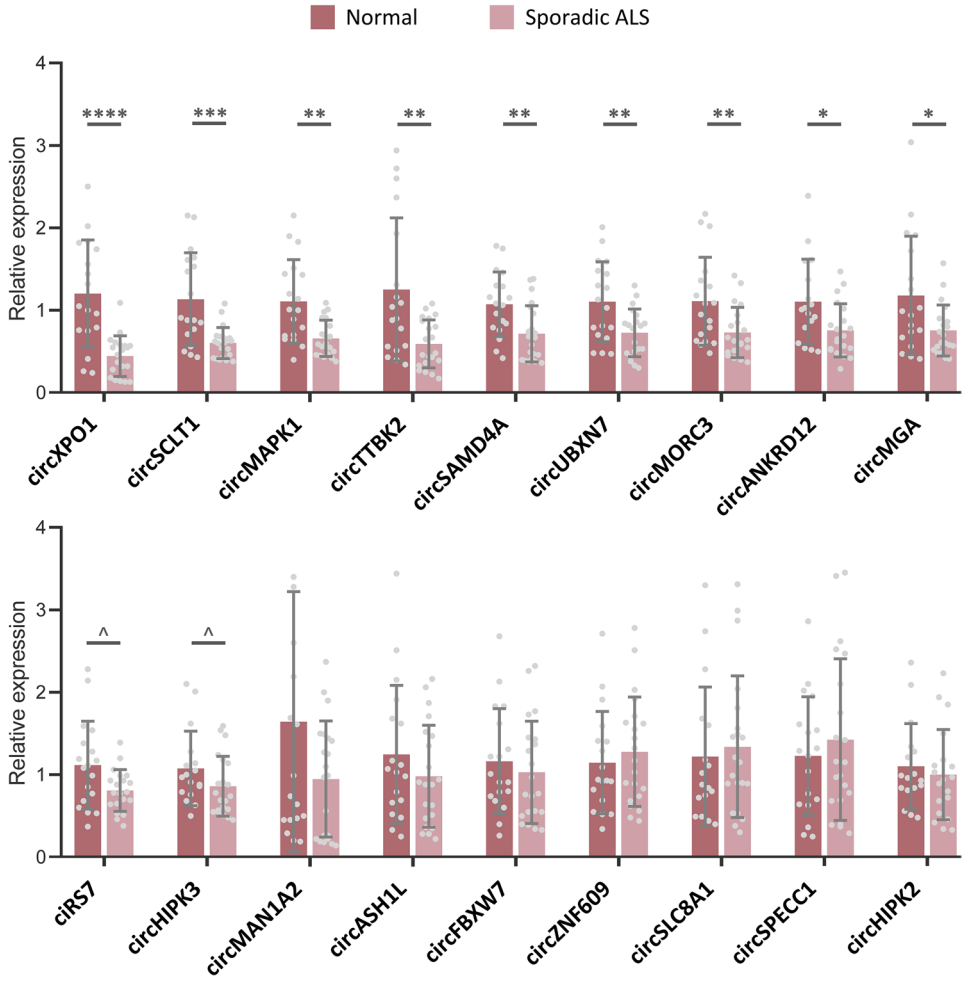


Fig. 6 Relative expression of selected circRNAs in brain of sALS patients ($n = 21$) compared to controls ($n = 18$). Expression was normalized using *GAPDH* as control. Grey dots represent 2^{-ddCt} value obtained for each sample. Bars represent mean ± standard deviation. Statistical significance was assessed by Student's t-test (unpaired two-tailed). ^$p < 0.1$ (close to significance), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The exact p-values are indicated in Fig. 7

misfolded proteins in different tissues, ALS-linked RBPs may exert distinct effects on CNS and muscle in ALS, which could differently affect circRNA biogenesis.

Notably, a possible therapeutic approach for ALS has been proposed based on the regulation of circRNA levels through DBR1 inhibition [25]. This enzyme catalyzes the debranching of lariat introns that are formed during pre-mRNA splicing, which may result in accumulation of cytoplasmic intronic circRNAs (ciRNAs) [3, 5]. The absence of DBR1 enzymatic activity is sufficient to rescue mutant TDP-43 toxicity in yeast, in human neuroblastoma cells and in rodent primary cortical neurons [25]. This likely occurs because the accumulated ciRNAs act as cytoplasmic decoys to titrate mutant TDP-43 relieving important cellular RNAs and RBPs that mislocalized TDP-43 normally sequesters from their normal function in ALS [25]. These results could support the hypothesis stated above, according to which neurons respond worse to misfolded proteins and would result in decreased physiological levels of circRNAs that could not be beneficial to the CNS. On the contrary, muscle may present an increase in the physiological levels of circRNAs, which could contribute to reduce the toxicity of misfolded proteins and show greater compensatory regenerative capacity to damage. In this regard, it is also possible that the observed decrease in circRNA levels reflects their increased degradation in the CNS of ALS model and patients. Although the degradation of circRNAs has hardly been studied, it has recently been shown that m6a methylation of RNA and activity of RNase L could promote the global elimination of circRNAs [3]. Interestingly, m6a hypermethylation of protein coding and non-coding RNA species have been observed in the spinal cord of sALS patients [78] and the expression of RNase L transcript is upregulated in iPSC-derived motor neurons from ALS patients [79]. In this respect, RNase L-mediated degradation of circRNA has been found to be necessary for the activation of protein kinase R (PKR) [80], which has

		Spinal cord (SOD1G93A mice)					Quadriceps (SOD1G93A mice)					Brain (sALS)	Conservation
		Male				Female	Male				Female		
		P50	P75	P105	P120	P120	P50	P75	P105	P120	P120		
circRNA-seq (SOD1G93A spinal cord)	circSOX5	0.2	0.6	0.08	0.004	NT	NQ	NQ	NQ	NQ	NQ	NT	92.1%
	circTTBK2	0.1	0.7	0.02	0.007	NT	NQ	NQ	NQ	NQ	NQ	0.002	89.8%
	circMGA	0.1	0.07	0.03	0.0003	0.004	NQ	NQ	NQ	NQ	NQ	0.03	88.4%
	circGRIP1	0.09	0.02	0.02	0.001	NT	NQ	NQ	NQ	NQ	NQ	NT	92.3%
	circGRID1	0.1	0.3	0.006	0.0009	NT	NQ	NQ	NQ	NQ	NQ	NT	91.2%
	circEGFEM1	0.2	0.2	0.01	0.005	NT	NQ	NQ	NQ	NQ	NQ	NT	69.7%
	circVPS13D	0.1	0.4	0.001	0.04	NT	NQ	NQ	NQ	NQ	NQ	NT	89.4%
	circUST	0.2	0.1	0.2	0.003	NT	NQ	NQ	NQ	NQ	NQ	NT	91.3%
	circSpg11	0.4	0.9	0.1	0.01	NT	NQ	NQ	NQ	NQ	NQ	NH	NH
	circAnks1b	0.1	0.1	0.03	0.006	NT	NQ	NQ	NQ	NQ	NQ	NH	NH
	circCog7	0.9	0.4	0.01	0.01	NT	NQ	NQ	NQ	NQ	NQ	NH	NH
	circPcdh15	0.002	0.3	0.04	0.001	NT	NQ	NQ	NQ	NQ	NQ	NH	NH
Literature search	circXPO1	0.9	0.04	0.01	0.03	NT	0.6	0.02	0.04	0.01	0.9	0.00009	95.1%
	circMORC3	0.3	0.03	0.04	0.008	NT	0.1	0.7	0.9	0.04	NT	0.009	89.5%
	circSPECC1	0.3	0.5	0.0009	0.000004	0.0003	NQ	NQ	NQ	NQ	NQ	0.6	82.4%
	circASH1L	0.7	0.2	0.006	0.001	0.0008	0.03	0.02	0.004	0.00008	0.008	0.3	84.4%
	circZNF609	0.4	0.7	0.07	0.0015	NT	0.5	0.5	0.2	0.02	0.2	0.5	97.3%
	circANKRD12	0.3	0.2	0.004	0.0007	0.0004	0.4	0.04	0.01	0.0006	0.5	0.01	90.0%
	circUBXN7	0.3	0.6	0.01	0.004	0.009	0.1	0.7	0.5	0.001	0.6	0.009	88.3%
	circMAPK1	0.01	0.006	0.005	0.0001	0.007	0.9	0.4	0.5	0.02	0.08	0.001	95.4%
	circSCLT1	0.1	0.4	0.2	0.00007	0.004	NQ	NQ	NQ	NQ	NQ	0.0005	83.3%
	circFBXW7	0.1	0.4	0.01	0.0009	0.007	0.6	0.4	0.04	0.01	0.3	0.4	87.1%
	circSAMD4A	0.4	0.6	0.03	0.0007	0.02	0.5	0.2	0.7	0.02	0.09	0.003	92.7%
	circSLC8A1	0.6	0.3	0.007	0.0002	0.0009	0.8	0.2	0.01	0.04	NT	0.6	88.0%
	circCWC27	0.2	0.04	0.03	0.0006	0.003	0.5	0.5	0.03	0.003	0.5	NT	77.6%
	circMAN1A2	0.1	0.1	0.4	0.008	NT	0.2	0.02	0.008	0.00007	0.008	0.1	87.1%
	circHIPK2	0.8	0.9	0.04	0.006	NT	0.5	0.03	0.04	0.005	0.01	0.4	94.2%
	circHIPK3	0.1	0.01	0.008	0.004	0.0006	0.01	0.08	0.1	0.03	0.3	0.07	93.7%
	ciRS7	0.02	0.02	0.005	0.00007	0.0005	0.9	0.03	0.02	0.01	0.3	0.06	69.5%
	circARF3	0.1	0.3	0.1	0.004	0.002	0.7	0.2	0.1	0.002	0.04	NT	93.3%

p<0.00001	p<0.0001	p<0.001	p<0.01	p<0.05	p>0.1	0.1<p>0.05	p<0.05	p<0.01	p<0.001	p<0.0001
SIGNIFICANT DOWNREGULATION					NOT SIGNIFICANT		SIGNIFICANT UPREGULATION			

Fig. 7 Summary of all the data obtained from the SOD1G93A murine model and sALS patients. A total of 30 circRNAs were studied, of which 18 circRNAs were selected from a literature search and 12 based on data from a circRNA-seq analysis in spinal cord of SOD1G93A mice. This figure shows all the p-values obtained, representing in blue a significant downregulation, in red a differential upregulation, and in ochre an unaltered expression or close to being significant. Taken together, these results suggest that circRNAs in ALS are deregulated in a global, progressive, tissue-dependent and sex-independent manner. Abbreviations: NQ (not quantifiable), NT (not tested), NH (not human homologous circRNA), P50 (early presymptomatic stage), P75 (late presymptomatic stage), P105 (symptomatic stage), and P120 (terminal stage)

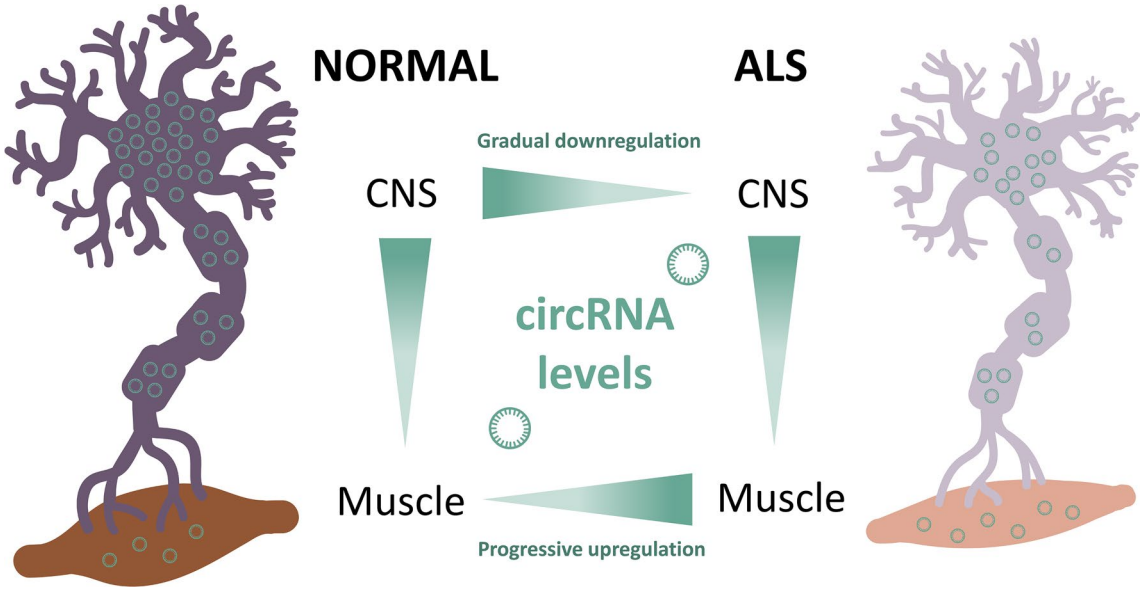


Fig. 8 Schematic representation of the differences in circRNA expression profiles in central nervous system and skeletal muscle of ALS. In spinal cord and brain the levels of circRNAs may be downregulated, whereas in quadriceps could be upregulated. Nonetheless, circRNAs are always more abundant in the CNS than in muscle, regardless of state (normal or ALS). These changes in the pattern of circRNA expression observed in ALS could be due to alterations in their biogenesis, in the stability of their structure and/or in their transport to the cellular exterior. These differences in circRNA levels may be increasingly pronounced with the course of the disease and could be a reflection of neuronal and muscle damage

been detected at higher levels in spinal cord [81] as well as in hippocampus from ALS patients [82], and its inhibition has been proposed as a promising therapeutic approach [82]. Therefore, increased levels of circRNAs in the CNS of ALS could be beneficial, as it could decrease TDP43-mediated toxicity [25] and PKR activation [80].

It is noteworthy that decreased expression of circRNAs in the CNS and increased levels in muscle have been observed in other pathologies and disease models. CircRNA levels are decreased in the spinal cord of spinal cord injury (SCI) models [83, 84] and in brain cortex of patients with schizophrenia [85], whereas increase is observed in myopathies such as DM1 [13, 14]. Based on these findings it may be plausible, regardless of the possible cause (RBPs, m6a methylation.), that changes in circRNA abundance could reflect a compensatory response to neuronal (e.g., neuroinflammation, reactive glia, oxidative damage, glutamate excitotoxicity, ADAR-mediated RNA editing) and muscle damage (muscle weakness and wasting) common to ALS and ALS mimic-pathologies. In this regard, it has been observed that there is a connection between the deregulation of global circRNA abundance and immune microenvironment [86, 87].

During the preparation of this manuscript, a comparable tissue-dependent pattern of circRNAs observed in CNS and muscle of ALS patients was reported by Tsitsipatis [21]. While the mentioned study examined different circRNAs from those investigated in the present article (with the exception of circFBXW7), their findings and conclusions aline closely with ours. Interestingly, some of the circRNAs that we selected through a literature review independent of the Tsitsipatis work overlap with the data of their sequencing analysis in muscle biopsies from ALS patients (circXPO1, circSCLT1, circSPECC1, circMGA, circHIPK3, circUBXN7, circSAMD4A and circHIPK2). Three of these circRNAs were not detected in the quadriceps muscle from SOD1G93A mouse model, possibly due to lower expression levels and different methodological sensitivity. Nevertheless, considerable overlap between the results and general conclusions of these two studies strengthen the value of these findings and reinforce the importance of circRNAs in ALS pathology.

Our results corroborate previous findings regarding the relatively high degree of conservation of the circRNAs between mouse and human, and how the altered expression profiles in most cases are captured in both species (Fig. 7) [21]. While it is true that many of the circRNAs differentially expressed in SOD1G93A mice did not have human homologs (or these had not yet been annotated due to the relatively recent discovery of this RNA class), it is important to note that in certain cases with human homologs, the findings obtained from the mouse model were also observed in human ALS samples. This emphasizes the potential value of animal models in translating experimental findings to the benefit of patients. It is also worth noticing that the circRNAs that were not observed to be dysregulated in the patient samples could be because they are altered in the spinal cord but not in the brain. Therefore, although the same trend of decreased circRNA levels in CNS tissues has been observed in ALS,

there could be some circRNAs that are downregulated only in the spinal cord or brain.

Finally, in this work we have also studied the differences in the expression of circRNAs according to tissue and sex. The levels of circRNA were found to be higher in the spinal cord than in the skeletal muscle, regardless of the mouse genotype (Fig. 5). This is reasonable since neurons are by far the cell type with the highest number and expression of circRNAs [9]. Therefore, although in ALS circRNA abundance could be decreased in the spinal cord and increased in muscle, their levels would still be higher in the CNS because neurons are enriched in these RNA molecules. This fact could also explain why all the circRNAs we have studied were expressed in the spinal cord but only slightly more than 50% could be quantified in the quadriceps (Fig. 7). The vast majority of circRNAs that we have only been able to quantify in the spinal cord come from circRNA-seq analysis, so it is possible that they could be tissue-specific or highly enriched in the CNS. Moreover, some circRNAs that we selected for their known relevance to the muscle (such as circZNF609 and circSAMD4A), were also found in spinal cord (Fig. 7). As for sex, the expression patterns of circRNAs were maintained in spinal cord and skeletal muscle of SOD1G93A females (Fig. 3), but these variations were more pronounced in males (Figs. 1 and 2). This could be due to sex-dependent differences observed in ALS, since SOD1G93A females tend to have a later disease onset and display modestly increased survival [88]. Consistently, the alterations of lesser extent observed in the end stage females could reflect their better functional status compared with males. Indeed, the levels of circRNAs in females at terminal stage (P120) are more comparable to those obtained for males at late symptomatic stage (P105) in both in quadriceps and spinal cord (Fig. 7).

From the 30 circRNAs studied, 16 were found to be particularly altered in ALS and may therefore be closely related to the etiopathogenesis of this disease (circSCLT1, circXPO1, circUBXN7, circSAMD4A, circMAPK1, circANKRD12, circMAN1A2, circMORC3, circTTBK2, ciRS7, circHIPK3, circASH1L, circHIPK2, circFBXW7, circMGA and circCWC27) (see Table 1 for more details about circRNAs). Some of these circRNAs have been previously associated with other neurodegenerative diseases or myopathies, such as AD [11, 62], PD [52, 66] and DM1 [13, 14]. Ten of these circRNAs have been mechanistically studied, being some involved in inflammation [51, 54, 56, 62, 65, 66, 68], myogenesis [8, 64, 67], and cell cycle and proliferation [39, 60]. In addition, many seem to be involved in the brain development [27]. The function of the remaining circRNAs is still poorly understood. Nevertheless, a relevant proportion of circRNAs have been shown to regulate the expression of their host genes through various mechanisms [89, 90]. In this study, we observed that the expression of most of the host genes analyzed was consistently deregulated with their corresponding circRNAs in both spinal cord and quadriceps, with many of them correlating positively (Fig. 4 and Fig. S2). This indicates that higher circRNA levels may be associated with increased expression of their host genes. These results could potentially be explained by the competing endogenous RNA (ceRNA) mechanism, in which an increase in circRNA levels leads to the sequestration of miRNAs that would otherwise inhibit the host gene, thereby increasing its expression [89–91]. To confirm such possible mechanism, further studies are needed to verify the interactions between the circRNAs of interest and their associated miRNAs, as well as to analyze the downstream targets of these miRNAs at the protein level. Since one of the selection criteria for circRNAs in this study was the function of their host genes, many of them are involved in processes that are altered in ALS, and some have been identified as modifiers of more than one ALS-causal gene [42]. Interestingly, proteins encoding some host genes have been proposed as therapeutic targets for ALS (*XPO1* [43], *MAPK1* [57], *TTBK2* [28] and *HIPK2* [92]). On the other hand, circASH1L was significantly upregulated in the quadriceps from the asymptomatic to the terminal stage (Figs. 2 and 7), so it could be a promising ALS biomarker. It is noteworthy that the expression of its host gene (*ASH1L*), which is involved in myoblast fusion [50], was also dysregulated in our ALS mouse model and showed a strong correlation with its circular isoform (Fig. 4). Other potential biomarkers of ALS or muscle damage could be circMAN1A2, circXPO1, circANKRD12, circHIPK2 and ciRS7. Regarding circMAN1A2 (hsa_circ_0000118), it should be noted that another circRNA from the same host gene (hsa_circ_0000119) was found previously altered both in muscle and in spinal cord [21].

Finally, it is worth noting that these circRNA alterations could not only distinguish between healthy and affected individuals but also reflect temporal changes. When normalizing our data to P50, we observed significant changes in circRNA expression over time in both analyzed tissues (Fig. S1). In the spinal cord (Fig. S1a), most circRNAs increased with age in WT animals, whereas in the ALS mouse model, their levels tended to decrease. This result aligns with previous studies describing circRNA accumulation in the CNS during aging, a trend that may be disrupted in neurodegenerative diseases, as observed in Parkinson's disease patients [61, 93, 94]. In the quadriceps muscle (Fig. S1b), circRNA expression generally increased with age in the ALS mouse model, whereas in healthy animals, it exhibited fewer variations. These findings may be consistent with the global increase in circRNA levels reported in myopathies and the relative

stability of circRNA expression in muscle from healthy animals, in contrast to the CNS [13, 94, 95].

Conclusions

In conclusion, the expression of circRNAs in ALS could be characterized by a progressive and tissue-dependent pattern that seems to be opposite in CNS and skeletal muscle (Figs. 7 and 8). These variations in circRNA levels could be a common signature to all or different forms of ALS and seem to be indicative of neuronal and muscle damage caused by disease progression. This phenomenon could be due to alterations in the biogenesis of circRNAs, being their main players dysregulated in ALS (RBPs [72] and ADAR-mediated RNA editing [96]). Other independent and non-exclusive possibilities to this hypothesis may be an alteration of circRNAs stability (DBR1 [25], RNase L, m6a methylation [78]) or an increase of circRNAs transport from the motor neuron to the muscle [21]. In view of all these findings, the role of circRNAs in the etiopathogenesis of ALS could bear relevance from the earlier stages of the disease, paving the way to consider them as potential diagnostic and prognostic biomarkers. More studies are also needed to further investigate the function of these circRNAs as therapeutic targets of new treatments, as well as their expression in more accessible tissues, such as peripheral blood or plasma, for use as molecular markers of ALS.

Materials and methods

ALS mouse model

All experimental procedures were approved by the Ethics Committee for Animal Experiments of Universidad de Zaragoza (Ref. PI52/15) and were performed accordingly with the institutional and the international guidelines for the use of laboratory animals. Mice were housed under a standard light: dark (12:12) cycle with food and water *ad libitum*. The transgenic mice (SOD1G93A) were obtained by crossbreeding hemizygous B6SJL-Tg SOD1G93A males (stock number 002726) purchased from The Jackson Laboratory (Bar Harbor, ME, USA) with B6SJL females from Janvier Labs (Saint- Berthevin Cedex, France).

Spinal cord and quadriceps muscle were isolated from a total of 33 TG mice and their respective controls (age-matched WT littermates) after euthanization with CO_2. These tissues were immediately frozen and stored at − 80∘C. The SOD1G93A murine model exhibits a much more pronounced affection in the spinal cord compared to the brain. For this reason, we considered the spinal cord to be the best option for investigating disease progression in the nervous system in this work.

Patients

Brain tissue samples from ALS patients and non-diseased controls were collected, processed and provided by the CIEN Tissue Bank, CIEN Foundation, Instituto de Salud Carlos III and Biobanco en Red de la Región de Murcia (BIOBANC-MUR). This study was approved by El Comité de Ética de la Investigación de la Comunidad de Aragón (CEICA) (Ref. PI17/0025) and El Comité Científico del banco de tejidos de la Fundación CIEN (Ref. CCS17003). Written informed consent was obtained from all subjects. Detailed clinical characteristics are shown in Table S1.

Gene expression

RNA extraction

For mice spinal cord and quadriceps, tissues were homogenized with QIAzol Lysis Reagent (Qiagen) using the TissueLyser LT (Qiagen). RNA was isolated with Direct-zol RNA Miniprep (Zymo Research) according to the manufacturer's instructions. For human brain samples, RNA purification was performed as previously described [97]. The quality and concentration of each extraction was measured with Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific).

circRNA-sequencing (circRNA-seq)

RNA from spinal cord samples of 50-day-old WT ($n = 8$) and SOD1G93A ($n = 8$) mice was quality controlled using Agilent Bioanalyzer (Fig. S3) and 2 µg of total RNA was used as input material for circRNA-seq analysis performed by Novogene Bioinformatics Technology Co., Ltd (Beijing, China). The experimental procedure consisted of ribosomal RNA depletion, RNase R digestion to eliminate non-circular RNAs, double-stranded cDNA synthesis, adapter ligation, second-strand cDNA elimination, first-strand cDNA purification for PCR, and finally sequencing with Illumina HiSeq™ 2500 (Illumina) using the 150 bp paired end sequencing strategy. The reads were cleaned by removal of adapter sequences, as well as those reads with $N > 10\%$ and low-quality reads where reads with quality values $Q <= 5$ and the number of bases accounting for more than 50% of the entire read. Finally, reads only observed in one mouse and those where average read count across all samples was less than one were filtered out. circRNAs were identified and annotated by CIRCexplorer [98], DESeq2 [99] was used for the differential expresssion analysis. The normalized expression of circRNAs in each sample are calculated based on the TPM method where the normalized expression equals to readCount * 1,000,000 / library size (total sample circRNA readcount). Data quality summary, raw counts and TPM normalized counts are given in Supplementary Material 2 and the FASTQ files are available in Gene Expression Omnibus (GSE289637).

cDNA synthesis and quantitative PCR (qPCR)

From each sample, 100 ng (CNS) or 200 ng (muscle) of total RNA was used as template for retro-transcription (RT) with SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific), following the manufacturer's protocol. Next, circRNA and mRNA levels were quantified by qPCR using PowerUp SYBR Green MasterMix (Thermo Fisher Scientific) and a set of mouse (Table S2) or human (Table S3) specific primers. Average cycle threshold (Ct) values for each circRNA across different tissues are provided in Table S4. *GAPDH* and *ACTB* were measured for sample normalization [100] and relative expression changes were determined by the 2^{-ddCt} method.

Statistical analysis

Statistical analysis and figure generation were conducted using GraphPad Prism version 8.0.1 software. ANOVA and Student's t-tests were applied to compare four (stages) and two (genotypes/tissues/control and ALS patients) groups, respectively. Pearson correlation analysis was performed to evaluate the relationship between circRNA levels and host gene expression. Differences were considered statistically significant at $p < 0.05$ (*) and highly significant at $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****).

Abbreviations

ACTB	Actin beta
AD	Alzheimer's disease
ADAR	Adenosine deaminases acting on RNA
ALS	Amyotrophic lateral sclerosis
ALS2	Alsin Rho guanine nucleotide exchange factor ALS2
C9ORF72	Chromosome 9 open reading frame 72
cDNA	Complementary DNA
CDR1	Cerebellar degeneration related 1
circRNA	Circular RNA
circRNA-seq	circRNA sequencing
ciRNA	Circular intronic RNA
CNS	Central nervous system
DBR1	RNA lariat debranching enzyme
DM1	Myotonic dystrophy type 1
DMD	DUCHENNE muscular dystrophy
DPR	Dipeptide repeat proteins
fALS	Familial ALS
FUS	Fused in sarcoma
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
iPSCs	Human induced pluripotent stem cells
miRNA	microRNA
ncRNA	non-coding RNA
PD	Parkinson's disease
QD	Quadriceps
qRT-PCR	Quantitive real time-PCR
RBPs	RNA-binding proteins
sALS	Sporadic ALS
SC	Spinal cord
SCI	Spinal cord injury
SOD1	Cu-Zn superoxide dismutase 1
TARDBP	TAR DNA binding protein
TG	Transgenic
TGFβ2	Transforming growth factor beta 2
WT	Wild type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11725-4.

Supplementary Material 1: Supplementary tables (characteristics of patients, primer sequences, and average Ct values for different tissues) and supplementary figures (relative circRNA expression normalized to 50 days, correlation analyses between 12 circRNAs and their host gene expression, and RNA quality control used for circRNA-seq).

Supplementary Material 2: Circular RNA-Seq Data Summary.

Acknowledgements

We thank Elena Tapia and the workers of the animal house in the Centro de Investigación Biomédica de Aragón for their help in maintaining the mice colony.

Author contributions

Conceptualization: A.C.C., J.M.T, and R.O. Methodology: A.C.C., J.M.T, L.M.-G., L.M.-M., M.d.l.T. and S.M.-R. Software: A.C.C., J.M.T. and L.M.-G. Validation: A.C.C., J.M.T, and R.O. Formal analysis: A.C.C., J.M.T and L.M.-G. Investigation: L.M.M, M.d.l.T, and L.M.-G. Resources: A.C.C. and R.O. Data curation: A.C.C., J.M.T. and L.M.-G. Writing (original draft preparation): L.M.-G. Writing (review and editing): all the authors. Visualization: all the authors. Supervision: A.C.C. and J.M.T. Project administration: A.C.C., J.M.T and R.O. Funding acquisition: A.C.C., A.G.-R. and R.O.

Funding

This work was supported by Instituto de Salud Carlos III [PI21/00372], Fondo Europeo de Desarrollo Regional (FEDER) "Una manera de hacer Europa" from the European Union, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED-612) [CB18/05/00037], Spanish Ministry of Science and Innovation with funds from the European Union NextGenerationEU from the Recovery, Transformation and Resilience Plan (PRTR-C17.I1), University of Zaragoza [UZ2018-BIO-03] and Consolidated Groups from Gobierno de Aragón. L.M.-G. was supported by Departamento de Industria e Innovación from Gobierno de Aragón and Fondo Social Europeo.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval

The corresponding certificate with reference PI08/20 and CP-CI P17/0025 have been approved and obtained through the Ethical Advisory Commission for Animal Experimentation of the University of Zaragoza and the Ethical Advisory Commission of Clinical Research.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

All authors consent to the publication of the manuscript.

Competing interests

The authors declare no competing interests.

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Received: 17 May 2024 / Accepted: 16 May 2025
Published online: 01 July 2025

References

1. Obi P, Chen YG. The design and synthesis of circular RNAs. Methods. 2021;196:85–103.
2. Eger N, Schoppe L, Schuster S, Laufs U, Boeckel JN. Circular RNA splicing. Adv Exp Med Biol. 2018;1087:41–52.
3. Zhou M, Xiao MS, Li Z, Huang C. New progresses of circular RNA biology: from nuclear export to degradation. RNA Biol. 2021;18:1365–73.
4. Shen H, An O, Ren X, Song Y, Tang SJ, Ke XY, et al. ADARs act as potent regulators of circular transcriptome in cancer. Nat Commun. 2022;13:1508.
5. Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, et al. Circular Intronic long Noncoding RNAs. Mol Cell. 2013;51:792–806.
6. Su M, Xiao Y, Ma J, Tang Y, Tian B, Zhang Y, et al. Circular RNAs in cancer: emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers. Mol Cancer. 2019;18:90.
7. Miao Q, Ni B, Tang J. Coding potential of circRNAs: new discoveries and challenges. PeerJ. 2021;9, e10718.
8. Sun X, Kang Y, Li M, Li Y, Song J. The emerging regulatory mechanisms and biological function of circular RNAs in skeletal muscle development. Biochim Biophys Acta Gene Regul Mech. 2022;1865:194888.
9. Rybak-Wolf A, Stottmeister C, Glažar P, Jens M, Pino N, Giusti S, et al. Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. Mol Cell. 2014;58:870–85.
10. Van Rossum D, Verheijen BM, Pasterkamp RJ. Circular RNAs: novel regulators of neuronal development. Front Mol Neurosci. 2016;9:74.
11. Zhang M, Bian Z. The emerging role of circular RNAs in Alzheimer's disease and Parkinson's disease. Front Aging Neurosci. Frontiers Media S.A. 2021.
12. Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, et al. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. Mol Cell. 2017;66:22–e379.
13. Czubak K, Taylor K, Piasecka A, Sobczak K, Kozłowska K, Philips A, et al. Global increase in circrna levels in myotonic dystrophy. Front Genet. 2019;10:649.
14. Voellenkle C, Perfetti A, Carrara M, Fuschi P, Renna LV, Longo M, et al. Dysregulation of circular RNAs in myotonic dystrophy type 1. Int J Mol Sci. 2019;20:1938.
15. Butti Z, Patten SA. RNA dysregulation in amyotrophic lateral sclerosis. Front Genet. 2019;9:712.
16. Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci. 2014;17:17–23.
17. Wu LS, Cheng WC, Chen CY, Wu MC, Wang YC, Tseng YH, et al. Transcriptomopathies of pre- and post-symptomatic frontotemporal dementia-like mice with TDP-43 depletion in forebrain neurons. Acta Neuropathol Commun. 2019;7:50.
18. Errichelli L, Dini Modigliani S, Laneve P, Colantoni A, Legnini I, Capauto D, et al. FUS affects circular RNA expression in murine embryonic stem cell-derived motor neurons. Nat Commun. 2017;8:14741.
19. Colantoni A, Capauto D, Alfano V, D'Ambra E, D'Uva S, Tartaglia GG, et al. FUS alters circrna metabolism in human motor neurons carrying the ALS-Linked P525L mutation. Int J Mol Sci. 2023;24:3181.
20. Dolinar A, Koritnik B, Glavač D, Ravnik-Glavač M. Circular RNAs as potential blood biomarkers in amyotrophic lateral sclerosis. Mol Neurobiol. 2019;56:8052–62.
21. Tsitsipatis D, Mazan-Mamczarz K, Si Y, Herman AB, Yang J-H, Guha A, et al. Transcriptomic analysis of human ALS skeletal muscle reveals a disease-specific pattern of dysregulated circRNAs. Aging. 2022;14:9832–59.
22. Hosaka T, Yamashita T, Teramoto S, Hirose N, Tamaoka A, Kwak S. ADAR2-dependent A-to-I RNA editing in the extracellular linear and circular RNAs. Neurosci Res. 2019;147:48–57.
23. D'Ambra E, Santini T, Vitiello E, D'Uva S, Silenzi V, Morlando M, et al. Circ-Hdgfrp3 shuttles along neurites and is trapped in aggregates formed by ALS-associated mutant FUS. iScience. 2021;24:103504.
24. Wang S, Latallo MJ, Zhang Z, Huang B, Bobrovnikov DG, Dong D, et al. Nuclear export and translation of circular repeat-containing intronic RNA in C9ORF72-ALS/FTD. Nat Commun. 2021;12:4908.
25. Armakola M, Higgins MJ, Figley MD, Barmada SJ, Scarborough EA, Diaz Z, et al. Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models. Nat Genet. 2012;44:1302–9.
26. Li A, Hooli B, Mullin K, Tate RE, Bubnys A, Kirchner R, et al. Silencing of the Drosophila ortholog of SOX5 leads to abnormal neuronal development and behavioral impairment. Hum Mol Genet. 2017;26:1472–82.
27. Gruner H, Cortés-López M, Cooper DA, Bauer M, Miura P. CircRNA accumulation in the aging mouse brain. Sci Rep. 2016;6:38907.
28. Liachko NF, McMillan PJ, Strovas TJ, Loomis E, Greenup L, Murrell JR, et al. The Tau tubulin kinases TTBK1/2 promote accumulation of pathological TDP-43. PLoS Genet. 2014;10:e1004803.
29. Van Damme P, Dewil M, Robberecht W, Van Den Bosch L. Excitotoxicity and amyotrophic lateral sclerosis. Neurodegener Dis. 2005;2:147–59.
30. Lai C, Xie C, McCormack SG, Chiang HC, Michalak MK, Lin X, et al. Amyotrophic lateral sclerosis 2-deficiency leads to neuronal degeneration in amyotrophic lateral sclerosis through altered AMPA receptor trafficking. J Neurosci. 2006;26:11798–806.
31. Leal SS, Gomes CM. Calcium dysregulation links ALS defective proteins and motor neuron selective vulnerability. Front Cell Neurosci. 2015;9:225.
32. Andrés-Benito P, Gelpi E, Jové M, Mota-Martorell N, Obis È, Portero-Otin M, et al. Lipid alterations in human frontal cortex in ALS-FTLD-TDP43 proteinopathy spectrum are partly related to peroxisome impairment. Neuropathol Appl Neurobiol. 2021;47:544–63.
33. Mizuno H, Warita H, Aoki M, Itoyama Y. Accumulation of chondroitin sulfate proteoglycans in the microenvironment of spinal motor neurons in amyotrophic lateral sclerosis Transgenic rats. J Neurosci Res. 2008;86:2512–23.
34. Hirano K, Ohgomori T, Kobayashi K, Tanaka F, Matsumoto T, Natori T, et al. Ablation of Keratan sulfate accelerates early phase pathogenesis of ALS. PLoS ONE. 2013;8:e66969.
35. Logan A, Belli A, Di Pietro V, Tavazzi B, Lazzarino G, Mangione R, et al. The mechanism of action of a novel neuroprotective low molecular weight dextran sulphate: new platform therapy for neurodegenerative diseases like amyotrophic lateral sclerosis. Front Pharmacol. 2022;13:983853.
36. Tripolszki K, Gampawar P, Schmidt H, Nagy ZF, Nagy D, Klivényi P, et al. Comprehensive genetic analysis of a Hungarian amyotrophic lateral sclerosis cohort. Front Genet. 2019;10:732.
37. Younis RM, Taylor RM, Beardsley PM, McClay JL. The ANKS1B gene and its associated phenotypes: focus on CNS drug response. Pharmacogenomics. 2019;20:669–84.
38. Martínez-Menárguez JÁ, Tomás M, Martínez-Martínez N, Martínez-Alonso E. Golgi fragmentation in neurodegenerative diseases: is there a common cause? Cells. 2019;8:748.
39. Li H, Shan CP, Wang JY, Hu CJ. CircRNA Hsa_circ_0001017 inhibited gastric Cancer progression via acting as a sponge of miR-197. Dig Dis Sci. 2021;66:2261–71.
40. Stankiewicz TR, Pena C, Bouchard RJ, Linseman DA. Dysregulation of Rac or Rho elicits death of motor neurons and activation of these GTPases is altered in the G93A mutant hSOD1 mouse model of amyotrophic lateral sclerosis. Neurobiol Dis. 2020;136:104743.
41. Bernardini C, Censi F, Lattanzi W, Barba M, Calcagnini G, Giuliani A, et al. Mitochondrial network genes in the skeletal muscle of amyotrophic lateral sclerosis patients. PLoS ONE. 2013;8:e57739.
42. Yanagi KS, Wu Z, Amaya J, Chapkis N, Duffy AM, Hajdarovic KH, et al. Meta-analysis of genetic modifiers reveals candidate dysregulated pathways in amyotrophic lateral sclerosis. Neuroscience. 2019;396:A3–20.
43. Wobst HJ, Mack KL, Brown DG, Brandon NJ, Shorter J. The clinical trial landscape in amyotrophic lateral sclerosis—Past, present, and future. Med Res Rev. 2020;40:1352–84.
44. Guo Y, Huang Q, Zheng J, Hsueh CY, Huang J, Yuan X, et al. Diagnostic significance of downregulated circMORC3 as a molecular biomarker of hypopharyngeal squamous cell carcinoma: A pilot study. Cancer Manag Res. 2020;12:43–9.
45. Ichimura Y, Matsushita T, Hamaguchi Y, Kaji K, Hasegawa M, Tanino Y, et al. Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy. Ann Rheum Dis. 2012;71:710–3.
46. Wei C, Peng D, Jing B, Wang B, Li Z, Yu R, et al. A novel protein SPECC1-415aa encoded by N6-methyladenosine modified circSPECC1 regulates the sensitivity of glioblastoma to TMZ. Cell Mol Biol Lett. 2024;29:127.

47. Lan P, Li M, Wang Y, Wang J, Li L, Zhang S, et al. Y-box protein-1 modulates circSPECC1 to promote glioma tumorigenesis via miR-615-5p/HIP1/AKT axis. Acta Biochim Biophys Sin (Shanghai). 2023;55:1902–12.
48. Bottero V, Santiago JA, Quinn JP, Potashkin JA. Key disease mechanisms linked to amyotrophic lateral sclerosis in spinal cord motor neurons. Front Mol Neurosci. 2022;15.
49. Cheon S, Culver AM, Bagnell AM, Ritchie FD, Vacharasin JM, McCord MM, et al. Counteracting epigenetic mechanisms regulate the structural development of neuronal circuitry in human neurons. Mol Psychiatry. 2022;27:2291–303.
50. Castiglioni I, Caccia R, Garcia-Manteiga JM, Ferri G, Caretti G, Molineris I et al. The trithorax protein Ash1L promotes myoblast fusion by activating Cdon expression. Nat Commun. 2018;9.
51. Karedath T, Ahmed I, Al Ameri W, Al-Dasim FM, Andrews SS, Samuel S, et al. Silencing of ANKRD12 circrna induces molecular and functional changes associated with invasive phenotypes. BMC Cancer. 2019;19:565.
52. Ravanidis S, Bougea A, Karampatsi D, Papagiannakis N, Maniati M, Stefanis L, et al. Differentially expressed circular RNAs in peripheral blood mononuclear cells of patients with Parkinson's disease. Mov Disord. 2021;36:1170–9.
53. Prudencio M, Belzil Vv, Batra R, Ross CA, Gendron TF, Pregent LJ, et al. Distinct brain transcriptome profiles in C9orf72-associated and sporadic ALS. Nat Neurosci. 2015;18:1175–82.
54. Wang S, Cheng Z, Chen X, Lu G, Zhu X, Xu G. CircUBXN7 mitigates H/R-induced cell apoptosis and inflammatory response through the miR-622-MCL1 axis. Am J Transl Res. 2021;13:8711–27.
55. Feng SY, Lin H, Che CH, Huang HP, Liu CY, Zou ZY. Phenotype of VCP mutations in Chinese amyotrophic lateral sclerosis patients. Front Neurol. 2022;13:790082.
56. Wu Z, Yuan J, Li J, Du Z, Yin M, Cheng X, et al. Hsa_circ_0008870 suppresses bone formation of growth plate through Inhibition of miR-185-3p/ MAPK1 axis in idiopathic short stature. Front Bioeng Biotechnol. 2022;10:1022830.
57. Sahu R, Upadhayay S, Mehan S. Inhibition of extracellular regulated kinase (ERK)-1/2 signaling pathway in the prevention of ALS: target inhibitors and influences on neurological dysfunctions. Eur J Cell Biol. 2021;100:151179.
58. Wang R, Xu X, Hao Z, Zhang S, Wu D, Sun H, et al. Poly-PR in C9ORF72-Related amyotrophic lateral sclerosis/frontotemporal dementia causes neurotoxicity by Clathrin-Dependent endocytosis. Neurosci Bull. 2019;35:889–900.
59. Franklin JP, Cooper-Knock J, Baheerathan A, Moll T, Männikkö R, Heverin M, et al. Concurrent sodium channelopathies and amyotrophic lateral sclerosis supports shared pathogenesis. Amyotroph Lateral Scler Frontotemporal Degener. 2020;21:627–30.
60. Yang Y, Gao X, Zhang M, Yan S, Sun C, Xiao F, et al. Novel role of FBXW7 circular RNA in repressing glioma tumorigenesis. J Natl Cancer Inst. 2018;110:304–15.
61. Hanan M, Simchovitz A, Yayon N, Vaknine S, Cohen-fultheim R, Karmon M, et al. A Parkinson's disease circRNAs resource reveals a link between circSLC8A1 and oxidative stress. EMBO Mol Med. 2020;12:e11942.
62. Song C, Zhang Y, Huang W, Shi J, Huang Q, Jiang M, et al. Circular RNA Cwc27 contributes to Alzheimer's disease pathogenesis by repressing Pur-α activity. Cell Death Differ. 2022;29:393–406.
63. Costa J, Streich L, Pinto S, Pronto-Laborinho A, Nimtz M, Conradt HS, et al. Exploring cerebrospinal fluid IgG N-Glycosylation as potential biomarker for amyotrophic lateral sclerosis. Mol Neurobiol. 2019;56:5729–39.
64. Yan J, Yang Y, Fan X, Tang Y, Tang Z. Sp1-mediated circrna circHipk2 regulates myogenesis by targeting ribosomal protein Rpl7. Genes (Basel). 2021;12:696.
65. Huang R, Zhang Y, Han B, Bai Y, Zhou R, Gan G, et al. Circular RNA HIPK2 regulates astrocyte activation via Cooperation of autophagy and ER stress by targeting MIR124–2HG. Autophagy. 2017;13:1722–41.
66. Zhang Y-J, Zhu W-K, Qi F-Y, Che F-Y. CircHIPK3 promotes neuroinflammation through regulation of the miR-124-3p/STAT3/NLRP3 signaling pathway in Parkinson's disease. Adv Clin Experimental Med. 2022;32:315–29.
67. Yao R, Yao Y, Li C, Li X, Ni W, Quan R, et al. Circ-HIPK3 plays an active role in regulating myoblast differentiation. Int J Biol Macromol. 2020;155:1432–9.
68. Zhao J, Zhou Y, Guo M, Guo M, Yue D, Yue D, et al. MicroRNA-7: expression and function in brain physiological and pathological processes. Cell Biosci. 2020;10:77.
69. Shoichet SA, Waibel S, Endruhn S, Sperfeld AD, Vorwerk B, Müller I, et al. Identification of candidate genes for sporadic amyotrophic lateral sclerosis by array comparative genomic hybridization. Amyotroph Lateral Scler. 2009;10:162–7.
70. Zhang Z, Zhang T, Feng R, Huang H, Xia T, Sun C. circARF3 alleviates Mitophagy-Mediated inflammation by targeting miR-103 / TRAF3 in mouse adipose tissue. Mol Therapy: Nucleic Acid. 2019;14:192–203.
71. Madruga E, Maestro I, Martínez A. Mitophagy modulation, a new player in the race against ALS. Int J Mol Sci. 2021;22:740.
72. Xue YC, Ng CS, Xiang P, Liu H, Zhang K, Mohamud Y, et al. Dysregulation of RNA-Binding proteins in amyotrophic lateral sclerosis. Front Mol Neurosci. 2020;13:78.
73. Galbiati M, Crippa V, Rusmini P, Cristofani R, Cicardi ME, Giorgetti E, et al. ALS-related misfolded protein management in motor neurons and muscle cells. Neurochem Int. 2014;79:70–8.
74. Crippa V, Galbiati M, Boncoraglio A, Rusmini P, Onesto E, Giorgetti E, et al. Motoneuronal and muscle-selective removal of ALS-related misfolded proteins. Biochem Soc Trans. 2013;41:1598–604.
75. Onesto E, Rusmini P, Crippa V, Ferri N, Zito A, Galbiati M, et al. Muscle cells and motoneurons differentially remove mutant SOD1 causing Familial amyotrophic lateral sclerosis. J Neurochem. 2011;118:266–80.
76. le Gall L, Duddy WJ, Martinat C, Mariot V, Connolly O, Milla V, et al. Muscle cells of sporadic amyotrophic lateral sclerosis patients secrete neurotoxic vesicles. J Cachexia Sarcopenia Muscle. 2022;13:1385–402.
77. Šušnjar U, Škrabar N, Brown AL, Abbassi Y, Phatnani H, Phatnani H, et al. Cell environment shapes TDP-43 function with implications in neuronal and muscle disease. Commun Biol. 2022;5:314.
78. McMillan M, Gomez N, Bekier M, Li X, Tank MR. RNA methylation influences TDP43 binding and disease pathogenesis in models of amyotrophic lateral sclerosis and frontotemporal dementia. Mol Cell. 2023;83:219–e2367.
79. Ziff OJ, Neeves J, Mitchell J, Tyzack G, Martinez-Ruiz C, Luisier R et al. Integrated transcriptome landscape of ALS identifies genome instability linked to TDP-43 pathology. Nat Commun. 2023;14.
80. Liu CX, Li X, Nan F, Jiang S, Gao X, Guo SK, et al. Structure and degradation of circular RNAs regulate PKR activation in innate immunity. Cell. 2019;177:865–e88021.
81. Hu JH, Zhang H, Wagey R, Krieger C, Pelech SL. Protein kinase and protein phosphatase expression in amyotrophic lateral sclerosis spinal cord. J Neurochem. 2003;85:432–42.
82. Zu T, Guo S, Bardhi O, Ryskamp DA, Li J, Tusi SK, et al. Metformin inhibits RAN translation through PKR pathway and mitigates disease in C9orf72 ALS/FTD mice. Proc Natl Acad Sci U S A. 2020;117:18591–9.
83. Qin C, Liu C, Yang bin, Gao DG, Zhang F, Zhang X. C, Circular RNA expression alteration and bioinformatics analysis in rats after traumatic spinal cord injury. Front Mol Neurosci. 2019;11.
84. Wu R, Mao S, Wang Y, Zhou S, Liu Y, Liu M et al. Differential circular RNA expression profiles following spinal cord injury in rats: A Temporal and experimental analysis. Front Neurosci. 2019;13.
85. Mahmoudi E, Fitzsimmons C, Geaghan MP, Shannon Weickert C, Atkins JR, Wang X, et al. Circular RNA biogenesis is decreased in postmortem cortical Gray matter in schizophrenia and May alter the bioavailability of associated MiRNA. Neuropsychopharmacology. 2019;44:1043–54.
86. He L, Qiu L, Chen F, Chen T, Peng F, Li Z, et al. Dysregulation of global circular RNA abundance regulated by spliceosomes predicts prognosis in hepatocellular carcinoma. Hepatol Commun. 2022;6:3578–91.
87. Yan L, Chen YG. Circular RNAs in immune response and viral infection. Trends Biochem Sci. 2020;45:1022–34.
88. Miana-Mena FJ, Muñoz MJ, Yagüe G, Mendez M, Moreno M, Ciriza J, et al. Optimal methods to characterize the G93A mouse model of ALS. Amyotroph Lateral Scler Other Motor Neuron Disorders. 2005;6:55–62.
89. Yuan H, Liao X, Hu D, Guan D, Tian M. Back to the origin: mechanisms of circRNA-Directed regulation of host genes in human disease. Noncoding RNA. Multidisciplinary Digital Publishing Institute (MDPI); 2024.
90. Shao T, Pan Y, hong, Xiong X. dong. Circular RNA: an important player with multiple facets to regulate its parental gene expression. Mol Ther Nucleic Acids. Cell Press; 2021;369–76.
91. Moreno-García L, López-Royo T, Calvo AC, Toivonen JM, de la Torre M, Moreno-Martínez L, et al. Competing endogenous RNA networks as biomarkers in neurodegenerative diseases. Int J Mol Sci. 2020;21:1–42.
92. Lee S, Shang Y, Redmond SA, Urisman A, Tang AA, Li KH, et al. Activation of HIPK2 promotes ER Stress-Mediated neurodegeneration in amyotrophic lateral sclerosis. Neuron. 2016;91:41–55.
93. Gruner H, Cortés-López M, Cooper DA, Bauer M, Miura P. CircRNA accumulation in the aging mouse brain. Sci Rep. 2016;6.
94. Niu R, Liu J. Circular RNA involvement in aging and longevity. Curr Genomics. 2022;23:318–25.
95. Abdelmohsen K, Panda AC, De S, Grammatikakis I, Kim J, Ding J, et al. Circular RNAs in monkey muscle: Age-dependent changes. Aging. 2015;7:903–10.

96. Hosaka T, Tsuji H, Kwak S. Roles of aging, circular RNAs, and RNA editing in the pathogenesis of amyotrophic lateral sclerosis: potential biomarkers and therapeutic targets. Cells. 2023;12:1443.
97. Oros D, Strunk M, Breton P, Paules C, Benito R, Moreno E, et al. Altered gene expression in human placenta after suspected preterm labour. Placenta. 2017;55:21–8.
98. Zhang XO, Wang H, Bin, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. Cell. 2014;159:134–47.
99. Love MI, Huber W, Anders S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15.
100. Calvo AC, Moreno-Igoa M, Manzano R, Ordovás L, Yagüe G, Oliván S, et al. Determination of protein and RNA expression levels of common housekeeping genes in a mouse model of neurodegeneration. Proteomics. 2008;8:4338–43.

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